Presentation Abstracts

138 GRAFT PRECONDITIONING WITH LOW-DOSE TACROLIMUS (FK506) AND NITRIC OXIDE INHIBITOR (AGH) REDUCES ACUTE REJECTION AFTER LIVER TRANSPLANTATION IN THE RAT

*E. Matevossian, H.D. Kern, N. Hueser; Klinikum rechts der Isar, Chirurgische Klinik und Poliklinik, Transplantation, München, Deutschland

Background/aim: The acute rejection (AR) is a main cause of primary dysfunction or non-function after liver transplantation (LTx). Recent evidence indicates that an increase in nitric oxide (NO) production after LTx is associated with AR. The aim of this study was to demonstrate that low-dose FK506 in combination with aminoguanidine (AGH), which leads to a reduction of NO levels, has a protective effect by reducing AR associated injury after LTx.
Materials and methods: Fortyone DA-(RT1av1) rats served as donors and recipients for allogeneic orthotopic arterialised LTx. They were divided into 3 groups: controls without pre-/treatment (I), pre-/treatment with AGH only (II), and pre-/treatment with low-dose FK506 in combination with AGH (III). After LTx the laboratory parameters and liver biopsy were performed.
Results: The levels of NO in groups I and II were significantly higher on day 10 after LTx compared to group III ($P = 0.001$, $P = 0.001$, *Fig. 1*). In group III the AR-associated liver necrosis rate was reduced significantly *(Fig. 2, 3a-c)*.
Conclusion: Our results demonstrated that a combined dual pharmacological pretreatment (group III) reduced AR of the graft after LTx in a rat model.

0147 DIFFERENCES BETWEEN BIG AND SMALL ISLETS OR WHY DOES SIZE MATTER?

*A. Steffen[1], A. Müller[2], S. Bornstein[1], M. Solimena[2], B. Ludwig[1]; [1]Universitätsklinikum Dresden, Medizinische Klinik III, Dresden, Deutschland; [2]Technische Universität Dresden, Molecular Diabetology, Dresden, Deutschland

Introduction: Hypoxia is a major factor limiting islet engraftment in the early post transplant period. Recent studies showed the relationship between islet size and function, suggesting that islets with smaller diameter and therefore smaller diffusion distance are superior to large islets regarding insulin secretion and survival rate. Our aim was to investigate the differences in small and large islets with regard to insulin biosynthesis, conversion of proinsulin to insulin, and secretion upon glucose stimulation.
Methods: Using COPAS (Complex Object Parametric Analyser and Sorter) isolated human islets were automatically sorted based on time of flight (TOF)relative to particle size in groups of small (<150 μm) and large (>150 μm) islets. Insulin and proinsulin secretion following glucose stimulation was analyzed by RIA. SDS page was performed using antibodies against PC1/3, PC2 and γ-tubulin. For electron microscopy, islets were chemically fixed and processed according to standard procedures.
Results: Insulin secretion was significantly different between large and small islets adjusted for islet particle number. Small islets released ~4 fold the amount of insulin compared with large islets. Following stimulation total insulin was increased in both groups. In contrast, total proinsulin was increased significantly in large islets compared to small islets. The insulin/proinsulin ration was significantly higher in smaller islets compared to large islets, suggesting a higher conversion rate from insulin to proinsulin in small islets, possibly due to the expression levels and/or activity of prohormone convertases (PCs). To test this hypothesis PC1/3 and PC2 were analyzed by western blotting. Both PCs appeared to be over-expressed in small islets compared to large islets. Electron microscopy studies showed that insulin granules of small islets display a pronounced halo phenomenon, which results from the crystallisation of insulin. In contrast, high proinsulin content prevents insulin crystallization. We observed a less pronounced halo in large islet granules consistent with their higher proinsulin rate and lower PC content.
Conclusion: Our data suggest a privilege of small islets in regard to shorter diffusion distance resulting in superior oxygen supply and insulin secretion upon glucose challenge. The conversion rate of proinsulin into insulin seems more effective in smaller islets compared to large islets, mediated by different levels of PC expression. Thus, putting more emphasis on islet size than islet volume might result in a higher survival rate and better islet transplantation outcome.

0154 FIBRIN-BASED ENGINEERED HEART TISSUE (FBEHT): A POTENTIAL FUTURE STRATEGY TOWARDS CARDIAC REGENERATION?

*L. Conradi[1], A. Hansen[2], A. Eder[2], M. Hirt[2], S. Schaaf[2], A. Reinsch[3], H. Reichenspurner[3], T. Eschenhagen[2]; [1]Universitäres Herzzentrum Hamburg, Klinik und Poliklinik für Kardiologie, Hamburg, Deutschland; [2]Universitätsklinikum Hamburg-Eppendorf, Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Hamburg, Deutschland; [3]Universitäres Herzzentrum Hamburg, Klinik und Poliklinik für Herz- und Gefäßchirurgie, Hamburg, Deutschland

Objectives: We have previously shown that force-generating collagen-based EHTs can be constructed in-vitro. Recently, the manufacturing process has been adapted to replace collagen I with fibrin resulting in more homogenous distribution of myocytes and standardization of the system. We sought to evaluate the potential of FBEHTs as future regenerative treatment option for diseased myocardium.
Methods: EHTs were generated from neonatal rat cardiomyocytes in a fibrin-based matrix and cast between two flexible silicone posts fitting 6- or 24-well-format. For implantation purposes, the geometry of EHTs was altered to obtain larger constructs. After 14–20 days in culture, EHTs were implanted onto the left-ventricular myocardium of Wistar rats. Hearts were harvested 3–28 days later for further analyses.
Results: Spontaneous contractions of the EHTs were observed after 3–5 days in culture. Contractile forces increased over the next 10–15 days resulting in coherently beating constructs. Histologically, EHTs displayed a differentiated cardiac phenotype exhibiting cross-striated α-sarcomeric-actinin and lectin-positive capillary-like structures. EHTs proved to be robust enough to be sutured onto rat hearts, covering approximately 2/3 of the anterolateral wall of the left ventricle. Upon explantation, EHTs were clearly distinguishable on the epicardial surface. Histological analyses revealed strong vascularization. Cells of a cardiac phenotype survived inside implanted EHTs for up to 4 weeks, but exhibited a lower degree of cardiac differentiation than native myocardium.
Conclusions: Preliminary data show that FBEHTs can be generated in size suitable for engraftment onto rat hearts. Further investigations are currently under way, addressing issues like cell-survival and maturation or electromechanical integration.

0193 ADULT LIVING DONOR LIVER TRANSPLANTATION (LDLT) WITHIN THE MELD PERIOD IN GERMANY. A MULTICENTERED RETROSPECTIVE ANALYSIS

*U. Settmacher[1], M. Götz[1], E. Baerthel[1], A. Rahmel[2]; [1]Universitätsklinikum Jena, Klinik für Allgemein-, Viszeral- und Gefässchirurgie, Jena, Deutschland; [2]Eurotransplant Foundation, Leiden, Niederlande

Objective: The aim of this analysis is to provide an update on the current trend of LDLT within the MELD period in Germany and to encourage to a broader implementation of LDLT in the transplantation process.
Method: We reviewed the data of LDLT in Germany from 15th December 2006 to 31st December 2009 by means of a multicentered retrospective analysis. The data were provided by Eurotransplant via a questionnaire that we previously developed for this purpose. The data were analyzed descriptively and with the Kaplan-Meier method.
Result: In Germany, there are 10 centres applying the LDLT program. 85 adult LDLTs were performed throughout Germany during the review period, ranging from 1 to 16 LDLTs per centre. We did not receive data of 15 patients, conclusively we analyzed 70 patients: 45 male (64.3%), 25 female (35.7 %). The mean (±SD) recipient age was 48.8 (±14.5) years. 52 of 70 suffered from liver cirrhosis without malignancy (74.3%) and 18 from cirrhosis with an associated malignancy (25.7%). 13 patients had a hepatocellular carcinoma (72.2%), 6 of them within the Milan criteria (33.3%), 7 beyond (38.9%) and 5 showed other malignancies (27.8%). The mean LAB MELD score was 15 (±8). The median time on the waiting list came to 67.5 [10.5–216.5] days. 6 retransplantations were performed (8.6%). 14 of 70 patients deceased.
Conclusion: LDLT is an established treatment option which may reduce waiting time, provide better timing and better organ quality compared to deceased donor liver transplantation.

0270 TREATMENT OF BRAIN DEAD DONORS WITH ANTI-IL-6R MONOCLONAL ANTIBODY ATTENUATES THE EARLY AND LATE INFLAMMATORY IMMUNE RESPONSE

*A. Reutzel-Selke[1], F. Beiglböck[1], M.P. Velikova[1], M. Marksteiner[1], D. Polenz[1], P. Neuhaus[1], K. Kotsch[2], J. Pratschke[1,3]; [1]Charité, Virchow-Klinikum, Universitätsmedizin Berlin, Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Berlin, Oesterreich; [2]Charité, Campus Mitte, Universitätsmedizin Berlin, Institut für Medizinische Immunologie, Berlin, Deutschland; [3]Medizinische Universität Innsbruck, Univ.-Klinik für Visceral-, Transplantations- und Thoraxchirurgie, Innsbruck, Oesterreich

Objective: We evaluated the efficacy of a single donor pretreatment with an anti-IL-6 receptor (IL-6R/CD126) mAb to attenuate brain death associated graft injury.
Methods: Kidneys from brain dead (BD) and living donor (LD) F-344 rats were transplanted into Lewis recipients. Brain death was induced 6 hour before transplantation (Tx). Both BD and LD were treated with anti-IL-6R mAb (500 μg/rat, iv; -5h pre Tx). Untreated donor grafts served as controls (con). The early (18 hour, d7) and late (5 months) immune response was analysed by flow cytometry, immunohistochemistry, real-time RT-PCR, and ELISA.
Results: IL-6R mAb significantly reduced the early and late intragraft IL-6, IFN-γ, and TNF-α expression in BD grafts ($P < 0.05$ vs. BD con), whereas treatment of LD had no effect or even promoted the expression of inflammatory cytokines. The number of intragraft APCs (OX62+ DC, CD45RA+ B cells,

ED1+ monocytes/macrophages) was significantly elevated in BD grafts early after Tx ($P < 0.05$ vs. LD). IL-6R mAb reduced the number of APCs in BD ($P < 0.05$ vs. BD con), but not in LD grafts. The number of NK cells was significantly higher in the BD group ($P < 0.01$ vs. LD). While NK cell counts were markedly reduced in the treated BD group ($P < 0.0001$ vs. BD con), donor treatment caused an increase of the NK cell frequency in the LD group ($P < 0.05$ vs. LD con). However, after 5 months proteinuria had increased in both, recipients of treated and untreated BD donor grafts ($P < 0.05$ vs. LD), thus indicating that a single donor treatment might be not sufficient to improve the long-term function of BD donor grafts.

Conclusion: Donor treatment with anti-IL-6R mAb attenuates the inflammatory immune response in recipients of grafts from BD donors, while recipients of LD grafts do not seem to benefit.

LONGTERM OUTCOME AFTER TRANSPLANTATION

0011 LOW PRETRANSPLANT SERUM ADIPONECTIN LEVELS ARE ASSOCIATED WITH ADVERSE ALLOGRAFT OUTCOMES IN KIDNEY TRANSPLANT RECIPIENTS

M. Roos[1], M. Baumann[1], D. Liu[1], P. Horn[2], F. Heinemann[2], M. Lindemann[2], J. Lutz[1], U. Heemann[1], O. Witzke[3], M. von Eynatten[1]; [1]Klinikum rechts der Isar, Nephrologie, München, Deutschland; [2]Universität Essen, Tranfsuionsmedizin, Essen, Deutschland; [3]Universität Essen, Nephrologie, Essen, Deutschland

Background: In kidney transplant recipients endothelial dysfunction is an almost universal risk factor for allograft failure. Adiponectin is a adipocyte derived hormone, circulating in different multimers. The high-molecular weight (HMW) form is believed to be the active form, exerting anti-inflammatory and anti-apoptotic properties on endothelial cells. This studied evaluated, whether low pretransplant adiponectin may reflect a proinflammatory milieu in patients undergoing kidney transplantation, thereby potentially influencing long-term graft survival.

Methods: In 206 renal transplant recipients pretransplant total- and HMW adiponectin levels were retrospectively measured in serum by ELISA and Western blot, respectively. The 24 months follow up revealed a 94% patient surival (13 patients died); 18 patients had graft failures within 2 years posttransplantation.

Results: Pretransplant total- and HMW adiponectin levels correlated with systemic inflammation, cardiovascular risk factors and markers of glucose metabolism at baseline. After 2 years follow-up pretransplant adiponectin levels were significantly inversely associated with the incidence of allograft loss (adiponectin: r = -0.216; $P = 0.002$: HMW: r = -0.218; $P = 0.002$). In multivariable adjusted Cox proportional hazard regression models patients in the lowest total- and HMW adiponectin quartile had a significant increased risk for allograft failure: Hazard ratio [95%CI]: 4.25 [1.27–14.24; $P = 0.019$], and 3.35 [1.04–10.76; $P = 0.042$], respectively.

Conclusion: Low circulating levels of total- and HMW adiponectin are indepenedently asspociated with an increased risk of allograft loss in kidney-transplanted patients. Measurement of pretransplant adiponectin levels may identify patients at risk for adverse allograft outcomes after kidney transplantation.

0019 SUSTAINED RENAL IMPROVEMENT TO MMF AND CNI TAPER PROMOTES LONG-TERM SURVIVAL IN LIVER TRANSPLANT PATIENTS WITH CNI-RELATED NEPHROPATHY

A. Kornberg[1], K. Thrum[2], B. Küpper[2], B. Krause[3], P. Büchler[1], A. Altendorf-Hofmann[3], H. Friess[1], J. Wilberg[3]; [1]TU München, Klinik für Chirurgie, München, Deutschland; [2]Klinikum Bad Berka, Chirurgie, Bad Berka, Deutschland; [3]Friedrich-Schiller-Universität, Jena, Deutschland

Background: Conversion from a calcineurin-inhibitor (CNI) based immunosuppressive regimen to mycophenolate mofetil (MMF) with subsequent CNI dose reduction may result in short-term renal function improvement in liver transplant patients with CNI-related nephropathy. There is, however, no data about long-term efficacy on kidney function and patient survival.

Methods: In 63 liver recipients with increased serum creatinine levels, 2 g MMF per day was administered, followed by a significant CNI dose reduction. Renal function was assessed by determination of serum creatinine levels and creatinine clearance (CCl). MPA trough levels were continuously determined. The impact of clinical parameters on long-term survival post-conversion was analysed by uni- and multivariate analysis.

Results: Follow-up after conversion to MMF and CNI taper ranges between 26 and 123 months (median: 85 months). There was no case of allograft rejection after switch to MMF. At 60 months post-conversion, mean creatinine level has significantly decreased from 197.1 ± 59.3 mmol/L at conversion to 160.1 ± 76.1 mmol/L, and mean creatinine clearance has significantly increased from 38.7 ± 13.4 ml/min at conversion to 47.9 ± 21.1 ml/minutes ($P < 0.001$). Forty-six patients (73.1%) demonstrated sustained renal function improvement after 5 years, while 9 patients suffered from progressive nephropathy and 8 liver recipients have died before. MPA trough levels were significantly higher in renal responders, when compared to renal non-responders ($P < 0.001$). Only full-dose (2g) MMF medication (HR = 18.6; $P = 0.006$) and the early posttransplant (≤24 months) conversion to MMF and CNI reduction (HR = 13.2, $P = 0.02$) were identified as independent predictors of sustained renal improvement. Persistent renal response to modified immunosuppression was identified as the only independent predictor of long-term survival post-conversion (HR = 7.8; $P = 0.001$). In patients with sustained renal response, 5-year survival rate post-conversion was significantly higher (93.9%) than in patients without kidney recovery (64.3%, $P < 0.001$).

Conclusion: Sustained renal response to MMF and CNI taper is an independent promoter of patient long-term survival in liver transplant patients with CNI-related nephropathy. It may be achieved by an early posttransplant conversion to full-dose MMF treatment.

0083 FULL DOSE MYCOPHENOLATE MOFETIL (CELLCEPT®) WITH LOW DOSE TACROLIMUS OPTIMIZES RENAL FUNCTION IN LONG TERM RENAL TRANSPLANT PATIENTS; RESULTS OF THE TRANCEPT STAY STUDY

U. Heemann[1], V. Kliem[2], A. Hamza[3], K. Budde[4], F.J. Juarez de la Cruz[5], W. Arns[6], J.S. Jürgensen[7], H. Haller[8]; [1]Klinikum rechts der Isar II. Medizinische Klinik und Poliklinik, Nephrologie, München, Deutschland; [2]Nephrologisches Zentrum Niedersachsen, Transplantationszentrum, Hann. Münden, Deutschland; [3]Universitätsklinikum Halle, Klinik u.Poliklinik f.Urologie, Halle, Deutschland; [4]CAMPUS CHARITÉ MITTE, CharitéCentrum 12 Med.Poliklinik, Berlin, Deutschland; [5]Hospital Angeles de Torreon, Torreon, Mexico; [6]Krankenhaus Merheim, Medizinische Klinik I, Köln, Deutschland; [7]Charité - Universitätsmedizin Berlin, Berlin, Deutschland; [8]Kliniken d.Med.Hochschule, Zentrum Innere Medizin Abt.Nephrologie, Hannover, Deutschland

Introduction: In recent years, large prospective studies have demonstrated the benefit of combining mycophenolate mofetil (MMF) and low dose tacrolimus on GFR of patients during the first year after renal transplantation. Data on MMF+ tacrolimus combination for patients beyond the first year of transplantation are more sparse. We analyzed data from the international TranCept STAY observational study on renal transplant patients to assess the effect of MMF+ tacrolimus dose combinations on GFR 6 months to several years after transplantation.

Methods: Renal allograft recipients more than 6 months post transplant, with GFR>20 ml/minutes, receiving MMF from the time of transplantation were enrolled into the TranCept STAY study and followed for 4 years. For this analysis GFR, calculated with the abbreviated MDRD formula, was correlated with doses of MMF and CNI recorded at study enrollment in a multivariate regression. The analyses were performed on all patients as well as a subpopulation more than a year after transplantation at observational study entry.

Results: A total of 2040 patients were enrolled into the STAY cohort (median enrollment 1.51 years after transplantation). Of these, 1173 (58%) were enrolled after the first year following transplantation (median time after transplantation to enrollment 3.44 years, maximum 15.9 years). 987were treated with ciclosporin (CSA), 921 with tacrolimus. At enrollment, average CsA and tacrolimus doses were 210 ± 88 mg/day and 5.3 ± 3.6 mg/day respectively. Average dose of MMF was 1482 ± 511 mg/day with concomitant CSA, and 1270 ± 504 mg/day with tacrolimus. In CsA+ MMF patients, no significant correlation between GFR and CsA weight normalized dose or between GFR and MMF dose was observed. However, there was a highly significant positive correlation between the GFR and MMF dose with concomitant tacrolimus (coeff. ± standard error. = +7.3 ± 1.32 ml/minutes/g, $P < 0.001$) and a significant negative correlation between weight normalized tacrolimus dose (coeff. -33 ± 14.75 ml/minutes/mg/kg, $P = 0.025$) for the whole population. This effect on GFR was also prominent in the subgroup with late post-transplant enrollment (median = 3.44 years) into TranCept (figure). MMF dose correlation to GFR in this subgroup was positive (coeff. + 9.9 ± 1.76, $P < 0.001$) while the effect of normalized tacrolimus dose was negative (coeff. -53 ± 23.2, $P = 0.022$).

Conclusion: Combination of full dose MMF with low dose tacrolimus may benefit GFR years after transplantation.

0103 HYPERLIPIDEMIA, AN ADJUSTABLE RISK FACTOR AFTER CARDIAC TRANSPLANTATION: ANALYSIS OF 1007 HEART TRANSPLANT RECIPIENTS

H.B. Lehmkuhl[1], C. Bara[2], A. Karpov[3], H. Schwende[3], A. Zuckermann[4], G. Junge[3]; [1]Deutsches Herzzentrum Berlin, Berlin, Deutschland; [2]Medizinische Hochschule Hannover, Hannover, Deutschland; [3]Novartis Pharma AG, Basel, Schweiz; [4]Universitätsklinik für Chirurgie, Vienna, Oesterreich

Introduction: Hyperlipidemia is a well known risk factor for cardiovascular events in the general population. However, there is limited data evaluating the impact of immunosuppression-related hyperlipidemia in heart transplant recipients (HTxR). We retrospectively evaluated the impact of six different immunosuppressive regimens (ISR) on lipid patterns, also accounting for the use of lipid modifying agents (LMA).

Methods: HTxR (safety population, N = 1007) from 3 randomized, multicenter studies were exposed to one of the following ISR: SD-CsA/AZA (N = 214); SD-CsA/MMF (N = 83); SD-CsA/h-EVR (N = 211); SD-CsA/l-EVR ($n = 209$); SD-CsA/TDM-EVR (N = 100); RD-CsA/TDM-EVR (pooled from two studies; N = 190) [SD = standard dose; RD = reduced dose; h-EVR = fixed dose 3.0 mg/day; l-EVR = fixed dose 1.5 mg/day; TDM-EVR = Co 3–8 ng/mL]. Lipid panels (TC, TG, LDL, HDL, TC/HDL ratio), use of LMA and specific risk factors were followed and analyzed for 6 and 12 months post HTx.

Results: Demographic and Tx specific basline (BL) characteristics were comparable across groups. Percentage of patients taking LMA at BL ranged from 27.3%–37.3% and increased to >70% in all groups at M6. Retrospective categorical analysis showed highest percentage of HTxR in the TC > 240 mg/dL category for SD-CsA/h-EVR (40.8%) and l-EVR (38.1%) compared to AZA (21.7%), MMF (11.1%), SD-CsA/TDM-EVR (26.6%), and RD-CsA/TDM-EVR (33.8%). Similar pattern was seen for categorical TG > 200 mg/dL and LDL > 130 mg/dL: h-EVR (60.9%; 41.3%), l-EVR (57.4%; 31.5%) compared to AZA (29.5%; 27.1%), MMF (19.0%; 27.0%) and SD-CsA/TDM-EVR (40.7%; 39.3%), and RD-CsA/TDM-EVR (43.0%; 39.7%). For categorical HDL > 60 mg/dL and TC/HDL <4 different distributions were observed (Table). Full categorical analyses and risk factors will be presented.

Conclusion: Hyperlipidemia is a known side effect of mTOR based immunosuppression. High exposure EVR in combination with SD-CsA showed the highest incidence of patients with increased lipid values. Consequently, reduction of both, EVR- and CsA exposure, faciltated by TDM-EVR, markedly improved the lipid profile in EVR-treated HTxR. Finally, TDM-EVR based immunosuppression showed better TC/HDL profiles compared to fixed dose EVR in combination with SD-CsA.

0166 ONE YEAR RESULTS OF AN INTENSIFIED DOSING OF ENTERIC-COATED MYCOPHENOLATE SODIUM IN RENAL TRANSPLANT PATIENTS RESULTS IN IMPROVED EFFICACY WITHOUT COMPROMISING SAFETY

C. Sommerer[1], W. Arns[2], P. Glander[3], T. Ariatabar[2], S. Kramer[4], E.-M. Vogel[4], M. Zeier[1], K. Budde[3]; [1]Nierenzentrum, University Hospital, Heidelberg, Deutschland; [2]Transplantationszentrum, Dept. of Nephrology, Köln-Merheim, Deutschland; [3]University Hospital Charité, Berlin, Deutschland; [4]Novartis Pharma, Nürnberg, Deutschland

Objective: We examined the influence of an intensified dosing (ID) regimen with enteric-coated mycophenolate sodium (EC-MPS) in comparison to EC-MPS standard dosing (SD) on efficacy and safety 12 months after renal transplantation

Methods: De-novo kidney transplant recipients were treated with basiliximab, steroids and cyclosporine and randomized to EC-MPS standard D (SD: 1440 mg/d) or to an intensified EC-MPS dosing regimen (ID: 2 weeks: 2880 mg/d; subsequent 4 weeks: 2160 mg/d; followed by 1440 mg/d). After completion of the core study at month 6, patients were included in an observational follow-up until month 12.

Results: One hundred and twenty-eight patients were randomized to either SD ($n = 65$) or ID ($n = 63$), 101 (78.9%) pts ($n = 49$ ID vs. $n = 52$ SD) completed the follow-up analysis at month 12. The incidence of BPAR was lower in the intensified compared to the standard group (SD $n = 12$ (23%) vs. ID $n = 3$ (6.1%); $P < 0.05$) from transplantation to month 12 with one additional BPAR in ID vs 2 BPAR in SD pts in the follow-up period. Patient survival was 98.0% in the ID and 96.2% in the SD group, and 3 graft losses were observed in the ID and 5 graft losses in the SD group. In the follow-up period (month 6 to month 12) the ID regimen was not associated with a higher rate of hematological side effects (11 ID pts vs. 15 SD pts). Slightly more infections (29 ID pts vs. 24 SD pts) and gastrointestinal symptoms (19 ID pts vs. 15 SD pts) were reported in the ID group. Important safety parameters are listed in the table.

Conclusion: An intensified dosing regimen of EC-MPS was associated with a significantly lower BPAR rate after 12 months without compromising tolerability and safety.

0171 RENAL FUNCTION OF AN EVEROLIMUS BASED THERAPY AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN MAINTENANCE RENAL TRANSPLANT RECIPIENTS ONE YEAR AFTER CONVERSION

P. Reinke[1], T. Rath[1], K. Budde[1], W. Arns[1], S. Kramer[2], E.-M. Vogel[2], F. Pietruck[1], H. Haller[1], C. Sommerer[1]; [1]APOLLO, Study Group, Deutschland; [2]Novartis Pharma, Nürnberg, Deutschland

Objective: Renal function, safety and efficacy of an Everolimus regimen was assessed 12 months after conversion to an Everolimus regimen after Calcineurin-Inhibitor (CNI) withdrawal in maintenance kidney allograft recipients.

Methods: In an open-label, randomized, controlled, multi-center study 93 renal maintenance patients with a stable renal function defined as serum creatinine <2.5 mg/dl and on a stable immunosuppressive therapy consisting of CNI, Enteric-coated mycophenolate sodium (EC-MPS) with or without corticosteroids were randomized to either continue CNI treatment (trough level (C0): Tacrolimus 5–10 ng/ml, Cyclosporine: 80–150 ng/ml) and EC-MPS ($n = 47$) or b) convert to an Everolimus (C0: 6–10 ng/ml) and EC-MPS ($n = 46$) regimen.

Results: Ninety-three pts were randomized to either Everolimus/EC-MPS or CNI/EC-MPS. In the CNI group 29 pts were treated with CsA and 17 pts received Tac (mean trough levels: CsA pts 98.0 ± 25.6 ng/mL; Tac pts 5.7 ± 2.2 ng/mL). Mean of years since the most recent transplantation was higher in the Everolimus/EC-MPS group compared to the CNI group (7.0 ± 5.2 vs. 5.8 ± 5.6). Renal function expressed as calculated GFR (Nankivell method) improved from randomization to Month 12 by 3.6 mL/ minutes / 1.73m2 in favor of the Everolimus/EC-MPS regimen (ns). The observed GFR slope from conversion to month 12 was +3.4[-0.2;+7.0] for Everolimus/EC-MPS and -0.2[-3.9;+3.5] mL/ minutes /1.73m2 for CNI/EC-MPS patients (ns). One death occurred in both groups and no graft loss or BPAR was observed in either group. The number and proportion of patients discontinued study regimen due to adverse events were 15/46 (32.6%) in the Everolimus/EC-MPS and 5/47 (10.6%) in CsA/EC-MPS group. Important safety parameters related to study medication are listed in the table.

Conclusions: The late conversion to an Everolimus/EC-MPS treatment in maintenance renal transplant patients with a stable renal function after CNI withdrawal leads to a better renal function. Overall late conversion was safe although more side effects were reported but for a complete risk/benefit assessment a long term follow-up is needed which will be attempted.

INFECTION AND PTLD AFTER TRANSPLANTATION

0050 A SINGLE DOSE OF AN ADJUVANTED INFLUENZA A H1N1 VACCINE (PANDEMRIX®) DOES NOT PROVIDE A PROTECTIVE IMMUNE RESPONSE IN THE MAJORITY OF RENAL TRANSPLANT RECIPIENTS

S. Brakemeier[1], B. Schweiger[2], P. Glander[1], F. Diekmann[1], H.-H. Neumayer[1], K. Budde[1]; [1]Charite, Nephrologie, Berlin, Deutschland; [2]RKI, Berlin, Deutschland

Introduction: In the course of the Influenza A H1N1 pandemic patients with an underlying chronical illness and especially immunocompromized patients were recommended to receive vaccination against novel Influenza A H1N1 (1). In healthy controls, between 80 and 95% of adults develop a sufficient immune response after a single vaccination with an adjuvanted split-virion formulation (2–4), thus a single vaccination is deemed appropriate and was recommended for patients under the age of 60. However no data are available for immunosuppressed patients so far, and therefore we evaluated the immune response to an adjuvanted Influenza A H1N1 vaccine (Pandemrix®) in renal transplant patients after a single vaccination.

Methods: All kidney transplant recipients in our centre who received a transplant at least 6 months ago were offered the vaccine and to participate in an observational study between November 2009 and January 2010. The study was approved by the local Ethics committee. A single dose of Pandemrix® (3.75 μg per dose, adjuvanted) was administered at day 0. After giving informed consent a total of 49 patients (9 female/40 male) aged 53 ± 14 years agreed to participate in the study. The median time after transplantation was 4.8 ± 5.7 years. 22 healthy subjects served as controls.

Results: One patient (2%) had an elevated titer before vaccination suggesting previous immunisation, although being completely asymptomatic. Of the remaining 48 patients, after a median of 21 days only 18 (37.5%) developed a titer of 1:40 or more, which is considered as a sufficient immune response. The other 30 patients (62.5%) showed no or only a weak response. In contrast to this 20 subjects (91%) of the control group developed a protective titer of 1:40 or more after 21 days. Immunosuppressive regimens consisted of mycophenolate (41/49, 83.7%) in combination with either tacrolimus (n = 15, 30.6%), cyclosporine (n = 14, 28.6%), everolimus (n = 16, 32.7%), or sirolimus (n = 3, 6.1%), with a median through level of 5.4 ng/ml, 94.5 ng/ml, 6.8 ng/ml, and 6.3 ng/ml, respectively. 23/49 (46.9%) of patients received a median dose of 4mg methylprednisolone, the remaining patients were off steroids. None of the included patients were diagnosed with Influenza A H1N1 until the end of the observation period.

Conclusion: These data suggest that in renal transplant patients a single dose of Pandemrix® is not sufficient to induce a protective immune response.

0069 RISK FACTORS FOR BK-POLYOMAVIRUS INFECTION AFTER RENAL TRANSPLANTATION AND DISCONTINUATION OF MYCOPHENOLATE AS A WAY OF TREATMENT

D. Steubl[1], M. Baumann[1], M. Fischereder[2], B. Krämer[3], U. Heemann[1], J. Lutz[1]; [1]Klinikum rechts der Isar der TU München, Abteilung für Nephrologie, München, Deutschland; [2]Medizinische Poliklinik Innenstadt, Klinikum der Universität München, Nephrologisches Zentrum, München, Deutschland; [3]Universitätsklinikum Mannheim, V. Medzinische Klinik, Mannheim, Deutschland

Background: BK-Virus (BKV)-replication in blood and urine after renal transplantation can lead to severe impairment of graft function and even graft loss. Some risk factors have been identified. Specific antiviral treatments are not well studied so far, there is no consensus of how to adapt the immunosuppressive therapy.

Patients and methods: This retrospective study was performed at three transplant centers of the university hospitals of Munich and Regensburg. We compared 57 renal transplant recipients with BKV-replication, detected through PCR performed on viral DNA in blood samples with 71 transplant recipients who had no BKV-replication detectable with this method to identify risk factors for the development of BKV-infection. Furthermore, we compared outcome and graft function in 14 patients with BKV-infection, in whom mycophenolate (MMF) was discontinued with an additional dose reduction of the remaining immunosuppressants versus 32 patients in whom MMF was maintained at a reduced dose of MMF together with also reduced dose of the other immunosuppressants.

Results: Patients with BKV-infection received significantly more often MMF ($P < 0.05$).

Conclusions: MMF and Tacrolimus seem to play a crucial role for BKV-infection after renal transplantation. Discontinuation of MMF with a reduction of the remaining immunosuppression could be an option to interfere the BKV-infection after renal transplantation. An exchange of MMF with antiviral agents could be evaluated in future studies.

0112 PROGNOSTIC FACTORS FOR AND PREVALENCE OF BK-VIRUS INFECTION IN A CAUCASIAN RENAL TRANSPLANT POPULATION

*V. Malyar[1], V. Busch[1], U. Hillebrand[1], S. Reiermann[1], K. Kliche[1], B. Breil[2], M. Dugas[2], *B. Suwelack[1]; [1]Uniklinikum Münster, Med. Klinik und Poliklinik D, Münster, Deutschland; [2]Uniklinikum Münster, Institut für Medizinische Informatik und Biomathematik, Münster, Deutschland*

Introduction: BK-virus (BKV) infection may lead to BK-virus nephropathy (BKN), which is a serious complication after renal transplantation (RTX) and may lead to allograft loss. Objective was the examination of the prevalence of BK-viremia and BKN in a Caucasian renal transplant population and the identification of risk factors associated with BKV-infection.

Methods: Single-center analysis. Patients (pts) undergoing RTX between 2005 and 2009 with a tacrolimus-based triple-drug immunosuppression (IS) aged ≥18 years with at least one assessment of serum-PCR for BKV post-RTX were included. PCR was considered positive if >500 copies (cps)/ml.

Results: Among 311 Caucasian kidney transplant recipients with at least one assessment of BK-PCR, PCR became positive in 58 (18.6%) pts. Nonparametric Mann-Whitney Test identified donor age (odds ratio 1.82, CI 1.00–3.29, P = 0.015), the total number of HLA mismatches (OR 2.51, CI 1.38–4.55, P = 0.013) and the presence of a mismatch on the HLA-DR locus (OR 2.52, CI 1.09–5.84, P = 0.013) to be significantly associated with post-RTX prevalence of BKV-infection. Among the 58 pts with BK viremia, 28 pts (48%) had virema with >7000 cps/ml, thereof 10 pts with histologically proven BKN (BKN+ group) and 18 pts without BKN or without biopsy (BKN- group). BK viremia appeared 153.6 ± 151.2 days after RTX (mean ± standard deviation) and reached its peak after another 119.5 ± 167.7 days. The median of peak viremia of all pts with copies > 7000/ml was 48068 cps/ml (range 7730–196000000 cps/ml). The peak viremia was significantly higher in the BKN+ group than in the BKN- group: median 762350 cps/ml (range 30217 to 196000000 cps/ml) vs 22866 cps/ml (range 7730 to 352000 cps/ml), P = 0.002 in a nonparametric Mann-Whitney Test. IS was reduced after diagnosis. In 11 of 28 pts (39%) with cps >7000/ml, BK virus was cleared. Clearance was reached 216.36 ± 187.7 (mean ± sd) days after peak viremia. Among pts with >7000 cps/ml, 2 pts lost their allograft function due to BKN. Interestingly, BKN was also present in 2 pts with low virus load <3500 cps/ml.

Conclusion: Significant BK-viremia and BKN occur frequently after RTX. Pts with a high number of HLA mismatches, particularly those with a mismatch on the HLA-DR locus, and pts with a high donor age are prone to post-TX BKV-infection. Mostly, viremia occurs within the first post-RTX year. In our population, graft loss happened in 2 pts, clearance was achieved in 39% of pts with significant viremia. IS should be reduced significantly at detection of BK-viremia >1000 cps/ml in order to avoid BKN and graft loss.

ISCHEMIA/REPERFUSION INJURY AND ORGAN PRESERVATION

0016 BOTH DOPAMINE AND ITS DERIVATE N-OCTANOYL-DOPAMINE PROTECT CARDIOMYOCYTES AGAINST COLD PRESERVATION INJURY

C. Hepp[1], C. Vettel[1,2], U. Benck[1], B.K. Krämer[1], P. Schnuelle[1], B. Yard[1], S. Höger[1]; [1]Universitätsmedizin Mannheim, V. Medizinische Klinik, Mannheim, Deutschland; [2]Universitätsmedizin Mannheim, Inst f. Experimentelle und Klinische Pharmakologie und Toxikologie, Mannheim, Deutschland

We have previously shown that the protective effect of dopamine (DA) in renal transplantation is likely due to mitigation of cold preservation (CP) injury. Recently, we have developed a non hemodynamic dopamine derivative, i.e. N-Octanoyl-Dopamine (NOD) that is approximately 40× more effective than dopamine to protect endothelial cells against CP. Since cold ischemia (CI) is considered as an important cause of early cardiac graft loss, the study was conducted to investigate the effect of DA and NOD on cold preserved cardiomyocytes. Cardiomyocytes were stored for 8 hours at 4 °C in the absence or presence of various concentrations of DA or NOD. ATP levels and LDH release were measured after CI. Hereafter cardiomyocytes were re-warmed, contraction frequencies and ATP regeneration were subsequently assessed. After CI ATP levels in DA or NOD treated cardiomyocytes were significantly higher compared to untreated cells. This was paralleled by a low LDH release in the former cells. NOD was more efficacious than DA in this regard. Regeneration of ATP after re-warming occurred in treated cells within 1 hour, while in untreated cells ATP regeneration was impaired. Accordingly, rates of contraction were significantly improved in DA ($P < 0.01$) and NOD ($P < 0.05$) treated cells.Our data demonstrate that DA or NOD treatment not only prevent CP injury of cardiomyocytes but also maintains functionality of cardiomyocytes after CP and rewarming. Hence this study suggests that similar to renal TX outcome, donor treatment with low dopamine might also have a salutary effect on heart Tx outcome.

0075 ALTERATIONS IN GENE EXPRESSION PROFILE ASSOCIATED WITH RENAL ISCHEMIA-REPERFUSION INJURY ARE ATTENUATED BY HYDROXYFASUDIL MEDIATED RHO-KINASE INHIBITION IN A RAT MODEL OF ACUTE RENAL FAILURE

D. Kentrup, S. Reuter, B. Edemir, A. Grabner, H. Pavenstädt, E. Schlatter, E. Büssemaker; Universitätsklinikum Münster, Medizinische Klinik und Poliklinik D, Experimentelle Nephrologie, Münster, Deutschland

Purpose: One of the main causes of acute renal failure following (renal) surgery, transplantation or trauma is renal ischemia-reperfusion injury (IRI). In IRI, cytoskeletal reorganizational processes mainly regulated by Rho GTP-ases play a crucial role. Therefore, we hypothesized that blockade of the Rho effector Rho-associated coiled-coil containing protein kinase (ROCK) may improve renal IRI outcome. IRI triggers complex mechanisms in the affected (organ) systems. Hence, we performed microarray analysis as an analytical approach to identify gene expression alterations and regulatory pathways involved.

Methods: Male Sprague Dawley rats were unilaterally nephrectomized 1 week before inducing IRI by clamping the left renal artery for 45 minutes. Rats were divided into two groups: One h before the ischemia procedure rats received either 10 mg/kg hydroxyfasudil (NxIRHF, i.p.) or vehicle (NxIRCTR, isotonic NaCl). During the reperfusion phase following the operation rats were housed for 1−4 d in metabolic cages; blood and urine samples were taken daily for analysis. Microarray analysis was performed on kidneys of both groups, which were harvested on post operative day 4, as well as on kidneys from unilaterally nephrectomized rats. Data are given as mean values ± SEM.

Results: Rats treated with HF showed a significantly improved creatinine-clearance (NxIRHF: 0.54 ± 0.03 ml/ minutes /100 g vs. NxIRCTR: 0.14 ± 0.02 ml/ minutes /100 g, $n = 6$, $P < 0.01$) as well as fractional Na^+-excretion (NxIRHF: 0.23 ± 0.04 % vs. NxIRCTR: 0.64 ± 0.22 %, $n = 6$, $P < 0.01$) when compared to sham treated controls. After IRI, 1115 genes were differentially expressed compared to unilaterally nephrectomized rats as shown by microarray analysis. These include, among others, overrepresented genes related to cell-cell signaling (151 genes), cellular movement (144 genes), cell death (200 genes), inflammatory responses (123 genes) and immune cell trafficking (84 genes). Alterations in gene expression were significantly reduced by HF-treatment.

Conclusion: Attenuation of renal IRI via hydroxyfasudil mediated ROCK-inhibition was paralleled by a reduced alteration of the gene expression profile. Therefore, ROCK-inhibition is a potential therapeutic target in renal IRI.

0125 PKC-INHIBITOR SOTRASTAURIN ACTS CYTOPROTECTIVE AND ENHANCES RENAL GRAFT FUNCTION AFTER PROLONGED COLD PRESERVATION

L. Chaykovska[1], F. Fuller[2], A. Kusch[1], R. Catar[1], J. Pützer[1], M. Haller[3], J. Troppmair[3], U. Hoff[1], D. Dragun[1]; [1]Charité, Nephrology and Intensive Care Medicine, Berlin, Deutschland; [2]Charité, Urology, Berlin, Deutschland; [3]Innsbruck Medical University, Daniel-Swarovski-Research Laboratory and Department of Visceral-, Transplant- and Thoracic Surgery , Innsbruck, Oesterreich

Background: Preservation injury in kidney transplantation is often aggravated by tubulotoxicity of standard immunosuppressive drugs. New therapeutic strategies are aimed at reduction of toxicity.

Methods: We studied a novel immunosuppressant, an inhibitor of proteinkinase C (PKC), sotrastaurin compared to mycophenolic acid (MPA) in a life-supporting rat model of syngeneic renal transplantation after 24 hour cold-storage in UW-solution. Recipients ($n = 6$/group) received sotrastaurin (30 mg/kg/b.i.d.), MPA (20 mg/kg/day) or vehicle daily after surgery. Function, structure, apoptosis (TUNEL), proliferation (PCNA) and inflammatory markers (ED1+ cells, mRNA of MCP-1, iNOS, ICAM-1 and VCAM-1)were studied on days 2 and 7. Stress-kinase Erk1/2 and p66Shc phosphorylation, which serves an adaptor protein for activation of stress signalling pathways via PKC were determined in protein extracts.

Results: Sotrastaurin-treated rats showed a significantly better graft function on days 2 and 7 posttransplant compared to MPA. While monocyte/macrophage infiltration and adhesion molecules were reduced to a similar extent by sotrastaurin and MPA at both time points, gene expression of the inflammatory markers MCP-1 and iNOS were lower in MPA treated groups. In contrast, sotrastaurin accounted for reduced tubular damage and improved regeneration at day 2 posttransplant reflected by lower rates of apoptosis and necrosis together with higher numbers of proliferating tubular cells. Reduced phosphorylation of the PKC adapter protein p66Shc and its downstream target Erk1/2 accounted for sotrastaurin mediated cytoprotection.

Conclusion: Protective effects on tubular epithelium during phases of acute injury and repair make sotrastaurin a favorable immunosuppressant in the setting of delayed graft function and severe preservation injury.

0129 IS CLOSED CIRCUIT DURING EX-VIVO LUNG PERFUSION FOR RECONDITIONING OF DAMAGED DONOR-LUNGS SUPERIOR TO OPEN CIRCUIT PERFUSION?

S. Wipper, Y. von Rittberg, A. Lüke, C. Pahrmann, H. Reichenspurner, F. Wagner; UHZ Hamburg Eppendorf, Herz- und Gefäßchirurgie, Hamburg, Deutschland

Purpose: To evaluate whether perfusion with a closed-circuit using centrifugal pump is superior to an open-circuit with rotary blood-pump and reservoir during ex-vivo reconditioning of donor pig-lungs.

Methods and Materials: Pig lungs were harvested after Perfadex preservation (40 ml/kg). Ex-vivo perfusion occurred for 6 hours in our basic heparin-coated circuit containing leucocyte filter and deoxigenator, primed with erythrocyte-concentrate and Steen-solution 1:1, Hb 5.0 mg/dl. We compared two study groups ($n = 6$ each): group I: closed circuit centrifugal pump, priming volume 1000 ml; gr.II: open circuit, reservoir, rotary blood pump, priming volume 2000 ml. Respiratory and hemodynamic parameters were monitored pre-harvest and hourly during reperfusion. We evaluated pre- and post-reperfusion wet-dry ratios and histology by a semiquantitative score. Completeness of deflation after ventilator disconnection was indexed (PDI: 1 = normal - 5 = no collaps+foamy edema). Transplantability was evaluated according to macroscopy (PDI < 3) and standard clinical donor criteria.

Results: All lungs were perfused for 6 hours. In gr.I four of six lungs reached transplantability at study end-point, while in gr.II all lungs were successfully perfused with transplantable status. No significant differences between the groups were seen for oxygenation index, pulmonary compliance and pulmonary vascular resistance throughout perfusion (gr.I vs. gr.II at 360 minutes: 349 ± 67 vs. 371 ± 52 mmHg; 44 ± 7 vs. 47 ± 7 ml/cmH_2O; 1126 ± 380 vs. 1224 ± 544dynes; P = ns respectively). Histological score and wet/dry ratios indicated physiological status in both groups. Lactate levels were elevated in both groups, but significantly lower using open-circuit (gr.II) (360 minutes:16.3 ± 1.7 vs 11.0 ± 2.7 mg/dl; $P < 0.05$). PDI was significantly lower in groupII (2.2 ± 0.3 vs. 1 ± 0 after 60 minutes; 2.4 ± 0.6 vs. 1.4 ± 0.5 after 6 hours).

Conclusion: Surprisingly a closed system with small priming volume did not result in any improvement of ex-vivo isolated lung perfusion. Both systems allowed safe longterm perfusion which should be sufficient to recondition predamaged lungs.

0332 TAT-CRMA PREVENTS APOPTOTIC TISSUE DAMAGE IN MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

*S. Krautwald[1], *A. Linkermann[1], K.C. Wollert[2], U. Kunzendorf[1]; [1]University Kiel, Division of Nephrology and Hypertension, Kiel, Deutschland; [2]Hannover Medical School, Department of Cardiology and Angiology, Hannover, Deutschland*

Question: Apoptosis of cardiomyocytes is the major pathophysiological factor in ischemia-reperfusion injury after therapy of myocardial infarction. In order to investigate the potential of crmA as apoptosis inhibitor in such a clinically important setting, we produced a TAT-crmA fusion protein and analyzed its anti-apoptotic potency *in vivo*.

Methods: Viruses produce anti-apoptotic proteins that have evolved to protect the infected host cell from apoptotic cell death. The cowpox virus protein crmA is capable of blocking both the intrinsic and extrinsic apoptotic pathway. To deliver crmA into eukaryotic cells, we fused the TAT protein transduction domain of HIV to the N-terminus of crmA.

Results: *In vitro*, the TAT-crmA fusion protein was efficiently translocated into target cells and inhibited apoptosis mediated through caspase-8, caspase-9 and caspase-3 after stimulation with etoposide, doxorubicin, staurosporine or a-Fas. To examine the intrinsic apoptotic pathway *in vivo*, we investigated the survival of mice treated with doxorubicin. Whereas all control mice died within 31 days, 40% of mice that concomitantly received intraperitoneal injections of

TAT-crmA survived. The extrinsic apoptotic pathway was investigated following a-Fas stimulation. 90% of TAT-crmA-treated animals survived an otherwise lethal dose of a-Fas induced by organ failure. To test these observations in a clinically relevant setting, we employed a murine cardiac ischemia-reperfusion model. TAT-crmA reduced infarction size by 40% and preserved left ventricular function, even when the fusion protein was injected at the time of reperfusion of the occluded coronary artery.

Conclusions: These results prove that TAT-crmA may be considered as a new drug for protecting organ tissue in several clinical situations of apoptotic injury, including ischemia-reperfusion.

HLA AND IMMUNOLOGY

0128 DONOR ANTIGEN SPECIFIC REGULATORY T CELL FUNCTION AND OUTCOME IN KIDNEY TRANSPLANT RECIPIENTS

M. Gasser[1], I. Tsaur[2], K. Lopau[3], C.-T. Germer[1], A. Chandraker[4], A.M. Waaga-Gasser[5]; [1]Universitätsklinikum Würzburg, Chirurgische Klinik I, Würzburg, Deutschland; [2]Universitätsklinikum Frankfurt, Klinik für Nephrologie, Frankfurt, Deutschland; [3]Universitätsklinikum Würzburg, Medizinische Klinik, Schwerpunkt Nephrologie, Würzburg, Deutschland; [4]Brigham and Women's Hospital, Harvard Medical School, Transplantation Research Center, Boston, Vereinigte Staaten von Amerika; [5]Universitätsklinikum Würzburg, Chirurgische Klinik, Molekulare Onkologie und Immunologie, Würzburg, Deutschland

Background: Chronic allograft dysfunction remains a major impediment to long-term allograft survival. Many different factors have been shown to contribute to deteriorating graft dysfunction, including an ongoing alloimmune response sometimes called chronic rejection.
Materials and Methods: Out of 623 patients transplanted at a single center we selected 107 patients who were mismatched with their donors for one of a number of relevant HLA DR antigens. Patients were categorized into groups according to immunosuppression treatment and then further divided into those with stable or deteriorating graft function (chronic allograft dysfunction or CAD). A total of 37 representative T cell lines were generated from these patients. In patients with CAD, only those with biopsy proven chronic allograft nephropathy were selected.
Results: Although cell lines generated from both stable CsA and Tac treated patients had much higher percentages of CD4+CD25+ regulatory T cells (Tregs) and Treg associated gene expression compared to the CAD groups, the Tac treated stable patients also had a considerably higher Treg population compared with the CsA treated group. Stable patients were associated with better longer term graft function and T cells from these patients showed increased production of IL-4 and IL-10 and were also able to regulate the *in vitro* proliferation and production of IFN-g by CAD cell lines to mismatched HLA-DR derived peptides in an IL-10 dependent fashion. CAD cell lines produced significantly more IL-2, IFN-g and IL-17.
Conclusion: This study highlights the potential functional importance of naturally occurring donor specific Tregs derived from kidney transplant recipients treated with conventional immunosuppression.

0140 DUAL NON-HLA ANTIBODIES SIMULTANEOUSLY TARGETING ENDOTHELIAL AT1-AND ETA RECEPTORS INDUCE VASCULAR PROLIFERATION AND COAGULATION

*M. Näther[1], A. Philippe[1], R. Catar[1], A. Eisenreich[2], G. Riemekasten[3], U. Rauch[2], *D. Dragun[1]; [1]Charité Universitätsmedizin, Medizinische Klinik mit Schwerpunkt Nephrologie und internistische Medizin, Berlin, Deutschland; [2]Charité Universitätsmedizin, Kardiologie, Berlin, Deutschland; [3]Charité Universitätsmedizin, Rheumatologie, Berlin, Deutschland*

Cardiac transplant recipients and patients with vascular autoimmune pathologies harbouring non-HLA-antibodies directed against Angiotensin II type 1 (AT_1R-Abs) and Endothelin-1 type A receptors (ET_AR-Abs) are at increased risk for early onset of microvasculopathy. We hypothesized that these non-HLA antibodies may actively contribute to vascular obliteration by induction of complement-independent mechanisms and sought to investigate mechanisms responsible for intravascular coagulation and proliferation responses. AT_1R-Ab and ET_AR-Ab positive IgG fraction isolated from patients with obliterative vasculopathy or control IgG served for signal transduction and transcription factor activation studies, as well as for coagulation and proliferation assays in human microvascular endothelial cells. Both autoantibodies were biologically active as they induced stress-kinase ERK1/2 phosphorylation which could be blocked by respective receptor inhibitors. AT_1R- and ET_AR-Abs specifically triggered activation of transcription factor Ets-1 down-stream from ERK1/2, as confirmed by phosphorylation, chromatin immunoprecipitation and electromobility shift assays, followed by cell proliferation. Pharmacological inhibition of the upstream kinases of ERK1/2 established a direct link between ERK1/2, Ets-1 and endothelial proliferation. We also detected increased tissue factor expression, a gene target of Ets-1. AT_1R- and ET_AR-Abs also enhanced procoagulatory activity of tissue factor in endothelium, determined in coagulation assay. Anti- AT_1- and ET_A receptor autoantibodies may directly contribute to the key mechanisms involved in pathogenesis of obliterative vasculopathy and represent a link between the increased vascular responsiveness, intravascular coagulation and proliferation responses. Dual receptor pharmacologic targeting should add to current immunosuppressive regimens in patients harbouring non-HLA antibodes directed against vascular receptors.

0188 ACTIVATION OF THE NKG2D-SYSTEM IN HUMAN RENAL CELLS - A POTENTIAL ROLE IN ORGAN TRANSPLANTATION

F.J. Putz, M.C. Banas, B. Stoelcker, A. Steege, B. Banas; Uniklinikum Regensburg, Innere Medizin II Nephrologie, Regensburg, Deutschland

The MHC class I-related Chains A and B (MICA and MICB) and UL-16 binding proteins (ULBP1−5) are distantly related homologs of MHC class I proteins that are induced upon cellular distress conditions. MICs and ULBPs are recognized by the NKG2D activating receptor, which activates NK cells and costimulates effector T-cell subsets, leading to cytotoxic lysis of the stressed target cells. The metalloproteinase ADAM-17 and the chaperon ERp-5 are able to shed MIC A/B from the cell membrane. Recently a role in graft versus host disease and transplant rejection could be shown. Three human renal cell lines, mesangial cells, podocytes and proximal tubular epithelial cells (HK2), were analysed for expression of NKG2D-ligands. MIC A/B expression was tested by real-time PCR and FACS-analyses. The presence of ADAM-17 and ERp-5 was confirmed by real-time PCR. The amount of soluble MIC A/B was measured by ELISA-experiments. In addition, to examine the interaction between renal cells and CD8+ T lymphocytes cytotoxicity-assays were performed. Mesangial cells (HMC) and podocytes expressed only low amounts of MIC A/B-RNA whereas HK2 cells showed a marked expression. Surface expression of NKG2D-ligands was analyzed by flow cytometry using monoclonal antibodies to MICA/B and ULBP1−3 and significant differences in NKG2D-L expression patterns among the cell lines and inducing stimuli could be observed. In HMC ULBP3 protein was most prominent. In podocytes ULBP2, ULBP3 and MICA was comparable high. In contrast, in HK2 cells MICA was the most prominent protein. In the supernatant of all cell lines a significant release of soluble MICA was noted. HK2 cells also released considerable amounts of MIC B. Investigation of ERp-5 and ADAM-17 showed a higher expression in HK2 compared to HMC and podocytes. In cytotoxicity-assays only HMC were lysed, in contrast podocytes and HK2 cells which were not easily affected. The specific lysis could be significantly blocked using antibodies directed against NKG2D and MIC A/B. In conclusion, we show for the first time a functional expression of NKG2D ligands by renal cells which might play a role for local activation of infiltrating immune cells during renal allograft rejection.

0203 HLA-SPECIFIC ANTIBODIES REDUCE LONG TERM GRAFT SURVIVAL AND INCREASE THE INCIDENCE OF PROTEINURIA EPISODES IN RENAL TRANSPLANTATION

R. Richter[1], S. Köhler[1,2], M. Schulz[1], E. Scheuermann[2,3], H.-G. Kachel[2,3], J. Gossmann[2,3], S. Gauer[2], E. Seifried[1], H. Geiger[2], C. Seidl[1], I.A. Hauser[2]; [1]DRK Blutspendedienst Baden-Württemberg-Hessen, Transplantationsimmunologie, Frankfurt, Deutschland; [2]Johann Wolfgang Goethe-Universität, Medizinische Klinik III: Kardiologie, Angiologie/Hämostaseologie, Nephrologie, Frankfurt a.M., Deutschland; [3]KfH Kuratorium für Dialyse und Nierentransplantation , Frankfurt a. M., Deutschland

Backround: The significance of antibodies for acute and chronic transplant graft injury is still under discussion. To test the significance of HLA specific- and/or cytotoxic antibodies (AB) for kidney graft survival, we performed a retrospective study with a total of 364 kidney transplant recipients, transplanted between 2003 and 2009. Additionally, in this time period blood creatinine concentrations and episodes of proteinuria were investigated in 43 transplant recipients without and 54 recipients with HLA specific- and/or cytotoxic AB.
Methods: AB were monitored by ELISA in combination with the lymphocyte cytotoxicity assay (LCT). To avoid hyperacute rejection of the transplanted kidney, no transplantation was performed against HLA-class-I or HLA-class-II AB Immunosuppression of HLA-class-I AB negative transplant recipients was performed with cyclosporine A, mycophenolate mofetil, and steroids. Patients with HLA-class-I AB received intensified immunosuppression with tacrolimus, mycophenolate mofetil, antithymocyte globuline, and steroids.
Results: Kaplan Meier estimates show a reduced long term kidney graft survival in patients with pre-transplant HLA-class-I AB detected by ELISA prior to transplantation (90% versus 79%). A significantly reduced long term graft survival was found in patients with a positive LCT prior to transplantation (91% versus 67%, $P = 0.004$, log rank test) and with immunization against HLA-class-II antigens (91% versus 69%, $P = 0.05$, log rank test). Also, immunisation post-transplantation was a risk factor for long-term graft survival. An increased number of episodes with proteinuria was found in HLA-class-I (7.7 ± 0.9 % / month vs. 5.7 ± 0.8 % / month (control), $P = 0.09$, Mann Whitney test) and HLA-class-II AB positive patients (14.0 ± 5 % / month, $P = 0.08$, Mann Whitney test). Also in LCT positive patients an increased number of proteinuria episodes was found (11.2 ± 4.0% per month). Contrary, in our patient cohort blood creatinine concentrations of immunized and non-immunized patients revealed no difference.
Conclusion: Our results confirm the significance of HLA-class-I and HLA-class-II AB for graft rejection, both detected in the ELISA and LCT. The increased number of episodes with proteinuria in immunized patients supports the immunological aetiology of transplant glomerulitis and transplant glomerulopathy. The avoidance of immunisation events may be of significance for the graft survival. A better understanding of the immunological events in transplantation might help to develop new strategies for preventing early graft loss in HLA-class-I- and HLA-class-II AB positive transplant recipients.

0227 SINGLE NUCLEOTIDE POLYMORPHISMS OF CHEMOKINE RECEPTORS AND ATP-BINDING CASSETTE TRANSPORTER AND RISK OF BILIARY STRICTURES AFTER LIVER TRANSPLANTATION

S. Beckebaum[1,2], S. Iacob[2], A. Dechene[2], C. Klein[1,2], A. Paul[1], G. Gerken[2], V. Cicinnati[1,2]; [1]Universitätsklinikum Essen, Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Essen, Deutschland; [2]Universitätsklinikum Essen, Klinik für Gastroenterologie und Hepatologie, Essen, Deutschland

Background: Ischemic type biliary lesions (ITBL) and anastomotic strictures (AS) are major complications following liver transplantation (LT), leading to

reduced graft and patient survival. Genetic polymorphisms in chemokine receptors which mediate leukocyte trafficking have been reported to be associated with ITBL. For late AS, others than surgical factors should be incriminated. Genetic polymorphisms of ATP-binding cassette (ABC) transporters which determine serum cholesterol level and cholesterol efflux into bile contribute to production of lithogenic bile.

Aim: To investigate the role of chemokine receptors and ABC transporters for development of biliary lesions after LT.

Material and Methods: We genotyped 3 chemokine receptors (CCR2, CCR5 and CX3CR1) and ABC transporter G8 (ABCG8) in 165 LT recipients (42 with ITBL, 36 with AS, 87 controls) by PCR or PCR-restriction fragment length polymorphism assay. Serum concentration of chemokines CCL3 and CCL5 as ligands of CCR5 and CX3CL1 as ligand of CX3CR1 were measured by enzyme linked immunosorbent assays.

Results: A 32-base pair deletion in the CCR5 gene (CCR5Δ32) was present in 33.3% of patients with ITBL compared to 14.9% in controls ($P = 0.01$). The following genotypes of CX3CR1 were found: G745A (wild type 44.2%, heterozygous 43.6%, homozygous mutant 12.1%) and C839T (72.1%, 24.8%, 3%). The risk of AS in the carriers of the 745A allele was significantly increased compared to controls ($P = 0.02$). CCL3, CCL5, CX3CL1 serum concentrations did not differ between ITBL and control patients, as well as between AS and control group. Analysis of ABCG8 exons 8 and 13 for single nucleotide polymorphisms (SNPs) revealed the following results: C1199A (wild type 69.1%, heterozygous 29.7%, homozygous mutant 1.2%) and C1895T (46.1%, 44.2%, and 9.7%). 16.7% of patients with ITBL had the T1895T genotype compared to 5.7% ($P = 0.04$) in controls. Patients homozygous for either SNPs of ABCG8 had a significantly higher risk to develop both ITBL and AS.

Conclusions: CCR5Δ32 hetero/homozygosity and ABCG8 T1895T mutations have shown to be risk factors for occurrence of ITBL. CX3CR1 745A allele carriers had increased risk of AS. These findings may have translational relevance for predicting the risk of occurrence of biliary lesions after LT.

0302 CORRELATION OF CD4+CD25+FOXP3+ T CELLS AND IL-17 EXPRESSING CD4+ T CELLS IN PATIENTS WITH CHRONIC ALLOGRAFT DYSFUNCTION AFTER KIDNEY TRANSPLANTATION

K. Timrott[1,2], F.W.R. Vondran[1], S. Kollrich[1], F. Lehner[1], J. Klempnauer[1], R. Schwinzer[1,2]; [1]Hannover Medical School, Transplant Laboratory, Department of General-, Visceral- and Transplantation Surgery, Hannover, Deutschland; [2]Hannover Medical School, Integrated Research and Treatment Center Transplantation (IFB-Tx), Hannover, Deutschland

Background: The parameters responsible for the development of chronic allograft dysfunction (CAD) after kidney transplantation are not well understood and the etiology remains unclear. Aim of this study is to analyze the role of regulatory T cells (T_{regs}) and IL-17 expressing $CD4^+$ T cells in CAD after kidney transplantation.

Methods: Peripheral blood mononuclear cells were separated from patients (5 to 10 years after kidney transplantation) who had stable graft function ($n = 13$) or had developed CAD ($n = 13$). All patients received a calcineurin inhibitor based therapy. The frequency of T_{regs} ($CD4^+CD25^+FoxP3^+$ cells) was determined by flow cytometry. IL-17 producing $CD4^+$ T cells were detected by intracellular staining of IL-17 after *in vitro* stimulation with PMA/ionomycin.

Results: The frequency of $CD4^+$ T cells did not differ between stable patients and patients suffering from CAD (36.6 ± 12.2% vs. 36.6 ± 14.0%, respectively). However, in the CAD group the $CD4^+CD25^+FoxP3^+$ subset was markedly expanded compared to patients with stable graft function (4.0 ± 1.3% vs. 2.4 ± 1.3%, $P = 0.00003$). In CAD patients with an expanded $CD4^+CD25^+Foxp3^+$ subset we found more $CD4^+IL\text{-}17^+$ cells after PMA/ionomycin stimulation than in patients with stable graft function (0.9 ± 0.4% vs. 0.5 ± 0.5%, respectively).

Conclusions: These data suggest that the development of chronic allograft dysfunction after kidney transplantation is accompanied with alterations in the composition of the $CD4^+CD25^+FoxP3^+$ T cell subset. It remains to be established whether the increased frequency of $CD4^+CD25^+FoxP3^+$ cells in CAD patients results from an expansion of IL-17 producing T_{regs}.

0336 THE PRESENCE OF DONORSPECIFIC LOW LEVEL, C3D FIXING ANTI-HLA ANTIBODIES BEFORE KIDNEY TRANSPLANTATION IS A RISK FACTOR FOR EARLY REJECTION EPISODES

T. Kauke[1], M. Guba[1], M. Stangl[1], U. Schönermarck[2], M. Spannagl[3], K.-W. Jauch[1]; [1]Klinikum der Universität, Chirurgische Klinik und Poliklinik Großhadern, München, Deutschland; [2]Klinikum der Universität, Med.I, Schwerpunkt Nephrologie, München, Deutschland; [3]Klinikum der Universität , Immungenetik, München, Deutschland

Introduction: At present there are diverging opinions concerning the clinical relevance of anti-HLA antibodies only detected in solid phase assays (SPA) before kidney transplantation. Additional conjugates (C4d, C3d, IgG1−4, IgM) were developed helping to describe the clinical impact of the detected antibodies. Initial publications on this issue were controversial. The aim of our study was to investigate the risk of early rejection in case of of donorspecific, low level, C3d fixing (DSA+/C3d+) HLA-antibodies in pretransplant sera.

Patients and Methods: We observed 219 consecutive patients transplanted the last 28 months with a kidney from deceased donors. All patients were transplanted with a negative crossmatch. Repeated mismatches to prior transplantations were forbidden. Patients with high immunological risk (eg. with cytotoxic HLA-antibodies or with high level SPA+ antibodies) were not recommended for transplantation. 55/219 patients were positive in SPA before transplantation. Sera of SPA positive patients were measured by means of Luminex Single Antigen (LSA) beads with standard IgG conjugate and additionally with anti-C3d conjugate. The rate of rejections within the first 3 weeks after transplantation was correlated with the presence of DSA+/C3d+ HLA-antibodies.

Results: Acute rejections (AR) occurred in 21 out of 219 (9.6%) patients within the first 3 weeks after transplantation. In the group of SPA positive patients 16/55 (29%) suffered AR. In 24/55 (43.6%) SPA positive patients DSA+/C3d+ HLA-antibodies were detected. 13 of these 24 patients had AR and 11 patients had none. In 31 out of 55 SPA positive patients who were DSA-/C3d- only 3 (9.7%) patients showed AR ($p < 0.05$).

Conclusion: According to our preliminary data low level DSA+/C3d+ HLA-antibodies are a risk factor for early rejection episodes. Further investigations using a combination of assays to determine specificity and function are needed to estimate the role of low level HLA-antibodies on graft outcome.

BASIC SCIENCE

0078 INVESTIGATION OF THE EFFECT OF LOCAL INTRA-GRAFT TREATMENT WITH LYMPHOCYTE MIGRATORY BLOCKERS AND A KV1.3 POTASSIUM CHANNEL BLOCKER TOGETHER WITH SHORT-TERM SYSTEMIC IMMUNOSUPPRESSION ON SKIN REJECTION IN RECONSTRUCTIVE TRANSPLANTATION

T. Hautz[1], B. Pulikkottil[2], J. Grahammer[1], R. Jindal[2], C. Krapf[1], V.S. Gorantla[2], B. Zelger[3], G. Brandacher[1,2], P. Petzelbauer[4], M.P. Schoen[5], H. Glossmann[6], R. Margreiter[1], A.W. Lee[2], J. Pratschke[1], S. Schneeberger[1,2]; [1]Innsbruck Medical University, Dept. Visceral, Transplant and Thoracic Surgery, Innsbruck, Oesterreich; [2]University of Pittsburgh Medical Center, Div. of Plastic Surgery, Pittsburgh, PA, Oesterreich; [3]Innsbruck Medical University, Dept. of Pathology, Innsbruck, Oesterreich; [4]Vienna Medical University, Vienna, Oesterreich; [5]Universität Göttingen, Dept. für Dermatologie, Göttingen, Deutschland; [6]Innbruck Medical University, Institute of Biochemical Pharmacology, Innsbruck, Oesterreich

Background: To overcome skin rejection in reconstructive transplantation we investigated the effect of lymphocyte migratory blockers (BBeta15–42, efomycine-M) and a Kv1.3-potassium-channel-blocker (correolide-C, Kv1.3 potassium channels on lymphocytes are involved in T-cell activation during rejection) on skin rejection in a composite tissue allograft model.

Methods: ALS was given on days -1/0 and 3 after BN-to-Lewis orthotopic rat-hind-limb-transplantation. Animals were either treated with cyclosporine+IL-2-fusion-protein (21 days) followed by daily intraperitoneal or subcutaneous-intragraft BBeta15–42, or tacrolimus (50 days) together with weekly subcutaneous-intragraft efomycine-M or tacrolimus (30 days) together with subcutaneous-intragraft correolide-C twice/week. Rejection was assessed by inspection, H&E-staining and immunohistochemistry. Tacrolimus-24 hour-trough-blood-levels, WBC and RBC counts were recorded.

Results: Untreated animals rejected grafts on day 9 ± 1. Treatment with Bbeta15–42, efomycine-M and correolide-C alone had no effect. Animals treated with ALS+IL-2/Fc+CyA rejected on day 50.6 ± 7.2. Additional daily intraperitoneal injections with BBeta15–42 had no effect, but local-subcutaneous BBeta15–42-therapy resulted in long-term allograft survival (>150 days; $P = 0.0374$). After discontinuation of BBeta15–42 on day 100 donor skin grafts transplanted on day 150 remained rejection-free while third-party-skin-grafts were rejected within 18 ± 2 days, indicating donor-specific-tolerance. After weaning tacrolimus on day 30 or 50 limbs were rejected within 10 days ± 1. Treatment with local efomycine-M resulted in long-term (150 days) allograft survival. Histology on day 150 showed a mild lymphocytic dermal infiltrate and single vacuolized keratinocytes. Local correolide-C therapy resulted in insignificant prolongation of graft survival (day 43 ± 4; $P = 0.24$). Tacrolimus-mean-blood-levels were 2.97 ± 0.98 ng/ml and undetectable 5 days after weaning.

Conclusion: Local subcutaneous treatment with lymphocyte migration blockers together with transient immunosuppressive/immunomodulatory regimen results in long-term limb allograft survival. A Kv1.3 blocker has no effect on skin rejection.

0081 IMPACT OF TOLL-LIKE RECEPTOR 2 EXPRESSION IN RENAL ALLOGRAFT REJECTION

T. Bergler[1], U. Hoffmann[1], C. Pace[1], B. Jung[1], S. Farkas[2], P. Rümmele[3], B.K. Krämer[4], B. Banas[1]; [1]Uniklinikum Regensburg-Innere Medizin II; Nephrologie/Transplantationsmedizin, Regensburg, Deutschland; [2]Uniklinikum Regensburg, Chirurgie, Regensburg, Deutschland; [3]Uniklinikum Regensburg, Pathologie, Regensburg, Deutschland; [4]Uniklinikum Mannheim , V. Medizinische Klinik, Mannheim, Deutschland

TLRs have been identified to augment innate immune defense mechanisms and play an important role in initiation and modulation of adaptive immune responses. An important role of TLR2 has been shown in various experimental models of renal ischemia/reperfusion injury, however, the role of TLR2 in allograft rejection is still inconsistent. To study the expression of TLR2 in renal allograft rejection systematically, we established an experimental rat transplantation model: Brown-Norway rats served as donors and Lewis rats as recipients and cyclosporine A (CsA) was used as immunosuppression (5mg/kg). To discriminate, whether regulation of TLR2 was following immunological processes after allogeneic transplantation or was a consequence from ischemia/reperfusion injury, control animals subjected to syngeneic transplantation or to ischemia/reperfusion damage were investigated. Additionally TLR2 expression was analyzed in 99 human renal allograft biopsies. TLR2 mRNA was significantly elevated in rat allografts with acute rejection on day 6 and decreased spontaneously towards day 28. There was no induction detected in control rats ± CsA, unilateral nephrectomized rats ± CsA or syngeneic transplanted rats. Enhanced TLR mRNA levels were significantly associated with higher creatinine concentrations. TLR2 staining was also significantly increased in human allografts with acute rejection. TLR2 protein could be localized in tubular epithelial cells, vascular endothelial cells, and in CD68 and CD4 positive infiltrating cells. In conclusion, TLR2 is markedly upregulated both in experimental and human acute renal allograft rejection. Our data suggest a role for TLR2 during allogen-dependent graft damage after renal transplantation.

0146 EXPERIMENTAL TRANSPLANTATION: NOVEL IMMUNOSUPPRESSION USING THE JAK3-INHIBITOR R348

O. Deutsch[1], T. Deuse[1,2], E. Masuda[3], V. Taylor[3], G. Park[3], D. Carroll[3], H. Reichenspurner[1], J.B. Velotta[2], R.C. Robbins[2], S. Schrepfer[1,2]; [1]University Heart Center, TSI-Lab, Department of Cardiac Surgery, Hamburg, Deutschland; [2]Stanford University School of Medicine, Department of Cardiothoracic Surgery, Stanford, USA, Deutschland; [3]Rigel Pharmaceuticals, South San Francisco, USA, Vereinigte Staaten von Amerika

Background: This is the first study to investigate the role of a novel JAK3 and Syk inhibitor, R348, in the prevention of chronic airway allograft rejection. Both kinases are vital for cytokine signal transduction and immune cell differentiation.

Methods: Trachea from Brown-Norway donors were heterotopically transplanted in the greater omentum of Lewis rats. Recipients were treated for 28 days with R348 (10, 20, 40, or 80 mg/kg) or rapamycin (0.75 or 3 mg/kg) or left untreated. Grafts were harvested and tracheal segments were processed for histological evaluation by computer morphometry determining degree of luminal obliteration and percentage of respiratory epithelium coverage. Thymus and spleen weights were quantified and compared between all groups. Side effects of R348 and rapamycin were assessed using animal weights calculated every week. Plasma levels of R333, the active metabolite of R348, were quantified by high-power liquid chromatography and pharmacokinetics were determined.

Results: R348 at 20, 40, and 80 mg/kg significantly inhibited luminal obliteration (69 ± 20%, 20 ± 13%, 15 ± 7%; $P = 0.003$ vs. no medication). Rapamycin in both concentrations significantly inhibited luminal obliteration (37 ± 15%, 11 ± 6%; $P < 0.001$ vs. no medication) similarly to R348 at 40 and 80 mg/kg and was more effective than R348 at 10 and 20 mg/kg (37 ± 15%, 11 ± 6% vs. 94 ± 10%, 69 ± 20%; $P = 0.003$). R348 at 40 and 80 mg/kg significantly preserved respiratory epithelium compared to R348 at 10 and 20 mg/kg (49 ± 35%, 76 ± 27% vs. 0 ± 0, 3 ± 7%; $P = 0.004$) and was superior to rapamycin in luminal preservation (49 ± 35%, 76 ± 27% vs. 27 ± 17%, 36 ± 15%; $P = 0.01$). All R348 treated recipient thymus and spleen weights were significantly lower compared to the non-treated group ($P = 0.001$). Animal weight gain over 28 days was similar between all groups with the exception that recipients treated with 80 mg/kg of R348 had significantly reduced weight gain compared to the rest ($P < 0.0001$). Plasma levels of R333 were more stable (6000 ng/ml at 2 hours, 6500 ng/ml at 8 hours) and showed a slower decrease.

Conclusions: R348 effectively prevented the development of obliterative airway disease (OAD) and significantly preserved respiratory epithelium with 40 mg/kg being the optimal dose. Rapamycin significantly inhibited luminal obliteration with minimal effects on respiratory epithelium preservation. R348 occupies a favorable pharmacokinetic profile compared to rapamycin and is highly effective at precluding chronic airway allograft rejection.

0223 TREATMENT OF ENDOTHELIAL CELLS WITH ATG PREVENTS TRANSENDOTHELIAL MIGRATION OF LEUKOCYTES

A. Beiras-Fernandez[1], I. Hartmann[1], I. Kaczmarek[1], B. Reichart[1], G. Eißner[1]; [1]Ludwig-Maximilians-Universität, Herzchirurgische Klinik, München, Deutschland

Objectives: Polyclonal antithymocyte globulins (ATGs) are agents employed for induction of immunosuppression after organ transplantation. We have previously shown that ATGs influence the expression of adhesion molecules in an animal model. We postulated that ATGs would have an isolated effect on endothelial cells (EC), reducing the leukocyte transmigration and the extent of endothelial response.

Methods: Cells from an immortalized human microvascular endothelial cell line (HMEC) were incubated with ATG (ATG-S ©, Fresenius Biotech, Germany) for 2 hours on collagen-coated polytetrafluorethylene (PTFE) filters. Endothelial transmigration of peripheral blood mononuclear cells (PMBC) was assessed in different wells ($n = 20$ in 4 independent experiments). Expression of ICAM-1 and MHC class I molecules on HMEC was studied by flow-cytometry. Unspecific rabbit IgG was used as control. Statistical analysis was done with the 2-tailed Student's t-test.

Results: PMBC transendothelial migration was significantly reduced after ATG treatment of HMEC as compared to untreated controls (1.55E+05 ± 1.00E+04 vs. 6.85E+05 ± 1.87E+05; $P < 0.05$). Incubation with rabbit IgG showed no significant differences. Incubation of EC with ATG significantly reduced the surface expression of ICAM-1 (73.3 ± 4.1 % vs. 33.4 % ± 4.1 positive cells, $P < 0.001$) and MHC class I (100.0 ± 3.5 % vs. 59.5 ± 4.6 %, $P < 0.001$) on HMEC.

Discussion: Incubation of isolated EC with ATGs prevented the transendothelial migration of PMBC. Furthermore, a decrease of the expression of endothelial adhesion molecules could be assessed. In addition to reduced endothelial activity, down-regulation of adhesion molecules by ATG may decrease graft cell infiltration after solid organ transplantation.

0261 DIFFERENTIAL EFFECTS OF CYCLOSPORIN A AND SIROLIMUS ON THE EXPRESSION OF NADPH OXIDASE ISOFORMS IN KIDNEY CELLS

J. Schreiber, A. Blumert, I. Hauser; Uniklinik Frankfurt, Nephrologie, Frankfurt am Main, Deutschland

Background: Increased formation of reactive oxygen species (ROS) has been attributed to increased activity of nadph oxidases in the kidney in the setting of chronic allograft nephropathy. It has been shown that the immunosuppressive regimen influences kidney redox status and that Cyclosporin A impairs endothelial function by increasing vascular superoxide production. NADPH oxidases are an important source of intracellular ROS production. There are several isoforms of this enzyme termed Nox1, Nox2, Nox4 which have been shown to be expressed in the kidney. These nadph oxidase isoforms differ in subcellular localization and probably also in their involvement in signal transduction pathways of pathophysiological processes. TGF beta, a major profibrogenic stimulus, for example, has been shown to strongly induce Nox4 in lung epithelial cells leading to lung interstitial fibrosis. Similar processes may be important in kidney tubular epithelial fibrosis. In this study, we investigated whether therapeutically relevant concentrations of immunosuppressive drugs, i. e. Cyclosporin A, Everolimus and Sirolimus directly influence the expression of distinctive NADPH oxidase subunits in primary human kidney epithelial cells and umbilical vein endothelial cells substitutional for kidney endothelial cells.

Methods: cultures of human renal epithelial cells and human umbilical vein endothelial cells were cultured overnight under serum free conditions and treated with different concentrations of sirolimus (SRL; 5−100 ng/mL) or cyclosporine A (CsA; 100−3000 mg/mL) for 24 hours. Gene expression of nadph oxidase subunits was analyzed using real-time polymerase chain reaction.

Results: Compared with control, treatment of kidney epithelial cells with 10 ng/mL Sirolimus over 24 hours decreased mRNA expression of Nox4 by 30 % ($P < 0.05$) without effect on the expression of Nox1 or Nox2 whereas 200 ng/mL CsA increased the expression of Nox1 and Nox4 3-fold ($P < 0.05$) and 1.6-fold ($P < 0.05$), respectively. In umbilical vein endothelial cells, treatment with Sirolimus and Everolimus decreased Nox2-Expression by 52% ($P < 0.05$), but Sirolimus dose dependently increased Nox4 expression 3.8-fold ($P < 0.05$).

Conclusions: Different immunosuppressive drug regimens individually influence ROS formation in the kidney by changing gene expression of Nox1 and Nox4 isoforms of nadph oxidases in a cell type specific way. These findings may in part explain the endothelial dysfunction caused by cyclosporine A and preventive effect of Sirolimus on renal fibrosis.

0275 INDOLEAMINE 2,3-DIOXYGENASE (IDO) AND TREG SUPPORT ARE CRITICAL FOR CTLA4IG MEDIATED TOLERANCE INDUCTION TO SOLID ORGAN ALLOGRAFTS

R. Sucher[1], R. Oberhuber[2], N. Fischler[3], I. Kronberger[2], R. Ollinger[2], D. Fuchs[1], E. Werner[1], B. Zelger[4], G. Tellides[5], N. Pilat[6], T. Wekerle[6], R. Margreiter[2], J. Pratschke[2], G. Brandacher[2]; [1]Medizinische Universität Innsbruck, Abteilung für Biochemie, Biocenter, Innsbruck, Oesterreich; [2]Medizinische Universität Innsbruck, Abteilung für Visceral- Transplantations und Thoraxchirurgie, Innsbruck, Oesterreich; [3]Medizinische Universität Innsbruck, Abteilung für Herzchirurgie, Innsbruck, Oesterreich; [4]Medizinische Universität Innsbruck, Abteilung für Pathologie, Innsbruck, Oesterreich; [5]Yale University, Department of Surgery, New Haven, Vereinigte Staaten von Amerika; [6]Medizinische Universität Wien, Abteilung für Transplantationschirurgie, Wien, Oesterreich

Purpose: Costimulatory blockade of CD28-B7 interaction with CTLA4Ig is a well established tolerance induction strategy. Although previous *in vitro* studies confirm that CTLA4Ig up-regulates IDO expression in DCs, the precise mechanisms of CTLA4Ig and IDO interaction remain unclear. Here we studied if concerted immunomodulation *in vivo* by CTLA4Ig, IDO and Tregs accounts for indefinite survival of murine cardiac allografts.

Methods: C57BL/6 IDO (WT/knock outs) mice received BALB/c hearts. Group 1 [No treatment], Group 2 [Donor-specific transfusion (DST)], Group 3 [CTLA4Ig], Group 4 [CTLA4Ig+DST], Group 5 [CTLA4Ig+DST+ IDO inhibitor 1-Methyl-Tryptophan (1-MT)] and Group 6 [CTLA4-Ig+DST+ aCD25 mAb]. 1-MT was delivered in slow release pellets (at surgery or POD 50). Serum enzyme activity of IDO (kyn/trp) was analyzed by HPLC. Quantitative PCR was used for mRNA expression of IDO1/IDO2, Foxp3 and granzyme B. Anti-donor Abs were screened by FACS. Histopathology (H&E) and immunohistochemistry (for IDO, Foxp3, CD4, CD8, CD20, CD68 and C4d) of tissues was performed.

Results: Graft survival: Group 1 [7.7 ± 1.9 d], Group 2 [10.7 ± 1.3 d], and Group 3 [47.7 ± 29.8 d]. Group 4: Indefinite graft survival [>100 d] and tolerance without chronic rejection in IDO WT but acute rejection in IDO knock out recipients. Group 5: IDO inhibition with 1-MT, either at transplant or at POD 50, *abrogated* CTLA4Ig+DST tolerance induction. Group 6: aCD25 mAb depletion of Tregs *prevented* CTLA4Ig+DST tolerance induction. Tolerant recipients had significantly higher IDO activity as compared to non-tolerant animals, which markedly correlated with intragraft IDO and Foxp3 levels on immunostaining. IDO1/IDO2 mRNA expression was similar in tolerant and non-tolerant recipients. Anti-donor Abs were absent in all long-term survivors.

Conclusion: This study provides the first direct *in vivo* evidence that CTLA4Ig induced tolerance to murine cardiac allografts is critically dependent on synergistic cross-linked interplay of IDO and Tregs. These results have important implications for the clinical development of this costimulatory blocker.

MESENCHYMAL STROMAL CELLS AND BONE MARROW TRANSPLANTATION

0085 IMMUNOGENICITY OF MESENCHYMAL STEM CELLS FROM THE UMBILICAL CORD LINE FOR REGENERATIVE THERAPIES

M. Stubbendorff[1], K. Tang-Quan[1], T. Deuse[1], N. Philipp[1], M. Kay[1], R. Robbins[2], H. Reichenspurner[1], S. Schrepfer[1]; [1]UHZ, TSI Lab, Hamburg, Deutschland; [2]Stanford University School of Medicine, CT Surgery, Stanford, Vereinigte Staaten von Amerika

Background: Mesenchymal stem cells are of particular interest for their potential application in cell therapies. In this study, we investigate the immunogenicity of human umbilical cord-derived MSCs (clMSCs) and their immunogenic differences to adult bone marrow-derived MSCs (BM-MSCs).

Methods: clMSCs and BM-MSCs were characterized for MSC markers, their multipotent capacity and their proliferation activity *in vitro*. Expression of immunogenic surface markers under normal and IFNγ-enriched culture conditions (MHC-I, β2-microglobulin, MHCII, costimulatory molecules) was analyzed by flow cytometry. Activity of Indolamine-Deoxygenase (IDO) was assessed by Western blot. Cells were transduced for firefly luciferase expression and transplanted for *in vivo* bioluminescence imaging (BLI). *In vivo* immune response was evaluated 5 days after clMSC and BM-MSC transplantation by ELISPOT for TH1 and TH2 response.

Results: clMSCs demonstrated significant higher proliferation activity than BM-MSCs ($P = 0.01$) and multipotent capacity by differentiating into osteo-, chondro-, and adipocytes as well as expression of MSC biomarkers comparable to BM-MSCs. Due to their immature state, clMSCs showed significantly lower expression of MHCI, β2-microglobulin, and MHCII under normal conditions as well as after IFNγ stimulation compared to BM-MSCs, suggesting a lower immunogenicity. Interestingly, after IFNγ-stimulation, clMSCs and BM-MSCs similarly produced IDO, an enzyme usually produced at the fetomaternal interface, to help avoid rejection of the fetus. Upregulation of IDO combined with lower immunogenic surface molecule expression on clMSCs correlated with decreased spot frequencies for IFNγ (42 ± 40 vs. 322 ± 78) and IL4 (59 ± 61 vs 220 ± 103) in the ELISPOT assay compared to BM-MSCs ($P = 0.001$ for IFN γ and IL4). BLI confirmed delayed rejection of clMSCs, resulting in increased cell survival (clMSC: 10.9 ± 1.2 days vs. 7.2 ± 0.9; $P < 0.01$).

Conclusions: Our study demonstrates that clMSCs elicit a lower immune response than BM-MSCs. Since clMSCs can be easily obtained at birth from the umbilical cord, these cells may provide a promising and less immunogenic stem cell source for allogeneic transplantation.

0118 GENERATION OF GENETICALLY ENGINEERED LOW-ANTIGENICITY MHC I KNOCK-DOWN HUMAN EMBRYONIC STEM CELLS TO PREVENT IMMUNE REJECTION

*T. Deuse[1,2], M. Seifert[3], A. Fire[2], N. Philipps[2], M. Kay[2], H. Volk[3], H. Reichenspurner[1], R. Robbins[2], *S. Schrepfer[1,2], [1]UHZ, TSI Labor, Hamburg, Deutschland; [2]Stanford Universität , CT Surgery, Stanford, Deutschland; [3]Charite, Immunologie, Berlin, Deutschland*

Introduction: The pluripotency of human embryonic stem cells (hESC) makes them a promising candidate for cell-based myocardial repair strategies. However, we show that hESC, in contrary to former beliefs, are not sufficiently immune privileged and undergo immune rejection after transplantation. To overcome this fundamental hurdle of hESC transplantation, we sought to generate a hESC line with reduced antigenicity that would be spared from a host immune response.

Methods and Results: The antigenicity of undifferentiated hESC, predifferentiated embryoid bodies (hEB) and mature adult human cardiomyocytes (hCM) was assessed. MHC I surface expression increased with differentiation state in the order hESC< hEB< hCM. All cells were negative for MHC II. The hESC line was stably transduced to express firefly luciferase for *in vivo* bioluminescence imaging (BLI). 110^6 hESC were transplanted into the thigh muscle of WT Balb/C or immunodeficient Balb/C nude mice. Despite their relatively low MHC I expression, hESC were completely rejected within 7 ± 1 days in WT mice, but not in nude mice, confirming the immunologic nature of their cell death. Immunocompetent Balb/C mice mounted a combined cellular and humoral immune response against hESC, as demonstrated by ELISPOT assays and the determination of hESC-specific antibodies, respectively. hESC MHC I expression was targeted on the transcriptional and translational level. Cells were first transfected with MHC class I siRNA and secondly underwent adenoviral gene transfer of anti-MHC class I intrabodies. MHC I surface expression on these genetically engineered hESC (ge-hESC) was successfully reduced after 36h. Transplantation of ge-hESC did not result in cell rejection in Balb/C mice and BLI confirmed steady cell signals for an observation period of 42 days. Only minor host cellular and no humoral immune activation was found.

Conclusion: We successfully generated low-antigenicity MHC I knock-down ge-hESC that did not undergo immune rejection after transplantation. Such barely immunogenic stem cell lines will be crucial for the success of future tissue regenerative approaches.

0118 MECHANISMS OF GROWTH FACTOR DETERMINED DIFFERENTIATION OF MESENCHYMAL STEM CELLS TO VASCULAR SMOOTH MUSCLE CELL OR FIBROBLASTIC LINEAGES

B. Hegner[1], R. Catar[1], M. Näther[1], P. Wagner[1], O. Sezer[2], M. Gollasch[1], A. Kusch[1], D. Dragun[1]; [1]Charité Universitätsmedizin Berlin, Med. Klinik m. S. Nephrologie u. intern. Intensivmedizin, Berlin, Deutschland; [2]Universitätsklinikum Würzburg, Zentrum Innere Medizin, Hämatologie und Onkologie, Würzburg, Deutschland

Dysfunctional differentiation of circulating bone marrow derived mesenchymal stem cells (MSC) may contribute to tissue fibrosis and vasculopathy in cardiac and renal transplants. We sought to investigate the impact of growth factors (GF) CTGF, b-FGF, PDGF-BB, and TGF-β on MSC differentiation along fibroblast and smooth muscle like lineages. Distinct differentiated lineages were identified by morphology. Expression of vascular smooth muscle and fibroblast marker proteins, proliferation responses, and signal transduction analyses were studied after long term exposure to individual GF. Functional L-type Ca-channels served to confirm a vascular smooth muscle cell like phenotype, while the SirCol-assay and qRT-PCR for collagen production implicated fibroblastic differentiation. b-FGF and PDGF-BB decreased expression of smooth muscle marker proteins and increased levels of fibroblast markers. CTGF and TGFβ had the opposite effect. Only b-FGF induced functional L-type Ca-channels in contrast to PDGF-BB and TGFβ that abrogated nimodipine sensitive Ca influx. PDGF-BB had the most prominent pro-proliferative effect. Upregulation of col1a1 transcripts and secretion of collagen was increased by PDGF-BB and TGFβ. We detected unique signal transduction signatures of the GF explaining the observed phenotypic differences. Our results implicate that b-FGF promotes a phenotypic switch towards proliferative vascular smooth muscle cells found in neointimal lesions. PDGF-BB and TGFβ induce fibroblast and myofibroblast differentiation, respectively. We propose that the individual GF dominated microenviroment is crucial for dysfunctional differentiation of MSC and can instead of tissue regeneration drive neointima lesion or fibrosis. Therapeutic targeting of GF signaling could be instrumental in controlling regenerative capacities of MSC.

0178 BONE MARROW MONONUCLEAR CELL TRANSPLANTATION IMPROVES VASCULARIZATION AND CARDIAC FUNCTION IN LEFT-VENTRICULAR HYPERTROPHY

C. Yeong-Hoon[1], K. Neef[1], A. Tenbrock[1], O. Liakopoulos[1], A.-C. Stabbert[1], C. Stamm[2], T. Wittwer[1], T. Wahlers[1]; [1]Uniklinik Köln / Herzzentrum, Klinik und Poliklinik für Herz- und Thoraxchirurgie, Köln, Deutschland; [2]Deutsches Herzzentrum, Klinik für Herz-, Thorax- und Gefäßchirurgie, Berlin, Deutschland

Objectives: Cardiac cell therapy represents a promising treatment strategy for cardiovascular regeneration in non-ischemic cardiomyopathy. Paracrine factors from bone marrow stem cells show pro-angiogenic and anti-apoptotic effects on the myocardium, resulting in increased myocardial perfusion and preservation of ventricular function. Here, systemic bone marrow mononucleated cell (MNC) transplantation is investigated for its regenerative potential in a mouse model of myocardial hypertrophy.

Methods: Left-ventricular (LV) hypertrophy was induced by transverse aortic constriction (TAC) in NOD-scid mice. Human bone marrow was processed by Ficoll gradient centrifugation. The cell product was characterized by FACS analysis (CD34, CD45, CD133) and colony forming units (CFU). $1 \times 10e6$ MNC were transplanted intravenously 1 week post-TAC ($n = 15$). Cardiac-MRI assessment was performed weekly and included determination of LV volumes, LV wall-thickness and LV ejection fraction. Capillary density was determined by quantitative immunohistochemistry (caveolin-1).

Results: Human bone marrow MNC for transplantation were verified by FACS analysis and differentiated into 12.3 ± 2.7 CFU per million cells. Human cells were detectable up 7 days post transplantion by rt-PCR analysis. Capillary density assessment revealed reduced capillary-to-myocyte-ratio in TAC animals which was ameliorated by cell transplantation (2938 ± 483 (Sham); 2187 ± 376 (TAC); 2553 ± 334 (MNC, $P < 0.02$ vs. TAC and Sham)). However, while onset of heart-failure was delayed, there was no change in LV-wall-thickness.

Conclusions: Bone marrow MNC transplantation induces proangiogenic effects non-ischemic cardiomyopathy with a significant delay of onset of heart-failure. These promising results can be easily translated into clinical application a may have a significant benefit for patients with non-ischemic heart-failure.

0180 LINEAGE CONVERSION OF SKELETAL MUSCLE DERIVED PRECURSOR CELLS INTO CARDIOMYOCYTES - A PROMISING AUTOLOGOUS CELL SOURCE FOR CARDIAC CELL THERAPY

Y.-H. Choi[1], K. Neef[1], P. Treskes[1], S. Kumar[1], O. Liakopoulos[1], C. Stamm[2], T. Wittwer[1], T. Wahlers[1]; [1]Uniklinik Köln / Herzzentrum, Klinik und Poliklinik für Herz- und Thoraxchirurgie, Köln, Deutschland; [2]Deutsches Herzzentrum, Klinik für Herz-, Thorax- und Gefäßchirurgie, Berlin, Deutschland

Objectives: Cardiac cell transplantation is a promising approach for cardiac regeneration in heart failure patients. However, the ideal cell source has not been found yet. In the presented work we induced a lineage conversion of skeletal muscle derived precursors into cardiomyocytes avoiding gene manipulation, which can be easily translated into a clinical application.

Methods: Skeletal precursor cells were isolated from adult C57/BL6 mice. Following a primary expansion and purification according to a skeletal myoblast isolation protocol, the cell product was further cultured under hanging drop culture conditions. The forming cell clusters were characterized by immunohistochemistry and single cell patch-clamping.

Results: Under hanging drop culture conditions the purified cells showed a high lineage conversion rate towards cardiomyocyte-like phenotype. Besides synchronous beating of the clusters, these cells were highly positive for cardiac troponin, connexin43, cardiac myosin heavy-chain. Electrophysiological assessment under 8 Hz stimulation showed cardiomyocyte like shape of the action-potentials.

Conclusions: Despite an ongoing controversial discussion about skeletal precursor cells a cell source for cardiac cell therapy, we confirmed successful lineage conversion of those cells into a cardiomyocyte-like phenotype. This provides an outstanding alternative cell source for cardiac cell therapy which can be easily translated into a clinical application.

TRANSPLANTATION PATHOLOGY AND PHARMAKODYNAMICS

0042 CLINICAL AND HISTOPATHOLOGICAL FINDINGS IN EXPLANTED BUT NOT TRANSPLANTED KIDNEYS - A RETROSPEKTIVE ANALYSIS

J.W. Rey[1], D. Bode[2], U. Wirges[2], H.-P. Dienes[3], J.U.W. Fries[3]; [1]Johannes Gutenberg University Hospital Mainz, Department of Internal Medicine I, Mainz, Deutschland; [2]German Foundation of Organtransplantation (DSO), Region of North Rhine-Westphalia, Essen, Deutschland; [3]University Hospital of Cologne, Institute of Pathology, Cologne, Deutschland

Background: Kidney transplantation is the treatment of choice for patients with end stage renal disease, an increasing problem of the Western World. Nevertheless there is still a growing discrepancy between the availability and the need for kidney transplants. Therefore clinical and histopathological findings in explanted but not transplanted kidneys could give answers which histopathological parameters should be evaluated to increase the numbers of kidneys for transplantation.

Methods: From 2005 to 2009, we investigated 59 kidneys of deceased organ donors, which were explanted but not transplanted. Clinical data were listed of all explanted kidneys. All grafts underwent photographical documentation, and were histopathologically analysed using an established pretransplant biopsy protocol.

Results: One hundred percent of kidney transplants from patients with hypertension, older than 50 years and diabetes had severe grades of glomerulosclerosis, tubular atrophy, interstitial fibrosis and arterial narrowing ($P = 0.029$). Moderate and severe grades were seen in cases when creatinine clearance rate fell below 90 ml/minutes. ($P = 0.023$). Additional histopathological changes observed representing in non-allocation included renal cyst and polycystic kidneys, renal infarcts, arteriosclerotic narrowing of renal arteries, thrombotic or embolic arterial occlusion, and any kind of renal tumors. 18 of 59 (30.5%) contralateral kidneys were transplanted. 7 recipients (38.9%) received hemodialysis one year after transplantation.

Conclusion: Kidney transplants from elderly donors with a past history of diabetes and hypertension should be used with care and not without profound diagnostic evaluation such as creatinine clearance and kidney biopsy. To avoid loss of a kidney graft for transplantation in cases of unsuccessful allocation for one kidney, allocation and acceptance for double kidney transplant should be seriously considered.

0049 INOSINE 5́-MONOPHOSPHATE DEHYDROGENASE (IMPDH) ACTIVITY IN CHILDREN AND ADOLESCENTS: PHYSIOLOGICAL REGULATION AND RESPONSE TO MYCOPHENOLIC ACID (MPA) THERAPY

*A. Rother[1], P. Glander[2], E. Vitt[3], R. Feneberg[1], N. von Ahsen[4], V.W. Armstrong[4], M. Oellerich[4], C. Budde[2], B. Tönshoff[1], *L.T. Weber[3]; [1]University Childreńs Hospital, Pediatric Nephrology, Heidelberg, Deutschland; [2]University Clinic, Department of Nephrology, Berlin, Deutschland; [3]University Childreńs Hospital, Pediatric Nephrology, München, Deutschland; [4]University Clinic, Department of Clinical Chemistry, Göttingen, Deutschland*

Introduction: MPA, the active moiety of mycophenolate mofetil (MMF), acts as a specific inhibitor of human lymphocyte proliferation by inhibiting IMPDH. Outcome after renal transplantation (RTx) as for acute rejection episodes and adverse events has been shown to depend on pretransplant IMPDH activity in adults. Since many drug targets and metabolizing enzymes are developmentally regulated during childhood and adolescence, we investigated a potential developmental regulation of IMPDH activity.

Methods: We analyzed IMPDH activity in peripheral blood mononuclear cells (PBMCs) in 80 healthy children (27 infants (2–5.9 years.); 31 school-age children (6–11.9 years.); 22 adolescents (12–17.9 years.)) in comparison to 106 healthy adults. Pretransplant IMPDH activity was obtained from 31 children (mean age 12.0 ± 5.4 years.) and 81 adults with end-stage renal disease (ESRD). In addition complete pharmacokinetic/pharmacodynamic profiles of MPA and IMPDH after RTx were obtained in 17 children as well as in 21 adults with sample collection before and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after MMF administration. IMPDH activity was measured by HPLC

and normalized to the adenosine monophosphate (AMP) content of the cells, MPA plasma concentrations were measured by HPLC.

Results: IMPDH activity displayed high interindividual variability (coefficient of variation 40.5%). Median IMPDH activity did not differ significantly in healthy infants (80.5 (range, 23.0–183.9) μmol/s/mol AMP), school-age children (60.6 (29.6–152.8)), adolescents (82.7 (42.6–154.2)) and healthy adults (83.1 (26.5–214.6)). Pretransplant IMPDH activity was comparable in children (median 83.8 (39.6–163) μmol/s/mol AMP) and adults with ESRD (92 (16.7–213.3)). IMPDH activity was inversely correlated with MPA plasma concentration in both children and adults.

Conclusions: There is no pronounced developmental regulation of IMPDH activity in PBMCs in children above the age of 2 years. MPA inhibits IMPDH activity in children and adults to a comparable extent. High interindividual variability of IMPDH activity is probably due to polymorphisms of IMPDH genes. The analysis of IMPDH activity prior to RTx may have the potential to optimize MPA therapy.

0054 LIVING-DONOR KIDNEY TRANSPLANTATION IN CROSSMATCH-POSITIVE PATIENTS WITH PERITRANSPLANT IMMUNOADSORPTION AND ANTI-CD20 THERAPY

C. Morath[1], J. Beimler[1], G. Opelz[2], J. Ovens[2], S. Scherer[2], J. Schmidt[3], B. Schmied[3], M.-L. Gross[4], V. Schwenger[1], M. Zeier[1], C. Süsal[2]; [1]Nierenzentrum Heidelberg, Nephrologie, Heidelberg, Deutschland; [2]Universität Heidelberg, Transplantationsimmunologie, Heidelberg, Deutschland; [3]Universität Heidelberg, Transplantationschirurgie, Heidelberg, Deutschland; [4]Universität Heidelberg, Pathologie, Heidelberg, Deutschland

Introduction and aims: Living-donor kidney transplantation in crossmatch positive patients is a challenge that requires specific measures.

Methods: Eight patients with either donor-specific antibodies against their living donor (DSA; $n = 1$) or DSA and a positive B-cell CDC crossmatch ($n = 4$) or DSA and a positive T- and B-cell CDC crossmatch with class I ELISA crossmatch positivity ($n = 3$) were transplanted after successful desensitization with immunoadsorption and administration of anti-CD20 antibody immediately pretransplant. Patients in addition had either basiliximab ($n = 5$) or thymoglobulin induction ($n = 3$) and maintenance immunosuppression consisting of tacrolimus, enteric-coated mycophenolic sodium and steroids. Patients were followed by posttransplant antibody monitoring and protocol biopsies.

Results: Graft and patient survival rates at 1 year were 100% with a median serum creatinine of 1.72 mg/dl on day 14 and 1.68 mg/dl at year one. Seven out of 8 patients had at least one biopsy-proven acute rejection episode (borderline changes in 11 biopsies and BANFF IA rejection in 2 biopsies). Antibody-mediated rejection without graft loss was diagnosed in 3 out of 8 patients. Delayed graft function was observed in one patient. Infectious complications were infrequent. Notably, one allograft was lost beyond year one in a patient with systemic lupus erythematosus and antiphospholipid syndrome due to glomerular thrombi.

Conclusions: Immunoadsorption in combination with anti-CD20 therapy is highly effective for desensitization of living-donor kidney allograft recipients, even in patients with positive CDC crossmatches.

0170 ASSESSMENT OF BIOMARKERS FOR THERAPEUTIC DRUG MONITORING OF MTOR-INHIBITORS AFTER HEART TRANSPLANTATION

S. Klein, H.B. Bittner, M.-T. Dieterlen, J. Garbade, M. Vollroth, S. Dhein, F.W. Mohr, M.J. Barten; Herzzentrum Leipzig, Klinik für Herzchirurgie, Leipzig, Deutschland

Objective: Therapeutic drug monitoring (TDM) of immunosuppressive drugs after heart transplantation (HTx) like the mTOR-inhibitors sirolimus (SRL) or everolimus (ERL) is based on measuring blood levels alone. But this often results in under- or over-immunosuppression leading to rejection or infection, respectively. Earlier studies have shown the potential value of measuring pharmacodynamic drug effects for TDM. Therefore we developed an assay to measure drug effects on the mTOR-pathway.

Methods: Blood from five volunteers was incubated with different clinical relevant concentrations of SRL (0.9–91.4 μg/L), cyclosporine A (CsA, 75.1–1202 μg/L), mycophenolate acid (MPA, 0.08–3.2 mg/L) or Dexamethasone (DEX, 0.5–200 ng/mL). Following activation whole-blood was analyzed by flow cytometry to measure phospho-S6 in T-cells, a downstream product of the mTOR-pathway. For validation we determined coefficient of inter-assay and intra-assay variability.

Results: Phospho-flow analysis revealed that addition of SRL suppressed phosphorylation of ribosomal-protein S6 in human T-cells, whereas CsA, MPA and DEX as known from their mechanism of action did not inhibit mTOR-related S6-phosphorylation. We determined the assay-specific IC_{50} for SRL at 23.5 nM. The maximum inhibitory effect (I_{max}%) of SRL on S6 phosphorylation in T-cells was obtained at 89%. Inter-assay and intra-assay coefficients of variation ranged from 0.12 to 0.25 and 0.03 to 0.05 respectively.

Conclusions: In this study, we established a specific whole-blood assay to assess drug effects on the mTOR-pathway. Future studies in HTx recipients will show if such an assay has the potential to dose mTOR-inhibitors SRL or ERL in combination with either CsA or MPA more safely without loosing the efficacy.

0301 THE ROLE OF B-CELLS, MONOCYTES AND T-CELLS IN TRANSPLANT GLOMERULOPATHY AND ACUTE REJECTION

E. Sobkowiak[1], E. Kiss[2], S. Gauer[1], E. Gröne[2], N. Obermüller[1], H. Geiger[1], I.A. Hauser[1]; [1]JWG-University Clinic , Nephrology, Frankfurt, Deutschland; [2]German Cancer Research Center, Cellular and Molecular Pathology, Heidelberg, Deutschland

Introduction: In acute rejection infiltrates of T- and B-cells are observed. Increased monocyte infiltrates were described, when patients received a T-cell depletion therapy. The contribution of immune cells to chronic allograft nephropathy is still unknown but it is hypothesised that the antibody mediated rejection could play a role in the pathogenesis of late graft failure. We studied cell infiltrates in kidney transplant biopsies and looked for correlations with clinical outcome.

Methods: Immunohistochemical stainings of 69 biopsies of kidney transplants with vascular rejection (vR, $n = 14$), interstitial rejection (iR, $n = 17$), acute tubular necrosis (ATN, $n = 16$), and chronic allograft nephropathy (CAN, $n = 22$) subdivided into transplant glomerulopathy (TG, $n = 16$) and interstitial fibrosis (IF, $n = 6$), were performed using monoclonal antibodies against CD20, CD68 and CD45RO to identify B-cells, monocytes and T-cells. We determined the number of positively stained cells in glomerulus (mean cell count/glomerulus)and tubulointerstitium (cells/high power field, 400×). The results of leukocyte infiltrates were compared between the groups using the Mann Whitney Test and correlated to serum creatinine and creatinine clearance using Kendall Tau correlation.

Results: We observed increased B-cell counts in glomeruli and T-cell counts in tubulointerstitium in biopsies with acute rejection compared to CAN (B-cells: vR vs CAN: mean rank 25.15 vs 11.70, $P < 0.0001$ and iR vs CAN: mean rank 23.00 vs 14.25, $P = 0.012$; T-cells: vR vs CAN: mean rank 24.71 vs 14.55, $P = 0.004$ and iR vs CAN: mean rank 26.29 vs 15.14, $P = 0.002$). There was no significant difference in T- or B-cell numbers between vR and iR, but an increased amount of monocytes in glomeruli was seen in vR and TG compared to iR (vR vs iR: mean rank 18.82 vs 12.59, $P = 0.052$; TG vs iR: mean rank 21.00 vs 12.00, $P = 0.006$) and TG compared to IF (mean rank 13.28 vs 6.75, $P = 0.033$). An increase of T-cells in glomeruli was observed in TG compared to iR (mean rank 20.69 vs 13.53, $P = 0.034$). Significantly less infiltrates of all 3 cell types were observed in biopsies with ATN. We observed a trend to worse clinical outcome in patients with vR with increased B-cell infiltrates in glomeruli.

Conclusions: These results indicate a role of B- and T-cells in acute rejection and T-cells and monocytes in TG. Interestingly the monocyte infiltrates in glomeruli are also increased in vR. These results and also an increase of monocytes in glomeruli in biopsies with TG compared to IF indicate an immunological pathogenesis of TG.

LIVER TRANSPLANTATION

0040 ANTIVIRAL TREATMENT OF HCV RECURRENCE AFTER LIVER TRANSPLANTATION: IMPACT ON COSTS AND QUALITY OF LIFE

*C. Logge[1], E. Vettorazzi[2], B. Nashan[1], *M. Sterneck[1]; [1]Universitätsklinikum Hamburg Eppendorf, Klinik und Poliklinik für Hepatobiliäre Chirurgie und Transplantationschirurgie, Hamburg, Deutschland; [2]Universitätsklinikum Hamburg Eppendorf, Insitut für Mdizinische Biometrie und Epidemiologie, Hamburg, Deutschland*

Introduction: Within 5 to 10 years after LTx 20 to 40% of HCV patients can be expected to develop cirrhosis with rapid decompensation and graft loss. Response to antiviral therapy with pegylated interferon-α (pegIFN) and ribavirin can stop disease progression. Aim of our analysis was to determine cost-effectiveness of antiviral treatment for HCV recurrence post LTx.

Methods: A markov model was constructed to simulate disease progression of recurrent HCV until graft loss (= death). Outcome and cost-effectiveness of treatment strategies in genotype 1/4 and 2/3 patients were analysed: Patients with HCV genotype 1/4 received pegIFN/ribavirin for 48 weeks and stopped treatment in case of EVR (negative HCV PCR after 12 weeks). Patients with genotype 2/3 received treatment for 48 or 24 weeks and also stopped treatment in case of EVR. Based on literature data we assumed a 40% EVR with a 21% SVR in patients with genotype 1/4 and a 90% EVR with a 75% and 73% SVR in the 48- and 24-week strategy for genotype 2/3 patients, respectively. Health-state utilities to assess quality of life and health-state costs were based on literature estimates. A societal perspective was used and a 3% annual discount rate applied.

Results: The probability of organ loss/death 10, 20 and 30 years post LTx was 0.41, 0.79 and 0.93 in a no treatment strategy and 0.34, 0.65 and 0.78 in the treatment strategy for genotype 1/4 patients, 0.18, 0.35 and 0.45 in the 48-week and 0.19, 0.36 and 0.46 in the 24-week treatment strategy of genotype 2/3 patients. In genotype 1/4 patients treatment resulted in a mean life-time cost increase of € 40.393.- and a gain of 1.23 quality adjusted life years (QUALYs). In patients with genotype 2/3 the 48- and 24-weeks treatment strategy resulted in a mean life-time cost increase of € 103.427.- and € 87.344.- and a gain of 4.42 and 4.3 QUALYs, respectively . The discounted incremental cost effectiveness ratio (ICER) was € 32.860/QUALY in patients with genotype 1/4 and € 23.385/QUALY and € 20.316/QUALY in patients with genotype 2/3 treated for 48- and 24-weeks, respectively. Therefore all treatments undercut the general accepted limit of cost-effectiveness of € 50.000/QUALY in

Germany. In a two-way sensitivity analysis costs of potential future antiviral therapies for genotype 1/4 patients improving EVR and SVR were calculated. Costs per QUALY will not exceed the ones of the EVR strategy (ICER € 32.860/QUALY) if additional drug costs are not higher than € 3.708.- per 10% improvement of EVR. Based of an ICER of €50.000/QUALY drug costs must not exceed € 27.249.- per 10% EVR improvement.

Conclusion: Based on this analysis treatment with pegIFN/ribavirin using EVR as stop criterion compared to no treatment improves outcome and is cost-effective in patients with HCV recurrence. In patients with genotype 2/3 the 24-week strategy dominates the 48-week strategy in terms of cost-effectiveness. Potential future more effective antiviral therapies in patients with genotype 1/4 remain on the same level cost effective if additional costs do not exceed € 3.708 per 10% EVR gain.

0095 RECURRENCE-FREE LONG-TERM SURVIVAL IN LIVER TRANSPLANT PATIENTS WITH ADVANCED HCC IS DEPENDENT FROM 18-F-FDG TUMOR UPTAKE

A. Kornberg[1], B. Küpper[2], K. Thrum[2], P. Büchler[1], A. Altendorf-Hofmann[3], B. Krause[4], H. Friess[1], J. Wilberg[4]; [1]TU München, Klinik für Chirurgie, München, Deutschland; [2]Klinikum Bad Berka, Chirurgie, Bad Berka, Deutschland; [3]FSU Jena, Tumorzentrum, Jena, Deutschland; [4]FSU Jena, Jena, Deutschland

Background: Patients with hepatocellular carcinoma meeting the Milan criteria may achieve recurrence-free long-term survival after liver transplantation (LT). There is, however, some evidence that a subset of patients with advanced HCC beyond the established criteria systems (Milan, UCSF) may, nevertheless, benefit from LT. The aim of this retrospective trial was to identify predictive variables for recurrence-free long-term survival in liver transplant patients with advanced HCC.

Patients and materials: A total of 77 patients with HCC undergoing LT were included. In all of them, diagnosis of HCC was confirmed at explant pathology. Since 1996, PET scanning was performed preoperatively to determine 18-F-FDG tumor uptake. We distinguished between PET + (increased FDG-uptake) and PET - (no FDG uptake) status of the tumor, when compared to the normal surrounding liver tissue. Explant tumors were retrospectively classified according to the Milan and UCSF criteria. Relevant clinical and pathohistological variables were correlated with tumor recurrence rate and recurrence-free survival by uni- and multivariate regression analysis.

Results: Current posttransplant follow-up is ranging between 5 and 159 months (median: 44 months). According to the explant pathology, 46 patients (59.7%) had tumors meeting the UCSF criteria, 33 of them fulfilling the Milan criteria (group 1) and 13 of them exceeding the Milan criteria but meeting the UCSF criteria (group group 2). Thirty-one patients (40.3%) suffered from advanced HCC beyond the UCSF criteria (group 3). Overall 5-year recurrence-free survival rate was 74%. It was significantly higher in group 1 (97%; Milan In) when compared to group 2 (73%, UCSF In) and group 3 (49%, UCSF Out; $P = 0.001$), respectively. One patient in group 1 (3%) and 2 patients in group 2 (15.4%) developed posttransplant tumor relapse, while 13 patients with advanced HCC of group 3 (42%) were suffering from tumor recurrence ($P = 0.001$). In univariate analysis, none of clinical parameters, but tumor grading, microvascular invasion (MVI) and 18F-FDG uptake of HCC had an impact on patient outcome. In multivariate analysis, only PET + status was identified as independent predictor of long-term survival (Hazard Ratio = 12.5, $P = 0.01$). Patients with 18-F-FDG nonavid advanced HCC ($n = 12$) had a significantly better 5-year recurrence-free survival rate (87%) than patients with 18-F-FDG avid tumors ($n = 15$, 26%, $P = 0.004$). The positive and negative predictive value of PET status to indicate MVI were 80% and 66.6%, respectively.

Conclusion: Liver transplantation provides excellent long-term outcome in patients with HCC meeting the Milan or the UCSF criteria. However, a subset of patients with advanced HCC beyond the UCSF criteria profit from LT too. Preoperative PET scanning seems to be a viable tool for identifying those patients with less aggressive biological tumor behaviour, and thereby for a careful expansion of indication criteria.

0143 EVALUATION OF THE EARLY IV AND PO PHARMACOKINETICS OF MYCOPHENOLATE MOFETIL (MMF) AFTER LIVER TRANSPLANTATION IN COMBINATION THERAPY WITH TACROLIMUS OR CYCLOSPORINE A - A PROSPECTIVE RANDOMIZED SINGLE CENTER TRIAL

A. Pascher[1], F. Selke[1], A. Reutzel-Selke[1], S. Bauer[2]; [1]Charité Universitaetsmedizin Berlin, Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Berlin, Deutschland; [2]Charité, Universitaetsmedizin Berlin, Campus Mitte, Department of Clinical Pharmacology and Toxicology, Berlin, Deutschland

Objective: The study was designed to evaluate the pharmacokinetics of mycophenolate mofetil (MMF) in the early postoperative phase (2 weeks) after liver transplantation (LTx).

Methods: In a prospective randomized single center trial (01/2003 to 05/2005), 43 patients undergoing orthotopic LTx with insertion of a t-tube were recruited of which 24 were finally analysed. The average age was 48.6 (20 to 62) years (4 female, 20 male). MMF (2×1g/day) was administered either orally (po; group 1), or intravenously (IV) until day 6 after LTx followed by po administration (group 2 and 3), respectively. Patients of groups 1 and 2 additionally received tacrolimus (TRL), those of group 3 received cyclosporine A (CsA).

Results: Variance analysis comparing groups 1 and 2 showed significant differences in the time response regarding the blood level of the pharmacon ($P = 0.039$). Patients receiving IV-administration started with higher blood levels of MPA (mycophenolic acid, the biologically active metabolite of MMF), that dropped down visibly after conversion to po-application at day 7.No difference was determined between group 2 and 3 regarding MPA levels ($P = 0.819$). Both, patients with TRL- and those with CsA administration showed a comparable time response with a drop after conversion to po-application. Further quantifications of MMF metabolites in blood and bile did not show any influence of CsA on the pharmacokinetics of MMF.

Conclusion: IV-application of MMF could be a strategy to prevent acute rejections in the first days after tranplantation. The bioavailability of MMF is not influenced by CsA in the first days after LTx.

0161 ETHYL GLUCURONIDE HAIR ANALYSIS IMPROVES ASSESSMENT OF LONG-TERM ALCOHOL ABSTENTION IN LIVER TRANSPLANT CANDIDATES WITH ALCOHOLIC LIVER CIRRHOSIS

K. Staufer[1], H. Andresen[2], S. Kjer[1], M. Yegles[3], K. Ziegler[4], K.-H. Schulz[4], B. Nashan[1], M. Sterneck[1]; [1]UKE Hamburg, Hepatobiliäre und Transplantationschirurgie, Hamburg, Deutschland; [2]UKE Hamburg, Rechtsmedizin, Hamburg, Deutschland; [3]University of Luxemburg, Laboratoire National de Santé - Toxicologie, Luxemburg, Luxembourg; [4]UKE Hamburg, Medizinische Psychologie, Hamburg, Deutschland

Background: In order to verify long-term alcohol abstention prior to listing patients (pts) for orthotopic liver transplantation (OLT) we here evaluated in a pilot study the use of detecting ethyl glucuronide (EtG) in pts' hair.

Methods: After signing written informed consent, prior to listing pts with previous alcohol consumption EtG was quantitatively assessed in a 0–3 cm and if available 3–6 cm hair segment, reflecting alcohol consumption within the last 3 months (m), or 3 to 6 m, respectively. EtG was extracted from hair by ultrasonication in water and analyzed by gas-chromatography-mass-spectrometry after derivatisation. Cut offs were: >30 pg/mg (excessive drinkers, >60 g ethanol/day), 7–30 pg/mg (moderate drinkers, >10–40 g ethanol/day) and <7 pg/mg (teetotallers or very moderate drinkers). In parallel a psychological evaluation - blinded to the results of alcohol tests - was done. Additionally, the blood alcohol markers ethanol (EtOH; cut off 0.1g/kg), methanol (MeOH; cut off 5mg/l), carbohydrate deficient transferrin (CDT; cut off 2.6%), as well as urinary EtG (uEtG; cut off 0.5 mg/l) were determined.

Results: Alcohol marker analyses were done in 26 pts (m/f: 18:8; median age: 55, range: 39–67). Sixty-two% ($n = 16/26$) of pts were positive for at least one alcohol marker: EtOH, MeOH, CDT and uEtG were elevated in 1, 2, 6 and 7 pts, respectively, whereas EtG in hair was positive for either excessive and/or moderate alcohol drinking in 13/26 (50%) of pts (positive at 0–3 m: 50%, $n = 13/26$; positive at 3–6 m: 59%, $n = 10/17$; pts positive at 3–6 m were always positive at 0–3 m, too: 38%, $n = 10/26$). Overall, EtG in hair was the only positive marker in 25% ($n = 4/16$) of pts with any positive alcohol marker. On the other hand, in also 25% of pts alcohol consumption was not detected by EtG in hair, but only by uEtG and/ or CDT. In these cases, hair strands were taken within 4–28 days prior to urine/blood testing, indicating a diagnostic time gap of EtG in hair. Psychological evaluation was done in 23 pts. Nine pts (39%) admitted and 14 pts (61%) negated alcohol consumption within the last 6m. However, in 29% ($n = 4/14$) of pts denying alcohol consumption EtG in hair was positive. In 2 of them also CDT was positive, confirming the hair results.

Conclusion: EtG hair analysis improves detection of alcohol consumption compared to psychological evaluation, since it was positive in 29% of pts denying alcohol intake. In a quarter of pts EtG in hair was the only positive marker, however, in the same proportion of pts alcohol intake was only detected by uETG and/or CDT. Therefore, EtG hair analysis is recommended additionally to EtOH, CDT and uETG in OLT candidates.

0229 NON-INVASIVE ASSESSMENT OF SEVERE GRAFT FIBROSIS AND INFLAMMATION IN PATIENTS TRANSPLANTED FOR NON-HCV RELATED LIVER DISEASES

S. Beckebaum[1,2], S. Iacob[2], C. Klein[1,2], A. Dechene[2], J. Varghese[1], H. Baba[3], G. Sotiropoulos[1], A. Paul[1], G. Gerken[2], V. Cicinnati[1,2]; [1]Universitätsklinikum Essen, Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Essen, Deutschland; [2]Universitätsklinikum Essen, Klinik für Gastroenterologie und Hepatologie, Essen, Deutschland; [3]Universitätsklinikum Essen, Institut für Pathologie und Neuropathologie, Essen, Deutschland;

Background: The diagnostic use of biochemical markers and multiparameter scores remains to be determined in liver transplant (LT) patients. Moreover, there are only a few published studies evaluating the predictability of fibrosis using transient elastography (TE) in the LT setting, all of which were performed in HCV transplant recipients.

Purpose: To assess the efficacy of TE, biochemical tests, and more complex scores in determining severe fibrosis ($F \geq 3$) after LT in non-HCV patients. Moreover, we assessed the diagnostic value of the ActiTest for predicting moderate to severe inflammation ($A \geq 2$).

Methods: One hundred seven patients transplanted for non-HCV related liver diseases [HBV infection ($n = 24$), alcohol-related liver disease ($n = 22$), autoimmune-related liver disease ($n = 14$), and other etiologies ($n = 47$)] who underwent liver biopsy, TE and blood tests on the same day were included in the study.

Results: The optimal TE cut-off values were 5.0 kPa for $F \geq 1$, 7.3 kPa for $F \geq 2$, 9.9 kPa for $F \geq 3$ and 12.6 kPa for $F = 4$, respectively. The corre-

sponding area under the receiver operating curves (AUROCs) for F ≥ 1, F ≥ 2, F ≥ 3, and F = 4 were 0.86, 0.85, 0.88 and 0.97, respectively. Univariate analysis identified the following significant variables for predicting F ≥ 3: INR (P = 0.01), total bilirubin (P = 0.005), AST (P < 0.0001), ALT (P = 0.007), AP (P = 0.004), GGT (P = 0.0007), total protein (P = 0.01), albumin (P = 0.03), cholesterol (P = 0.01), gamma globulin (P = 0.0005), haptoglobin (P = 0.02), alpha 2 macroglobulin (P = 0.01), hyaluronic acid (P = 0.01), TE value (P < 0.0001). Independent predictors of severe fibrosis were cholesterol (P = 0.03), AST (P = 0.03), ALT (P = 0.02), GGT (P = 0.04), total protein (P = 0.04), haptoglobin (P = 0.03) and TE value (P = 0.002). Results from multiparameter scores revealed a significant difference between patients with F0-F2 and F ≥ 3 [APRI (P < 0.0001), Benlloch score (P = 0.01), Fibrotest (P = 0.0006), Hepascore (P < 0.0001), Fib4 (P = 0.0005), Lok score (P = 0.02), Fibroindex (P = 0.001)]. For the ActiTest, optimal cut-off value was 0.45 for prediction of A ≥ 2 with corresponding AUROC curve of 0.69 indicating a lower performance as compared to results from studies in viral liver disease.

Conclusion: Transient elastography as well as multiparameter scores can be reliably used for predicting F≥3 in non-HCV transplant recipients.

KIDNEY TRANSPLANTATION

0008 FAS LIGAND ON RENAL TUBULAR CELLS MEDIATES FRATRICIDE IN ACUTE KIDNEY FAILURE

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

Question: Acute toxic kidney injury as mediated by cisplatin (CIN) depends on Fas-mediated apoptosis. Fas ligand (FasL) is expressed on tubular epithelial cells and infiltrating immune cells. Whereas its role in T-cells has been investigated in detail, the functional relevance of FasL expression in primary kidney cells remains to be explained.

Methods: We investigated acute renal failure in wildtype mice and immunodeficient SCID/beige mice and blocked FasL with the monoclonal antibody MFL3. Above this, primary tubular segments (PTCs) and freshly isolated segments of thick ascending limb (TALs) were employed upon cisplatin-mediated apoptosis.

Results: Blocking FasL using MFL3 protects mice CIN in a dose-dependent manner. Whereas all cisplatin-treated C57/B6N mice died within 6 days, B-cell-, T-cell- and NK-cell-deficient SCID/beige mice exhibited marked renal failure at that time point but showed a significant survival benefit, with only 55% mortality after cisplatin injection. Interestingly, treating SCID/beige mice with MFL3 completely restored survival after otherwise lethal CIN, suggesting another functionally relevant source of FasL in renal homeostasis besides immune cells. Therefore, we isolated TALs from mouse kidneys to determine direct FasL-mediated apoptosis after incubation with cisplatin, which could be blocked with MFL3 in the complete absence of immune cells. Furthermore, cisplatin-stimulated PTCs induced apoptosis in TALs freshly isolated from GFP-transgenic mice. This fratricide could be blocked by MFL3.

Conclusion: We conclude that CIN is mediated though FasL, which is functionally expressed on fratricide-inducing tubular cells. Our observations reveal an additional mechanism that significantly contributes to organ failure besides the infiltration of FasL-bearing immune cells into the kidney.

0076 BELATACEPT VS CYCLOSPORINE IN KIDNEY TRANSPLANT RECIPIENTS: TWO-YEAR OUTCOMES FROM THE BENEFIT STUDY

C. Larsen[1], J. Grinyo[2], B. Charpentier[3], J. Medina Pestana[4], N. Kamar[5], Y. Vanrenterghem[6], C. Lin[7], G. Di Russo[7], P. Garg[7], F. Vincenti[8]; [1]Emory University, School of Medicine, Atlanta, Deutschland; [2]University Hospital Bellvitge, Barcelona, Spanien; [3]Bicetre Hospital, Kremlin Bicetre, Frankreich; [4]Unifesp, Hospital do Rim e Hipertensao, Sao Paulo, Brazilien; [5]University Hospital, Toulouse, Frankreich; [6]University Hospital, Leuven, Belgien; [7]Bristol Myers Squibb, Princeton, Vereinigte Staaten von Amerika; [8]UCSF, San Francisco, Vereinigte Staaten von Amerika

Introduction: Belatacept-based regimens were associated with superior renal function and similar patient/graft survival vs cyclosporine (CsA) at 1 year in the BENEFIT study, despite an increase in acute rejection (AR) in the early post-transplant period. The current analysis assesses pre-specified outcomes from BENEFIT in the intent-to-treat population after 2 years of treatment.

Methods: BENEFIT is a 3-year, randomized, phase III study in adults receiving a kidney transplant from a living or standard criteria deceased donor. Patients were randomized 1:1:1 to a more intensive (MI) or less intensive (LI) regimen of belatacept or CsA; all patients received basiliximab induction, MMF, and corticosteroids.

Results: Six hundred and sixty-six patients were randomized and transplanted; 493 (n = 164 MI; n = 176 LI; n = 153 CsA) completed 2 years on treatment. Patient/graft survival was similar across groups (94% MI; 95% LI; 91% CsA) at Year 2. The superior renal benefit of belatacept-based regimens was sustained through Year 2, as evidenced by a 15−17 mL/minutes higher measured GFR (P < 0.0001 MI or LI vs CsA) or calculated GFR in the belatacept groups vs CsA. There were 8 additional patients with an AR episode between Year 1 and Year 2 (n = 4 MI; n = 4 CsA). The improvements in the cardiovascular and metabolic risk profile for belatacept vs CsA were sustained, and an additional beneficial effect on LDL-cholesterol emerged at Year 2 ($P \leq 0.002$ MI or LI vs CsA). The overall incidence rate of malignancies and serious infections remained comparable across groups. There were 2 previously reported cases of PTLD between Year 1 and Year 2 in the MI group (total cases in BENEFIT through July 2009: n = 3 MI; n = 2 LI; n = 1 CsA). The overall safety profile remained similar across groups.

Conclusions: At 2 years, a belatacept-based regimen demonstrated sustained superior renal function and similar patient/graft survival vs CsA. There was no additional efficacy gained by using the MI regimen vs the LI regimen. No new safety signals emerged. Belatacept is a promising therapeutic option in kidney transplant patients.

0089 BELATACEPT VS CYCLOSPORINE IN ECD KIDNEY TRANSPLANTS: TWO-YEAR OUTCOMES FROM THE BENEFIT-EXT STUDY

*A. Durrbach[1], C. Larsen[2], J. Medina Pestana[3], Y. Vanrenterghem[4], F. Vincenti[5], S. Florman[6], J. Xing[7], A. Block[7], *P. Garg[7], J. Grinyo[8]; [1]Bicetre Hospital, Kremlin Bicetre, Frankreich; [2]Emory University, School of Medicine, Atlanta, Vereinigte Staaten von Amerika; [3]Unifesp, Hospital do Rim e Hipertensao, Sao Paulo, Brazilien; [4]University Hospital, Leuven, Belgien; [5]UCSF, San Francisco, Vereinigte Staaten von Amerika; [6]Mount Sinai Medical Center, New York, NY, Vereinigte Staaten von Amerika; [7]Bristol Myers Squibb, Princeton, NJ, Vereinigte Staaten von Amerika; [8]University Hospital, Bellvitge, Barcelona, Spain, Spanien*

Introduction: Belatacept-based regimens were associated with better renal function, with comparable patient/graft survival and acute rejection (AR) vs a cyclosporine (CsA)-based regimen in extended criteria donor (ECD) kidney transplant recipients at 1 year in the BENEFIT-EXT study. The current analysis assesses pre-specified outcomes from BENEFIT-EXT in the intent-to-treat population after 2 years of treatment.

Methods: BENEFIT-EXT is a 3-year, randomized, Phase III study in adults receiving an ECD kidney transplant. Patients were randomized 1:1:1 to a more intensive (MI) or less intensive (LI) regimen of belatacept or CsA; all patients received basiliximab induction, MMF, and corticosteroids.

Results: 543 patients were randomized and transplanted; 347 completed 2 years on treatment (n = 116 MI, n = 119 LI; n = 112 CsA). Patient/graft survival was similar across groups (83% MI, 84% LI, 83% CsA) at 2 years. The renal benefit of belatacept was sustained at Year 2 as assessed by the measured GFR (52 mL/minutes MI, 50 mL/minutes LI, and 45 mL/minutes CsA; P = 0.028 MI vs CsA; P = 0.108 LI vs CsA) and by the calculated GFR (8−10 mL/minutes higher in the belatacept groups vs CsA). There were 3 additional episodes of acute rejection after the first year (n = 1 LI; n = 2 CsA). The cardiovascular and metabolic risk profile benefits of belatacept vs CsA on serum lipids and blood pressure were sustained. The overall incidence rates of malignancies and serious infections remained comparable across groups. There were two previously reported cases of PTLD between Years 1 and 2 (n = 1 each MI and LI; total cases through July 2009 in BENEFIT-EXT: n = 2 MI; N = 3 LI; n = 0 CsA). The overall safety profile remained similar across groups.

Conclusions: A belatacept-based regimen maintained better renal function, a better cardiovascular/metabolic risk profile, and similar patient/graft survival vs CsA at 2 years. There appeared to be no additional efficacy gained by using the MI regimen vs the LI regimen. No new safety signals emerged. Belatacept is a promising option in patients receiving ECD kidneys.

0092 SAFETY PROFILE OF BELATACEPT IN KIDNEY TRANSPLANT RECIPIENTS FROM A POOLED ANALYSIS OF PHASE II AND PHASE III STUDIES

J. Grinyo[1], B. Charpentier[2], J. Medina Pestana[3], Y. Vanrenterghem[4], F. Vincenti[5], R. Shi[6], M. Agarwal[6], D. Thomas[6], C. Larsen[7]; [1]University Hospital, Bellvitge, Barcelona, Spain, Spanien; [2]Bicetre Hospital, Kremlin Bicetre, France, Frankreich; [3]Unifesp, Hospital do Rim e Hipertensao, Sao Paulo, Brazil, Brazilien; [4]University Hopsital Leuven, Leuven, Belgium, Belgien; [5]UCSF, San Francisco, CA, Vereinigte Staaten von Amerika; [6]Bristol Myers Squibb, Princeton, NJ, Vereinigte Staaten von Amerika; [7]Emory University, School of Medicine, Atlanta, Vereinigte Staaten von Amerika

Introduction: Belatacept, a selective co-stimulation blocker, is associated with better renal function and an improved cardiovascular/metabolic risk profile vs cyclosporine (CsA) in kidney transplant recipients. The current analysis focuses on pooled safety data for belatacept vs CsA used in combination with basiliximab, MMF, and steroids through July 2009.

Methods: Patients in the three core studies were randomized to a more intensive (MI) or less intensive (LI) regimen of belatacept or CsA. The pooled analysis included 1425 intent-to-treat patients (MI = 477; LI = 472; CsA = 476). Median follow-up was ∼2.4 years; some patients were followed for ∼7 years.

Results: The incidence of deaths (MI: 7%; LI: 5%; CsA: 7%) and serious adverse events (MI: 71%; LI: 68%; CsA: 69%) were lowest in the belatacept LI group. The overall incidence of malignancies remained low, but was slightly higher in the MI group (MI: 10%; LI: 6%; CsA: 7%). 15 cases of PTLD occurred (n = 8 MI; n = 5 LI; n = 2 CsA) across the three studies, including eight cases involving the CNS (n = 6 MI; n = 2 LI). The excess PTLD risk was

concentrated in EBV negative recipients and in patients receiving the MI regimen. No PTLD cases occurred after 18 months in the belatacept groups. The frequency of serious infections was 37%, 32%, and 36% in the MI, LI, and CsA groups, respectively. Rates of polyoma (MI: 7%; LI: 3%; CsA: 6%) and fungal infections (MI: 22%; LI: 17%; CsA: 21%) were lower in the LI group vs the MI or CsA groups. 1 case of progressive multifocal leukoencephalopathy was reported in the MI group. Rates of herpes infections were higher in the belatacept groups (MI: 15%; LI: 13%; CsA: 10%). Tuberculosis occurred in 10 patients (n = 5 MI; n = 4 LI; n = 1 CsA); mostly in endemic areas. There were no reports of anaphylaxis or hypersensitivity to belatacept.

Conclusions: Longer-term treatment with belatacept-based regimens was generally safe. PTLD in the CNS was higher in belatacept vs CsA, especially in EBV- patients and with the MI dose. The incidence of deaths and serious infections was lowest in the belatacept LI regimen. The overall balance of safety favored the LI regimen over the MI regimen.

0097 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], K. Pressmar[3], B. Krämer[4], J. Andrassy[1], P. Reinke[5], C. Hugo[6], O. Hakenberg[7], M. Fischereder[8], A. Pascher[9], M. Stangl[1], B. Banas[10], K.-W. Jauch[1]; [1]Klinikum der Universität München, Campus Grosshadern, Chirurgische Klinik und Poliklinik, München, Deutschland; [2]Universität Innsbruck, Klinik für Transplantation, Viszeralchirurgie und Thoraxchirurgie, Innsbruck, Oesterreich; [3]Universität Erlangen, Innere Medizin, Schwerpunkt Nephrologie, Erlangen, Deutschland; [4]Universität Heidelberg, Innere Medizin V, Schwerpunkt Nephrologie, Mannheim, Deutschland; [5]Charite, Innere Medizin, Schwerpunkt Nephrologie, Berlin, Deutschland; [6]Technische Universität Dresden, Innere Medizin III, Dresden, Deutschland; [7]Universität Rostock, Urologische Klinik, Rostock, Deutschland; [8]Klinikum der Universität München, Campus Grosshadern, Innere Medizin I, Schwerpunkt Nephrologie, München, Deutschland; [9]Charite, Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Berlin, Deutschland; [10]Universität Regensburg, Innere Medizin II, Schwerpunkt Nephrologie, Regensburg, Deutschland

The SMART study showed that at 12 months posttransplant, early conversion to a CNI-free regimen with SRL in combination with MMF resulted in a better renal function compared to a CsA-based regimen in combination with MMF (Transplantation 2010). This in an observational follow-up for additional 2 years with the same endpoints as the core study. Follow-up was documented in overall 113 patients at 24 months and 99 patients at 36 months. During the study, many patients changed their immunosuppressive regimen (e.g. switched from sirolimus to cyclosporine), but the vast majority remained on MMF. At 24/36 months 46.8%/40.7% of patients were on treatment in the SRL arm and 77.8%/61.4% of patients in the CsA arm. The SRL and MMF arm continued to have a trend towards a lower median S-creatinine (m24:1.40 vs.1.60 and m36: 1.40 vs.1.60 mg/dl) and a higher eGFR (m24:62.46 vs.55.09 and m36: 60.69 vs.54.82 mL/minutes /1.73 m^2), but results in the ITT analysis did no longer reach significance at m24 and m36 follow-up. However, in the subset of patients who were able to stay on their designated therapy for at least 12 months renal function was still significantly better in the SRL arm as compared to the CsA arm. Patient- and graft survival at 24 months (SRL 99% vs. CsA 97%) and 36 months (SRL 97% vs. CsA 94%) was excellent in both arms. Three late (>12 months) biopsy-proven rejections were recorded in the CsA arm, none in the SRL arm. De novo malignancy developed in 5 patients in the CsA arm, no malignancy was recorded in the SRL arm (P = 0.0239). There were no notable differences in late infections or adverse events during follow-up beyond month 12. Due to high rate of treatment changes in the SRL arm, advantages in renal function at 12 months were gradually diluted at 24 and 36 months follow-up in the ITT analysis. Further analysis are warranted to develop prognostic tools in order to determine which individual patient would benefit of a CNI-free, SRL-based therapy long-term.

0172 CONVERSION TO AN EVEROLIMUS/ENTERIC-COATED MYCOPHENOLATE SODIUM REGIMEN AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN DE NOVO RENAL TRANSPLANT PATIENTS IMPROVES RENAL FUNCTION: 2 YEARS FOLLOW-UP OF THE ZEUS TRIAL

C. Sommerer[1], T. Becker[1], K. Budde[1], F. Pietruck[1], U. Eisenberger[1], W. Fischer[2], S. Kramer[2], P. Reinke[1], W. Arns[1]; [1]The ZEUS, Study Group, Deutschland; [2]Novartis Pharma, Nürnberg, Deutschland

Objective: In de novo kidney allograft recipients renal function, efficacy and safety was assessed after conversion to an Everolimus/Enteric-coated mycophenolate sodium (EC-MPS) regimen after Cyclosporine (CsA) withdrawal at month 24 post-transplantation.

Methods: In this prospective, open-label, controlled, multi-center study renal allograft recipients were randomized to an immunosuppressive regimen consisting of either Everolimus/EC-MPS or CsA/EC-MPS at Mo 4.5 after transplantation. After completion of the core study at Month 12, patients were included in an observational 12 Month follow-up study.

Results: Three hundred patients (pts) were randomized to either Everolimus/EC-MPS (n = 155) or CsA/EC-MPS (n = 145), 244 (81.3%) pts completed the 24 month visit. Renal function expressed as calculated GFR (Nankivell method) was similar in both groups at baseline (randomization 4.5 Month post tx) with an improvement by 7.16 mL/minutes /1.73 m^2 in favor of the Everolimus/EC-MPS regimen (P = 0.017) at Month 24 (61.7 ± 17.1 vs. 68.9 ± 19.4 mL/minutes/1.73m^2) The observed GFR slope from randomization to Month 24 was +6.7 [+3.2,+10.2] for Everolimus/EC-MPS and -0.8 [-4.5,+2.9] mL/minutes /1.73m^2 for CsA/EC-MPS pts. Similarly GFR slope with MDRD (+9.4 [+4.0,+14.9]) and Cockcroft-Gault formular (+7.0 [+3.4,+10.6]) were significantly better (P < 0.001) in the CNI-free regimen (CsA/EC-MPS-treated pts: MDRD: -0.8[-6.2,+4.6] mL/minutes; Cockcroft-Gault: -1.2[-5.0,+2.6] mL/minutes). Fewer pts in the Everolimus group had a decline of GFR compared to renal function at randomization (Nankivell: 24.7% vs 41.4%; P = 0.0034) compared with Cyclosporine. BPAR was reported in 17 (11.0%) Everolimus/EC-MPS-treated vs. 7 (4.8%) CsA/EC-MPS treated patients between randomization and Mo 24. After 12 months two additional BPAR occurred in each group. Three death and one graft loss was observed in the CsA/EC-MPS group none in the Everolimus group. The number of patients with infections (35 pts (22.6%) in the Everolimus vs. 30 pts (20.7%) in the CsA group) and hospitalization (43 pts (27.7%) in the Everolimus vs. 51 pts (35.2%) in the CsA group) in the follow-up period (Mo 12 to Mo 24) was comparable in both groups.

Conclusions: The conversion to Everolimus/EC-MPS in de novo renal transplant patients after CNI withdrawal early after transplantation significantly maintains renal function over a period of 24 months without compromising efficacy and safety.

0202 INFLUENCE OF PREFORMED DONOR-SPECIFIC NON-LYMPHOCYTOTOXIC ANTIBODIES AGAINST HUMAN LEUKOCYTE ANTIGENS ON RENAL ALLOGRAFT SURVIVAL

M. Ziemann[1], C. Schönemann[2], C. Bern[1], N. Lachmann[2], M. Nitschke[3], L. Fricke[3], S. Görg[1]; [1]Universitätsklinikum Schleswig-Holstein, Institut für Transfusionsmedizin, Lübeck, Deutschland; [2]Charité, Campus Virchow Klinikum, Institut für Transfusionsmedizin, FB Gewebetypisierung, Berlin, Deutschland; [3]Universitätsklinikum Schleswig-Holstein, Transplantationszentrum Lübeck, Lübeck, Deutschland

Background: The prognostic value of HLA antibodies in recipients of kidney transplants detected solely by solid phase assays is still unclear. Contrary to many studies using expensive tests for every sample, in routine practice the specificity of HLA antibodies is only determined for sera reactive in screening tests. The aim of this study was to examine the effect of HLA antibodies detected by routine methods on antibody-mediated rejection (AMR) and graft survival.

Methods: All available pretransplant sera from kidney transplant patients in Luebeck between 1998 and 2000 were screened for HLA antibodies, and the antibody specificity of reactive sera determined using bead array techniques. The occurrence of donor-specific HLA antibodies (DSA) was correlated with AMR within 90 days and graft survival.

Results: DSA were found in 19 out of 143 patients and correlated with the incidence of impaired graft function and AMR within the first 90 days after transplantation (60% and 20% versus 20% and 5%, respectively, P = 0.04 and 0.02). Kaplan-Meier-analysis revealed shorter death-censored graft survival in patients with DSA and AMR (P < 0.001). Median graft survival was 1.7 years for patients with DSA and AMR, 10.5 years for patients with DSA but no AMR, and 11.0 years for patients without DSA. Specifying the antibody specificity only in sera reactive in a screening test reduced material costs by about 65%.

Conclusions: The combined screening was a cost-effective strategy to detect DSA and prevent early AMR and shortened graft survival due to preformed HLA antibodies.

0272 PROSPECTIVE IMMUNOLOGIC RISK STRATIFICATION IN DE NOVO KIDNEY TRANSPLANT RECIPIENTS - EARLY OUTCOME EVALUATION

L. Schewior[1], R.A.K. Stahl[1], T.H. Eiermann[2], T. Strauss[1], M. Koch[3], B. Nashan[3], F. Thaiss[1]; [1]Universitätsklinikum Hamburg Eppendorf, III. Medizinische Klinik und Poliklinik, Nephrologie, Nierentransplantation, Rheumatologie, Endokrinologie, Hamburg, Deutschland; [2]Universtitätsklinikum Hamburg Eppendorf, Institut für Transfusionsmedizin / HLA- Labor, Hamburg, Deutschland; [3]Universitätsklinikum Hamburg Eppendorf, Klinik und Poliklinik für Hepatobiliäre Chirurgie und Transplantationschirurgie, Hamburg, Deutschland

Background: Early impairment of renal allograft function is due to various immunologic and non-immmunologic mechanisms (KTx). Pre-transplant immunologic risk stratification might help to predict renal transplant outcome and may allow tailored immunosuppression (IS). There is still a lack of specific single prognostic markers which predict a defined immunologic risk for the recipient. In order to better define immunologic risks we applied a pattern of potential immunologic immunologic parameters and analysed the clinical outcome according to our classification.

Methods: Prior to renal transplantation all recipients (KTxR) were allocated into for 4 immunologic risk groups (gr 1−4). Criteria included number of former renal Tx, potential immunologic renal disease of KTR, PRA, HLA-Antibodies, Donor-Specific-Antibodies, ABO-incompatible KTx, European senior donor progamm and HLA-Mismatch [Fig.1]. Primary outcome parameters were functioning graft (fKTx) and estimated glomerular filtration rate (eGFR) at discharge. Secondary outcome parameters were delayed graft function (DGF) and KTx related complications (lymphocele, wound infection, urinary lekage). All patients received induction therapy with steroids and Basiliximab and a Calcineurin Inhibitor (CNI) in combination with either MPA or everolimus. Initial CNI trough levels: Tacrolimus (TAC, 4−6 ng/dL) resp. Cyclosporin (CsA,

80–120 ng/dL). KTR in risk group 3 and 4 did receive additional IS with Thymoglobulin or Rituximab.
Results: Seventy-nine KTx recipients were enrolled. Patient numbers in 4 immunologic risk groups were: low (gr 1: $n = 23$); normal (gr 2: $n = 44$); elevated (gr 3: $n = 8$); high (gr 4: $n = 4$). Baseline demographics (gender, BMI, diabetes mellitus, hypertension, coronary artery disease, cold ischemic time, operation time) were comparable amongst groups (P = ns). More patients did receive Thymoglobulin and/or Rituximab in groups 3 and 4. Total KTS was 84% and mean eGFR was 57.8 ± 26 mL/minutes. Better KTx function and eGFR were observed in group 1 and 2 (fKTx 95.7% and 86.4%, eGFR 60.9 ± 24 and 57.2 ± 28 mL/minutes, DGF 17.4% and 22.7%). Patients in the higher immunized risk groups 3 and 4 showed lower KTx function (fKTx 62.5% and 25%, eGFR 50.2 ± 12 and 53.0 ± 0 mL/minutes, DGF both 25%). Peri- and postoperative surgical and infectious complications were rare and did not differ amongst groups (total $n = 4$). Two deaths occurred, both due to acute cardiovascular events (gr 1 and gr 2). Hospital and IS treatment costs were higher in group 3 and 4 compared to group 1 and 2.
Conclusions: Pretransplant immunologic risk stratification allows to identify patients with increased- and high immunologic risk (15%) with high risk for early transplant failure (50%). This approach might be useful to assign KTR to specific IS regimens to improve early postransplant outcome and resource allocation

PANCREAS TRANSPLANTATION

0110 OUTCOME OF PANCREAS AND KIDNEY GRAFTS IN PANCREAS TRANSPLANT (PAK/SPK) RECIPIENTS REQUIRING OPERATIVE REINTERVENTION FOR PANCREAS GRAFT-RELATED COMPLICATIONS

M. Müller, C. Wimmer, M. Rentsch, H. Arbogast, M. Stangl, K.-W. Jauch, M. Guba; Klinikum der Universität München - Campus Großhadern, Chirurgische Klinik, München, Deutschland

Pancreas transplantation frequently requires surgical reinvervention due to graft-related complications. This retrospective study of 311 pancreas transplantation in 276 recipients examines the impact of pancreas graft-related complications on patient survival and graft survival (both pancreas and kidney). A cohort of 276 consecutive pancreas recipients, transplanted between 1995–2009, were analyzed. Of 311 pancreas transplantations (271 (Re-)NP; 40 (Re-)PAK/SP) 162 required surgical reintervention for pancreas graft-related complications (57 graft thrombosis/Rx, anastomosis leakage 10, intaabdominal septic complications/graft pancreatitis 39, 32 bleeding, 17 wound-healing and 7 other), in 92 cases (57%) reintervention was indicative for an early graft loss (<40d). One and 5 year patients survival was 98.6/94.9% for uncomplicated and 93.1/85.1% for reoperated patients. Pancreas graft survival at 1 and 5 years was 90.5/83.3% for uncomplicated and 38.8/32.9% for reoperated patients; corresponding 1 and 5 year survival for kidney grafts 95.9/89.3% and 88.8/78.7% respectively. Pancreas graft-related complications requiring operative reintervention predicts poor outcome of both pancreas and kidney grafts. All measurements should be taken to avoid the requirement of surgical reintervention after pancreas transplantation, which when necessary, minimizes the success of pancreas transplantation and significantly endangers patient's life.

0144 AGONIST OF GROWTH HORMONE RELEASING HORMONE AS A POTENTIAL EFFECTOR FOR SURVIVAL AND PROLIFERATION OF PANCREATIC ISLETS

B. Ludwig[1], C. Ziegler[1], A.V. Schally[2], C. Richter[1], A. Steffen[1], N.L. Block[2], M. Ehrhart-Bornstein[1], S. Bornstein[1]; [1]Universitätsklinikum Dresden, Medizinische Klinik III, Dresden, Deutschland; [2]University of Miami Miller School of Medicine, Departments of Pathology and Medicine, Miami, USA, Vereinigte Staaten von Amerika

Background: Therapeutic strategies for transplantation of pancreatic islet cells are urgently needed to expand ß-cell mass by stimulating islet cell proliferation and/or prolonging islet cell survival. Control of the islets by different growth factors provides a potential venue for augmenting ß-cell mass.
Methods and results: In the present study, we demonstrated the expression of the biologically active splice variant-1 (SV-1) of GHRH receptor in rat insulinoma (INS-1) cells as well as in rat and human pancreatic islets. In studies *in vitro* of INS-1 cells, the GHRH agonist JI-36 caused a significant increase in cell proliferation and a reduction of cell apoptosis. JI-36 increased islet size and glucose-stimulated insulin secretion in isolated rat islets after 48–72 hours. At the ultrastructural level, INS-1 cells treated with agonist JI-36 revealed a metabolic active stimulation state with increased cytoplasm. Co-incubation with the GHRH antagonist MIA-602 reversed the actions of the agonist JI-36, indicating their specificity. *In vivo*, the function of pancreatic islets was assessed by transplantation of rat islets under the kidney capsule of streptozotocin-induced diabetic NOD-SCID mice. Islets treated with GHRH agonist JI-36 were able to achieve normoglycemia earlier and more consistently than untreated islets. Furthermore, in contrast to diabetic animals transplanted with untreated islets, insulin response to an intraperitoneal glucose tolerance test (IPGTT) in animals receiving islets treated with agonist JI-36 was comparable to that of normal healthy mice. In conclusion, our study provides evidence that agonists of GHRH represent a promising pharmacological therapy aimed at promoting islet graft growth and proliferation in diabetic patients.

0196 KOMBINED KIDNEY-PANCREAS TRANSPLANTATION FOR PATIENTS WITH DIABETES MELLITUS TYPE II - A SINGLE-CENTER REPORT

H.M. Hau, D. Uhlmann, M. Bartels, S. Jonas; Uniklinikum Leipzig, Chirurgie, Leipzig, Deutschland

Introduction: The purpose of this study was to examine the benefits of pancreas transplantation in patients with type II diabetes mellitus (DM) - especially regarding to glucose/insulin metabolism and long-term outcome.
Patients&Methods: From 2001 to 2006 we performed 50 simultaneous pancreas/kidney transplantations (SPK). Among these patients, 5 had a DM type II and received a SPK. The diagnosis of DM type II was based on the guidelines of the American Diabetes Association. The pancreas was transplanted in standard technique with a venous anastomosis by using the caval vein of the recipient and an enteric drainage by doing a duodenojejunostomy.
Results: The mean recipient age was 53.6 ± 3.6. Recipient median BMI was 27.6 ± 1.59 kg/m^2. DM type II was diagnosed at a mean age of 33.8 ± 3.23 years. The SPK was performed at a mean of 13.2 ± 3.67 years after the beginning of a medical treatment. Median insulin demand of the patients was 72.8 ± 13.95 units. All patients had a retinopathy and nephropathy, and 60% had evidence of neuropathy. For all 5 patients deceased donors were used. The postoperative results after pancreas transplantations for DM type II diabetics have not been significantly different from those for type I. The average duration of hospital stay was 24 days. One patient needed a relaparatomy because of a transplant pancreatitis with intra-abdominal abscess. A wound infection was found in a further patient. There were no cardial or respiratory complications. In-hospital mortality rate was 0 %. The patient with pancreatitis had a delayed pancreas graft function and required insulin substitution. All other recipients had good initial function of both grafts and are free of insulin at a mean follow-up of 5.4 years (HbA1C 6.06%) without severe complications. The patient with severe pancreatitis needs further insulin substitution.
Conclusion: SPK can provide excellent glucose control in recipients with type 2 DM. Long-term results were comparable with those seen in transplant recipients with type 1 DM. Considering the increasing number of young type II diabetics without obesity SPK could be a possibility to prevent macrovascular und microvascular complications of diabetes mellitus.

0260 INCIDENCE OF HYPERFIBRINOLYSIS DURING SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION

Z. Mathe[1], D. Dirkmann[2], E. Malamutmann[1], J. Treckmann[1], A. Paul[1], K. Görlinger[2]; [1]Universitätsklinikum Essen, Klinik für Allgemeinchirurgie, Viszeral- und Transplantationschirurgie, Essen, Deutschland; [2]Universitätsklinikum Essen , Department of Gastroenterology and Hepatology, Essen, Deutschland

Introduction: Bleeding complications are the second most common cause for relaparotomy following simultaneous pancreas-kidney transplantation (SPKT) and are frequently associated with graft loss and even patient death.
Patients and methods: Aim of our retrospective study was to evaluate the incidence of hyperfibrinolysis as a possible reason for bleeding complications, during and after SPKT and its impact on transfusion requirements, graft survival, and death. We performed card review of 38 patients (17 female and 21 male), mean age 41.2 years (range, 26–57 years), who underwent SPKT between 2002 and 2009 in a single center. Forthcoming thromboelastometry (ROTEM) data were reviewed for variables (i.e. clot lysis indices after 30, 45, and 60 minutes) and morphological patterns indicating hyperfibrinolysis. Furthermore, groups of patients with and without hyperfibrinolysis were compared regarding to their mean transfusion requirements, as well as incidence of graft loss and death.
Results: Point of care coagulation monitoring by ROTEM was performed in 14 patients due to observed diffuse clinical bleeding. Incidence of hyperfibrinolysis as confirmed by ROTEM was found to be 10.5% (4 out of 38 patients) throughout the whole population and 28.6% (4 out of 14 patients) with diffuse clinical bleeding. Differences regarding lysis onset time, clinical relevance and consequence were found among these cases. Intraoperative transfusion requirements for packed red blood cells, fresh frozen plasma and platelet concentrates were found to be identical in both groups.
Conclusion: We are the first to report on the detection of hyperfibrinolysis during SPKT. Incidence was found to be 10.5% in our population. According to our data thromboelastometry is helpful to detect possible reasons of diffuse bleeding like hyperfibrinolysis and enable reasonable decisions concerning antifibrinolytic therapy.

PEDIATRIC TRANSPLANTATION

0055 IMPLEMENTATION OF A NOVEL WEB-BASED REGISTRY FOR PAEDIATRIC KIDNEY TRANSPLANTATION IN CENTRAL EUROPE

B. Höcker[1], R. Feneberg[1,2], L. Plotnicki[1,3], K. Krockenberger[2], B. Tönshoff[1], German Society of Paediatric Nephrology[4]; [1]University Children's Hospital, Division of Paediatric Nephrology, Heidelberg, Deutschland; [2]Coordinating Center for Clinical Studies, University of Heidelberg, Heidelberg, Deutschland; [3]University of Medical Informatics Heilbronn and Heidelberg, Heilbronn, Deutschland; [4]on behalf of the German Society of Paediatric Nephrology, GPN, Deutschland

Background: Because of the partially artificial setting and relatively short-term endpoints of controlled clinical trials, long-term data collection for paediatric renal transplant (RTx) recipients is vital for clinical research, quality assurance and improved patient care. Due to lack of such a registry in Central Europe, the GPN Paediatric RTx Working Group, in 2009, decided its introduction, applying novel information technologies.

Implementation and data protection: This entirely internet-supported registry is to fulfil, or even exceed, applicable legal data protection and security requirements, using the sophisticated security concept of the Telematikplattform für Medizinische Forschungsnetze (www.tmf-ev.de). Its data protection concept envisages a strict separation of personal and medical data (e.g., two-server storage), additionally following stringent data access-controlling policies.

Data exchange: Is planned with Eurotransplant (ET), the Collaborative Transplant Study (CTS) and the European Society for Pediatric Nephrology (ESPN) registry. Data import from existing structures will reduce manual data input efforts significantly, and data transfer to ET, CTS and ESPN decrease workloads of participating centres.

Clinical Trials: An additional module for conducting registry platform-internal prospective controlled clinical trials is under design. Registry-internal implementation of clinical trials will meet national and international laws and guidelines to ensure conformity with Good Clinical Practice (GCP).

Summary: Until mid-2010, the GPN will establish a registry for paediatric RTx patients allowing long-term outcome analyses in paediatric RTx. A module for registry-internal clinical trials will be realized by 2011. Unlike existing registries, it will interface to databases such as ET, CTS and ESPN, reducing manual data input volumes considerably.

0062 EFFECT OF (VAL-)GANCICLOVIR ± CMV HYPERIMMUNOGLOBULIN ON EBV VIREMIA IN PEDIATRIC RENAL TRANSPLANTATION: SUBGROUP ANALYSIS OF A PROSPECTIVE MULTICENTER TRIAL

B. Höcker[1], U. Küsters[2], S. Böhm[2], H. Fickenscher[2], M. Pohl[3], M. Holder[4], U. John[5], M. Kemper[6], H. Fehrenbach[7], M. Wigger[8], M. Schröder[9], E. Wühl[1], B. Tönshoff[1], German Society of Pediatric Nephrology[10]; [1]University Children's Hospital, Division of Pediatric Nephrology, Heidelberg, Deutschland; [2]Hygiene Institute of the University Heidelberg, Department of Virology, Heidelberg, Deutschland; [3]University Children's Hospital, Freiburg, Deutschland; [4]Olga Hospital, Stuttgart, Deutschland; [5]University Children's Hospital, Jena, Deutschland; [6]University Children's Hospital, Hamburg, Deutschland; [7]Children's Hospital, Memmingen, Deutschland; [8]University Children's Hospital, Rostock, Deutschland; [9]Clementine Children's Hospital, Frankfurt/Main, Deutschland; [10]for the, German Society of Pediatric Nephrology (GPN), Deutschland

Objective: Retrospective studies have shown an association between EBV serostatus, antiviral prophylaxis and the occurrence of EBV-associated PTLD in kidney allograft recipients. However, data on the potential effects of antiviral prophylaxis on the development of EB viremia (primary infection and/or reactivation) in pediatric renal transplant recipients are lacking.

Method: In the framework of a prospective trial in 114 pediatric renal transplant patients, aged 11.1 ± 5.9 years, we performed a subgroup analysis of the influence of antiviral prophylaxis with (val-)ganciclovir ± CMV hyperimmunoglobulin on the occurrence of EB viremia.

Results: Out of 73 patients with known donor (D)/recipient (R) EBV serostatus, 23 (aged 7.2 ± 5.5 years) were at high risk (D+/R-) for EBV viremia, and 50 at moderate (D+/R+, $n = 39$) or low risk (D-/R+, $n = 8$; D-/R-, $n = 3$). Prophylaxis with (val-)ganciclovir +/– CMV hyperimmunoglobulin led to a significantly ($P = 0.015$) lower incidence of EBV primary infection in high-risk patients (Fig. 1). Patients on (val-)ganciclovir only, experienced EBV primary infection numerically less often than patients without prophylaxis (5/13 vs. 3/3, $P = 0.051$). A potential additive effect of CMV hyperimmunoglobulin on the development of EB viremia could not be shown so far (3/7 *vs.* 7/13, $P = 0.651$). (Val-)ganciclovir did not affect the incidence of EB viremia in moderate or low-risk patients.

Conclusion: Antiviral prophylaxis with (val-)ganciclovir significantly reduces the emergence of EBV primary infection in high-risk patients, thereby potentially lowering the risk of EBV-associated PTLD.

0289 DEFINITION AND OUTCOME OF SMALL- AND LARGE-FOR-SIZE LIVER TRANSPLANTATION IN CHILDREN

U. Herden, R. Ganschow, T.Y. Tsui, J. Pollok, B. Nashan, L. Fischer; Universitätsklinikum Hamburg-Eppendorf, Hepatobiliäre und Transplantationschirurgie, Hamburg, Deutschland

Introduction: Both small- and large-for-size liver grafts are supposed to take an elevated postoperative risk. Due to an age depended variation of percentual liver volume in children, other definitions have to be used compared to adults. Based on autopsy findings and a literature review of pediatric standard liver volume definitions we analyzed the outcome following pediatric LTX depending on the graft weight to recipient standard liver weight ratio.

Methods: All pediatric LTX between 2000 and 2008 were retrospectively analyzed. Patients were divided into 3 groups depending on the ratio graft weight to recipient standard liver weight (calculated by the Hamburg formula: standard liver weight[g] = -149.37 + (4.85 × body height[cm]) + (11.49 × body weight[kg]) × 1.08).

Group 1 < 0.5 (small-for-size)
Group 2 ≥ 0.5–≤ 1.25 (size-matched)
Group 3 > 1.25 (large-for-size)

Results: Between 2000 and 2008 we performed 333 pediatric LTX, thereof a complete follow up was available in 311 children. 28 children received a small-for-size graft (median age 7.5y, range 0.8–15.3 years), 202 children a size-matched graft (median age 2.1y, range 0–16 years) and 81 children a large-for-size graft (median age 0.6y, range 0–15.2 years). Overall living donation was performed in 79 patients (75 left-lateral grafts, three full-left grafts, one full-right graft) and cadaveric LTX was performed in 232 children (46 whole organs, 22 reduced organs, 132 left-lateral splits, 11 full-left splits, 10 right-extended splits); distribution of different graft types was comparable between the three groups. 1-and 5-year patient survival rates were 96%/92%, 95%/92% and 95%/92%, and 1- and 5-graft survival rates were 85%/66%, 84%/73% and 83%/71% for group 1 to 3, respectively. Statistical analysis showed no significant difference in patient and graft survival between the three groups. Graft loss during the first 3 month was 10.7% in group 1 (primary non function (PNF) $n = 2$, hepatic artery thrombosis (HAT) $n = 1$), 9.4% in group 2 (PNF $n = 9$, HAT $n = 6$, intra-/postoperative patient death due to cardiopulmonary or infectious complications $n = 4$) and 14.8% in group 3 (PNF $n = 3$, HAT $n = 5$, patients death $n = 4$) without significant difference between the groups.

Conclusion: Based on few age- and size-matching donors in pediatric LTX mostly left-lateral lobes from adult livers (cadaveric split or living donation) were used for transplantation, causing large-for-size grafts in small and small-for-size grafts in older children. In our study group we found no difference in patient and graft survival between pediatric patients with size-matched organs in contrast to small- or large-for-size organs. Also rates for early graft loss due to PNF or vascular complications were equal. Actually the use of size mismatched organs seems to be a feasible procedure.

0310 DISTINCT CHANGES IN LYMPHOCYTE SUBPOPULATIONS AND EXPRESSION OF BAFF/BAFF-R AFTER PEDIATRIC KIDNEY TRANSPLANTATION

A. Lehnhardt[1], F. Dunst[1], M. van Husen[1], S. Loos[1], M. Koch[2], M.J. Kemper[1]; [1]Universitätskrankenhaus Hamburg-Eppendorf, Pädiatrische Nephrologie, Martinistr. 52, Deutschland; [2]Universitätskrankenhaus Hamburg-Eppendorf, Hepatobiliär- und Transplantationschirurgie, Hamburg, Deutschland

Introduction: Standard immunosuppressive regimes after pediatric kidney transplantation (PKTx) mainly target T-cells. Only little is known about its influence on FoxP3+ regulatory T-cells (T-Regs) and even less about B-cell immunity. B-cell activating factor (BAFF, BlyS) and its receptor BAFF-R (BR3) are integral for B-cell activation, survival, and homeostasis. Aim of our study therefore was a cross-sectional analysis of lymphocyte subpopulations and BAFF/BAFF-R in a cohort of children after renal transplantation.

Patients and Methods: Forty-four children (17 girls) with a median age of 12.3 (range 2–17.8) years were studied 2.9 (range 1–12.4) years after PKTx, three after combined liver and kidney transplant. Most patients received immunosuppressive triple therapy with steroids, calcineurin inhibitors and mycophenolic acid. 22 age- matched healthy children served a s controls. FACS analysis for lymphocyte surface antigens CD3, CD4, CD8, CD19, CD25 and BAFF-R as well as intracellular FoxP3 was performed. Serum-BAFF was measured with commercially available ELISA.

Results: While absolute numbers of lymphocytes as well as relative numbers of CD8+ T-cells and CD4+CD25+FoxP3+ regulatory T-cells were not different, the frequency of CD4+ T-cells (mean ± SE) was increased compared to healthy controls ((49.1 ± 9.3 vs 40.2 ± 5.1%, $P < 0.001$). We found significantly less CD19+ B-cells in transplanted patients (10.2 ± 3.8 vs 15.0 ± 5.0%, $P = 0.001$). Serum-BAFF was significantly higher in patients (1375 ± 418 vs 895 ± 178 pg/ml, $P < 0.001$), whereas BAFF-R expression (MFI) on CD19+ cells was lower (median 434, range 108 -1683 vs 730, range 280 -1153, $P < 0.04$). Within the group of renal transplant patients serum BAFF was inversely correlated with BAFF-R expression ($P < 0.004$, r = 0.43) and calculated GFR ($P < 0.03$, r = 0.33). The relative number of T-Regs correlated with BAFF-R expression on CD19+ B-cells ($P < 0.017$, r = 0.36).

Conclusion: Distinct alterations in lymphocyte subpopulations as well as BAFF and BAFF-R are present after PKTx. High BAFF levels may be secondary to reduced B-cell count and lead to chronic immunological allograft injury, possibly by promoting survival of alloreactive B-cells. A higher percentage of T-Regs together with higher BAFF-R expression on B cells,

as seen in some patients during follow-up, may be indicators of partial tolerance. Future studies into the regulation of T- and B-cell immunity after renal transplantation are urgent and may allow for a more individual tailoring of immunosuppression.

TRANSPLANT PSYCHOLOGY AND PSYCHOSOMATIC MEDICINE

0001 IMPACT OF DONOR-TRANSMITTED CORONARY ATHEROSCLEROSIS ON QUALITY OF LIFE (QOL) AND QUALITY-ADJUSTED LIFE YEARS (QALY) AFTER HEART TRANSPLANTATION

O. Grauhan[1], H. Chang[2], W. Albert[1], N. Hiemann[1], R. Meyer[1], H. Lehmkuhl[1], M. Dandel[1], C. Knosalla[1], M. Hübler[1], M. Pasic[1], Y. Weng[1], R. Hetzer[1]; [1]Deutsches Herzzentrum Berlin, Thorax-, Herz- und Gefäßchirurgie, Berlin, Deutschland; [2]Taipei Veterans General Hospital, Cardiac Surgery, Taipeh, Taiwan

A prevalence of significant coronary artery sclerosis (CAS) in the donor pool of about 20% and an insufficient routine donor screening without angiography result in accidental transmission of significant CAS in 5–10% of heart transplantations. The purpose of this study was to evaluate the impact of donor-transmitted coronary artery sclerosis (DCAS) on quality of life (QOL) and quality-adjusted life years (QALY) after transplantation. In 1253 consecutive transplantations single-vessel donor-transmitted CAS was found in 53 patients (DCAS1 group) and double- or triple-vessel donor-transmitted CAS in 26 patients (DCAS2/3 group). QALY were calculated and the SF-36 questionnaire was used to analyze QOL. Patients without DCAS, who were matched for sex, age and time after transplantation, served as control (NDCAS). Thirty-day mortality in groups NDCAS, DCAS1 and DCAS2/3 was 12.2%, 13.2% and 61.5%, respectively. However, beyond the first year the annual decrease in all groups was comparable (5.4%/year, 4.3%/year, and 5.0%/year). The SF-36 questionnaire showed no significant difference between the groups in the long-term survival, and despite the fact, that more coronary interventions have been performed in DTCA groups than in NDTCA group. QALY were comparable in groups NDCAS (8.0 QALY) and DCAS1 (8.5 QALY), respectively, but worse in DCAS2/3 (2.2 QALY). Donor-transmitted coronary atherosclerosis represents a risk for early graft failure but does neither impair long-term survival thereafter nor quality of life (QOL). On the other hand, donor screening by angiography seems to be a good investment to avoid the loss of about 6 quality-adjusted life years (QALY) by transmitted coronary atherosclerosis.

0107 FOR THE INVESTIGATORS OF THE WAITING FOR A NEW HEART STUDY: FLUID AND SALT INTAKE PREDICT HIGH-URGENCY TRANSPLANTATION IN THE WAITING FOR A NEW HEART STUDY

H. Spaderna[1], G. Weidner[2], S.L. Connor[3], D. Zahn[4], K.A. Bramstedt[5], J.M.A. Smits[6]; [1]Universität Mainz, Psychologie, Mainz, Deutschland; [2]San Francisco State University, Biology, San Francisco, Vereinigte Staaten von Amerika; [3]Oregon Health & Science University, Clinical Nutrition, Portland, Vereinigte Staaten von Amerika; [4]Ruhr-Universität , Klinik für Psychosomatische Medizin und Psychotherapie, Bochum, Deutschland; [5]California Pacific Medical Center, Transplant Ethics, San Francisco, Vereinigte Staaten von Amerika; [6]Eurotransplant, Thoracic Advisory Committee, Leiden, Niederlande

Objective: Patients with advanced heart failure are recommended to restrict fluid and salt intake. We investigated the role of these health behaviors for the prognosis of patients awaiting heart transplantation (HTx).
Methods: A multi-site prospective study was conducted with 318 (53.5 ± 11.4 years of age, 18% female) newly listed HTx candidates enrolled at 17 German-speaking hospitals between April 2005 and December 2006. Baseline demographics, daily fluid intake and frequency of consumption of salty foods were assessed by questionnaire. Eurotransplant provided medical characteristics at time of listing and waiting list outcomes (death, delisting due to clinical deterioration, high-urgency HTx, elective HTx, delisting due to improvement). Applying a competing risk approach, cause-specific Cox proportional hazard models were used to investigate the association of fluid intake (adjusted for cardiac index) and salty foods with each of the competing outcomes.
Results: By December 2008 (median = 338 days, range 13–1394 days) 54 patients died, 110 received high-urgency HTx, 15 were delisted due to deterioration, and 30 due to improvement. Higher fluid intake was related to a frequent salt consumption ($r = 0.22$, $P < 0.001$). Fluid intake adjusted for cardiac index and frequent consumption of salty foods had additive effects on high-urgency HTx ($X^2(2) = 11.1$, $P < 0.01$), independent of age, sex, body mass index, disease severity, and diuretics. High liquid intake and frequent consumption of salty foods both increased the hazard for this outcome (hazard ratio [HR] = 1.51, 95% confidence interval [CI] 1.06–2.14; HR = 1.97, 95% CI 1.02–3.81). Both health behaviors were unrelated to any competing outcomes.
Conclusion: Patients who do not restrict their salt and fluid intake appear to experience a health decline prompting high-urgency HTx. Behavioral interventions focusing on adherence to the recommended fluid and salt intake could help to reduce the relatively high number of patients placed in high-urgency status.

0115 LIVING KIDNEY DONORS' QUALITY OF LIFE IN THE FIRST YEAR: A PROSPECTIVE LONGITUDINAL STUDY WITH AN APPROPRIATE REFERENCE GROUP

S. Kröncke[1], B. Nashan[2], L. Fischer[2], K.-H. Schulz[1]; [1]Universitätsklinikum Hamburg-Eppendorf, Hepatobiliäre und Transplantationschirurige & Medizinische Psychologie, Hamburg, Deutschland; [2]Universitätsklinikum Hamburg-Eppendorf, Hepatobiliäre und Transplantationschirurige, Hamburg, Deutschland

Objective: Prospective studies on the course of living kidney donors' quality of life (QOL) are still rare. Furthermore, the existing studies do not consider an adequate reference group. Instead, they compare healthy donors with the general population, which includes subjects with diseases. This is the first prospective study comparing living donors not only to the general population but also to a healthy sample, which constitutes a more adequate comparison group.
Methods: In a prospective design, we investigated QOL (Short-Form 36-Item Health Survey, SF-36) as well as anxiety and depression (Hospital Anxiety and Depression Scale, HADS) in living kidney donors ($n = 35$) before donation and at 3 postoperative time points (1 week, 3 months, 1 year).
Results: Our results show an impaired physical QOL 1 week and 3 months post donation. Mental QOL as well as anxiety did not demonstrate any significant changes across time. The depression score, however, increased at 1 week post donation with a subsequent decrease later.
Conclusions: The postoperatively unaltered mental QOL might be due to a careful psychological evaluation and postoperative support of the donors. The physical impact of living donation, on the other hand, seems to be severe and persisting for at least 3 months postoperatively. Further research is needed to determine the length and nature of the postoperative QOL impairment as well as possible influencing factors. The first postoperative year should be monitored closely to provide medical or psychosocial care when indicated.

0207 ARE LIVER TRANSPLANTED CHILDREN MORE IMPULSIVE? ATTENTION AND EXECUTIVE FUNCTIONING AFTER PEDIATRIC LIVER TRANSPLANTATION

*T. Kaller[1], *N. Langguth[1], R. Ganschow[2], B. Nashan[3], K.H. Schulz[1,4]; [1]UKE, Medizinische Psychologie, Hamburg, Deutschland; [2]UKE, Pädiatrische Hepatologie und Lebertransplantation, Hamburg, Deutschland; [3]UKE, Hepatobiliäre Chirurgie, Hamburg, Deutschland; [4]UKE, Transplantationszentrum, Hamburg, Deutschland*

Aims: Liver transplanted children have an increased risk to develop serious developmental problems. We examined attention and executive functioning and their relation to intelligence, behavior, quality of life, and several disease-related variables after transplantation.
Methods: Participants mean age at transplantation was 3.7 ± 3.8 years ($n = 137$, aged 10.3 ± 3.7 years). Assessment included: attention and executive functioning (TAP/KITAP), intelligence (WISC/K-ABC), behavior (SDQ), and quality of life (Kidscreen-52).
Results: In most TAP ($n = 67$) and KITAP ($n = 70$) subscales children scored in the lower normal range, but reaction times were significantly below the population mean, i.e., TAP- and KITAP-Alertness. However, the TAP-Go/NoGo reaction time was significantly above the population mean. Reaction times in Alertness and Go/NoGo tasks differed significantly. Most TAP and KITAP subscales, especially Alertness and Go/NoGo, were highly correlated with WISC and K-ABC subscales indicating that liver transplanted children with lower intelligence scores display longer reaction times. Moreover, significant correlations were obtained between Alertness and Go/NoGo and several SDQ subscales. Children with longer reaction times suffered from more behavioral problems. Additionally, KITAP-Alertness was significantly correlated with Bullying (Kidscreen-52). The TAP-Go/NoGo subscale correlated significantly with age at transplantation and duration of illness implying that younger children and children with a shorter duration of illness revealed higher T-scores.
Conclusion: Results provide evidence suggesting that liver transplanted children are at risk of developmental deficits and behavioral problems. Deceleration at Alertness tasks but acceleration at Go/NoGo tasks indicate that children skipped the decision-making process required for Go/NoGo performance and treated Alertness and Go/NoGo tasks as equal. That implies liver transplanted children display a deficient control of non-adequate reactions or impulsive behavior. In addition, high correlations between Go/NoGo performance and intelligence, mental health, and quality of life emphasized the relevance of attention and executive functioning, especially response inhibition, for several life domains. In summary, results demonstrate the need for an early and comprehensive developmental screening after pediatric liver transplantation.

HEART AND LUNG TRANSPLANTATION

0004 COMPLIANCE UNDER MODIFIED RELEASE TACROLIMUS IN CHRONIC STABLE PATIENTS AFTER HEART TRANSPLANTATION

A. Dösch[1], S. Mueller[1], I. Gueler[1], S. Celik[1], M. Konstandin[1], L. Frankenstein[1], A. Koch[2], P. Ehlermann[1], H.A. Katus[1]; [1]Uniklinik Heidelberg, Abteilung für Kardiologie, Heidelberg, Deutschland; [2]Uniklinik Heidelberg, Abteilung für Herzchirurgie, Heidelberg, Deutschland

Background: Modified release tacrolimus is a new, once-daily oral formulation of the established immunosuppressive agent tacrolimus. This study evaluated patient compliance under modified release tacrolimus vs. conventional tacrolimus or cyclosporine A in patients after heart transplantation (HTX).
Methods: Fifty-four chronic stable patients (41 male, 13 female) were switched to modified release tacrolimus from conventional tacrolimus or cyclosporine A according to manufacturer's recommendations. Compliance was assessed at baseline and 4 months after switch with a validated questionnaire (The Basel Assessment of Compliance with Immunosuppressive Medication Scales [BAASIS]) including a *Visual* Analogue Scale (*VAS*). Noncompliance is defined as any self reported noncompliance (response score 1 to 5) on any of the 4 items.
Results: Fifty patients were available for statistical analysis, as modified release tacrolimus was discontinued due to patients' preference in one patient and gastrointestinal disturbances in three patients. After 4 months, BAASIS score improved significantly from 2.9 ± 0.5 to 1.5 ± 0.5 ($P < 0.0001$). Compliance was improved in 28 patients (56.0%), unchanged in 18 (36.0%), and impaired in 4 patients (8.0%). VAS score improved statistically significant from 82.3 ± 2.6% to 97.5 ± 4.8% ($P < 0.0001$). No significant changes regarding hematological, renal or liver function parameters were observed after 4 months (all P = NS).
Conclusions: Therapeutic regimens for transplant recipients are often complex, contributing to a high incidence of medication noncompliance. This study in chronic stable patients after HTX demonstrates a significant improvement in patient compliance after switch to modified release tacrolimus. Modified release tacrolimus was generally well tolerated. Further studies are currently underway to investigate long-term safety after HTX regarding prevention of rejection and occurrence of side effects.

0022 MYOCARDIAL WALL MOTION AND DEFORMATION IMAGING ALLOW THE PROOF OF FUNCTIONALLY RELEVANCE FOR CARDIAC ALLOGRAFT REJECTIONS WHICH ARE UNDERESTIMATED BY MORPHOLOGIC GRADING

M. Dandel, H. Lehmkuhl, C. Knosalla, D. Kemper, R. Hetzer; Deutsches Herzzentrum Berlin, Herz- Thorax- u. Gefäßchirurgie, Berlin, Deutschland

Background: Although the ISHLT scoring system constitutes the basis of therapeutic decisions during heart transplant acute rejection (AR), it does not always reflect the progressive severity of functional alterations associated with AR. Myocardial wall motion and deformation assessment by tissue Doppler (TD) and 2D-strain imaging reveal alterations of contractile function undetectable by conventional echocardiography. To improve therapeutic decisions, we compared the endomyocardial biopsy (EMB) data with functional data provided by TD and 2D-strain imaging.
Methods: Between 1/2006 and 6/2009, echocardiographic examinations including TD and 2D-strain imaging were performed prior to all EMBs. Alterations of left ventricular (LV) TD and 2D-strain parameters were tested for relationships to the morphologic grade of AR.
Results: Of 136 cellular ARs detected in EMBs obtained from 95 patients, 104 (76.5%) were mild (grade 1A, 1B or 2), 28 (20.6%) were moderate (3A) and 4 (2.9%) were severe (3B or 4). Of 104 histologically mild rejections, 26 (25.0%) were clinically manifested (fatigue, dyspnea etc.). Whereas only 2 of 104 histologically mild ARs were associated with LV changes detectable by conventional echocardiography, all 26 symptomatic plus 9 asymptomatic histologically mild ARs were accompanied by reduction with >15% of both, LV systolic wall motion peak velocity and global systolic strain-rate (circumferential and longitudinal). TD and 2D strain parameter changes were reversible after AR therapy in all patients. Among the 79 ARs with reversible TD and/or 2D strain parameter alterations, 55.7% were moderate or severe (3A, 3B, 4), the other 44.3% were histologically mild (< 3A). TD and 2D strain velocity parameter changes detected during symptomatic mild AR episodes, were equivalent to those found during cellular higher graded ARs (3A or higher).
Conclusions: TD and 2D strain imaging allow the proof of functionally relevance for ARs which are underestimated by morphologic grading. The relatively high prevalence of histologically mild rejections accompanied by myocardial dysfunction and the reversibility of functional alterations after therapy suggests the importance of TD and 2D strain imaging as potential guides for therapeutic decisions.

0028 ANTIBODY RESPONSE AFTER A SINGLE DOSE OF AN AS03-ADJUVANTED SPLIT-VIRION INFLUENZA A (H1N1) VACCINE IN HEART TRANSPLANT RECIPIENTS

S. Meyer[1], M. Adam[1], C. Ilchmann[2], C. Eulenburg[3], E. Sattinger[1], H. Runte[1], M. Schlüter[1], T. Deuse[4], H. Reichenspurner[4], A. Costard-Jäckle[1]; [1]Universitäres Herzzentrum Hamburg, Klinik für Allgemeine und Interventionelle Kardiologie, Hamburg, Deutschland; [2]Universitätsklinikum Hamburg-Eppendorf, Institut für Med. Mikrobiologie, Virologie und Hygiene , Hamburg, Deutschland; [3]Universitätsklinikum Hamburg-Eppendorf, Institut für Med. Biometrie und Epidemiologie, Hamburg, Deutschland; [4]Universitäres Herzzentrum Hamburg, Klinik für Herz- und Gefäßchirurgie, Hamburg, Deutschland

Introduction: Vaccination against the novel influenza A (H1N1) virus has been advocated for programs in cardiothoracic transplantation, but there is limited experience on the use of AS03-adjuvanted A/H1N1 pandemic influenza vaccines in immunosuppressed patients.
Materials and Methods: We conducted an observational, non-randomized single-center study to assess antibody response, vaccine-related complications and adverse effects in 47 heart transplant recipients (44 men; 56 ± 13 years). The AS03-adjuvanted, inactivated split-virion A/California/7/2009 H1N1v pandemic vaccine was administered. Immunoglobulin G (IgG) response was determined using a new pandemic influenza A IgG/IgA ELISA test kit. Adverse effects of vaccination were assessed by a standardized questionnaire.
Results: Eight patients (17%) had relevant pre-vaccination antibody concentrations. In the remaining 39 patients, vaccination resulted in a statistically significant increase of antibody concentration at 20 ± 2 days, with 16 patients (41.0%; 95% CI, 25.6% to 57.9%) reaching a relevant IgG concentration. Age, time post transplantation, and immunosuppressive regimen did not impact antibody response. Vaccination was well tolerated; at 4 months, no patient had reported influenza-like symptoms; serious adverse effects were not observed.
Conclusion: Single-dose administration of an AS03-adjuvanted vaccine against the novel influenza A (H1N1) virus induces a relevant antibody response in a substantial proportion of heart transplant recipients.

0029 PRECONDITIONING REGIMES FOR THE INDUCTION OF TOLERANCE IN PORCINE ALLOGENEIC LUNG TRANSPLANTATION: PERSPECTIVES FOR CLINICAL APPLICABILITY

K. Dreckmann, G. Warnecke, M. Avsar, A.-K. Knöfel, N. Madrahimov, W. Sommer, J. Gottlieb, J. Karstens, A. Haverich, M. Strüber; Medizinische Hochschule Hannover, HTTG Chirurgie, Hannover, Deutschland

Purpose: The preconditioning regime is essential for the induction of peripheral tolerance in our porcine allogeneic lung transplantation model. In order to further develop the protocol for tolerance induction, a combination of non-myeloablative irradiation and splenocyte infusion was performed in minipigs.
Methods: Allogeneic, left-sided single lung transplantation was performed in 29 MHC-mismatched minipigs. Intravenous pharmacologic immunosuppression was withdrawn after 28 postoperative days. In order to define the most effective treatment, different irradiation regimes were applied: 0.5 Gy whole body irradiation (WBI) and 7 Gy thymic irradiation (TI); 1.5 Gy WBI and 7 Gy TI; 1.5 Gy total lymphoid irradiation (TLI) and 7 Gy TI; 8.5 Gy TI and no irradiation. In all but the control group animals were treated with a donor splenocyte co-transplantation.
Results: Applied doses and character of non-myeloablative irradiation had a direct effect on the outcome of the experimental group, namely longterm survival of the graft. Shielding of the bone marrow from irradiation led to reduced side effects but also resulted in a decreased allograft survival rate. Selective irradiation of the thymus without WBI, and WBI at a reduced dose proved to be ineffective for reliable tolerance induction. Furthermore, long term survival in individual animals was associated with high levels of circulating CD4+CD25high+ regulatory T cells.
Conclusion: A combination of donor splenocyte co-transplantation and preoperative irradiation is effective once a cytoreductive threshold dose is exceeded. Further modification of the protocol may reduce the toxicity of the irradiation regime to balance efficient lymphocyte depletion with potential clinical applicability.

0066 SURGICAL WOUND COMPLICATIONS IN DE NOVO HEART TRANSPLANT RECIPIENTS TREATED WITH EVEROLIMUS: INCIDENCE AND MANAGEMENT

M. Barten[1], J.M. Arizon[2], G. Dong[3], H. Eisen[2], J. Kobashigawa[2], H.B. Lehmkuhl[2], C. Pellegrini[2], H. Ross[2], A. Zuckermann[2]; [1]Herzzentrum, Leipzig, Deutschland; [2]Study group, RAD001B253/A2411/A2403, Oesterreich; [3]Novartis Pharma, East Hanover, Vereinigte Staaten von Amerika

Introduction: The use of the mTOR inhibitors sirolimus and everolimus (EVR) in heart transplant recipients (HTxR) offers effective immunosuppression but the antiproliferative mode of action might increase the risk for surgical wound complications. For sirolimus, an incidence of deep surgical wound healing complications in 35% of HTxR was reported. Thus, the analysis of incidence and management of wound complications with EVR is of interest.

Methods: A post-hoc analysis identified wound complications in the adverse event (AE) databases of 3 randomized multicenter studies in 1007 de novo HTxR: B253 ($n = 634$), A2403 ($n = 199$), A2411 ($n = 174$). HTxR received fixed-dose (EVR 1.5 mg, 3 mg) or TDM-EVR (C0 3 8 ng/mL), azathioprine (AZA) or mycophenolate mofetil (MMF) with standard- or reduced-exposure cyclosporine A (CsA). Treatment was initiated within the first 72 hours post-Tx. Wound complication AEs up to Day 90 in the pooled EVR group were analyzed overall and per wound complication category for incidence, time to onset, and action taken and compared to AZA and MMF groups.

Results: Baseline characteristics were balanced across the three studies. Wound complications occurred overall in 14.8% of EVR-treated HTxR compared to 13.1% of AZA- and 8.4% of MMF-treated HTxR. Wound and sternal dehiscence occurred in less than 2.0% of HTxR in all groups. The majority of events were wound infections, which were classified to their site as incisional, mediastinal or other (Table). In 3.7% of EVR-treated patients, 4.2% of AZA-, and 2.4% of MMF-treated patients the wound complication required no action. Co-medication or non-drug therapy was initiated in 9.7%, 8.9%, 7.2% and 7.3%, 4.2%, and 4.8% of patients in the EVR, AZA, and MMF groups, respectively. Hospitalization for treatment was reported in 7.0% of patients with EVR compared to 5.2% in the AZA and 2.4% in the MMF group. Wound complication events occurred in the first 30 days post-Tx in 10.3%, 11.0%, and 7.2% of patients treated with EVR, AZA, or MMF, respectively. HTxR with initiation of EVR within 24 hours post-Tx had a higher incidence of wound complications than HTxR who started EVR treatment 48–72 hours post-Tx (24.3% versus 12.7%).

Conclusion: Our cross-study analysis of 1007 HTxR showed a notably lower incidence of wound complications in HTxR treated with EVR compared to previous reports with sirolimus. The majority of wound complications occurred in the early post-operative phase and did not require or prolong hospitalization. Delaying the initiation of EVR to 48–72 hours post-Tx might help to reduce the risk of wound complications.

0104 EMPHASIS OF THERAPEUTIC DRUG MONITORING: PROTON PUMP INHIBITORS IMPACT CYCLOSPORINE DOSE/ EXPOSURE RELATION IN DE NOVO HTXR TREATED WITH MMF OR EVEROLIMUS

C. Bara[1], G. Laufer[2], M.G. Crespo[3], T. Dengler[4], A. Karpov[5], L. Potena[6], H. Schwende[5], S. Varnous[7], G. Junge[5]; [1]Medizinische Hochschule Hannover, Hannover, Deutschland; [2]Universitätsklinik für Chirurgie, Vienna, Oesterreich; [3]Servicio de Cardiologia, Hospital A Coruna, A Coruna, Spanien; [4]SLK-Kliniken Heilbronn GmbH, Bad Friedrichshall, Deutschland; [5]Novartis Pharma AG, Basel, Deutschland; [6]Istituto di Malattie dell' apparato cardiovasculare, Bologna, Italien; [7]Groupe Hospitalier La Pitié-Salpêtrière, Paris, Frankreich

Introduction: Gastro-intestinal disorders are a well known side effect after heart transplantation (HTx) and are often treated with proton pump inhibitors (PPI). Drug-drug interactions with PPI have been described for CsA and MMF but not for the mTOR inhibitor everolimus (EVR). Here, the impact of PPI co-administration on cyclosporine (CsA) and EVR exposure is evaluated in immunosuppression with CsA and MMF versus CsA and EVR.

Methods: Data of 373 de novo HTxR from two randomized studies (A2403 [$n = 199$]; A2411 [N = 174]) were reviewed retrospectively. HTxR received TDM-EVR (target C0, 3–8 ng/mL) with standard dose CsA (SD-CsA/EVR, N = 100) or reduced dose CsA (RD-CsA/EVR, N = 190; pooled from 2 studies) or MMF 3g/d (SD-CsA/MMF, N = 83). Data of HTxR with or without PPI (PPI+/PPI-) were reviewed. The impact of PPI administration on dose-normalized (DN) blood exposure of both, CsA (C0, C2) and EVR (C0 [ng/mL]/ dose [mg]) was analyzed for MMF and EVR groups up to Month (M) 6 post-HTx.

Results: More HTxR took PPI at M6 in the MMF group (84.3%) compared to the SD-CsA/EVR (45.0%) and RD-CsA/EVR groups (64.7%). Comparison for PPI+ versus PPI- HTxR in the individual treatment groups showed that both, DN-CsA C0 and C2-levels, were lower for PPI+ HTxR between M1–6 in the MMF group (Table). In the pooled SD and RD-CsA/EVR groups PPI+ HTxR showed higher DN-CsA C0 and C2-levels compared to PPI- HTxR ($P = 0.04$; <0.01). Comparison of PPI+ HTxR between the treatment groups during M1–6 showed that DN-CsA C0 and C2-levels were higher for both, SD-CsA/ EVR and the pooled EVR groups vs the MMF group (C0[C2] $P = 0.04$[<0.01]). DN-EVR C0 levels were not affected by PPI intake ($P = 0.60$).

Conclusion: A higher percentage of HTxR on MMF needed PPI treatment compared to EVR treated HTxR. PPI dependent changes in DN-CsA C0 and C2-levels were seen with MMF and EVR, suggesting an interaction of PPI with CsA. Whereas MMF led to lower DN-CsA exposure in PPI+ HTxR, the results were inverse with EVR. This underlines the benefit of TDM to control the effects of such interactions. In contrast to recent reports on MMF-PPI interaction, our observations show that EVR exposure is not affected by PPI intake.

0133 EXTRACORPOREAL MEMBRANE OXYGENATION EXPERIENCE FOR LUNG FAILURE AFTER TRANSPLANTATION

G. Färber, A. Mühle, T. Doenst, S. Lehmann, C. Binner, J. Garbade, F.W. Mohr, H.B. Bittner; Herzzentrum Leipzig, Herzchirurgie, Leipzig, Deutschland

Introduction: Graft failure after lung transplantation (LTX) is a life-threatening complication. Extracorporeal membrane oxygenation (ECMO) is a treatment option as bridge to graft recovery or retransplantation. However, little is known about outcome. We reviewed our LTX experience and associated ECMO use.

Methods: This retrospective study is an analysis of all LTX patients ($n = 104$) between 2004 and 2009 in our department. Incidence of ECMO-implantation, 30-days and 1-year mortality as well as morbidity were investigated.

Results: ECMO-implantation was performed in 20 cases (19%, single-LTX $n = 11$, double-LTX $n = 9$). Indications were mainly primary graft dysfunction (PGD, $n = 10$), rejection ($n = 2$), acute respiratory distress syndrome (ARDS, $n = 3$), sepsis ($n = 2$) and low cardiac output during resuscitation ($n = 3$). ECMO-support ranged from 0.5 to 21 days (mean-duration = 2). Nine patients died within 30 days (47.4%) and additional five patients did not survive the first year (73.7% cumulative mortality). Patients with PGD showed the highest lung recovery potential, but seven of them died in the following course because of cerebral bleeding ($n = 1$), sepsis ($n = 1$ in 30 days, plus 3 in the first year) and multiorgan failure ($n = 2$). One of the two rejection-patients died 1 week after ECMO-explantation with a history of retransplantation due to PGD and ECMO-support. All Patients suffering from ARDS ($n = 3$), sepsis ($n = 1$) or cardiogenic shock ($n = 2$) died within 33 days. Thus, one-year-survival was 26.3%.

Conclusion: For lung transplanted patients with primary graft dysfunction ECMO-support is an alternative therapeutic strategy with limited but acceptable outcome. Post-LTX ECMO-support correlates with poor outcome for ARDS, Sepsis or cardiogenic shock.

0145 LEVOSIMENDAN FOR PRIMARY GRAFT FAILURE AFTER HEART TRANSPLANTATION: A THREE-YEAR FOLLOW UP

A. Beiras-Fernandez[1], F. Weis[2], F. Kur[1], M. Weis[2], I. Kaczmarek[1], B. Reichart[1]; [1]Ludwig-Maximilians-Universität, Herzchirurgische Klinik, München, Deutschland; [2]Ludwig-Maximilians-Universität, Klinik für Anästhesiologie, München, Deutschland

Background: Primary graft failure (PGF) is a severe complication responsible for 42% of in-hospital mortality after heart transplantation. It has been postulated that once 30-day survival is achieved, patients with PGF have no increased risk of death. Levosimendan increases 30-day survival in patients with PGF. We report the 3-year follow up of a single-centre cohort of patients with PGF treated with levosimendan.

Methods: Fifty-three patients underwent heart transplantation at our institution from 9/2005 to 12/2006. Twelve patients (22.6%) presented with PGF and were treated with levosimendan in a 24-hour continuous infusion (0.10 μg/kg/ minutes). Risk factors for 1-year and three-year mortality were analyzed. 30-day, 1-year and three-year survival were determined and compared with the patients without PGF ($n = 41$).

Results: There were no significant differences in donor's age, weight, height, and sodium within both groups. However, the ischemia time (259 + 53 vs. 227 + 50 minutes; $P = 0.06$) and the recipient's age (51.6 + 15 vs. 41.5 + 21 years; $P = 0.07$) were higher in the PGF patients. 30-day survival was 92 % in both groups. After one and 3 years, survival was significantly lower in the PGF cohort (50% vs. 80.6% and 41.7% vs. 80.6%; $P < 0.05$). 86.5% of the PGF patients died of non-cardiac reasons, predominantly infections.

Conclusions: Although treatment of PGF with levosimendan increases the 30-day survival, one year and three-year survival are reduced in this cohort of patients. PGF is associated with a poor long-term outcome, which might be a consequence of systemic malperfusion during cardiac low-output after transplantation.