TRANSPLANT
INTERNATIONAL

LECTURES

NOVEL IMMUNOSUPPRESSANTS AND HUMORAL REJECTION

V01 DOES THE OCCURENCE OF POSTTRANSPLANT *DE NOVO* DONOR-SPECIFIC HLA ANTIBODIES DEPEND ON THE IMMUNOSUPPRESSIVE REGIMEN IN IMMUNOLOGICALLY LOW-RISK PATIENTS?

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Background: The important role of humoral immunity in the pathogenesis of chronic allograft nephropathy has prompted research assessing the role of anti-HLA antibody (Ab) monitoring as a tool to predict allograft outcome.
Methods: We evaluated 106 immunologically low risk renal allograft recipients for *de novo* Ab occurrence. DSA were assessed by Luminex technology. Initial immunosuppression consisted of basiliximab as induction therapy, CsA, MPA and steroids.
Results: Altogether, sera of 106 immunologically low-risk patients were included in the present analysis. At month 3 after transplantation, 86 patient were eligible for randomization either to continue standard immunosuppression (S: CsA+MPA, $n = 28$) or to be converted to a CNI-free regimen with the mTOR inhibitor everolimus (CNI-free: EVE+MPA, $n = 29$) or to a CNI-low regimen (CNI-low: CsA+EVE, $n = 29$). At 4-year follow up, 17/79 patients (22%) had developed *de novo* DSA with 4/24 (17%), 5/28 (18%) and 8/27 (30%) in the standard, CNI-free and CNI-low group, respectively (n.s.). DQ *de novo* DSA was much more likely to appear compared with other loci antibodies. In the follow-up period, four patients developed active C4d+ antibody-mediated rejection (AMR). In all four patients classical *de novo* DSA directed to HLA-A, -B or -DR antigens were observed.
Conclusions: In a 4-year follow-up period, 22% of immunologically low-risk renal allograft recipients developed *de novo* DSA. Incidence of *de novo* DSA was higher in the CNI-low group without reaching statistical significance. Most of the DSA were directed to the HLA-antigen DQ. AMR was only noticed in patients demonstrating *de novo* DSA directed to "classical" HLA-antigen A, B or DR.

V02 ECULIZUMAB BRIDGING AND PLASMA CELL-DIRECTED THERAPY – A NOVEL CONCEPT IN SEVERE ANTIBODY MEDIATED REJECTION IN KIDNEY TRANSPLANTATION

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Rationale: Acute antibody mediated rejection (AMR) is a severe complication following kidney transplantation with profound impact on allograft survival. Human leukocyte antigen (HLA) antibody mediated cytotoxicity involves terminal complement activation. We report complement C5b inhibition by eculizumab to rapidly interrupt renal injury in AMR, thereby providing a window for antibody-directed therapy to become effective.
Methods: A case series of three patients with severe AMR and high titres of donor specific antibodies (DSA) is presented. Eculizumab was administered followed by plasma cell therapy with bortezomib or cyclophosphamid, respectively.
Results: In all patients, renal function stabilized rapidly despite unchanged DSA titres. Between one and 9 doses of eculizumab were administered until plasma cell therapy effectively reduced DSA titres over time. After a follow up of 2.5–21 months, median serum creatinine is 1.7 mg/dl and median eGFR is 31 ml/min/1.73 m^2.
Conclusion: Complement C5b blockade by eculizumab is highly effective in interrupting HLA antibody-mediated cell injury in AMR. As a novel concept, eculizumab bridging creates a therapeutic window for antibody-directed B- or plasma cell therapy to become effective and may improve outcome of AMR in kidney transplantation.

V03 EARLY EVEROLIMUS PLUS REDUCED TACROLIMUS VERSUS STANDARD TACROLIMUS IN DE NOVO LIVER TRANSPLANT RECIPIENTS RESULTS IN SUPERIOR RENAL FUNCTION AND SUSTAINS OVER 24 MONTHS: RESULTS OF A RANDOMIZED TRIAL

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Introduction: mTOR inhibitors have the potential to reduce calcineurin inhibitor nephrotoxicity by minimizing or eliminating the need for their use. The 12 month (M) results of H2304 study demonstrated superior renal function with everolimus (EVR) plus reduced tacrolimus (rTAC) vs. standard TAC (TAC-C) in de novo liver transplant recipients (LTxR). Presented here are 24M renal function results.
Methods: This 24M, multicenter, open-label study randomized (1:1:1) 719 de novo LTxR after a 30-day (±5 days) run-in period with TAC (±mycophenolate mofetil), to receive either EVR (C0 3–8 ng/mL) with rTAC (C0 3–5 ng/mL; EVR+rTAC, $N = 245$) or EVR (C0 6–10 ng/mL) with TAC withdrawal (TAC-WD; $N = 231$) at M4 or TAC-C (C0 6–10 ng/mL; TAC-C, $N = 243$); all arms included corticosteroids. Enrollment in TAC-WD arm was stopped early due to higher rejection rates. Main endpoints at M24 included composite efficacy failure rate of treated biopsy proven acute rejection, graft loss or death, and evolution of renal function from randomization (RND) to M24 measured as eGFR by MDRD4.
Results: At M24, composite efficacy failure rate in EVR+rTAC arm was comparable to TAC-C (10.3% vs. 12.5%, $P = 0.452$). Evolution of renal function from RND to M24 was superior for EVR+rTAC vs. TAC-C with an adjusted mean difference in eGFR change of 6.66 mL/min/1.73 m2 ($P = 0.0018$; ITT population). Significantly higher eGFR with EVR+rTAC was achieved at M2 post-LTx and was maintained until M24. On-treatment data showed a decrease in mean eGFR from RND to M24 of 6.6 mL/min/1.73 m2 with EVR+rTAC vs. 13 mL/min/1.73 m2 with TAC-C and 2.5 mL/min/1.73 m2 gain with TAC-WD. Urinary protein:creatinine ratio (mg/g) at M24 was higher with EVR+rTAC vs. TAC-C (Mean ± SD: 194 ± 280 vs. 159 ± 284, $P = 0.006$).
Conclusion: Early introduction of EVR at 1M post-LTx with rTAC showed superior renal function sustained for 24M compared to TAC-C, without compromising efficacy in de novo LTxR.

V04 EVEROLIMUS PLUS REDUCED TACROLIMUS IN *DE NOVO* LIVER TRANSPLANT RECIPIENTS ACHIEVES COMPARABLE OVERALL EFFICACY WITH FEWER BIOPSY-PROVEN ACUTE REJECTIONS VERSUS STANDARD TACROLIMUS: 24-MONTH RESULTS OF A RANDOMIZED TRIAL

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Purpose: The H2304 study compared the efficacy and safety of concentration-controlled everolimus (EVR) to eliminate or to reduce tacrolimus (TAC) versus standard TAC (TAC-C) in *de novo* liver transplant recipients (LTxR). Here, we present the 24M results.
Methods: In this 24M, multicenter, open-label study, 719 *de novo* LTxR were randomized (1:1:1) on day 30 ± 5 to receive EVR (C0 3–8 ng/mL) with reduced TAC (C0 3–5 ng/mL; EVR+rTAC) or EVR (C0 6–10 ng/mL) with TAC withdrawal (TAC-WD) at M4 or TAC-C (C0 6–10 ng/mL), all with steroids. Composite efficacy failure rate (treated BPAR [tBPAR], graft loss, or death) and its components, renal function (eGFR estimated by MDRD4 formula) and safety were assessed at M24. The TAC-WD arm was prematurely terminated due to higher rate of acute rejection (EVR+rTAC vs. TAC comparison is presented).
Results: At M24, the composite efficacy failure rate was comparable between EVR+rTAC and TAC-C (10.3% vs. 12.5%; difference: −2.2% [97.5% CI: −8.8%, 4.4%]). BPAR was significantly lower and less severe with EVR+rTAC vs. TAC-C (Table). At M24, EVR+rTAC maintained superior renal function vs. TAC-C (difference in eGFR change from randomization: 6.66 mL/min/1.73 m^2 [97.5% CI: 1.9, 11.42] $P = 0.0018$ for ITT population and 8.69 mL/min/1.73 m^2 [97.5% CI: 4.01, 13.36] $P < 0.0001$ for on-treatment patients). In the EVR+rTAC group 29.8% patients discontinued study drug due to AEs/infection compared to 21.5% in the TAC-C group. No new safety signals were identified during the study.
Conclusion: Early TAC reduction facilitated by EVR at 1M in LTxR maintains antirejection efficacy and leads to superior renal function versus standard TAC.

V05 THE PROTECT STUDY: EFFECT OF 35 MONTH EVEROLIMUS MONOTHERAPY VS. CALCINEURIN INHIBITOR-BASED THERAPY ON EFFICACY AND RENAL FUNCTION IN LIVER TRANSPLANT RECIPIENTS

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Post-transplant immunosuppression with calcineurin inhibitors (CNIs) is associated with impaired renal function. The PROTECT study showed that conversion from CNI to the mTOR-inhibitor everolimus (EVR) at 4 weeks after liver transplantation (LTx) achieves better renal function at 11 months (M) without compromising efficacy. Results of the PROTECT extension period up to 35 M are presented.

In this study LTx patients with initial good renal function (GFR ≥50 mL/min) 4–8 weeks after liver transplantation (LTx) were randomized to either continued CNI treatment ($n = 96$; standard CNI dose ± steroids) or switch to EVR ± steroids ($n = 98$). EVR was adjusted to target trough level of 5–12 ng/mL and CNI was withdrawn stepwise until week 16 post randomization. Patients who completed the 11 month core study were followed up to month 35 in the extension phase.

A total of 81 patients (EVR, $n = 41$; CNI, $n = 40$) continued in the follow-up phase. From M12 to M35 further renal function deterioration was observed in the CNI-arm while renal function remained stable in patients receiving EVR. Difference in eGFR between EVR and CNI: Cockcroft-Gault M11: −6.8 mL/min [$P = 0.240$];; M23: −9.8 ml/min [$P = 0.104$]; M35: -10.5 mL/min [$P = 0.096$) and Nankivell formula (M11: -6.6 mL/min [$P = 0.084$]; M23: -8.8 ml/min [$P = 0.039$]; M35: −10.5 mL/min [$P = 0.015$]). At M35 there were no significant differences in rates of mortality (EVR: 4.3% vs. CNI: 10.0%, $P = 0.535$), biopsy-proven acute rejection (24.4% vs. 15.8%, $P = 0.434$), and efficacy failure [BPAR, graft loss, death or loss to follow-up] (29.8% vs. 28.2%, $P = 0.903$) were similar. Discontinuation of study treatment due to an AE during the follow-up period was observed in 5 (12.2%) patients on EVR vs. 6 (15.0%) in the CNI group.

Conversion from CNI-based to EVR-based immunosuppression can be a safe alternative post-LTx and potentially reduces the risk of end-stage renal disease.

V06 SELECTIVE INHIBITION OF NFATC1 ACTIVATION IN RECIPIENT T-CELLS PROLONGS GRAFT SURVIVAL IN A MOUSE MODEL OF HETEROTOPIC HEART TRANSPLANTATION

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Introduction: The transcription factor NFATc1 whose expression is strongly induced by immune receptor signals in T-cells plays a key role in differentiation and function of peripheral T-cells. Calcineurin (CN) inhibitors are widely used in organ transplantation to prevent graft rejection. However, these inhibitors cause severe side effects, likely because of multiple CN targets. Here, we investigated the alternative T-cell specific ablation of NFATc1 expression to prevent allograft rejection.
Material and Methods: *Nfatc1$^{flx/flx}$/CD4-cre* mice on C57BL/6 background with the conditional inactivation of the *Nfatc1* gene in T-cells were used as recipients for BALB/c heart allografts. Allograft survival, the number of graft infiltrating cells (GICs) and the cytotoxicity of GICs were determined.
Results: Allogeneic grafts in *Nfatc1$^{flx/flx}$/CD4-cre* mice showed prolonged survival compared to allografts in wild type mice ($P = 0.0002$). The median allograft survival time in wild type mice was 8 days. In *Nfatc1$^{flx/flx}$/CD4-cre* mice, 56% of the allografts survived long term (>100 days). To analyse the local immune activation, the number of GICs was determined 5 days after transplantation. Allografts in *Nfatc1$^{flx/flx}$/CD4-cre* mice showed a lower total count of GICs ($p < 0.05$) and lower T-cell mediated cytotoxicity in comparison to wild type recipients.
Discussion and Conclusion: The proof of concept experiment with *Nfatc1$^{flx/flx}$/CD4-cre* mice shows that the selective ablation of NFATc1 expression in T-cells decreases immune activation and prolongs allograft survival. Therefore, selective targeting of NFATc1 expression in immune cells with designed small molecules may be an alternative to CN inhibitors with potentially fewer side effects.

MARGINAL ORGANS AND EXPANSION OF THE DONOR POOL

V08 THE PROGNOSTIC VALUE OF GLOMERULAR AND TUBULAR PROTEINURIA IN DECEASED DONOR KIDNEYS IN PREDICTING THE OUTCOME TWELVE MONTHS AFTER TRANSPLANTATION ESPECIALLY OF MARGINAL DONOR KIDNEYS

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Purpose: To find out whether analyzing the extent and profile of donor proteinuria could aid in predicting the success or failure of a (marginal) renal allograft.
Methods: Laboratory measurements of albumin, immunoglobulin G (IgG) and alpha-1-microglobulin (a-1-M) were performed in urine samples of brain dead donors at time of procurement using the ratio of protein (mg) to creatinine (g).
Results: Urine samples of 496 donor kidneys were analyzed. Mean donor age was 51(+16) years, applying UNOS expanded donor criteria 42% of all kidneys were marginal. Univariate analysis found both urinary IgG (beta 1, [95% CI] 0.171 [0.060–0.28] and alpha-1-M (0.110 [0.036–0.184] to be significantly associated with creatinine 12 months after tx ($P = 0.0025$ and 0.0036). Multivariate analysis still identified IgG to remain a significant predictor ($P = 0.02$). Performing ROC curves the AUC for IgG in discriminating poor kidney function with GFR below 30 ml/min after one year was 0.8, sensitivity and specificity were 77% and 68% with for a discriminating urinary IgG of 12 mg/g creatinine. Increasing excretion of urinary IgG is associated with a rising probability of graft impairment (clearance below 30 ml/min after 12 months).
Conclusion: High urinary IgG in kidney donors is a risk factor for poor graft function and might improve assessment of (marginal) donor kidneys.

V09 "OLD- TO OLD" ALLOCATION IN PANCREAS TRANSPLANTATION- IMPACT OF DIFFERENT CUTOFF AGES

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Objective: The group of „older" patients is growing significantly in Germany compared to US- registry data, where a significant amount of donors and recipients is still younger than 40 years,. Therefore the impact of different "cutoff ages" (CA) on recipient survival and organ fuction was studied in a single center analysis.
Material and methods: The registry of our pancreas transplant program ($n = 445$, 437 SPK, 8 PTA) were analyzed in different groups depending on the age of donor and recipient defining young (Y) and old (O) as below or above CA: 30, 40 or 50 years. Thus 4 groups were created (donor/ recipient): YY, OY, YO, OO.
Results: CA of 30y showed no difference in all qualities, CA of 40y showed slightly inferior pancreas function in both Y/O and O/O, CA of 50Y showed increased loss of pancreas function in O/Y and O/O. Patient survival was not affected in all settings.
Conclusion: The demographic challenge in pancreas transplantation can be faced in a „high load" center with individual decision making. However these data have to be validated with greater cohorts.

V11 SUBNORMOTHERMIC EX VIVO LIVER PERFUSION (SNEVLP) REDUCES ISCHEMIA/REPERFUSION INJURY IN LIVERS RETRIEVED AFTER CARDIAC DEATH (DCD)

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Methods: 45 min cardiac arrest was induced in pigs as a model of DCD organ retrieval. We compared 10 hr cold static preservation with 7 hr cold preservation plus 3 hr SNEVLP. Pig LT was performed after preservation. Hepatocyte, endothelial cell (EC), and bile duct injury were assessed by H&E staining, immunohistochemistry (CD31, Caspase 3, TUNEL) and AST. Beta galactosidase was determined as a marker of Kupffer Cell (KC) activation. Hepatic artery thrombus formation was assessed by MSB staining. ATP and Glycogen were determined.
Results: 45 min of warm ischemia reduced liver ATP and glycogen levels to 20% and 15% of baseline. After 2 hr of SNEVLP preservation ATP and glycogen content increased to 90% and 60% of baseline. 8 hr after LT SNEVLP vs cold stored livers had decreased serum AST levels (387 + 151 vs 934 + 459U/L, $P < 0.01$) and reduced hepatocyte necrosis (15% vs 45% $P < 0.01$). SNEVLP preservation resulted in decreased caspase 3 (16 cells/HPF vs. 46 cells/HPF, $P < 0.01$) and TUNEL (14 cells/HPF vs. 47.25 cells/HPF, $P < 0.01$) positive cells as markers of apoptosis. Microthrombosis within the peribiliary arteries was observed in cold stored organs (4/5 livers) but not in SNEVLP treated grafts (0/5 livers). CD31 staining demonstrated severe EC injury in cold stored grafts, while EC lining was preserved in SNEVLP treated DCD livers. After 3 hr of reperfusion beta galactosidase (KC activation) was significantly reduced in SNEVLP (95.65 + 41.61 vs 325.99 + 187.75, $P = 0.012$) stored grafts. Hourly bile production after transplantation was significant higher in SNEVLP treated grafts (9.58 + 3.83 ml vs 6.43 + 3.42 ml; $P < 0.01$). Bile ducts necrosis (75%) was present in 3 out of 5 cold stored livers, while no bile duct injury was detected in SNEVLP preserved ($P < 0.01$).
Conclusion: SNEVLP improves hepatic energy content in DCD liver grafts during preservation and reduces hepatocyte, endothelial cell and bile duct injury after LT.

V12 MELD SPECIFIC OUTCOMES BEFORE AND AFTER INTRODUCTION OF MELD-BASED ALLOCATION

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Background: As waiting list mortality dropped under a MELD-based allocation, outcome after liver transplantation deteriorated to 1-year survival rates below 80%. The aim of this study was to investigate whether the MELD allocation system per se results in inferior outcome due to inadequate patient selection.
Methods: Patients' (labMELD) and graft (DRI) characteristics were obtained from our liver transplant database. Graft survival of 306 consecutive patients before ($n = 153$) and after ($n = 153$) the cut-off date (December 16, 2006) of a MELD based allocation system in the Eurotransplant (ET) zone were analyzed.
Results: Overall graft survival was similar before and after the introduction of a MELD based allocation system. 3- and 12-months graft survival incrementally decreased with higher MELD score categories. In each MELD strata there was no difference in 3- and 12-months graft survival before and after the introduction of MELD. Recipient MELD Scores did not increase after changing the allocation system. The percentage of transplanted organs with a DRI >1.3, however, increased from 71% to 90%.
Conclusion: The introduction of a MELD based allocation system was not associated with detrimental organ survival or transplantation of sicker patients. The higher DRI reflects organ shortage but did not affect organ survival. Thus, the introduction of a MELD based allocation itself cannot be blamed for inferior patient survival after December 2006.

V13 PREVALENCE OF HEPATITIS C IN GERMAN ORGAN-DONORS

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Hepatitis C Virus (HCV) transmission occurs when blood tests reactive for anti-HCV or HCV-PCR. The European *Guide to safety and quality assurance for the transplantation of organs, tissues and cells* recommends to test every donor for anti-HCV and to extend this by PCR in case of an increased risk for HIV- or HCV-window period infection (WPI). We reviewed the rate of HCV-infection in realised organ donors with regard to an increased risk of WPI.
Methods: 6426 realised donors (2006-2010) were divided into 2 groups: *Increased-Risk-Donors* ($n = 106$) were at an increased risk for WPI with HIV-PCR and HCV-PCR performed prospectively and *Standard-Risk-Donors* ($n = 6320$) were without such risk.
Results: The rate of HCV-infection was 37.7% in *Increased-Risk-Donors* and 1.4% in *Standard-Risk-Donors* ($P < 0.001$). HCV-Viraemia existed in HCV infected donors in 62.5% of the *Increased-Risk-Donors* cases (anti-HCV+/PCR-: 15, anti-HCV+/PCR+: 24, anti-HCV-/PCR+: 1) and in 40% of the *Standard-Risk-Donors* (anti-HCV+/PCR-: 31, anti-HCV+/PCR+: 21, anti-HCV-/no PCR: 33). In *Standard-Risk-Donors* no transmission has been reported yet. HCV-infection was known before donor evaluation in 60% of the *Increased-Risk-Donors* and in 25.6% of the *Standard-Risk-Donors* ($P < 0.001$). In 23% oft he donors with intravenous, intranasal or oral drug-abuse HCV-infection existed compared to 1.4% in donors without abuse reported ($P < 0.001$).
Conclusion: The probability of HCV-infection was elevated in cases at an increased risk for WPI or in cases with known drug abuse. The recommended screening algorithm seemed to be sufficient in a general population with low prevalence of HCV and the option of targeted screening by PCR in case of an increased risk for WPI.

INFECTIONS AND COMPLICATIONS AFTER ORGAN TRANSPLANTATION

V14 POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: CLINICOPATHOLOGICAL ANALYSIS OF 54 CASES IN A SINGLE CENTER

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Introduction: A post transplant lymphoproliferative disorder (PTLD) is an infrequent but serious complication following solid organ transplantation. The incidence varies and depends on the type of organ, degree of immunosuppression and patient's immune status to Epstein Barr Virus.
Material & Methods: This is a retrospective data analysis in 5133 patients following kidney (n = 3440), liver (n = 1145), pancreas (n = 519) and intestinal/multivisceral (n = 29) transplantation. In this patient cohort, 54 cases of PTLD have been observed and correlated with induction therapy, maintenance immunosuppression, EBV status, CMV status, antiviral therapy, acute rejection, graft survival, retransplantation and death.
Results: The overall cumulative incidence of PTLDs was 1.05% (54/5133); 5 year survival was 25.9% (14/54); (highest in PTX 40% (2/5) and lowest in LTX: 20% (3/15). Overall survival was 2.9 years (0-13). PTLD occurred significantly earlier in patients transplanted after 2000 (101 months vs 23 months). Patient with a higher immunological risk received induction therapy and showed decreased patient and graft survival after kidney transplantation as well as a significantly higher risk for PTLD after liver transplantation. Donor age had an impact on graft survival, PTLD onset and patient survival following pancreas transplantation. Interestingly, prognosis was poor in early PTLD and more favorable in late PTLD (all $P < 0.05$).
Discusssion: We have identified a correlation between organ, patient age and induction therapy with PTLD development in a large single center analysis. A shift of PTLD occurrence towards later time points after transplantation with a more favorable outcome was observed in this study.

V15 UREA-MODIFIED PROTEIN ANTIGENS FOR SIMULTANEOUS ASSESSMENT OF CMV-SPECIFIC CD4 AND CD8 T-CELL RESPONSES USING A NEW CMV-ELISPOT ASSAY AND FLOW-CYTOMETRY

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To improve CMV-specific T-cell-monitoring in transplant recipients, we analysed the use of urea-modified CMV-proteins (IE-1 and pp65) for their potential to simultaneously stimulate CD4- and CD8-T-cells.

CMV-specific T-cells were quantified by IFNγ-induction using an ELISPOT-assay (CMV T-Track, Lophius Biosciences) or flow-cytometry and compared with a non-modified CMV-lysate. To assess validity in serologically defined immunocompromised patients, 40 healthy controls, 40 hemodialysis patients and 40 kidney recipients were screened.

Median ELISPOT-counts in CMV-IgG-positive individuals were low for IE-1 (3.8 (IQR 10.5)), medium for pp65 (65 (179.5)) and highest for CMV-lysate (120 (160)) without significant difference between patients and controls. IgG-negative individuals had almost no spots (0-0.5 (0-0.5)). Flow-cytometry revealed substantially lower median CMV-specific CD4-T-cell-frequencies after stimulation with urea-modified pp65 (0.09% (0.30%)) compared to non-modified CMV-lysate (1.93% (3.57%)). In contrast, induction of CD8-T-cells was stronger with pp65 (0.21% (0.94%) versus 0.14% (0.46%) for CMV-lysate). Median IE-1-specific CD4- and CD8-T-cell-frequencies were low (0.02% (0.03%) and 0.04% (0.17%)). Correlation between ELISPOT and flow-cytometry was highest with pp65 (r = 0.83) and CMV-lysate (r = 0.86).
Conclusion: While the non-modified CMV-lysate is well-suited for detection of CD4-T-cells, urea-modified protein antigens serve for concomitant stimulation of CMV-specific CD8-T-cells. Both ELISPOT and flow-cytometry are feasible readouts for monitoring of CMV-specific cellular immunity.

V16 MICAFUNGIN AS ANTIFUNGAL PROPHYLAXIS IN HIGH-RISK LIVER TRANSPLANTATION: A RANDOMISED MULTICENTRE TRIAL

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Background: Invasive fungal disease (IFD) after liver transplant (LTx) has significant morbidity and mortality.
Methods: Open-label, randomised, multicentre trial, to compare the efficacy and safety of micafungin with site approved standard care prophylaxis (SC) for invasive fungal disease (IFD) in high risk LTx patients (pts). After LTx, pts were randomised 1:1 to iv micafungin or iv SC (fluconazole; liposomal AmB; or caspofungin). Primary endpoint was clinical success (absence IFD and no additional antifungals) at end of prophylaxis (EOP). Non-inferiority of micafungin vs SC was assessed in the per protocol set (PPS) and confirmed in the full analysis set (FAS). Safety assessments were adverse events (AE) and liver and kidney function.
Results: The FAS comprised 172 micafungin and 172 SC pts (mean age 51.2 years, 67.4%male, MELD score >20 in 48.0%, $\geq$ 30 in 19.8%). Most common risk factors for IFD were post-operative renal impairment (31.4%), reoperation within 5 days of LTx (21.5%) and pre-operative renal impairment (20.3%). 60 (17.4%) pts had intra-operative transfusion of $\geq$20 units cellular blood product, liver re-transplant rate was 13.4%. At EOP clinical success was 98.6% for micafungin (n = 140) and 99.3% for SC (n = 137) (Δ [95% CI]: 0.7 [$-$2.7, 4.4]) in the PPS and 96.5% and 93.6% ($-$2.9 [$-$8.0, 1.9]) in the FAS. There were 4 *Aspergillus* and 8 *Candida* infections at EOP. 70% pts completed prophylaxis. Incidences of drug-related AE for micafungin and SC were 11.6% and 16.3%, leading to discontinuation in 6.4% and 11.6%, respectively. Liver and kidney function was similar between groups.
Conclusions: Micafungin was shown to be safe and non-inferior to SC for antifungal prophylaxis in high risk LTx pts.

V19 DAILY LOW-DOSE TACROLIMUS (TAC) IS A SAFE AND EFFICIENT IMMUNOSUPPRESSIVE REGIMEN DURING HCV TRIPLE DRUG THERAPY POST LIVER TRANSPLANT - A 24 WEEKS INTERIM ANALYSIS

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Background: Graft loss from hepatitis C virus (HCV) recurrence remains a major problem after liver transplantation (LT), and the response to pegylated interferon (PEG) and ribavirin (RBV) is poor. Triple therapy opens a new perspective for better graft and patient survival but severe drug interactions are a potential limitation in LT patients. We discuss an interim analysis after 24 weeks of treatment.
Methods: 16 patients with HCV genotype (GT) 1 recurrence after liver transplant were treated with TVR, pegylated interferon-alpha (PEG) and ribavirin (RBV) for 12 weeks followed by 12 or 36 weeks of dual therapy. After initiating TVR, TAC dosage was skipped until start of decline of therapeutic level and then administered as 0.1 mg with once or twice daily dosing. Trough levels of TAC as well as general condition and Lab values were monitored closely. Safety and efficacy data were gathered for time of treatment.
Results: All of the 13 male and 3 female patients completed the 12 weeks of triple therapy. At week 4, 15 of the patients were found to be HCV-RNA <12 IU/mL (RealTime-HCV-Assay). No viral break through has been experienced so far. With the above dosing strategy we were able to avoid any clinically evident TAC toxicity. 70% of the patients exhibited hematological side effects requiring RBV dose reduction, the administration of erythropoietin or blood transfusions. All patients tolerated the medications, no one stopped early.
Conclusion: We report a large single center experience for treating HCV recurrence after LT with TVR based triple therapy. With substantial dose reduction of TAC and intensive monitoring of blood levels, TVR-based triple therapy is effective in LT patients suffering from HCV genotype 1 recurrence. Adequate dosage adjustment is possible, avoiding toxicity or rejection in these patients, together with a high early viral response (EVR).

V20 A MODEL TO IDENTIFY PATIENTS AT RISK FOR POST-TRANSPLANT KIDNEY DISEASE AFTER LIVER TRANSPLANTATION

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Following liver transplantation (LT) a subgroup of the recipients develops chronic kidney disease (CKD) ranging from mild impairment of kidney function to need for hemodialysis. So far no reliable models exist to predict the development of CKD in liver graft recipients. The goal of this study is the development of a predictive model to estimate the development of renal function after LT based on parameters known before LT.

We retrospectively analysed clinical and biochemical pre- and perioperative parameters of 328 liver recipients transplanted between 2004–2008 and developed ordinal logistic regression models which predict CKD stage 3 or CKD stage 4 or worse after LT. The five most important variables of this model were taken to build a more simplified model for clinical use.

The full model allowed with high accuracy the prediction of CKD stage 3 or CKD stage 4 or worse. The simplified model includes 5 parameters (diabetes mellitus, primary sclerosing cholangitis, hepatitis C, recipient-age, cystatin-c based glomerular filtration rate before LT) and showed a good accuracy in the prediction of CKD $\geq$ stage 3 (AUC = 0.739) resp. CKD $\geq$ stage 4 (AUC = 0.774). To validate these data we tested the model in a prospective cohort and confirmed an acceptable accuracy for the prediction of CKD $\geq$ stage 3 (AUC = 0.716) resp. CKD $\geq$ stage 4 (AUC = 0.639).

In conclusion our model allows based on five parameters known before LT the identification of patients at risk to develop CKD after LT.

LIVING DONATION

V21 DROP-OUTS IN ABO-INCOMPATIBLE (ABOI) LIVING-DONOR RENAL TRANSPLANTATION MAY BE PREVENTED - A SINGLE CENTER EXPERIENCE

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Background: ABOi living-donor renal transplantation using a single dose of rituximab and blood-group specific immunoadsorption (IA) for desensitization, has become an established treatment modality to increase the donor pool and lower patient mortality. However, 10-20% of patients are high responders and and doe not reach the target anti-ABO titers enabling successful ABOi transplantation.
Methods: We retrospectively analyzed 30 patients who were desensitized for ABOi LD-Rtx (rituximab 375 mg/m^2, basiliximab (ATG $n = 1$), tacrolimus/MPA/prednisolone, IA and IVIG).
Results: Despite elevated donor-specific anti-ABOi titers (median 1:128; >1:128: 33% of patients) all patients reached the target of 1:8 pretransplant, using repeated administration of rituximab in high-responder patients (2, 3 and 4 rituximab infusions, respectively; one pretransplant drop-out for medical reasons). 29 of the 30 patients were successfully transplanted (1-year graft survival 95%, S-Cr: 1.5 $\pm$ 0.1 mg/dl, at discharge; 1.8 $\pm$ 0.2 mg/dl, last follow-up (25 $\pm$ 3 months)). The number of pretransplant IA treatments was significantly related to the pretreatment anti-ABOi titers ($r = 0.80$, $P < 0.0005$) as was the number of rituximab infusions ($r = 0.82$, $P < 0.0005$). Although preliminary data of our prospective study in ABOi versus compatible transplants indicate an increased risk of BK viremia after rituximab treatment ($P = 0.025$), repeated rituximab infusions did not increase the risk of BK viremia compared with a single infusion ($P = 0.470$).
Conclusion: Our data suggest that repeated rituximab infusions in high-responder patients may enable successful ABOi living-donor renal transplantation without increasing the risk of BK viremia.

V22 POST HOC SUBGROUP ANALYSIS FROM ZEUS: OUTCOME ON RENAL FUNCTION, EFFICACY AND SAFETY IN LIVING-DONOR KIDNEY TRANSPLANT RECIPIENTS AFTER CONVERSION FROM A CALCINEURIN INHIBITOR TO AN EVEROLIMUS BASED REGIMEN

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Background: To study renal function and patient outcome in living donation (LD) subgroup of kidney de novo transplant recipients after conversion from calcineurin inhibitor (CNI) therapy to an everolimus (EVR) based regimen.
Methods: Post hoc subgroup analysis from a prospective, open-label, controlled, multi-center study. 300 renal transplant (Tx) patients were randomized at month (Mo)4.5 post Tx to either EVR+enteric coated-mycophenolate sodium (EC-MPS) or cyclosporine(CsA)+EC-MPS regimen, among them 80 LD recipients (EVR $n = 42$;CsA $n = 38$).
Results: Adjusted eGFR (Nankivell) in LD subpopulation at Mo12 was 74.2 (95%CI[70.5;78.0]) mL/min/1.73 m^2 in EVR vs 63.6 (95%CI[59.5;67.8]) mL/min/1.73 m^2 in CsA pts, resulting in a difference of 10.6 mL/min/1.73 m^2 in favor of EVR pts ($P < 0.001$). BPARs: 6 in EVR vs 1 in the CsA group ($P = 0.109$). One graft loss in CsA, none in EVR subpop. Discontinuation due to AEs: 6 EVR pts (14%) and 5 CsA pts (13%) from RDZ to Mo12. Overall safety profile was similar between treatment groups.
Conclusions: EVR-based regimen with early elimination of CNI therapy in living-donor kidney transplant recipients is associated with a significant renal benefit at 12Mo post Tx without compromising safety and efficacy.

V23 DONOR QUALITY OF LIFE UP TO TWO YEARS AFTER LIVING DONOR LIVER TRANSPLANTATION: A PROSPECTIVE STUDY

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Objective: There is a lack of longer-term prospective data on living liver donors' quality of life (QOL). This is the first prospective study examining QOL up to 2 yr after donation.
Methods: A consecutive sample of living donors ($n = 40$) was compared with a sample of potential donors ($n = 27$) with respect to QOL, anxiety, and depression. Performing mixed-effects model analysis, both groups were assessed before donation/transplantation (T0) and at 3 postoperative data points: 3 months (T1), 1 yr (T2), and 2 yr (T3). Subsequently, both groups were compared with reference data of the general population and healthy individuals.
Results: At T1, living donors' physical QOL was impaired. At T2 and T3, physical QOL was slightly lower than the preoperative level but within the range of healthy individuals in both living donors and potential donors. Neither mental QOL nor depression showed significant changes across time, while anxiety decreased in both groups. Subgroup analysis of adult-to-adult (AA) donors and adult-to-pediatric (AP) donors revealed different trajectories of mental QOL, anxiety, and depression. AP donors experienced more preoperative psychological strain, which improved after donation, whereas AA donors showed unchanged anxiety and depression, and a slight decrease in mental QOL 2 yr after surgery. Two AA donors, whose recipients had died, reported persisting depressive symptoms after donation.
Conclusions: One and 2 yr after donation, QOL is not substantially impaired in the majority of donors. Future research needs to provide an even longer prospective follow-up and should more rigorously explore risk factors for a negative donor outcome.

V24 EXPERIENCE WITH LIVING DONOR LIVER TRANSPLANTATION (LD-LTX) FOR RE-TRANSPLANTATIONS

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Background: During the last years, the shortage of postmortal donor organs and donor risk index increased. LD-LTx is one option for candidates on the liver waiting list sustaining good graft quality.
Methods: We analyzed the cohort of LD-LTx at UKSH, Campus Kiel in the period from 30.11.2010 to 08.06.2013. A special focus was set on living donor grafts used for re-LTx.
Results: A total of 20 LD-LTx were performed including 10 SII+III (LLS), 2 SI-IV (LL) and 8 SV-VIII (RL). Median LLS-recipient age was 0.6 (0.3-11.8) y and donor age was 32.9 (21.0-42.7) y. Median recipient age in adults was 45.7 (16.5-63.6) y and donor age was 42.4 (26.7-51.6) y.

LD-re-LTx included 1 child and 3 adults. Indications for LD-re-LTx were: (I, waiting time (WT) 2968 d, 47 y, MELD 27) secondary sclerosing cholangitis after 1.LTx for PSC, (II, WT 1711 d, 26 y, MELD 22) biliary sepsis after 2.LTx with thrombosis of reconstructed hepatic artery anastomosed to the aorta, (III, WT 111 d, 7 y, PELD 30) chronic graft dysfunction (CDF) after 1.LTx for biliary atresia and Kasai operation and (IV, WT 399 d, 16 y, MELD 24) CDF after LD-LTx for metabolic liver disease.

All LD-LTx donors and recipients are alive after a median follow-up of 313 (6-918) d. None of the donors had a major complication (Clavien >2). Four recipients required re-LTx after LD-LTx. Cause for re-LTx were HAT including 2 LD-re-LTx with complex arterial re-reconstruction (1 adult, 1 child), 1 HAT in a patient with coagulopathy and primary Budd-Chiari-syndrome and 1 HAT in a 1 year old child.
Conclusions: Living donor liver transplantation is an option for waiting list patients being not reflected by the current allocation system. The risk for graft loss is increased in case of LD-re-LTx and must be faced against donor risk and mortality of the waiting list candidate. Moreover, the higher risk of graft failure has to be discussed open with the living donor couple prior to the operation.

V26 DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING BEFORE AND AFTER DONOR NEPHRECTOMY: LONGITUDINAL FOLLOW-UP REVEALS FUNCTIONAL CHANGES OF THE REMAINING KIDNEY IN DONORS

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Purpose: Uninephrectomy in living renal donors induces compensatory mechanisms, like glomerular hyperfiltration and hypertrophy, to overcome renal mass reduction. We performed a prospective longitudinal study to determine, if diffusion weighted imaging (DW-MRI) in living renal allograft donation allows monitoring potential changes in the non-transplanted remaining kidney of the donor due to unilateral nephrectomy.
Methods: Study protocol was approved by local ethics committee; written informed consent was obtained. Thirteen healthy kidney donors and their corresponding recipients were examined. DW-MRI was performed in donors before donation, and in donors and recipients at day 8, month 3 and 12 after donation. Total apparent diffusion coefficient (ADC_T) values were determined and the contribution of micro-circulation quantified in the "perfusion fraction" (F_P).
Results: ADC_T-values in the remaining kidney of donors increased from a pre-explantation value of 188 ± 9 to $202 \pm 11 \times 10^{-5}$ mm^2/s in the medulla and from 199 ± 11 to $210 \pm 13 \times 10^{-5}$ mm^2/s in cortex as early as one week after donation ($P < 0.01$). Medullary, but not cortical ADC_T values stayed elevated up to one year. ADC_T-values in allografts in recipients were stable. Compared with the values obtained pre-transplantation in donors, the cortico-medullary difference was reduced in allografts ($P < 0.05$). Cortical ADC_T-values correlated with estimated glomerular filtration rate in recipients ($R = 0.56$, $P < 0.001$), but not in donors. Cortical ADC_T-values in the same kidney before transplantation in donors correlated with those in recipients on day 8 after transplantation ($R = 0.77$, $P < 0.006$).
Conclusion: DW-MRI is able to detect early adaptations in the remaining kidney of donors after nephrectomy.Our results suggest potential monitoring utility of the method for donors.

V27 LIVING RENAL DONATION: IMPACT ON THE EMOTIONAL STATUS OF THE DONOR

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Background: The benefits of living kidney donation for recipients are well-known. Nevertheless, donor safety is of importance at all time. Data on the impact on the emotional status including depression and anxiety are rare.
Methods: In an open, prospective observational study, renal allograft donors were evaluated with respect to psychosocial and emotional outcome after living donation. Standardized questionnaires (HADS) as well as additional questions related to living donation were used.
Results: Altogether, 128 renal allograft donors were evaluated (86 male, median age 49.1 ± 11.4 years, mean time after transplantation 3.7 ± 3.8). None of the donors had any serious post-transplant complications and the renal function was stable. Concerning the emotional status, most of the patients demonstrated normal HADS anxiety scores, with pathological results in 6 patients (5%). Depression scale was within the normal range in 79% of the donors, 15 donors (13%) showed pathological results. Patients with pathological anxiety scores were more likely to be parents, foreign origin and living alone. There was no difference concerning gender, age or renal function. Concerning the HADS depression scale no difference in the patient cohort with and without depression symptoms could be noticed. In both scales health status was more often judged as worse compared to prior donation in patients with pathological results. There was no difference in graft function or recipient health.
Conclusion: A minority of renal donors demonstrated anxiety or depression symptoms assessed by the HADS scale. Health status was impacted by pathological results. Parenthood, foreign origin and living alone were identified as risk factors for anxiety symptoms.

ETHICAL ISSUES IN TRANSPLANTATION MEDICINE /PSYCHOSOMATIC MEDICINE

V29 DETECTION OF ALCOHOL CONSUMPTION IN LIVER TRANSPLANT RECIPIENTS USING DETERMINATION OF ETHYL GLUCURONIDE CONCENTRATION IN URINE AND HAIR

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Early detection of alcohol abuse in OLT recipients is essential to offer patients support and counseling to prevent organ damage. Here the diagnostic value of alcohol marker determination for assessment of alcohol consumption was evaluated.
Methods: In 104 transplant recipients, 31 with alcoholic liver disease (ALD) and 73 with non-ALD, hEtG was determined additionally to urine EtG, blood ethanol, methanol and carbohydrate deficient transferrin. The results were compared with patients' self-reports in a questionnaire and physicians' assessments.
Results: By physicians' assessments 22% of patients were suspected to consume potentially harmful amounts of alcohol, while only 6% of patients admitted consumption of >2 drinks/week. Alcohol markers revealed alcohol consumption in 17% (18/104) of patients. In all but 2 cases (89%) consumption was only detected by a positive hEtG. Compared to patients with non-ALD, patients with ALD were significantly more often suspected to consume alcohol by their physicians (35% vs 16%; $P = 0.03$), had significantly more often positive alcohol marker (35% vs 10%; $P = 0.001$) and significantly more often a positive hEtG (32% vs 8%; $P = 0.002$). Furthermore their mean proximal hEtG concentration was significantly higher (248.6 pg/mg+293.7 vs 25.1 pg/mg+14.3; $P = 0.049$). Correlation of hEtG results of the proximal or distal hair segment with physicians' assessment and patients' self report was poor (Spearman correlation ranging from 0.119 to 0.441).
Conclusion: Hair-EtG is a sensitive marker for detection of alcohol consumption in the transplant setting.

V30 PREDICTION OF MORTALITY AFTER LIVER TRANSPLANTATION IN THE MELD-ERA: A MULTIVARIATE RISK ASSESSMENT

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Background: In the era of MELD-based allocation, liver transplantation (LT) has a relevant mortality that might depend on both donor and recipient specific variables. Thus, this study evaluates those variables to identify risk factors for post-LT mortality being suitable for the development of a prognostic scoring system.
Methods: From December 2006 to March 2011, a total of 429 patients underwent LT in our department. Patients aged <18 years, HU-listings, split- (including living related), combined, and re-transplantations were excluded from the analysis. Univariate and multivariate analysis was employed to identify risk factors for post-LT mortality in 266 LTs. A risk score was calculated and its correlation to 90-day and 1-year mortality after LT was analyzed.
Results: The overall survival was comparable to the outcome data of patients after LT within the Eurotransplant area. A labMELD ≥20, female sex, coronary heart disease, warm ischemic time ≥50 min, donor risk index >1.5 and donor Na^+ >145 mmol/L were identified as independent predictors for both 90-day and 1-year mortality after LT. With an increasing number of these risk-factors the 90-day and 1-year mortality after LT escalated (0-1: 0% and 0%; 2: 3% and 17%; 3: 6% and 17%; 4: 22% and 33%; 5-6: 61% and 66%).
Conclusion: This analysis reveals a simple risk score for mortality after LT. Further subgroups of patients have a higher predicted mortality with compared to without LT.

V31 VOLUME AND OUTCOME RELATION IN GERMAN LIVER TRANSPLANT CENTERS: MUCH ADO ABOUT NOTHING?

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Background: Volume and outcome relationship for transplant procedures has become a major topic in discussions within the organ transplantation scandal in Germany.
Methods: The homepage of the Deutsche Stiftung für Organtransplantation (DSO) was screened for the annual reports of transplant programs for the years 2007 to 2010 (http://www.dso.de/infocenter/krankenhaeuser/transplantationszentren.html). From these reports the results of German liver transplant programs were extracted. Additionally, an analysis of volume per million people per number of transplant centers for each individual German federal state was made to give an overview of the concentration and density of transplant programs in each particular state for the years 2009 to 2011.
Results: In house mortality for German liver transplant centers ($R^2 = 0.005$, $P = 0.518$) and 3-year survival ($R^2 = 0.068$, $P = 0.085$) as well as a ROC analysis for in house mortality (AUC 0.55, CI: 0.41; 0.68, $P = 0.53$) did not show volume outcome relation. A definition of a threshold for a good center was not possible. One-year survival indicated better outcome in high volume centers albeit reduced quality of data. $R^2 = 0.106$, $P = 0.009$. The factor of transplants per year per million people per transplant centers is 0.6 for Germany. Some Federal States (Bavaria and Northrhine Westfalia) have an oversupply with transplant centers considering the number of transplants per million people per centers in the particular federal state.
Discussion: We propose the definition of a quality catalogue for liver transplant centers. A data basis for a time period of e.g. 3 to 5 years should be collected prospectively and decisions influencing the landscape of liver transplant centers should be made based upon the findings, respecting federal state sovereignty and regional medical requirements.

V32 OUTCOME OPTIMIZATION OR URGENCY - WHAT MATTERS MORE IN THE ALLOCATION OF DONOR LIVERS. A SURVEY AMONG OUTPATIENTS OF A MEDICAL UNIVERSITY DEPARTMENT

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Background: The sickest-first principle has been pursued with the introduction of the MELD-score as primary mode of allocation for donor livers. In Germany outcomes of liver transplantation may be negatively influenced by the transplantation of patients with very high MELD-scores and the use of donor organs with lower quality. Therefore some have claimed, allocation should be based more on outcome-oriented criteria.
Methods: A survey with binary questions (yes/no) regarding the appreciation of values concerning the allocation of donor livers was performed among general medical outpatients of a university hospital. End-stage liver disease patients were excluded. 204 returned forms were analyzed. Percentages of valid answers are given.
Results: Of 204 respondents, 47% were aged 50 yrs or younger, 46% were male. 88%, 73% and 41% of subjects answered, they would be willing to undergo transplantation for themselves with an estimated outcome of 20%, 50% and 80% one-year-mortality, respectively. Being asked, which of two case-examples should receive a donor organ, 68% of valid answers said the case with higher urgency and lower long-term survival should receive the organ, 60% said the case with better outcome but lower urgency. 70% said urgency was more important than long-term outcome as criteria for organ allocation. Under the assumption, urgency-based allocation would decrease results of liver transplantation, 58% refused to deny even the sickest patients transplantation. 78% said, patients likely to achieve 50% long-term survival should be transplanted.
Conclusion: It appears that a majority of subjects prioritize urgency over efficiency per procedure. Representative surveys should be performed.

V33 THE GERMAN TRANSPLANT SCANDAL: REFLECTIONS OF AN INSIDER

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In July 2012 a transplant scandal was made public in Germany. Mainstream publicity incriminated corrupt physicians and profit oriented hospital operators for manipulation of waiting list data. To elucidate an alternate position the following aspects are discussed:

Organ shortage was present before the scandal.

The implementation of a MELD- score based guideline for liver transplantation was inadequate due to the degree of organ shortage and lead to a progressive decline in patient and organ survival.

This development lead to the desire to help patients successfully with the consequence of severe violation of guidelines.

Inadequate publicity caused a dramatic reduction of organ donation rates.

Legislation is going to criminalize transplant physicians severely.

In consequence it will be extremely difficult to recruit talents for a career in transplant medicine.

Further the interaction between patient and physician is moved from hippocratic qualities to the technical handling of waiting list management.

Organ transplantation may become a model system fort legislative handling of every field of medicine replacing physicians responsibility by „public control" which may be in contradiction to the patients welfare.

Therefore the system must come back to sufficient self control and adequate transparency instead of becoming the subject of political discussions.

V35 QUALITY OF LIFE AFTER LIVER TRANSPLANTATION

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Aim of the study: Quality of life (QoL) is one of the major therapeutic goals after liver transplantation (LTx) besides patient and graft survival. The aim of the study was to evaluate the impact of transplant relevant paramters on QoL after LTx.
Methods: Quality of life was assessed by cross-sectional analysis using the EORTC-QLQ-C30 questionaire that was send to 238 liver transplant recipients. Investigated transplant relevant paramters included time after LTx, era (MELD or ELAS), hospitalization after LTx (> or <28 d), status (HU or T) and distance to transplant centre (> or <150 km).
Results: The questionaire was returned by 173/238 (72.68%) liver recipients. After LTx, the QoL - function scales increased over time. Higher disatnce to transplant centre (>150 km) was associated with higher rates of fatigue, dyspnea, sleep disturbances and financial difficulties. Patients with HU-status had a higher rate of financial difficulties after LTx compared to recipients with T-status. Comparison of MELD- ($n = 94$) and ELAS-era ($n = 79$) showed differences in physcial function, fatigue, and pain. Fatigue, pain, dyspnea, insomnia and finacial difficulties were associated with a longer hospitalization period.
Conclusion: Liver recipients have better results in function scales and less complains in symptom scales the longer the period after LTx. Era after LTx, duration of hospitalization >28 d after LTx, HU-status and distance >150 km to transplant centre have an impact on QoL after LTx.

V36 BEHAVIOURAL PROBLEMS AFTER PEDIATRIC LIVER TRANSPLANTATION (PLTX) - RELATED TO CALCINEURIN INHIBITOR (CNI) THERAPY?

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Parents repeatedly describe behaviour problems (aggressive behaviour, hyperactivity) in their children after liver transplantation. The change of immunsuppression from CNI to mycophenolate mofetil (MMF) often leads to marked improvement of symptoms. Neurologic and psychiatric side effects of CNI are described. Systematic evaluation of behavioural problems in relation to immunosuppression is missing.

Parents and teachers of 65 livertransplanted patients (4-17 years, median 10 years) received the Child Behaviour Check List (CBCL). Clinically and borderline relevant results were analyzed together.

46/65 parents (71%) and 28/65 teachers (43%) responded. Of 46 patients with parent reports, 11 received ciclosporin, 19 tacrolimus, 8 MMF and 8 combinations. 48% of their parents reported overall clinically relevant behaviour, differentiated into externalizing (33%) and internalizing (52%) behaviour. Most reported problem scales were somatic complaints, attention problems and social problems. Teachers reported markedly less problematic behaviour on all scales. There was no correlation with immunosuppressive medication.

Parents report a markedly elevated incidence of behaviour problems after pLTX compared to the normal population. Unexpectedly, externalizing behaviour problems did not correlate with CNI immunosuppression. The high percentage of internalizing behaviour problems reported is alarming. Further studies are needed to evaluate parent reports in relation to their children's and to psychological assessments.

KIDNEY

V37 C-TERMINAL AGRIN FRAGMENT (CAF) - A NEW BIOMARKER FOR EVALUATING KIDNEY FUNCTION IN RENAL TRANSPLANT RECIPIENTS

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Background: The C-terminal agrin fragment (CAF) is a proteolytic cleavage product of agrin, the major proteoglycan of the glomerular basement membrane. The role of CAF in evaluating kidney function has never been evaluated.
Material & Methods: We measured serum CAF and creatinine levels and calculated eGFR (MDRD) in 96 healthy individuals and in 110 end-stage renal disease (ESRD) patients undergoing kidney transplantation before and after transplantation. Correlation between CAF and creatinine levels/eGFR was calculated as within-patient- (cWP) and between-patient correlations (cBP). We evaluated the association of CAF with delayed graft function (DGF). The value of CAF for early detection of DGF compared to creatinine was evaluated by receiver operating characteristics (ROC) analysis.
Results: CAF levels strongly correlated with creatinine ($r = 0.86$(cWP), $r = 0.74$(cBP)) and eGFR (MDRD) ($r = 0.86$(cWP), $r = 0.77$(cBP)). Pretransplant (pre-Tx) CAF levels were 19-fold higher than in healthy individuals (1115.0 (258.4;3990.0) vs. 56.6 (20.0;109.5) pM). After transplantation, CAF levels decreased significantly faster than creatinine levels (postoperative day 1-3 (POD 1-3): 562.8 (101.6;2113.0), creatinine: pre-Tx 6.9 (3.1;15.7) mg/dl, POD 1-3: 6.4 (1.7;12.7), $P < 0.001$). Stable serum levels were reached 1-3 months after transplantation for CAF and creatinine (CAF: 145.1 (6.7;851.0) pM; creatinine: 1.6 (0.7;8.0) mg/dl). CAF-levels at POD 1-3 were significantly associated with DGF and outperformed creatinine in early detection of DGF (area under the curve (AUC)-CAF: 80.7% (95%-CI: 72.3%-89.1%) vs. AUC-Creatinine: 71.3% (95%-CI: 61.8%-81.1%, $P = 0.061$)).
Conclusion: CAF is a promising new and fast biomarker for kidney function and may serve as a new tool for the early detection of DGF.

V38 URINARY CALPROTECTIN DIFFERENTIATES BETWEEN PRERENAL AND INTRINSIC ACUTE RENAL ALLOGRAFT FAILURE

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Urinary calprotectin has recently been identified as a promising biomarker for the differentiation between prerenal and intrinsic acute kidney injury (AKI) in the non-transplant population. Calprotectin is highly increased in intrinsic AKI, whereas it is comparable to healthy controls in prerenal disease. The present study investigates, whether calprotectin is able to differentiate between these two entities in transplant recipients as well.

Urinary calprotectin concentrations were assessed by ELISA in 256 subjects including 82 cases of intrinsic acute allograft failure, 21 cases of prerenal graft failure, 112 patients with stable graft function, and 41 healthy controls without any history of renal disease. Acute graft failure was defined as AKI stage 1-3 (AKIN criteria), exclusion criteria were obstructive uropathy and metastatic cancer. The clinical differentiation of prerenal and intrinsic graft failure was performed either by biopsy or by a clinical algorithm including response to fluid repletion, history, physical examination, and urine dip stick examination.

Reasons for intrinsic graft failure comprised rejection, acute tubular necrosis, pyelonephritis, and viral nephritis. Calprotectin concentrations of patients with stable graft function (100 $\pm$ 120 ng/ml) were comparable to healthy controls (102 $\pm$ 229 ng/ml, p 0.94) and prerenal graft failure (99 $\pm$ 118 ng/ml, p 0.99). Mean urinary calprotectin was 45 times higher in intrinsic AKI 4532 $\pm$ 7428 ng/ml) than in prerenal AKI ($P < 0.01$). ROC-curve analysis revealed a high accuracy of calprotectin (AUC 0.98) in the differentiation of intrinsic vs. prerenal AKI. A cut-off level of 250 ng/ml provided a sensitivity of 96.3% and a specificity of 90.5% for the diagnosis of intrinsic AKI.

Urinary calprotectin is a promising biomarker for the differentiation of prerenal and intrinsic acute renal allograft failure.

V40 CLINICALLY RELEVANT BKV-REPLICATION EVENTS IN RENAL TRANSPLANT RECIPIENTS ARE ASSOCIATED WITH SIGNIFICANT CHANGES IN T-CELL FUNCTIONALITY

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Question: Infection with BK virus (BKV) may cause nephropathy and graft loss in renal transplant recipients. We wanted to know, if BKV-specific functional T-cell properties correlate with active BKV replication.
Methods: We first performed a flow-cytometric analysis of BKV-specific CD4 T-cell functionality after stimulation with overlapping peptides of BKV large T antigen and VP1 in 122 healthy controls (range 1-84 years), assessing intracellular IFNγ, IL-2 and TNFα. Potential changes related to BKV-associated complications were analysed in age-matched groups of 38 renal transplant recipients with (RTx-BKVpositive) and without (RTx-BKVnegative) confirmed clinically relevant BKV-reactivation, and 25 hemodialysis patients.
Results: Median frequencies of BKV-specific CD4 T-cells in controls showed an age-dependent decline ($P = 0.006$). Of note, transplant recipients showed a significantly higher prevalence of BKV-specific T-cell responses (57.9%) compared to age-matched healthy controls (21.7%) or hemodialysis patients (28%, $P = 0.017$). Interestingly, percentages of BKV-specific T-cells concurrently expressing all three cytokines was lowest in RTx-BKVpositive patients (mean 43.92 $\pm$ 17.59%) compared to immunocompetent individuals (65.99 $\pm$ 20.05%), hemodialysis (67.04 $\pm$ 11.10%) and RTx-BKVnegative patients (56.28 $\pm$ 22.43%; $P = 0.011$).
Conclusion: Active BKV replication is associated with an increased percentage of BKV-specific CD4 T-cells. Unlike primary infection in young healthy persons, clinically relevant BKV replication in renal transplant recipients is associated with constricted cytokine expression of BKV-specific CD4 T-cells. Together this may help to identify patients at risk for BKV nephropathy.

V42 INFLUENCE OF DONOR NEPHRON MASS INDEX OF KIDNEY FUNCTION AFTER TRANSPLANTATION - A PROSPECTIVE STUDY

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Introduction: Transplanted nephron mass is an important factor of long-term allograft survival, but accurate measurement is challenging. The aim of this study was to evaluate nephron mass index (NMI) and its relationship to creatinine clearance (CrCl) posttransplantation.
Methods: After approval of the local ethic committee we included 20 patients with a minimum follow up of 3 months so far. Beside donor and recipient data, donor kidney volume, size and weight were determined.
Results: We performed 7 living donor and 13 cadaveric donor kidney transplantations. The mean donor age was 57.4 years. Mean NMI (ratio of donor nephron mass to recipient BMI) was 9.87 and median creatinine level was 1.87 mg/dL 3 months after transplantation. There was no graft loss. After this short observation period neither donor kidney volume, NMI, cold ischemia time, donor age or operation time had a significant influence on CrCl.
Conclusion: The donor NMI did not significantly influence the CrCl at 3 months after transplantation. Since this is an on-going study with a recruitment target of 50 patients, results are preliminary.

V43 PREDICTION OF CARDIOVASCULAR EVENTS AFTER RENAL TRANSPLANTATION

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Background: Pulse wave velocity (PWV) is a marker of arterial stiffness and predicts cardiovascular events in the non-transplant population. Cardiovascular events are the leading cause of death and one of the leading causes of graft failure in renal transplant recipients. The present prospective study investigates, whether there is a correlation between PWV and cardiovascular events in renal transplant recipients as well.
Methods: A prospective study assessing the incidence of a composite cardiovascular endpoint within ≥3 years after pulse wave analysis was performed in 65 stable renal transplant recipients. Measurement of PWV was conducted by the SphygmoCor (AtCor^{R}) device. Composite endpoint of the study was the incidence of either myocardial infarction, stroke, occlusion of peripheral artery, admission to hospital due to decompensation of congestive heart failure, or death.
Results: 15 patients (23%) reached the composite endpoint during a follow-up of 4.4 ± 0.5 years. Binary logistic regression using PWV, systolic, diastolic and mean blood pressure as covariates revealed, that PWV (10.1 ± 3.6 m/s in subjects reaching vs. 8.5 ± 1.5 m/s in subjects not reaching the endpoint) was significantly associated with cardiovascular incidents ($P = 0.017$). Systolic (130.5 ± 29.9 vs. 131.7 ± 17 mmHg) and diastolic pressure (68.2 ± 18.6 vs. 75.2 ± 14.1 mmHg) as well as mean blood pressure (89 ± 21.5 vs. 94 ± 13.9 mmHg) did not show any significant correlation.
Conclusion: Increased arterial stiffness as assessed by PWV predicts cardiovascular events in renal transplant recipients and may be regarded as a footprint of the accelerated arteriosclerosis in this group of patients.

V44 SIGNIFICANT IMPACT OF PROLONGED BRAIN DEATH DURATION ON PATIENT SURVIVAL AFTER KIDNEY TRANSPLANTATION

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Introduction: In renal transplantation, graft survival using organs from deceased donors is inferior to results after living donation. Little however is known about the effect of brain death duration (BDdur) on long term outcome after kidney transplantation (KTx).
Methods: A single-center retrospective analysis of 1245 consecutive deceased donor KTx, performed between January 2000 and December 2010, was carried out. BDdur was calculated as the period between brain death declaration and start of cold perfusion. All recipient-, donor- and transplant-factors, known at the timepoint of KTx, were investigated for their impact on delayed graft function (DGF), acute rejection (AR), „graft loss" and „death". Uni- as well as multivariate statistical analysis were performed using binary logistic- and Cox-regression analysis.
Results: Mean BDdur was 12.01 ± 6.26 hours. DGF was associated with a significantly longer BDdur (12.13 ± 5.815 vs. 11.82 ± 6.177 hours, $P = 0.0034$). No significant correlation of AR and BDdur was seen (AR 11.85 ± 6.33 hours vs.12.04 ± 5.997 hours no AR, $P = 0.156$). BDdur did not affect long term graft survival, but had a significant impact on patient survival. Apart from recipient's age, BDdur was the most important independent factor for „death" after KTx (Hazard Ratio (95%CI): 1.041 (1.015-1.067); $P = 0.002$).
Conclusion: Taken together our results highlight the importance of BDdur as an independent factor negatively influencing long term survival after kidney transplantation. Strategies to optimize time management prior as well as during organ retrieval are essential to improve outcome after kidney transplantation.

KIDNEY/PANCREAS

V45 SINGLE CENTER STUDY: OUTCOME AFTER 428 COMBINED PANCREAS-KIDNEY-TRANSPLANTATIONS

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Objective: Diabetes mellitus has severe consequences including death due to organ failure. Beside necessary Insulin therapy and dialysis, a combined pancreas-kidney-transplantation is one option to improve life expectancy and quality. Today recepients are older and with a longer diabetes/dialysis anamnesis. Additionaly donor criteria were extended regarding age and BMI.
Method: During 1/1994 till 7/2012 428 combined pancreas-kidney-transplantations were performed.
Results: The follow-up time was 92 month, the overall survival was 91.6%. Patients 1/5/10-year survival was 96%/90%/85%. 36 Patients died, six of them in coherence with transplantation procedure. The 1/5/10-year pancreas function rate was 82%/72%/66%, the 1/5/10 year kidney function rate was 90%/82%/69%. Morbidity refers mainly to infections and rejections. 14% developed a graft thrombosis (1.8% kidney graft, 12% pancreas graft). 1-year rejection rate was 35%. The average donor-BMI increased (1994-1999: BMI 24; 2000- 2005: BMI 25; 2006-2012: BMI 26). Likewise donor age increased (1994-1999: 39 years; 2000- 2005: 45 years; 2006-2012: 50 years). Fraction of Donor Age >45 raised from 36% to 68%. Yet there was no increase of complications in context of the demographic changes observed.
Conclusion: In spite of demographic changes and consecutive expanded donor criteria, combined pancreas- kidney-transplantation within a competent transplantation center became a reliable treatment for diabetes mellitus type 1.

V47 125 CASES OF DUODENODUODENOSTOMY IN PANCREAS TRANSPLANTATION: A SINGLE-CENTER EXPERIENCE OF AN ALTERNATIVE ENTERIC DRAINAGE

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Backround: Different technical procedures have been developed successfully to perform pancreas transplantation (PT) with regard to exocrine drainage. Retroperitoneal graft placement behind the right colon allows exocrine drainage by direct side-to-side duodenoduodenostomy. This technique provides easy access of the graft for endoscopic hemostasis and rejection monitoring via endoscopic guided graft biopsy.
Methods: 241 pancreas transplantations (217 SPK, 17 PAK, 7 PTA) were performed at our center between 2002 and 2012. Out of 241 PT, Duodenoduodenostomy (DD) was performed in 125 patients and Duodenojejunostomy (DJ) was performed in 116 patients. In this retrospective analysis we compared our experience with these two types of enteric drainage, focused on graft-and patient survival as well as postoperative complications.
Results: Donor and recipient characteristics were similar except for recipient BMI (25.1vs.23.5 kg/m2) and duration of dialysis (37.5vs.32.1 months). Cumulative patient survival (DD vs. DJ) was 96% vs. 96.5% after 1 year and 95% vs. 92% after 3 years ($P = 0.62$,log-rank test). Pancreas transplant survival after 1 and 3 years was 83% and 82% in DD-group and 78% and 73% in DJ-group without significant difference ($P = 0.20$). There were 13/125(10%) cases of pancreas graft loss in the DD-group and 21/116 (18.1%) in the DJ-group ($P = 0.08$). Relaparotomy rate was slightly higher in the DJ group (48.2% vs.41.6%), $P = 0.09$.
Conclusion: DD is a feasible and safe technique in PT. It is equivalent to other established techniques and extends the possibility of anastomotic sites, especially in recipients who have underwent a second transplant. Further studies are required to confirm the benefits of a retroperitoneal graft position using DD.

V48 TRANSPLANTING ALLO-ISLETS WITHOUT IMMUNOSUPPRESSION

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Introduction: Pancreatic islet transplantation is currently restricted to patients with critical metabolic lability due to long-term need for immunosuppression and a persistent shortage of donor organs. To overcome these obstacles we have developed a strategy for islet macroencapsulation that provides sufficient immune-isolation whereas regulated islet graft function is maintained.
Case report and methods: A 63 year old patient with type 1 diabetes and severe metabolic lability was transplanted with isolated islets (2000 islets/kgBW) encapsulated in an oxygenated chamber system composed of immune-isolating alginate and polymembrane covers. Via a small abdominal incision, a pre-peritoneal pocket for the chamber was dissected, connected oxygen ports were implanted subcutaneously. No immunosuppressive therapy was applied.
Results: The procedure was surgically straightforward and without complications. We could demonstrate persistent graft function by detection of endogenous insulin and c-peptide secretion proving islet viability and function. This observation was accompanied by persistent lowering in HbA1c despite reduction in insulin requirement.

For oxygenation of the non-vascularized and therefore immune-shielded islet graft, the chamber-integrated gas reservoir was replenished daily via the implanted ports without complications.
Conclusion: This encapsulation strategy was for the first time applied to allogeneic human islet transplantation in man. We demonstrated a persistent graft function with regulated insulin secretion without any immunosuppressive therapy. This novel concept may allow for future widespread application for cell-based therapies.

V49 ISLET AUTOTRANSPLANTATION AFTER COMPLETE PANCREATECTOMY FOR COMPLICATIONS OF UPPER GI-TRACT SURGERY

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Objective: Anastomotic leakage after upper gastrointestinal tract surgery is associated with high morbidity and mortality. Complete pancreatectomy is representing a rescue procedure if major complications at a pancreatojejunostomy or duodenal anastomosis occurred or will be expected and the patient is in a condition that does not allow for drainage or reanastomosis. This procedure leads to complete endo- and exocrine deficiency with high metabolic lability and risk of life threatening hypoglycemia. Islet autotransplantation is a method to preserve endogenous insulin secretion avoiding immunosuppression.
Patients and Methods: Following complete or subtotal pancreatectomy in 6 patients islets were isolated using a modified Ricordi-method and transplanted into the liver via open surgery through a portal vein catheter infusion. Glucose, insulin and C-peptide levels were used for metabolic monitoring after autotransplantation for up to 6 months.
Results: 6 patients were autotransplanted with 170.000 ± 40.000 islet equivalents. The autotransplantation procedure was performed without complications. 2/6 patients died during hospital stay due to septic complications with functioning autografts. The remaining four patients showed good primary and 6-months graft function without or minimal requirement for exogenous insulin (4 to 10 I.U. per day).
Conclusion: Islet autotransplantation is an effective tool in the treatment of pancreatectomized patients to avoid long term complications of pancreoprive diabetes.

V50 MICB – A LIGAND FOR THE ACTIVATING NATURAL KILLER RECEPTOR NKG2D – IS A MARKER FOR THE PREDICTION OF GRAFT REJECTION AFTER COMBINED KIDNEY-PANCREAS TRANSPLANTATION

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Objective: Deregulated expression of ligands for the activating natural killer receptor NKG2D is implicated in the pathogenesis of type 1 diabetes mellitus (T1DM). We therefore analyzed whether soluble NKG2D ligands (sNKG2DL) e.g. MICA, MICB, and ULBP2, are present in blood samples of T1DM patients awaiting kidney-pancreas transplantation and whether these molecules are associated to transplantation-related complications.
Methods: 76 T1DM patients, undergoing combined kidney-pancreas transplantation between February 2008 and February 2012, and 46 healthy controls were included. The patients' blood samples were procured before transplantation. Soluble MICA, MICB and ULBP2 were quantifed by ELISA. The results obtained were associated to clinical- and biopsy-proven rejection, infections and loss of graft functions.
Results: Soluble ULBP2 was increased 10-fold in T1DM compared to healthy controls ($P < 0.0001$), whereas soluble MICA and MICB were in range of controls. Regarding the clinical outcome only the presence of MICB in blood samples procured before transplantation was associated with acute rejection after transplantation ($P = 0.0004$; RR = 2.159; Odds ratio = 5.867). No association of sNKG2DL levels with infection (bacterial, viral or fungal) or loss of graft function was found.
Conclusion: Thus, the presence of sMICB may indicate an immune activation of the T1DM patients favoring acute rejection after kidney-pancreas transplantation.

LIVER

V51 TWENTY-YEAR LONGITUDINAL FOLLOW-UP AFTER ORTHOTOPIC LIVER TRANSPLANTATION: A SINGLE-CENTER EXPERIENCE OF 313 CONSECUTIVE CASES

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With excellent short-term survival in liver transplantation (LT), we now focus on long-term-outcome and report the first European single-center 20-year survival data.

337 LT were performed in 313 patients (09/88-12/92). Impact on long-term-outcome was studied and a comparison to life expectancy of matched normal population was performed. A detailed analysis of 20-years-follow-up concerning overweight (HBMI), hypertension (HTN), diabetes (HGL), hyperlipidemia (HLIP) and moderately or severely impaired renal function (MIRF, SIRF) is presented.

Patient and graft survival at 1, 10, 20 years were 88.4%, 72.7%, 52.5% and 83.7%, 64.7%, 46.6%, respectively. Excluding one-year mortality, survival in the elderly LT recipients was similar to normal population. Primary indication ($P < 0.001$), age ($P < 0.001$), gender ($P = 0.017$), impaired renal function at six months ($P < 0.001$) and re-transplantation ($P = 0.034$) had significant impact on patient survival. Recurrent disease (21.3%), infection (20.6%) and de-novo malignancy (19.9%) were the most common causes of death. Prevalence of HTN (57.3 to 85.2%, $P < 0.001$), MIRF (41.8 to 55.2%, $P = 0.01$) and HBMI (33.2% to 45%, $P = 0.014$) increased throughout follow-up, while prevalence of HLIP (78.0 to 47.6%, $P < 0.001$) declined.

LT has conquered many barriers to achieve these outstanding long-term results. However, much work is needed to combat recurrent disease and side effects of immunosuppression (IS).

V52 LONG TERM SURVIVAL AFTER LIVER TRANSPLANTATION WITH AORTIC CONDUITS

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Introduction: Anastomosis of donor and recipient hepatic artery is the standard technique in orthotopic liver transplantation. In case of insufficient access to the recipient hepatic artery, revascularization using a donor iliac arterial interposition graft to the recipient aorta can be necessary. We analyzed the outcome of patients with aortic conduits in our center.
Methods: Patients undergoing liver transplantation at the Department of Surgery, Innsbruck, between 1977 and 2012 were included in the study. Donor, recipient and procedure related factors were retrospectively analysed focusing on the type of arterial reconstruction. Endpoint was 1- and 5-year graft and patient survival after transplantation.
Results: In the observational period 1148 liver transplants were carried out, 18 patients were lost to follow up. Median 1- and 5 year patient survival with standard hepatic artery anastomosis and conduit grafts was 90.5% vs 74.2 and 83.3% vs 68.5% ($P < 0.0005$), respectively. In 53 (4.6%) patients a conduit to the recipient aorta or iliac artery was conducted according to the surgeons preference. Among them, 15 (28.3%) were re-transplants as compared to 38 (3.5%) retransplantations among patients with a standard arterial anastomosis.
Conclusion: Long term survival is inferior in patients with aortic conduits. However, survival beyond 1 year was comparable between the groups. Of note, the retransplantation rate was higher in the aortic conduit group.

V53 DO WE HAVE RELIABLE CRITERIA TO AVOID RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER LIVER TRANSPLANTATION?

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Introduction: Tumour recurrence of hepatocellular carcinoma (HCC) after liver transplantation (LT) is still a relevant problem.
Method: In a retrospective study we included all patients with LT for HCC in our institution (observation period: January 2007-August 2012). Beside demographic data, we analysed course, laboratory results, bridging therapies as well as the correlation of imaging and histopathology of our recipients.
Results: During the study period we performed 91 LT because of HCC. The average waiting time on the liver recipient list was 349 days. Fifteen (16.5%) patients developed a recurrent HCC after LT. Our subgroup with tumour recurrence after LT presented with a mean disease-free survival of 11.9 months (3-37 months) and an overall survival of 22.7 months. Nine of the 15 patients died after a mean period of 5.6 months after diagnosis of tumour recurrence. Their tumour-specific therapy included chemotherapy, resection of metastasis and radiofrequency ablation of a recurrent tumour in the liver. In 8 of 15 patients the immunosuppressive therapy was switched from tacrolimus to everolimus. Obviously, radiologic underestimation of the tumour stage was an unresolved problem in 10 (66.6%) patients with recurrence.

In our study group neither Milan criteria, waiting time, bridging therapies, AFP blood levels nor pathologic grading had an influence on the tumour recurrence. Only microvascular invasion was a risk factor for tumour recurrence.
Conclusion: Despite careful selection of patients, an early recurrence of HCC after LT cannot be avoided completely. Reliable prognostic markers related to tumour biology are still missing.

V54 PRE-TRANSPLANT TREATMENT OF HEPATOCELLULAR CARCINOMA WITH RADIOEMBOLIZATION USING YTTRIUM-90 MICROSPHERES

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Background: Liver transplantation remains the only curative treatment option for patients with unresectable hepatocellular carcinoma (HCC). Pre-transplant treatment is imperative because of persisting donor organ shortage, prolonged waiting time and increased risk of tumor progression. Radioembolization is mostly employed in the control of large HCCs when other local ablation treatments are not indicated.
Methods: 20 patients received pre-transplant treatment of HCC with radioembolization using yttrium-90 microspheres at our transplant center since December 2006. The explanted livers were examined histopathologically for treatment response.
Results: Radioembolization was applied to the right liver lobe in 14 patients and to the left liver lobe in one patient. In five patients radioembolization of both lobes was performed. Patients underwent liver transplantation 100 [8–526] days after radioembolization. Histopathological examination of the explanted livers revealed complete necrosis of tumor cells in four patients, partial necrosis in 12 patients and viable tumor cells were detected in four patients. Three patients died of bone metastases and two patients died of recurrent HCC to the liver within 2 years after liver transplantation.
Conclusion: Histopathological assessment of the explanted livers from patients undergoing pre-transplant radioembolization demonstrated at least partial necrosis in 80% of patients treated in our center. We propose that radioembolization is a valuable and safe treatment option for bridging to liver transplantation.

V55 ROUTINE CHOLANGIOSCOPY AS PART OF THE PERCUTANEOUS TRANSHEPATIC MANAGEMENT OF BILIARY STRICTURES AFTER LIVER TRANSPLANTATION UNTREATABLE BY ENDOSCOPIC RETROGRADE CHOLANGIOGRAPHY – A PROSPECTIVE SINGLE CENTRE EVALUATION

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With an incidence of up to 30% biliary strictures are a major source of morbidity after liver transplantation (LT). While the majority of patients is treated by endoscopic retrograde cholangiographic (ERC) interventions, in some patients this approach fails and a percutaneous transhepatic biliary drainage (PTBD) is placed. In this prospective study we evaluated the diagnostic and therapeutic benefit of percutaneous cholangioscopies (PC) performed routinely during the PTBD changing procedure.

From 10/2007 to 01/2011 all LT recipients at Hannover Medical School with a PTBD were asked to participate. PC could be performed when a PTBD of at least 14F was in place. Endoscopic and radiological findings as well as follow-up data after PTBD removal (at least 6 months) were recorded in a prospective database.

From 10/2007 to 02/2012 we performed 111 PCs in 25 patients. Main cholangioscopic findings were biliary stones in 16, stenoses in 11 and bile duct inflammation in 19 patients. All stones could be removed cholangioscopically.

Stenoses were treated with balloon dilation or bougienage. 4 patients remained cholestatic despite of a successfully placed PTBD and were re-transplanted, 1 patient died during follow-up and in 20 patients the PTBD was removed after successful therapy (however in 4 of them recurrent strictures were found during follow-up).

In conclusion percutaneous cholangioscopy is a useful diagnostic and therapeutic supplementation of percutaneous interventions after LT.

V56 COMPLICATIONS OF ENDOSCOPIC SPHINCTEROTOMY (EST) IN PATIENTS AFTER LIVER TRANSPLANTATION (LT)

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Aim: The aim of this study was to evaluate the complication rate of EST up to one week post-interventionally in liver transplant patients.
Methods: We conducted a retrospective analysis of endoscopic retrograde cholangiopancreaticography (ERCP) procedures after LT at the University Hospital of Münster, Germany. A total of 107 consecutive posttransplant recipients who received EST during ERCP were included.
Results: A total of 10 complications accounting for a morbidity rate of 9.3% occurred. Three patients (2.8%) developed post-ERCP pancreatitis (PEP); defined as abdominal pain requiring therapy combined with increasing lipase levels $\geq$ three times the upper limit of normal. PEP was mild (Imrie score $<$3) in all cases. Post-ERCP bleeding occurred in 5 patients (4.7%). Perforation was observed in one patient (0.9%). One patient developed an acute post-ERCP cholangitis (0.9%).
Conclusion: Complication rate for EST in liver transplant patients did not differ considerably from that reported in the non-transplant setting. Remarkably, the risk of PEP (2.8%) was lower than that found in literature (5–7%), whereas the risk of bleeding was higher (4.7% versus 2%).

Thus, EST is a safe procedure after LT. Morbidity related to this procedure is similar to that reported among the general population. Immunosuppressive drugs given after LT may have a protective effect against PEP.

V57 TRANSTHYRETIN ASSOCIATED HEREDITARY AMYLOIDOSIS IN A GERMAN COHORT

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Background: The highly variable phenotype of transthyretin (TTR) associated hereditary amyloidosis has significant effects on the outcome of liver transplantation, currently the only curative treatment option. To optimize patient selection for transplantation, emphasis should be placed on the specific mutation involved.
Aim: The mutational variation of TTR-amyloidosis in german patients was studied.
Methods: 84 patients with histologically confirmed TTR-amyloidosis from 44 unrelated families were included. The mutation was identified by genomic DNA extraction from patient's blood and direct sequencing. Patients were examined for clinical signs and symptoms.
Results: The most prevalent mutation was Val30Met, detected in 45 patients from 26 families. Although all patients with this mutation presented primarily with polyneuropathy, a significant variation of age of onset was seen, ranging from 20 to 60 years. The remaining patients were found to have one of the following variants: Gly47Ala, Thr59Lys, Leu58His, Ile107Val, Gly53Ala, Asp39Val, Arg34Thr, Val20Ile, Thr60Ala, Glu89Gln. 16/84 patients received a liver transplant, 21/84 patients are currently being treated with Tafamidis. Outcomes are still being followed.
Conclusions: This is the first study to describe the mutational variants of TTR-amyloidosis in Germany. Similar to Portugal, Sweden and Japan, Val30Met was found to be the most common variant. Our continuously growing cohort of patients with hereditary amyloidosis and ongoing analysis, may enable us to categorize predominant mutations in Germany. Furthermore, it may facilitate the identification of prognostic factors for those patients treated with Tafamidis or liver transplantation.

LIVER/ SMALL INTESTINE

V58 TELAPREVIR BLOOD LEVELS ARE UNALTERED IN PATIENTS WITH CICLOSPORIN AND TACROLIMUS BASED IMMUNOSUPPRESSION AFTER ORTHOTOPIC LIVER TRANSPLANTATION

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Background/Aims: The protease inhibitor telaprevir may enhance virologic response rates in patients after OLT in combination with pegylated-interferon-alfa and ribavirin. Pharmacokinetic studies have shown significant drug-drug interactions between telaprevir and immunosuppression (IS), but telaprevir blood levels kinetics in OLT-patients with IS are unkown. Aim of the present study was to analyze telaprevir blood levels in patients with HCV genotype 1 infection after OLT in comparison to patients without OLT.
Methods: Five patients with HCV genotype 1 infection after OLT were treated with telaprevir, ribavirin and pegylated-interferon-alfa-2a and compared to 37 HCV genotype 1 infected patients without OLT treated with triple therapy. In patients with ciclosporin ($n = 3$) based IS, dose was reduced 4-fold, and 35-fold in patients with tacrolimus based IS ($n = 2$), and adjusted to blood trough levels if appropriate. Telaprevir blood levels were analyzed approximately 4 hours after intake by liquid chromatography electrospray-ionization-tandem mass spectrometry.
Results: Mean ± SD telaprevir blood levels were 3920 ± 733 ng/mL and 2201 ± 256 ng/mL in patients after OLT and ciclosporin or tacrolimus based IS, respectively, compared to 2686 ± 1157 ng/mL in non-OLT patients ($P = 0.3$). Moreover, telaprevir blood levels were steady at week 4, 8, and 12 in patients with and without IS (Figure 1). Mean ± sd ciclosporin and tacrolimus trough levels in patients after OLT were 77.92 ± 34.39 ng/mL and 6.0 ± 1.79 ng/mL, respectively. No acute graft rejection was observed during triple-therapy.
Conclusions: Telaprevir blood levels are not altered in patients with ciclosporin or tacrolimus based IS in patients with HCV re-infection after OLT. Interaction between telaprevir and ciclosporin/tacrolimus are clinical significant, but can be managed by close drug level monitoring.

V60 BRAIN DRAIN OUT OF LIVER TRANSPLANT SURGERY IN GERMANY

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A rapid turnover of liver transplant surgeons in Germany may unnecessarily bind resources and compromise on patient's safety. Here, we have documented a brain drain of surgeons out of liver transplantation in our institution over a period of 22 years.

The careers of 25 surgeons who engaged in liver transplantation during this observational period were followed.

The median (minimum-maximum) time of a surgeon from the first to the last liver transplantation was 6 (1–21) years for all surgeons, and 8 (3–21) years for surgeons, who completed training. Corresponding total numbers of liver transplant procedures were 56 (2–162) and 72 (37–162), respectively.Eight surgeons stopped before termination of their liver transplant training, 8 trained surgeons dropped out to become head surgeons in a non-transplant community hospital, only 1 surgeon has continued liver transplantation (as the chairman), but will stop his surgical career (to become CEO) 4 years prior retirement. Currently, liver transplantation is performed by 4 surgeons on call and 4 surgeons in training.

The vast majority (75%) of surgeons prematurely dropped out of liver transplantation to follow alternative surgical careers. Structural changes in academic transplant surgery have to be made to allow long-term commitments of interested surgeons and to avoid futile "transplant" careers.

V61 ADULT TO ADULT LEFT LDLT (AA-LEFT-LDLT): SINGLE CENTRE EXPERIENCE AT UNIVERSITY HOSPITAL TÜBINGEN

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Background: AA-Left-LDLT is under-represented (< 10% of LDLT worldwide and < 2% in Germany) mainly because of the fear of small for size syndrome in recipients.
Aim: Single centre retrospective analysis of 8 AA-left-LDLT to demonstrate the feasibility and safety of the procedure for both donor and recipient.
Methods: Left lobe consisted of Seg 2–4. Data analyzed: demographics, volumetry, outcome (reported as median value).
Results: September 2008–March 2013 we performed 36 LDLT: 9 left (8 AA-LDLT) , 3 right and 24 left lateral.
Recipient:
Age 48 (17–60)
Gender M:F = 2:6
Diagnosis 2 PSC, 2 Krypto, 1 AIH, 1 HCV+HCC, 1 C2-OH , 1 Caroli
Lab-MELD 15 (5–19)
GBWR 0.7 (0.5–0.9)
Flow Modulation 3 SAL , 1 HPC-Shunt, 7 PGI2 i.v.
Complication (Dindo-Clavien): III = 13/6 , IV = 4/2 , V = 1
Graft Survival: 75%. 2 graft losses because of SFFS in 1 and HAT in 1 (both re-transplanted with whole DDLT)
Pat Survival: 87.5% (median FUP 31 mo. 3–46). One patient died because of hyperkaliaemia at POD 6 after re-tx (35 POD after LDLT)
Donor:
- Age 49 (29–62)
- Gender M:F = 6:2
- RLVBWR 1.87 (1.39–2.04)
- Complications: only 1: IIIb psychological (Nadalin classification)
- Survival: 100%

Conclusion: Shifting the risks from donor to the recipient, AA-left-LDLT with small grafts in not decompensated patients by means of graft inflow modulation is feasible and safety.

V62 DOPPLER ULTRASOUND FINDINGS IN CHILDREN WITH EARLY LIVER GRAFT FAILURE

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Introduction: Doppler ultrasound (DU) is frequently used to evaluate graft perfusion after liver transplantation (LTx). Aim of our study was to analyze routine DU findings in the early postoperative period in children with subsequent liver graft failure.
Methods: Retrospective analysis of a prospective LTx data base (2000–2010). DU data were collected at 3 time points within the first 7 pod including arterial/portal flow measurements by a single investigator.
Results: Overall 377 pediatric LTx were performed, including 15 children (median age 3.5(0.2–15.2)yrs) with early liver graft failure (median time to graft failure 4(1–28)days) after primary LTx who had a complete follow-up including DU data. Pathological findings in these children were observed in only 2.7 % for systolic flow (peak <20 cm/s) and portal flow (max. < 10 cm/s) whereas an abnormal resistance index (<0.55 or >0.8) was noted in 51.4%. Comparison of the systolic peak flow (56.5 versus 40 cm/s) and the resistance index (both 0.7) between children with early liver graft failure due to hepatic artery thrombosis ($n = 8$; measurements performed between LTX and DU proven hepatic artery thrombosis) in contrast to primary liver graft non function ($n = 7$) revealed no significant difference ($P = 0.098/0.499$).
Conclusion: Doppler indices in the posttransplant course of children with early liver graft failure revealed a high rate of abnormal resistance indices (about 50%) whereas arterial and portal flow indices showed normal values. Before the event of hepatic artery thrombosis the cause of graft failure had no impact on the Doppler examination in the early postoperative course.

V64 SMALL BOWEL TRANSPLANTATION: INDICATIONS AND LIMITATIONS

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Small bowel transplantation (SBTx) can be performed for short bowel syndrome. The correct indication and ideal immunosuppression are crucial. Here we report on our experience with SBTx.
Methods: From 2007 until 2012 we have transplanted 10 patients (28–57 years) with short bowel syndrome because of complications related to total parenteral nutrition. Four patients received a combined liver and small bowel graft. The immunosuppressive regimen consisted of tacrolimus, prednisolone, mycophenolate mofetil and daclizumab ($n = 4$) or alemtuzumab ($n = 6$) for induction therapy.
Results: Two patients with a combined graft died within three months after the transplantation due to sepsis. Both patients received daclizumab for induction therapy, their grafts had the longest cold ischemia and the highest donor age. One patient died because of PTLD and one graft was lost due to chronic rejection. Six patients present with a functioning small bowel graft.
Conclusions: The ideal time point for SBTx appears to be before onset of vascular problems and irreversible liver disease. Alemtuzumab induction therapy for SBTx followed by triple immunosuppression with tacrolimus, prednisolone and mycophenolate mofetil is a safe regimen. Our experience suggests to choose a donor younger than thirty years of age and to aim for less than eight hours of cold ischemic time.

THORACIC ORGANS

V65 HIGH NEGATIVE PREDICTIVE VALUE OF CARDIAC MRI IN DETECTION OF SUB-CLINICAL ACUTE REJECTION AFTER HEART TRANSPLANTATION

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Objective: Sub-clinical acute cellular rejection (ACR) after heart transplantation (HTx) is a major cause of cardiac allograft vasculopathy. But with endomyocardial biopsy (EMB) the detection of sub-clinical ACR is limited by sampling error, interobserver variability or the wide variability of frequency and duration to use this invasive interventional tool. Thus, we used cardiac magnetic resonance imaging (CMRI) to identify sub-clinical ACR after HTx.
Methods: In total 73 patients with a mean age of 53 $\pm$ 12 years were scanned 167 times with a 1.5T-MRI scanner using a standard myocarditis protocol to detect sub-clinical ACR. Myocardial edema and myocyte damage were determined by using specific "cut-off" values to determine inflammatory parameters of early- and delayed- enhancement as well as to determine water content. CMRI-results were correlated with results of EMB performed on the same day using the original ISHLT EMB guidelines to more accurately determine sub-clinical ACR.
Results: In 87 (52%) out of 167 biopsies a rejection grade of 1A was diagnosed, whereas 16 biopsies (10%) were graded 1B, 2 biopsies (1.1%) 2A and 1 biopsy (0.5%) 3A, respectively. No rejection was diagnosed in 61 biopsies (36%).

Sensitivity, specificity and negative predictive value for rejection grade $\geq$1B were for (1) early-enhancement 64%, 70% and 93%; (2) delayed-enhancement 43%, 38% and 82%; (3) water content: 60%, 79% and 93%. In combination of all 3 parameters sensitivity, specificity and negative predictive value were: 71%, 66% and 94%.
Conclusion: We showed that a combination of specific CMRI parameters have a high negative predictive value in early detection of sub-clinical ACR. Future clinical studies need further to evaluate CMR as an evolving tool in ACR.

V67 C4D DEPOSITION CORRELATES TO MICROVASCULOPATHY IN CORONARY ANGIOGRAPHY AND BIOPSY AFTER HEART TRANSPLANTATION

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Purpose: Antibody-mediated rejection (AMR) has been shown to correlate to epicardial vasculopathy after heart transplantation (HTx). If AMR correlates to microvasculopathy in coronary angiography or endomyocardial biopsy was not studied yet.
Methods: Prospective analysis was done in 199 biopsies obtained from 78 HTx pts at 4 wks, 1 yr and 3 yrs after HTx. Vasculopathy was assessed in coronary angiography (ISHLT classification; remodeling type and peripheral obliterations according to the Task Force for Thoracic Organ Transplantation of the German Cardiac Society). Endomyocardial biopsy was studied for C4d deposition (immunohistochemistry) and microvasculopathy.
Results: Microvasculopathy in biopsy correlated with C4d deposition at 4 wks post HTx (35% vs. none; $P = 0.016$) and with persisting microvasculopathy at 1 year post-transplant (40% vs. none; $P = 0.037$). Peripheral obliterations at 4 wks post-transplant were more frequent in pts with C4d deposition during the same (36% vs. 10%; $P = 0.047$) and the consecutive follow-up (67% vs. 20%; $P = 0.009$). Peripheral obliterations at 1 year post-transplant correlated significantly with C4d deposition during the same (39% vs. 10%; $P = 0.020$) and the previous follow-up (25% vs. none; $P = 0.007$). All pts with negative remodeling at 1 year post-transplant (100% vs. 24%; $P = 0.006$) had more C4d deposition in the biopsy and the majority of these pts (47% vs. 19%; $P = 0.047$) also had more C4d deposition in the 3-year biopsy. Severe ISHLT CAV class correlated to C4d deposition only at 3 years after HTx ($P = 0.036$).
Conclusions: C4d deposition correlates to microvasculopathy as evident in coronary angiography or endomyocardial biopsy and emphasizes the role of AMR in diffuse vessel involvement.

V68 MANAGEMENT OF THORACIC TRANSPLANT PATIENTS WITH HEPARIN-INDUCED THROMBOCYTOPENIA (HIT-II): EXPERIENCE WITH PREOPERATIVE PLASMAPHERESES PLUS INTRAOPERATIVE HEPARIN

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Rationale: Heparin-induced thrombocytopenia (HIT) poses a tremendous surgical challenge, specifically in thoracic transplant surgery as heparin is the only anticoagulant drug that can be antagonized. Alternative anticoagulants (lepirudin, argatroban, danaparoid, bivalirudin) are not approved for CPB, and may pose great bleeding risks. Based on the antibody-mediated nature of HIT, we reasoned that it may be possible to eliminate HIT antibodies by plasmaphereses, allowing heparin and protamin to be used during surgery.
Objective: We here report our first experience with plasmaphereses in 6 HIT II-positive patients undergoing thoracic transplantation using heparin/protamin.
Methods: Four Patients underwent heart-transplantation, one patient lung-transplantation and one patient heart-lung-transplantation. HIT II was confirmed in all 6 patients and anticoagulant treatment was performed with argatroban until the time of surgery. The patients received a single run of plasmaphereses immediately after the donor organ was accepted and before transplantation. The surgical procedures were then performed using standard heparin/protamin. Postopoerative anticoagulation was again conducted with agratroban.
Results: All patients survived the operation and are still alive. There were no complications or side effects during the plasma exchange. The use of heparin during the transplantation was free of complications. No thromboembolic or bleeding complications were observed.
Conclusions: The results suggest that preoperative plasma exchange in HIT-II positive patients and using heparin during thoracic transplantation is safe and efficient. However, more experience is needed to verify this suggestion.

V69 CLINICAL OUTCOME IN HEART TRANSPLANT RECIPIENTS RECEIVING EVEROLIMUS IN COMBINATION WITH THE CALCINEURIN INHIBITOR TACROLIMUS OR CYCLOSPORIN A

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Objectives: It is currently not known whether the combination of everolimus (EVL) plus tacrolimus (TAC) is superior to the combination of EVL plus cyclosporine (CSA) regarding clinical outcomes in heart transplant recipients.
Methods: We compared 5-year clinical outcomes in 111patients receiving EVL plus CSA with 67 patients receiving EVL plus TAC. The primary endpoint was a composite of death and drug discontinuation. Secondary endpoints were cytomegalovirus (CMV) infections and kidney function.
Results: Groups were comparable regarding age, sex and primary diagnosis, but body mass index (BMI) was significantly higher and the time from transplantation to EVL plus CNI-inhibitor conversion was significantly longer in group A compared to group B (BMI: 27.0 $\pm$ 3.5 kg/m^2 vs. 25.8 $\pm$ 3.4 kg/m^2; $P < 0.001$; Time to conversion: 8.6 $\pm$ 4.7 years vs. 3.5 $\pm$ 3.1 years, $P = 0.020$). In the CSA and TAC group, the primary endpoint was reached by 56.8% and 52.2%, respectively ($P = 0.485$). Survival rates were 65.2% (CSA group) and 71.2% (TAC group), respectively ($P = 0.459$), and freedom from drug discontinuation was 60.2% and 54.1% ($P = 0.422$) in the respective groups. In covariate-adjusted Cox regression analysis, survival was similar in both groups (relative risk of the CSA group = 1.08 (95% CI: 0.48–2.13; $P = 0.984$). Compared to the CSA group, kidney function improved in the TAC group over time (P = 0.008). No case of CMV infection occurred, either in the CSA or in the TAC group.
Conclusions: In both groups, EVL discontinuation rate was relatively high. Compared to EVL plus CSA, EVL plus TAC seems to improve kidney function but does not improve overall survival.

V70 BRIDGE TO LUNG TRANSPLANTATION WITH A BI-CAVAL DUAL LUMEN CATHETER FOR VENO-VENOUS EXTRACORPOREAL MEMBRANE OXYGENATION IN NON-INTUBATED PATIENTS: A SINGLE CENTER EXPERIENCE

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Objective: Veno-venous extracorporeal membrane oxygenation (vvECMO) is an established therapy for bridging patients to lung transplantation (LTX). vvECMO-support additionally immobilizes patients due to the use of two cannulas. We here report our experience in four patients awaiting LTX with vvECMO support established by bi-caval dual lumen catheter (Avalon Eliteâ).
Methods: Since 01/2012 four patients supported with vvECMO using the Avalon-cannula implanted in the right internal jugular vein. Before ECMO-implantation all patient presented in refractory respiratory failure (pH 7.2 $\pm$ 0.1; PaO_2 8.5 $\pm$ 2.6 kPa; $PaCO_2$ 13.5 $\pm$ 4.7 kPa).
Results: All Avalon-catheters were implanted without complications and providing mean flow-rates of 3.5 $\pm$ 0.3 l/min. Full vvECMO-support normalized blood gas values (pH 7.4 $\pm$ 0.1, PaO_2 13.0 $\pm$ 3.4 kPa; $PaCO_2$ 6.4 $\pm$ 1.7 kPa) and eliminated shortness of breath or allowed deescalation mechanical ventilation. Mean vvECMO-support duration was 11 days ($\pm$ 4.4 days) while one patient is still on support. This single cannulation technique allowed our three extubated patients nearly independent food intake, mobilisation and physiotherapy with assistance. In one patient minor bleeding at the insertion site occurred and could be treated by purse-string suture. No further complication occurred.
Conclusion: Bridging high LAS lung transplant candidates with vvECMO-support established by a bi-caval dual-lumen catheter is safe and provides seriously-ill patients with desperately needed mobility and independence.

V71 CARDIOGONIOMETRY: A POTENTIAL NON-INVASIVE SCREENING TOOL FOR THE DIAGNOSIS OF CARDIAC ALLOGRAFT VASCULOPATHY IN HEART TRANSPLANT RECIPIENTS

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Background: Cardiac allograft vasculopathy (CAV) is an independent risk factor for late graft failure following heart transplantation. Due to the lack of symptoms and the nature of disease early diagnosis is difficult. Currently available non-invasive screening tools are either not always available or have a low sensitivity or specifity.
Methods: In this nonrandomized, single-institutional feasibility trial we examined the potential application of Cardiogoniometry (CGM) as a non-invasive screening tool for detection of cardiac allograft vasculopathy. CGM is a vectorcardiographic method utilising computer-aided three-dimensional analysis of cardiac potentials which was recently proven to detect coronary artery disease and myocardial ischemia with a sensitivity of 73% and a specificity of 84%. CGM measurements were correlated to coronarangiographic, echocardiographic and electrocardiographic findings of transplant recipients.
Results: CGM was performed in 30 transplant recipients 12 years (range 1–29 years) after heart transplantation. Median age was 62 years (range 44–79 years). According to coronarangiography CAV was present in 14 patients (47%) which correlated fully to pathological CGM-measurements (100%). In the remaining 16 patients a CAV was angiographically excluded and true-positive CGM findings were documented in 88% of these cases.
Conclusions: Cardiogoniometry could potentially be a useful, non-invasive screening tool for the diagnosis of cardiac allograft vasculopathy.

ORGAN DONATION

V74 LEVEL OF INFORMATION OF STUDENTS AT THE UNIVERSITY OF REGENSBURG CONCERNING ORGAN DONATION AND TRANSPLANTATION - INFORMED OR NOT INFORMED CONSENT IN ORGAN DONATION?

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Background: As a result of the actual amendment of the German transplantation law, every citizen will be regularly asked by health insurance companies about his attitude towards post-mortem organ donation - without the obligation to decide. The aim is to increase the willingness of donations as well as the availability of organs. Therefore, we investigated the level of information of students at the University of Regensburg and their agreement to organ transplantation regarding an informed consent.
Methods: Using an interdisciplinary developed questionnaire (Medicine, Theology, Educational Science) the level of information concerning process and possibilities of organ donation, the possession of an organ donor card, as well as the active or passive consent to donate organs was investigated.
Results: Out of 1225 respondents 31.5 % had an organ donor card, 49.1 % wanted to donate organs, 32.1 % were unsure. 98 % generally favoured organ donation. However, serious information deficits about brain death were identified: 37.4 % did not know that brain death is a prerequisite for a post-mortem organ donation, 18 % thought brain death is reversible, 52.7 % were not aware of the necessity of intensive medical care. Furthermore, providing information about other potential donor organs including lungs, pancreas, small intestine, and tissue is required.
Conclusion: Health insurance companies and responsible authorities need to close the identified gaps in knowledge in order to achieve „informed" consent with organ donation, which might increase the availability and number of donor organs.

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V75 EVALUATION OF IFNγ RELEASE ASSAYS FOR DETECTION OF CELLULAR IMMUNITY TOWARDS *M. TUBERCULOSIS* AND CMV IN SAMPLES FROM DECEASED DONORS

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No data exist on the feasibility of blood-based IFNγ release assays (IGRAs) for risk assessment in deceased donors. Therefore, we evaluated the performance of an ELISA-, an ELISPOT- and a flow-cytometric assay (FACS) to determine T-cell-immunity towards *M. tuberculosis* and CMV in deceased donors.

100 donors (52 ± 17 yrs) were screened at the time of organ procurement. A CMV-IgG-ELISA was used as a gold standard for CMV-infection. Specific stimulation was performed using PPD, ESAT-6/CFP-10, and a CMV-lysate in combination with commercial assay formats (QFT-TB/CMV (ELISA), T-SPOT.*TB* (ELISPOT)).

Indeterminate results were observed in 49.0% of ELISA-, 13.3% of FACS- and 0% of ELISPOT-assays. CMV-specific immunity was detected in 26.0%, 46.9%, and 54.1% of QFT-CMV-, FACS-, and ELISPOT-samples, respectively. Agreement with serology was highest for FACS (93%, K = 0.85), followed by ELISPOT (81%, K = 0.61), and ELISA (81%, K = 0.62). The percentage of PPD-positive results differed between assays (27.3% (ELISA), 27.6% (FACS), and 48.9% (ELISPOT)). Among PPD-positive samples, 8.3% were QFT-TB positive, 16.7% were positive in an ESAT-6/CFP-10-specific FACS-assay, and 25.6% were positive in the T-SPOT.*TB*-test. Neither delayed processing nor steroids had a significant effect on indeterminate results.

In conclusion, cellular immunity may be analysed from samples of deceased donors, although indeterminate results are more frequent than in healthy individuals.

IMMUNOSUPPRESSION

V76 5 YEARS FOLLOW-UP ON RENAL FUNCTION - ZEUS TRIAL: IMPROVED RENAL FUNCTION OF AN EVEROLIMUS/ ENTERIC-COATED MYCOPHENOLATE SODIUM REGIMEN AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN DE NOVO RENAL TRANSPLANT PATIENTS

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Aim: To follow-up (FU) on renal function (RF), efficacy and safety after conversion to an Everolimus (EVE)/Enteric-Coated Mycophenolate Sodium (EC-MPS) regimen after cyclosporine A (CsA) withdrawal in de novo kidney allograft recipients at month (Mo)60 after transplantation (Tx).
Methods: Prospective, open-label, controlled, multi-center study on renal Tx patients (pts) randomized at Mo4.5 post Tx to an immunosuppressive (IS) regimen consisting of either EVE/EC-MPS or CsA/EC-MPS. After completion of core-study (Mo12) pts entered into an observational FU.
Results: 300 pts were randomized to either EVE+EC-MPS ($n = 155$) or CsA+EC-MPS ($n = 145$), of whom 227(76%) pts completed Mo60 visit. RF expressed as eGFR (Nankivell) was similar in both groups at baseline (4.5Mo post Tx) with an improvement by +3.6 mL/min/1.73 m2 (95%CI:[+0.1;+7.2]; $P = 0.045$) in favour of the EVE regimen at Mo60 compared to +9.8 mL/min/ 1.73 m2 at Mo12 (ITT population). All pts who remained on the assigned EVE treatment (on-therapy) had a higher improvement of RF by +7.3 mL/min/ 1.73 m2 (95%CI:[+3.4;+11.3] $P < 0.001$) at Mo60. In the CsA group 3 deaths and 3 graft losses were observed vs 4 deaths and 4 graft losses in the EVE group. The number of pts with infections was 32(21%) in EVR vs 21(15%) in CsA group; hospitalisation was reported for 36pts (23%) in the EVE vs 21pts (15%) in the CsA group between Mo48 and Mo60. No significant difference in BPAR was reported with 21(14%) in the EVE vs. 11(8%) in the CsA group ($P = 0.095$) from randomization to Mo60.
Conclusion: Early conversion to EVE in de novo KTx pts after CNI withdrawal maintains a better RF over a period of 5 years without compromising efficacy and safety.

V77 HERAKLES AT MONTH 24: FOLLOW-UP RESULTS ON EFFICACY AND SAFETY OF THREE DIFFERENT TREATMENT REGIMENS IN DE NOVO RENAL TRANSPLANT PATIENTS DEMONSTRATE OPTIONS FOR INDIVIDUALIZED IMMUNSOSUPPRESSION

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Aim: To follow-up on safety and efficacy of 3 different immunosuppressive (IS) regimen 24 months (Mo) after renal transplantation (Tx).
Methods: 802 patients (pts) were included in this 1 year, prospective, open-label, randomized, controlled, multi-center study. After induction therapy with basiliximab all pts received cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) +steroids. At 3Mo post Tx 499pts were randomized 1:1:1 to either a) continue standard (STD) treatment CsA(100–180 ng/ml) + EC-MPS($n = 166$) or convert b) to a calcineurin inhibitor (CNI)-free regimen with everolimus(EVE; 5–10 ng/ml) + EC-MPS($n = 171$) or c) to CNI-low regimen with EVE (3-8 ng/ml) + reduced CsA(50-75 ng/ml) ($n = 162$). All pts continued on steroids. Mo24 follow-up (FU) visit was performed by 131 (96%) STD, 132 (96%) CNI-free and 125 (93%) CNI-low regimen pts of the ongoing FU population (pop).
Results: From randomization to Mo24 BPAR was reported in 17/144(12%) STD, 20/146(14%) CNI-free and 17/141(12%) CNI-low pts (ITT). 2 deaths(1%) occurred in the CNI-low, none in the other groups. 1(1%) graft loss was observed in the STD and 4(3%) in CNI-free group. Composite failure (BPAR/ death/graft loss/lost to FU) was measured in 19(13%) STD, 22(15%) CNI-free, 20(14%) CNI-low treated pts. Premature discontinuation due to adverse events occurred in 1/147(1%) of STD, 3/148(2%) of CNI-free and 1/141(1%) of CNI-low pts (safety-pop) from Mo12 to Mo24. Renal function expressed as cGFR (Nankivell) was significantly improved by +4.8 mL/min/1.73 m2 (95%CI: [+1.0;+8.6]) in favour of the CNI-free regimen at Mo24 (ITT; $P = 0.014$).
Conclusion: After 24Mo, the IS regimen using EVE with low-dose or without CNI-exposure is an efficacious and safe therapeutic approach offering the opportunity for an individualized IS.

V78 A POST HOC ANALYSIS OF 2 PROSPECTIVE, OPEN-LABEL, MULTICENTER, RANDOMIZED TRIALS: ONSET AND PROGRESSION OF DIABETES IN KIDNEY TRANSPLANT PATIENTS RECEIVING EVEROLIMUS OR CYCLOSPORINE. RESULTS FROM ZEUS AND HERAKLES

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Aim: To compare incidence of new-onset diabetes mellitus after transplantation (Tx) (NODAT) and progression of pre-existing diabetes mellitus (DM) in *de novo* kidney allograft recipients after conversion to everolimus (EVR) based regimen and withdrawal or reduction of cyclosporine A (CsA) combined with EVR.
Methods: *Post hoc* analysis from ZEUS and HERAKLES, 12 month (Mo), prospective, open-label, multicenter, randomized (RDZ) trials. De novo KTx pts received standard-exposure CsA + EC-MPS + steroids since Tx and were RDZ to either continue CsA regimen or convert to EVE/EC-MPS at Mo4.5 post Tx (ZEUS) or at Mo3 post Tx (HERAKLES) offering 3rd RDZ arm reduced CNI+EVR. *Post hoc* categorization and analyzis was done accordingly to NODAT development or DM progression.
Results: ZEUS: 8% (25/300) of pts had DM at Tx. At Mo12 NODAT had developed in 8% (22/275) of non-DM pts (EVR 14/142 vs CsA 8/133); of these 7%(20/275) had developed NODAT already at RDZ(EVR 13/142 vs CsA 7/ 133), incidence of NODAT after RDZ was similar between groups ($P = 0.97$). Mean blood glucose change from RDZ to Mo12: in NODAT($P = 0.10$) and DM ($P = 0.52$) subgroups similar between EVR and CsA. eGFR (Nankivell; ml/min) was similar at RDZ and significantly higher at Mo12 for EVR vs CsA pts within all subpop (NODAT: EVR +14.0(11.4) vs CsA -9.2(15.9); pre-existing DM: EVR +5.5(5.9) vs +0.7 (10.0) CsA). HERAKLES results: At Mo12 NODAT in 6.8% (30/438) of pts (EVR 6.7%(10/149) vs CsA 8.3%(11/133) vs reduced-CsA 6.3% (9/143). No different incidence of NODAT between the 3 regimen ($P = 0.62$).
Conclusion: Within 12Mo post Tx no difference in NODAT incidence or DM progression after CNI withdrawal and conversion to EVR were found by *post hoc* analysis. However, the benefit on renal function from early conversion to EVR translated as for total study pop into the NODAT subpop.

V79 4 YEAR DATA OF THE APOLLO TRIAL: OUTCOME ON RENAL FUNCTION OF AN EVEROLIMUS BASED THERAPY AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN MAINTENANCE RENAL TRANSPLANT RECIPIENTS

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Aim: Assessment of renal function, safety and efficacy of an Everolimus (EVE) regimen after Calcineurin-Inhibitor (CNI) withdrawal in maintenance kidney (K) allograft recipients.
Methods: Open-label, randomized, controlled, multi-center study. 93 patients (pts) on stable immunosuppressive therapy consisting of CNI, Enteric-Coated Mycophenolate Sodium (EC-MPS) ±corticosteroids were randomized (RDZ) to either continue CNI treatment +EC-MPS or convert to an EVE +EC-MPS based regimen; after 12Mo core study pts were included in an observational follow-up (FU).
Results: 93 pts with mean time of 6.4 years since most recent transplantation (Tx) were RDZ to either EVE ($n = 46$) or CNI ($n = 47$) treatment, 72 (77.4%) pts completed Mo48 visit. At Mo48 after RDZ mean adjusted GFR (Nankivell) in EVE group was higher by +5.6 mL/min/1.73 m2 (95%CI:[-0.6;+11.8];$P = 0.08$) compared to CNI group (ITTpts); mean GFR change from RDZ to Mo48 was +5.7(95%CI:[-0.1;+11.5]) for EVE and +0.1(95%CI:[-5.1;+5.3]) mL/min/ 1.73 m2 for CNI pts ($P = 0.08$). Mean eGFR of patients who remained on the assigned EVE treatment (on-therapy pop.) was higher by +8.7 ml/min (95% CI:[+16.0;+1.4] $n = 20$; $P = 0.02$) compared to CNI pts ($n = 31$) at Mo48. Mean trough levels: 84 ± 44 ng/ml in CsA, 5.9 ± 2.4 ng/ml in Tacrolimus and 6.7 ± 2.0 ng/ml in EVE treated pts. 2 deaths and 1 graft loss were observed in CNI group, 1 death and 3 graft losses in EVE group; no BPAR in either group. Any infection after Mo12 was reported for 23(50%) pts in EVE vs 20(43%) pts in CsA group, of these 3(7%) severe in EVE group and 1 (2%) severe in CNI group; none lead to hospitalization. No malignancy occurred in EVE group, 1 (2%) in CNI group.
Conclusion: Late conversion to an EVE/EC-MPS treatment in maintenance KTx patients after CNI withdrawal is safe and associated with a tendency towards better renal function maintained for 4 years.

V80 MTOR INHIBITION AND EVOLUTION OF URINARY PROTEIN EXCRETION IN NON-RENAL TRANSPLANT PATIENTS

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Background: The interplay of glomerular filtration and tubular absorption of proteins with various molecular weights defines pattern/magnitude of daily urinary protein excretion(UPE). Increased UPE serves as surrogate marker for renal injury and progressive nephron damage. mTOR-inhibitor treatment has also been associated with increased UPE not only in KTx but also non-renal Tx recipients.
Method: Data were retrieved from study H2304 (NCT00622869), a 24-month (M), randomized, multicenter study in 719 de novo LTx recipients comparing everolimus (EVR, C0 3–8 ng/mL) plus reduced tacrolimus (rTAC, C0 3–5 ng/mL) to standard TAC (sTAC-C, C0 6–10 ng/mL). The total daily UPE, measured as urinary protein-to-creatinine ratio, as well as a set of differently sized urinary proteins is described in order to allow a more detailed investigation of the origin and course of UPE in de novo LTx patients receiving EVR.
Results: UPE was higher with EVR+rTAC compared to sTAC with highest values at M6 (290 mg/day) followed by decreasing values at M12 and a further decrease to 194 mg/day at M24. Daily UPE maintained stable in TAC Control at 158 mg/day. UPE >500 mg/day occurred in 18.1% of patients in TAC-C vs. 23.6% in EVR+rTAC (18.9% when EVR C0 was in the range of 3–8 ng/mL). Analysis of urinary protein electrophoresis determining the distribution pattern of alpha1 MG(26 kDa), albumin(70 kDa), transferrin(80 kDa), and IgG (150 kDa) are shown in Fig1 demonstrating similar patterns for EVR and TAC.
Discussion: Clinical observations suggest that mTOR inhibition might be associated with increased UPE, potentially due to enhanced cell wall permeability and podocyte dysregulation. However, in case of mTORi facilitated CNI reduction the improvement in glomerular blood flow and consequently higher overall protein filtration in combination with mTOR-dependent reduction in tubular protein reabsorption may also contribute to increased UPE.

V81 EFFECT ON AN EVEROLIMUS- *VS.* MMF-BASED STEROID-FREE IMMUNOSUPPRESSIVE REGIMEN ON LONGITUDINAL GROWTH IN PAEDIATRIC RENAL TRANSPLANT RECIPIENTS

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Concerns have been raised that mTOR inhibitors might interfere with longitudinal bone growth by inhibition of growth plate chondrocyte proliferation and signalling of growth factors such as IGF-I and VEGF. Two published clinical studies with conflicting results are difficult to interpret because of concomitant administration of glucocorticoids. We therefore undertook a case-control study on longitudinal growth over 2 years in steroid-free paediatric patients after RTx by comparison of an EVR- *vs.* an mycophenolate mofetil (MMF)-treated patient cohort.

Data of 14 patients on an EVR/low-dose CsA-based regimen (age 6.9 ± 5.1 years; 8 prepubertal) were compared to 14 patients on an MMF-based regimen in conjunction with TAC ($n = 10$) or CsA ($n = 4$) (age 7.8 ± 4.2 years; 8 prepubertal). Matching criteria were: (i) age at RTx, (ii) age at study entry, and (iii) eGFR at study entry. Data documentation and analysis were performed within the platform of the CERTAIN Registry (Cooperative European Pediatric Renal Transplant Initiative;www.certain-registry.eu).

Mean height SDS at baseline was comparable in both groups (EVR group, -0.82 ± 1.01 SDS; MMF group, -1.14 ± 0.89 SDS; $P = 0.38$). One year after steroid withdrawal, mean height SDS in the EVR group increased to −0.50 ± 0.96 SDS compared to -0.90 ± 1.23 SDS in the MMF group ($P = 0.34$). At 2 years the respective height data were -0.40 ± 0.93 SDS in the EVR group vs. -0.50 ± 1.21 SDS in the MMF group ($P = 0.77$). The percentage of prepubertal patients experiencing catch-up growth, defined as an increase of height SDS ≥0.5 in 2 years, were similar in the EVR (5/8, 62%) and the MMF group (6/8, 75%; $P = 1.00$).

These data demonstrate that longitudinal growth 2 years after steroid withdrawal in an EVR treated cohort is comparable to that of a matched cohort treated with MMF. Hence, low-dose EVR does not appear to negatively impact growth in children after RTx.

V82 PHARMACODYNAMIC MONITORING OF MTOR INHIBITION BY PHOSPHOFLOW CYTOMETRIC DETERMINATION OF P70S6 KINASE ACTIVITY

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Background: Immunosuppressive therapy with mTOR inhibitors requires the maintanance of an effective inhibition of the alloimmune response while reducing drug-related nephrotoxicity. Therapeutic monitoring is based on mTOR inhibitor trough levels, which do not necessarily reflect biological effects on the PI3K/Akt/mTOR pathway and hence may often result in under- or over-immunosuppression.
Methods: Here, phosphorylation of p70S6 kinase was studied by phospho-flow cytometry and by western-blot in both PBMCs and CD3 + T cells of 84 renal transplant recipients (RTX) and 16 healthy volunteers. To further clarify whether p70S6K-activity is varying among CD4 + T cell subsets, cell sorted CD4 + $CD25^{hi}$ Tregs and CD4 + CD25- T cells were analysed for p70S6K phosphorylation.
Results: Simultanous analysis of p70S6K phosphorylation by phospho-flow cytometry and western blot showed high correlation in PBMCs of renal transplant patients ($r = 0.91$, $P < 0.001$). MTOR inhibition was associated with marked reduction of p70S6K-phosphorylation ($n = 26$) compared to healthy volunteers ($n = 16$) or RTX patients receiving calcineurin inhibitors ($n = 58$; all $P < 0.001$). p70S6K-phosphorylation in CD3 + T cells did not correlate with mTOR inhibitor trough levels. Cell sorted CD4 + $CD25^{hi}$ Tregs were found to exhibit significant lower phosphorylation of p70S6K in contrast to CD4 + CD25- T cells ($n = 3$). Furthermore, in RTX recipients receiving mTOR inhibition, p70S6K phosphorylation was selectively reduced in CD4 + CD25- T cells leaving CD4 + $CD25^{hi}$ Tregs unimpaired.
Conclusion: Phospho-flow cytometric quantification of p70S6K-phosphorylation may play an adjunct role to pharmacodynamically guide an individualized mTOR inhibitor based immunosuppression. It may have in addition potential to be employed in purity testing of Treg suspensions generated for adoptive tolerogenic therapies.

IMMUNOLOGY AND HLA ANTIBODIES

V83 COMPARISON OF THE SINGLE ANTIGEN BEAD LUMINEX ASSAY AND ELISA IN PATIENTS ON THE KIDNEY WAITING LIST AND WITH A KIDNEY TRANSPLANTATION

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The single antigen bead (SAB) assay (luminex) is highly sensitive for detection of HLA antibodies (Ab). To estimate the cut off value (normalized fluorescence intensity [nMFI]) for definition of clinically relevant Ab we compared the ELISA and SAB results from 145 patients (pat.) on the kidney waiting list and 51 pat. with a renal transplant. Using the ELISA, the 145 pat. on the waiting list included 30 pat. (20.7%) with HLA class I Ab, 23 pat. with HLA class II Ab (15.9%) and 45 pat. with HLA class I and/or HLA class II Ab (31%). In the SAB, using >1000/ >2000/ >3000 nMFI cut off values for definition of HLA Ab, 60/46/38 pat. (41.4%/31.7%/26.2%) revealed HLA class I-, 62/43/34 pat. (42.8%/29.7%/ 23.5%) HLA class II-, and 68/57/48 pat. (46.9%/39.3%/33.1%) revealed both HLA class I and/or II Ab. Moreover, in the SAB the percentage of immunized pat. with PRA values > 50% is strongly increased. Considering both HLA class I and II with a nMFI > 3000 the percentage of highly immunized pat. (PRA > 85%) is increased to 9.7% compared to 4.8% in the ELISA. Since immunisation with both HLA class I and II Ab, detected by ELISA is a risk factor for long term graft survival we next investigated SAB in a cohort of 51 transplanted pat., 15 pat. with and 36 patient without graft loss. When DSA were defined with a cut-off value of >2000 nMFI or >3000 nMFI, ELISA Ab positive pat. revealed an increased frequency of SAB-detected DSA. Pat. with transpant rejection revealed HLA class-I and/or II DSA with nMFI > 3000. In conclusion, using SAB cut off values > 3000 and considering HLA class II Ab relevant for transplantation will significantly increase the number of immunized and highly immunized pat. which may increase the time for the pat. on the waiting list. But the use of SAB with a cut off value of >3000 can improve the detection of DSA prior to transplantation and thus may decrease HLA Ab associated graft loss.

V84 CLINICAL OUTCOME IN MAJOR HISTOCOMPATIBILITY COMPLEX ANTIBODY POSITIVE HEART TRANSPLANT RECIPIENTS RECEIVING THYMOGLOBIN FOR INDUCTION THERAPY

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Background: Major Histocompatibility Complex (MHC) antibody positive heart transplant (HTx) recipients have a high risk for perioperative complications, including cellular and humoral rejections.
Methods: We assessed clinical outcomes in 39 MHC-antibody positive heart transplant recipients who received thymoglobin for induction therapy (group A). Results were compared with 39 MHC antibody negative HTx patients who received standard immunosuppressive therapy (group B). Patients were followed for up to one year.
Results: Age and diagnosis were comparable between the two groups. However, compared to group B the percentage of female patients was significantly higher in group A (41.0% vs. 15.4%; $P = 0.022$). Sex- and BMI-adjusted Cox regression analysis revealed no relative risk for 1-year mortality in group A [1.76 5%CI:0.59–5.28)] compared to group B. Freedom from biopsy-proven rejection was similar in group A and B (94.0% and 94.4%, respectively, $P = 0.925$). Freedom from CMV infection was 89.7% (group A) and 97.4% (group B; $P = 0.253$). During follow-up, 34 other infections occurred in group A and 35 in group B ($P = 0.189$). In the surviving patients, white blood counts were comparable between groups, whereas C-reactive protein levels were significantly higher in group A compared to group B ($P = 0.002$).
Conclusions: Our data suggest similar sex- and BMI-adjusted 1-year mortality in MHC positive HTx recipients receiving thymoglobuline in comparison with MHC negative patients receiving standard immunosuppression.

V85 ANTI-HLA ANTIBODY-DEPENDENT INDUCTION OF VASCULAR CELL ADHESION MOLECULE-1 EXPRESSION IS MODULATED BY HEME OXYGENASE-1 IN HUMAN ENDOTHELIAL CELLS

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Chronic antibody-mediated rejection (ABMR) is the key limiting factor for long-term graft survival after kidney and heart transplantation. The endothelium of allografts plays a major role in the pathogenesis of ABMR, because it is directly targeted by antibodies (abs) against anti-human leukocyte antigens (HLA). To examine the effects of anti-HLA abs on endothelial cells (ECs), cell cultures of human umbilical vein ECs (HUVECs), human aortic ECs and human dermal microvascular ECs were treated with the monoclonal anti-HLA class I ab w6/32. Binding of w6/32 to ECs markedly up-regulated gene expression of the pro-inflammatory adhesion molecule vascular cell adhesion molecule (VCAM)-1 and that of intercellular adhesion molecule (ICAM)-1, interleukin-8 (IL-8) and MCP-1. This up-regulation was mediated via the phosphatidylinositol-3-kinase (PI3K)/Akt signaling cascade as indicated by pharmacological inhibitor studies. To investigate the potential role of the anti-inflammatory endothelial enzyme heme oxygenase (HO)-1 in this pathway, HO-1 was modulated by pharmacological compounds and by a small interfering (si)RNA knockdown approach. Blocking of HO-1 activity by zinc-protoporphyrin (PPIX) and siRNA-mediated HO-1 knockdown enhanced VCAM-1 gene expression, whereas up-regulation of HO-1 with the HO-1 inducer cobalt-PPIX markedly inhibited w6/32-mediated VCAM-1 induction. Accordingly, w6/32 increased adhesion of THP-1 monocytes to ECs in an *in vitro* adhesion assay, which was counteracted by pharmacological up-regulation of HO–1. These findings suggest that the anti-HLA class I ab-dependent induction of pro-inflammatory adhesion molecules in ECs is down-regulated by HO-1, which may be a therapeutic target in ABMR.

V86 HIGHLY PURE HUMAN ANTIGEN-SPECIFIC TREGS WITH SUPERIOR FUNCTION IN PREVENTING ALLOGRAFT REJECTION

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Adoptive transfer of CD4 + FOXP3 + regulatory T cells (Tregs) might be an alternative option to achieve tissue specific tolerance without perturbance of general immunocompetence. Polyspecific Tregs can control graft versus host diseases under lymphopenic conditions. However, under non-lymphopenic conditions as in patients after organ transplantation polyspecific Tregs were so far largely ineffective in controlling immune responses. Herein, we describe that the surface molecules latency associated peptide (LAP) and glycoprotein A repetitions predominant (GARP) can specifically identify Tregs activated by their T cell receptor and not in bystander fashion. Using these markers we could show for the first time that the human natural Treg repertoire contains about 10% of alloreactive Tregs. In addition we show that CD154 is neither expressed on resting nor on activated Tregs and can therefore be used to increase the purity of isolated Tregs. The combination of CD154-LAP+ or CD154-GARP+ markers allowed the isolation of highly pure antigen-specific Tregs (purity>83%). The purity, assessed by TSDR methylation analysis, exceeds all other published Treg isolations and identifies furthermore just antigen-specific Tregs. Furthermore, we demonstrated that those LAP+ allospecific Tregs are highly capable in the prevention of potent allospecific DTH responses in humanized mice and in the prevention of rejection of allogeneic cells in immune reconstituted humanized mice.

V87 AUTOIMMUNE TARGETING OF PROTEASE ACTIVATED RECEPTOR-1 (PAR-1) DEREGULATES VEGF AND DISTURBS NEOANGIOGENESIS

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Background: Early loss of peritubular capillaries (PTC) is central to progressive nephron loss in native kidneys and transplants. VEGF is crucial for endothel mediated PTC homeostasis. Thrombin, a serin protease which elicits its cellular effects via GPCR PAR-1 is closely involved in VEGF regulation. Functional anti-GPCRs autoantibodies are able to induce endothelial dysfunction. We hypothesized that autoimmune GPCR targeting process may disturb VEGF induced angiogenesis and identified PAR-1 as a novel activating autoantibody target.
Methods: Human microvascular endothelial cells (HMEC-1) were stimulated with IgG isolated from patients' sera with transplant peritubular pathology (KTx-IgG). VEGF transcriptional regulation was studied by promoter deletion assay, transcription factor activation by qRT-PCR, western blot, EMSA and knockdown, VEGF secretion by ELISA. Tube formation on matrigel served to study endothelial neoangiogenic response.
Results: Treatment with KTx-IgG reduced ERK1/2 dependent VEGF secretion and tube formation. VEGF secretion and endothelial tube formation could be only normalized by pretreatment with specific PAR-1 inhibitor and PAR-1 2^{nd} extracellular loop peptide. KTx-IgG increased cFos protein expression and its binding to VEGF-promoter contributing to deregulate neoangiogenesis by reducing VEGF-promoter activity. AP-1 inhibition or cFos siRNA-induced knockdown reconstituted VEGF levels and increased its promoter activity.
Conclusions: We identified the PAR-1 receptor as a new target for functional antibodies in the context of kidney transplantation with disturbed PTC homeostasis. We showed that KTx-IgG disturbs VEGF transcriptional regulation resulting in reduced VEGF secretion and inability of endothelial cells to form tubes. PAR-1 mediated VEGF regulation could offer new possibilities for treatment of kidney transplants to obviate loss of PTCs.

V89 SILENCING HLA CLASS I EXPRESSION IN HUMAN CORNEAS TO DECREASE THE RISK OF GRAFT REJECTION AFTER KERATOPLASTY

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Purpose: The variability of the HLA complex contributes to elicit an immune response after allogeneic keratoplasty. This study aims to stably reduce HLA expression in cornea transplants to decrease the risk of rejection. This, represents a new therapeutic concept and may contribute to prevent graft rejection.
Methods: Lentiviral vectors encoding short hairpin RNAs (shRNA) targeting β2-microglobulin were used to induce the downregulation of HLA class I expression. Levels of β2-microglobulin mRNA and cell surface HLA class I expression were determined by Real-Time PCR and flow cytometry, respectively. Cell viability tests using 7-AAD and PI staining were performed after transduction. Microscopy analyses were carried out to evaluate the integrity of the corneal endothelium.
Results: A mean of 92%±7% of the total cornea cells were transduced. The delivery of the β2-microglobulin-specific shRNA caused a reduction by up to 95% in β2-microglobulin transcript levels as detected at day 15 after cornea transduction. The reduction of β2-microglobulin levels caused a decrease in HLA class I protein expression by up to 90%. Microscopic analysis of the cornea endothelium showed that this cell layer was not affected by the transduction procedure. Integrity of the HLA class I silenced endothelium and the absence of a positive signal upon 7-AAD/PI staining indicated the absence of off target effects that might impair the endothelial function.
Conclusion: This data demonstrates the feasibility of silencing HLA class I expression in the original 3D tissue-structure by RNAi-mediated nucleic acid targeting. Silencing HLA expression has the potential to overcome alloimmunization and tissue rejection caused by HLA mismatches keratoplasty.

BASIC SCIENCE I

V90 INTRAGRAFT CD11C$^+$ DENDRITIC CELLS TRIGGER A MORE POTENT IL-17 MEDIATED IMMUNE RESPONSE WHEN TRANSPLANTATING ORGANS FROM OLDER DONORS

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Organs from older donors are increasingly utilized for transplantation. Clinically, organ age has been linked to more frequent acute rejections. We dissected the impact of donor age on the recipient's immune response.

Hearts from young or old C57BL/6 (B6) mice were transplanted into young DBA/2 recipients. Old hearts were rejected significantly faster than young hearts (MST: 9 vs. 11, $P = 0.002$). Significantly higher ISHLT-rejection scores ($P < 0.05$) were observed in old allografts. Of note, IL-17 cytokine mRNA levels were dramatically increased in old allografts ($P = 0.0357$). Moreover, recipients of old grafts displayed increased frequencies of alloreactive IFN-γ producing splenocytes, higher percentages of CD8$^+$effector and CD8$^+$ IFN-γ^+ T cells ($P < 0.05$ for all experiments).

After confirming that organ age is critically influencing recipient's immune responses we dissected the role of old passenger-leukocytes. Chimeric animals were generated by transplanting bone marrow from young B6 mice into lethally irradiated B6 mice. Transplantation of chimeric hearts resulted in comparable survival rates (MST: 10 vs. 10), rejection scores and recipient systemic immune responses ($P > 0.05$ for al experiments).

Next, we depleted cardiac DC's using liposomal-clodronate. Depletion of CD11c$^+$ cells resulted in comparable survival rates and rejection scores. Moreover, systemic immune responses were not different after the engraftment of these grafts. Furthermore, IL-17-mRNA levels were significantly reduced in these old allografts. Systemic treatment with anti IL-17 resulted in a significantly prolonged survival of old allografts.

These results show that donor age enhances CD11c$^+$ cell immunogenicity through a potent Th-17 specific response. Blocking IL-17 in recipients of old organs abolishes donor age-related compromised survival.

V91 DENDRITIC CELLS AS BIOMARKERS FOR ACUTE CELLULAR REJECTION AFTER HEART TRANSPLANTATION

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Objective: Over the last decade manystudies evaluated the potential of certain biomarkers to predict acute cellular rejection (ACR) without finding their way in the clinical routine. Thus, in our study we monitored dendritic cells (DCs) inheart-transplantrecipients (HTxR) treated either with a tacrolimus (TAC) or cyclosporine A (CsA) -based immunosuppressive regimen in the context of ACR.
Methods: Both groups consisted of 14 maintenance HTxR. At different study time points (0, 3 and 6 months after study start) peripheral bloodfromall HTxRwas drawntoanalysefor (1)blood CsA or TACconcentration(trough value) and (2) to analyse myeloid and plasmocytoid (m and p) DCs with FACS. Histological rejection grading was performed of endomyocardial biopsies.
Results: TAC treated HTxR had significantly higher values of pDCs (CsA-group 53.9 $\pm$ 13.0%, TAC-group 67.5 $\pm$ 8.4%, $P < 0.05$) and significantly lower values of mDCs than CsA treated HTxR (CsA-group 57.9 $\pm$ 19.0%, TAC-group 45.2 $\pm$ 10.7%, $P < 0.05$). In general, HTxR with rejection grade of$\geq$2 ISHLT 1990 had significant ($P < 0.05$) lower values of pDCs (55.1 $\pm$ 16.2%) compared to HTxR without rejection (63.6 $\pm$ 10.5%). TAC treated HTxR had significantly less rejections compared to CsA treated HTxR (CsA-group 0.86 $\pm$ 0.95%, TAC-group 0.2 $\pm$ 0.4%, $P < 0.05$).
Conclusions: Our results showed that HTxR with high pDCs have a lower risk of rejection and that TAC treated HTxR had higher pDCs values compared to CsA treated HTxR. Future studies are needed to confirm if monitoring pDCs and mDCs have the potential value as biomarkers to identify HTxR at risk to develop ACR.

V92 PPARγ-DEPENDENT INHIBITION OF CYTOTOXIC T CELLS

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Background: Despite their essential role in adaptive immunity attacking tumor- or virus-infected cells, cytotoxic T cells (CTLs) are involved in mediating pathologic conditions such as liver failure during sepsis. Therefore inhibiting CTL activity is an important therapeutic aim.
Methods: Taking their desrtructive role into consideration we used an allogenic cytotoxicity assay with P815, a mouse mastocyoma cell line derived from the DBA/2 strain, as target and CTLs derived from the spleen of C57BL/6 mice as effector cells, to characterize the role of PPARγ in CTL cytotoxicity.
Results: Using this experimental approach we found that the most effective target vs. effector cell ratio was 1:10. Using this proportion 50% of the target cells were killed after 24 h. Interestingly adding the selective PPARγ agonist to the reaction reduced CTL-dependent cytotoxicity to 15%. Analyzing established factors causing CTL cytotoxicity we observed granzyme B, Fas and Fas-L downregulation at mRNA level. Experiments using CTLs derived from T-cell specific PPARγ-knockout mice proved that in these cells rosiglitazone treatment did not alter granzyme B, Fas and Fas-L expression. Accordingly, cytotoxicity remained unchanged at 50% even following rosiglitazone addition.
Conclusion: From these results we conclude that PPARγ activation in CTLs possibly can be considered a new therapeutic principle.

V95 DEVELOPING A BIOHYBRID LUNG - ENDOTHELIALIZATION WITH ALLOGENEIC MHC-SILENCED ENDOTHELIAL CELLS TO PREVENT TRANSPLANT REJECTION

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Purpose: Due to insufficient autologous cell harvesting, gas exchange membranes (PMP) are endothelialized with allogeneic human cord blood derived endothelial cells (EC) aiming towards development of a biohybrid lung. To prevent cell rejection caused by Major Histocompatibility Complex class I (MHC) incompatibility, expression of MHC in ECs was stably silenced. Capacity/functionality required for endothelialization of PMP was investigated.
Methods: MHC expression was silenced by RNA interference technology. Lentiviral vector system was used for delivery of shRNAs targeting β2-microglobulin (β2 m) transcripts. Non-specific shRNA sequences were used as negative control. Efficiency of MHC-silencing/endothelial phenotype were analyzed by qRT-PCR/flow cytometry. Cytokine secretion profiles were evaluated by Luminex technology. Endothelial specific activation and thrombogenic state markers (ESATS) were quantified by real-time RT-PCR. IFNγ-/TNFa-stimulation were used to examine biologic reactivity. Cell growth/seeding efficiency were assessed by microscopy.
Results: Transduction efficiency of 95% was achieved. Delivery of β2 m-specific shRNAs induced reduction of 90% of β2 m mRNA levels, causing knockdown of MHC surface expression of 92%. No significant changes were observed in cytokine secretion profiles. Upon IFN-γ stimulation, non-transduced ECs showed a 3-fold upregulation of MHC surface expression, whereas expressing β2 m-specific shRNA blocked upregulation. ESATS remained unaffected by MHC-silencing. Microscopy showed a confluent endothelial monolayer on PMP.
Conclusions: These results may bring the development of a bioartifical lung closer to reality.

V96 PHOSPHORYLCHOLINE-MODIFIED MACROMOLECULES INHIBIT THE ATP-DEPENDENT IL-1β RELEASE IN HUMAN MONOCYTES

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The secretion of bioactive interleukin-1β (IL-1β) requires two independent danger signals. Lipopolysaccharide (LPS) induces pro-IL-1β synthesis. Extracellular ATP binds to the P2X7 receptor, induces inflammasome activation and rapid secretion of IL-1β. We demonstrated effective suppression of ATP-induced IL-1β secretion by ligands of nicotinic receptors containing subunit α9 (nAChRα9). We postulate that phosphorylcholine (PC)-modified macromolecules, which are produced by numerous pathogens, are sensed by nAChRα9 and modulate ATP-induced inflammasome activation.

Human monocytic U937 cells were primed with LPS and stimulated with BzATP in the presence and absence of free PC, PC-modified bovine serum albumin (BSA), PC-modified LPS purified from *Haemophilus influenzae* wild type strains as well as with unmodified LPS from corresponding mutant strains defective in the biosynthesis of PC-LPS. IL-1β release was measured by ELISA.

PC, PC-modified BSA and PC-modified LPS inhibited BzATP-induced IL-1β release. Inhibition was abolished by nAChR antagonists specific for the subunit α9. In contrast to their PC-modified counterparts, unmodified BSA and LPS from mutant bacteria were not effective.

We propose a novel mechanism of immune evasion involving suppression of ATP-induced IL-1β release via nAChR containing subunit α9 by a broad range of PC-modified macromolecules produced by eukaryotic parasites and bacteria.

BASIC SCIENCE II

V97 THE EFFECT OF PRESERVATION SOLUTIONS HTK, HTK-N AND TIPROTEC ON VARIOUS TISSUES AFTER RAT HIND-LIMB ALLOTRANSPLANTATION

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Background: Ischemia/reperfusion (I/R) injury is an early factor damaging grafts in solid organ and composite tissue transplantation. We herein investigate the effect of the novel preservation solutions HTK-N and TiProtec on tissue preservation and damage in a vascularized composite allotransplantation (VCA) model.
Methods: Orthopic hind-limb transplantations were performed in male Lewis-rats following 10 h of CI. Limbs were flushed and stored in HTK-N, TiProtec, HTK or NaCl-solution. Skin, muscle, nerve, vessel and bone-samples were taken at the 10th post-operative day (POD) for histology, confocal and electron-microscopy.
Results: The confocal microscopy of the muscle viability showed an inferiority of TiProtec, whereas no significant differences were observed in the other groups. Histology showed a superiority ($P = 0.08$) of HTK in muscle preservation displaying a diffuse inflammatory infiltrate and only localized necrosis contrary to mainly major necrosis in the HTK-N, TiProtec and NaCl groups. In all other tissues no significant differences concerning tissue damage were observed. The majority of skin alterations included a mild inflammatory infiltrate in the dermis and rarely interface reactions, infiltration of the epidermis and sporadic epithelial necrosis. Nerve samples revealed mostly severe perineural inflammatory infiltrate, vacuolization and mucoid degeneration. Vessels showed intact endothelial cells and only a mild infiltrate. Electron microscopy revealed that vessel-preservation was equaly good in all groups.
Conclusion: Nerve and muscle are most susceptible to I/R injury in a VCA model. Skin and vessels on the other hand are relatively unaffected by I/R. HTK has the best preservation ability for muscle tissue, which is a major component of a VCA and crucial to gain function after limb transplantation.

V98 EFFICACY OF HEPATOCYTE TRANSPLANTATION IS INCREASED BY PHARMACOLOGICAL PRECONDITIONING OF THE HOST LIVER

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Background/Aim: Hepatocyte transplantation (HT) is only partially effective in correcting metabolic liver diseases. We investigated the hypothesis that pharmacological modification of barriers such as hepatic sinusoidal endothelium, low sinusoidal blood flow, instant blood-mediated immune response and cyclooxygenase-mediated inflammation should enhance donor cell engraftment in mice/rats, and improve the metabolic effect of HT in the UDP-UGT-1 deficient Gunn rat model of Crigler-Najjar-syndrome type 1 (CN1).
Methods: First series: C57/Bl6 mice received dextransulfate, nitroglycerin, naproxen, cyclophosphamide or hepatic X-irradiation prior to HT with donor cells from (Rosa)26 C57BL/6 mice. Cell engraftment was morphometrically measured three days post transplantation. Second series: Gunn rats received cyclophosphamide, naproxen or cyclophosphamide/naproxen prior to HT with donor cells from congeneic normal Wistar RHA rats. Serial serum bilirubin levels were followed for six months.
Results: Mice: Cyclophosphamide, HIR, and naproxen enhanced hepatocyte engraftment by 97%, 92%, 52% compared to control ($P < 0.001$). No significant effect was seen with dextransulfate/nitroglycerin. Rats: Four months post transplantation, mean reduction of serum bilirubin levels compared with sham-controls were: no drug: 32.0%, cyclophosphamide: 54%, naproxen:44%, cyclophosphamide + naproxen 68%.
Conclusion: A single preparative dose of HIR, cyclophosphamide or naproxen significantly increased hepatocyte engraftment in mice. Serum bilirubin level reduction is increased by a single injecton of cyclophosphamide or Naproxen, and doubled by combination of both drugs. These findings may be relevant in HT-based therapies of CN1 and other inherited metabolic liver disorders.

V99 ENGINEERED MSCS: INNOVATIVE HCC THERAPY AND POTENTIAL BRIDGING PROCEDURE BEFORE LIVER TRANSPLANTATION?

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Introduction: Mesenchymal stem cells (MSCs) have the capability to actively recruit to the stroma of growing epithelial tumours and transdifferentiate into tumor-associated cells. The aim of this study was the establishment of an MSC based suicide gene therapy against HCC and potential bridging procedure before transplantation.
Methods: MSCs isolated from bone marrow of C57/Bl6 p53-/- mice were stably transfected with Red Fluorescent Protein (RFP) or Herpes simplex virus thymidine kinase (HSV-Tk, "suicide gene") gene driven by the CCL5-promoter, respectively. MSCs were injected intravenously once per week into mice with orthotopically growing xenografts of hepatocellular carcinoma.
Results: MSC injections lead to increased proliferation rate and microvessel density. MSCs demonstrated homing to the hepatic xenografts and activation of the CCL5 promoter as demonstrated by RFP signals within the tumor. CCL5 + signals only became evident in tumors after intravenous injection of MSCs and were situated in close proximity to RFP+ MSCs. CCR1 and CCR5, both receptors for CCL5, were detectable in vicinity of CCL5-expressing MSCs. Application of HSV-TK transfected MSCs in combination with GCV significantly reduced tumor growth by 41% as compared to the control group and by 64% as compared to non-therapeutic MSC injections.
Conclusions: We are able to demonstrate active recruitment of systemically applied MSCs to growing HCCs. Stem cell mediated introduction of suicide genes into the tumor followed by prodrug administration proved as an effective treatment option for HCC and could potentially serve as individualized bridging procedure for patients awaiting organ transplantation in the future.

V100 DONOR BRAIN DEATH RESULTS IN DIFFERENTIALLY MODULATED IMMUNE ACTIVATION IN SOLID ORGANS

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Donor brain death (BD) and its pathophysiological changes have been shown to influence graft quality, therefore accelerating the immune response post transplantation. However, detailed information regarding immune activation of distinct lymphocyte subsets in the periphery and in BD donor organs is still missing in order to explain enhanced immunogenicity. For this purpose, C57BL/6 mice underwent BD induction and were followed for 3 hrs under continuous ventilation, whereas ventilated mice were used as sham group (SH) ($n = 5$). By cell isolation and flow cytometry, we observed a change for CD3 + T cell frequencies as well as an induction of activated CD25 + CD3 + CD4 + T cells in BD donor derived hearts compared with SH ($P < 0.05$). Moreover central memory T cells were significantly upregulated in liver compared to kidney ($P < 0.001$). Moreover, we detected enhanced levels of CD3-NKp46 + NK cells in BD donor kidneys, but not in liver ($P < 0.05$) which appeared significantly activated reflected by their NKG2D expression. In addition, NCR as well as NKG2D were upregulated on graft infiltrating NK cells on a single cell level. In summary, our results gain novel insights into the pathophysiology of BD induced immune activation revealing significant differences between various organs and the periphery. This indicates distinct mechanisms of activation which needs consideration for future treatment strategies.

V101 INFLUENCE OF NORMOTHERMIC, MILD- AND SEVERE HYPOTHERMIC CONDITIONS ON TRANSENDOTHELIAL MIGRATION: WHAT ABOUT THE REWARMING DURING REPERFUSION?

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Objective: Organs explanted for transplantation are subjected to cold ischemia. Peripheral blood mononuclear cells (PBMC) under hypothermic conditions evoke anti-inflammatory, whereas subunits of PBMCs show pro-inflammatory effects. The role of hypothermia on transendothelial-migration of leukocytes is not clarified yet. We investigated transendothelial-migration of PBMCs through human microvascular endothelial cells (HMEC-1) at different transmigration and cell-activation temperatures including the reperfusion-rewarming-process.
Methods: Following experimental set-ups were analyzed according to trans-endothelial-migration: 1)activated-PBMC; 2)activated-HMEC-1; 3)activated-HMEC-1/activated-PBMCs. Cells were activated at either 37°C, 30°C, 18°C or

4°C. For transendothelial-migration, the cells were either rewarmed to 37°C or transmigration was directly performed at 30°C, 18°C or 4°C.
Results: Rewarming from low (18°C, 4°C) to normothermic (37°C) conditions caused no change in transendothelial-migration of activated-PBMCs through non-activated-HMEC-1. But, significantly more non-activated-PBMCs transmigrated through activated-HMEC-1 when activated at 18°C and 4°C and rewarmed to 37°C. The same significant effect is observed when both cell types were activated.
Conclusion: Our results show a significant influence of temperature on transendothelial-migration. Our results show that transendothelial-migration of PBMCs through endothelial cells is not only modulated by cell-activation itself but that the activation temperature and the rewarming processes are essential. Endothelial protection prior to warm reperfusion should be considered.

V102 TWO SEPARATE PATHWAYS OF REGULATED NECROSIS CONTRIBUTE TO ISCHEMIA-REPERFUSION INJURY

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Regulated necrosis (RN) may result from cyclophilin D (CypD)-mediated mitochondrial permeability transition (MPT) and receptor-interacting protein kinase 1 (RIPK1)-mediated necroptosis, but it is currently unclear whether there is one common pathway in which CypD and RIPK1 act in or if separate RN pathways exist. Here, we demonstrate that necroptosis in ischemia-reperfusion injury (IRI) in mice occurs as primary organ damage, independent of the immune system, and that mice deficient for RIPK3, the essential downstream partner of RIPK1 in necroptosis, are protected from IRI. Protection of RIPK3-ko mice was significantly stronger than of CypD-deficient mice. Mechanistically, *in vivo* analysis of cisplatin-induced acute kidney injury and hyperacute TNF-shock models in mice suggested the distinctness of CypD-mediated MPT from RIPK1/RIPK3-mediated necroptosis. We therefore generated novel CypD-RIPK3 double-deficient mice that are viable and fertile without an overt phenotype and that survived prolonged IRI which was lethal to each single knockout. Combined application of the RIPK1 inhibitor necrostatin-1 (Nec-1) and the MPT inhibitor sanglifehrin A (SfA) confirmed the results with mutant mice. The data demonstrate the pathophysiological co-existence and co-relevance of two separate pathways of RN in IRI and suggest that combination therapy targeting distinct RN pathways can be beneficial in the treatment of ischemic injury.

V103 EX-VIVO INHIBITION OF RHO-KINASE DURING RENAL PRESERVATION IMPROVES GRAFT FUNCTION UPON REPERFUSION

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Background: Activation of the Rho-Rho-kinase pathway has been proven to cause vasoconstriction in renal afferent arterioles. Vascular dysfunction plays a pivotal role in triggering reperfusion injury after kidney transplantation. Therefore, the effect of a Rho-kinase inhibitor, added to the preservation solution, on renal function after cold storage was evaluated.
Methods: Porcine kidneys were preserved with cold HTK-solution for 18 h at 4°C. In the study group, HTK was supplemented with Fasudil (HA1077), a Rho-kinase inhibitor, whereas the control group received no further treatment ($n = 6$, resp.). Postischemic kidney function was evaluated by 90 min of isolated reperfusion in vitro.
Results: Rho-Kinase inhibition (RKI) was associated with significantly higher renal perfusate flow compared to the control group. In our model, RKI also significantly improved glomerular function in terms of renal clearances of creatinine as well as tubular cell integrity as reflected by reduced fractional sodium excretion and release of fatty acid binding protein, a specific tubular cell marker. Endothelial function, as measured by perfusate levels of nitric oxide and gene expression eNOS, was significantly increased in the study group.
Conclusion: Our results indicate that blocking the Rho-kinase pathway during cold preservation may lead to a better graft function upon reperfusion.