POSTER PRESENTATIONS

BASIC SCIENCE

P001 VALIDATION OF A NEW LUNG TRANSPLANTATION TECHNIQUE USING DIFFERENT ORGAN DONOR MODELS

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Objective: Lung transplantation (LTX) is a valuable therapeutic option for selected patients with end-stage pulmonary disease. However, the success of this treatment modality is limited by the reduced number of qualitative donor organs and complicated by ischemia–reperfusion injury (IRI) and graft dysfunction following transplantation. We developed a left single porcine lung transplant model to study graft IRI in lungs procured from a variety of different donors ranging from brain-dead heart beating to donation after cardiac death models.

Methods: Twenty four donor pigs (38–48 kg) were used for three different LTX-groups: Group 1, normal donor, normal recipient ($n = 8$) and Group 2 Donor brain death through the rapid inflation of a transcranially inserted balloon, and transplantation into a normal recipient ($n = 8$). Group 3: Non-heart beating donors (NHBD) with preceding brain death followed by circulatory arrest: Maastricht classification IV, normal recipient ($n = 8$). Donor lungs were procured following hypothermic low potassium dextran solution flush and hypothermically preserved for 3 hours. The size and weight matched recipients were pre-treated by standard immunosuppression protocols. Following a left lateral thoracotomy and removal of the left lung, a single left LTX was performed anastomosing the bronchus end-to-end, the donor left pulmonary vein to the recipient left atrial appendage, and the left pulmonary artery (PA) end-to-side to the main PA of the recipient. The chest was closed, the animal repositioned for sternotomy allowing clamping of the right lung hilum in order to perform single left LTX isolated ventilation and functional assessment. Data were required at baseline and 1 and 2 hours after LTX.

Results: The ease of the technical aspects of the surgical procedure led to a very stable large animal LTX setting associated with a very low experimental failure rate. There were no marked differences in the pre-post-LTX dry/wet-weight ratio, alveolar thickness, mean airway pressure, and compliance and between the three groups. However, significant differences were observed in oxygenation and alveolar-arterial differences in the NHBD group following LTX.

Conclusion: The modification of the pulmonary artery and vein anastomosis led to a stable standardized large animal single lung transplantation model. This allows to study objectively transplanted lung graft function from different donors with various donor lung quality. Lungs procured from NHBD with preceding brain death (Maastricht classification IV) performed poorly post-lung transplantation and are questionable suitable for donor graft pool expansion.

P002 DECOMPRESSION OF INFLAMMATORY EDEMA TOGETHER WITH ENDOTHELIAL PROGENITOR CELL TREATMENT EXPEDITES REGENERATION FROM ACUTE TUBULAR NECROSIS FOLLOWING RENAL ISCHEMIA–REPERFUSION INJURY

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Purpose: We have recently shown that increased pressure secondary to inflammatory edema may aggravate ischemia–reperfusion (I/R) injury and that prophylactic surgical decompression improves kidney dysfunction (Herrler et al., Transplantation 2010). Here we provide mechanistic insight how surgical decompression and endothelial progenitor cell (EPC) treatment may work together in the recovery from acute tubular necrosis (ATN).

Methods: A murine model of 45 minute warm renal ischemia was applied. Decompression of the kidney was achieved by microcapsulotomy. EPC derived from umbilical cord blood were intravenously injected 24 hours post-ischemia (10^6 cells/mouse). Renal function was assessed by ^{99m}Tc-MAG3 scintigraphy and laser Doppler perfusion 18 days after I/R. Histological analysis included H&E stains and immunohistology for panendothelial marker MECA-32 and cell proliferation marker Ki-67.

Results: Combined microcapsulotomy and EPC treatment exhibited synergistic effects resulting in significantly improved tubular and vascular function as compared to controls. H&E stains revealed reduced tubular necrosis and postischemic inflammation. Monotherapy using microcapsulotomy and EPC, respectively, was less pronounced in terms of functional efficacy. Histologically, EPC-treated ischemic kidneys showed severe inflammatory infiltration, while microcapsulotomy was associated with reduced structural damage. MECA-32 labeling detected distinct peritubular capillaries in cell-treated groups. Ki-67 stains showed a higher proliferation rate after ATN in animals treated with microcapsulotomy and/or EPC by contrast with non-treated ischemic kidneys.

Conclusion: Our data suggest that microcapsulotomy combined with a cell-based EPC treatment reconstitutes a regenerative environment enabling rapid proliferation of tubular cells and the repair of microcirculation by increased angiogenesis of peritubular capillaries.

P003 INTERLEUKIN-33 REDUCES ANTIBODY-MEDIATED REJECTION DURING CHRONIC CARDIAC REJECTION

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Background: Interleukin-33 (IL-33) is a member of the IL-1 family and stimulates the generation of cells, cytokines and immunoglobulins characteristic of a type 2 immune response. In this study, we demonstrate the effect of IL-33 on allograft function and antibody-mediated rejection during chronic cardiac rejection in mice.

Material and methods: B6.C-H2bm12/KhEg hearts were transplanted into wild-type MHC class II-mismatched C57BL/6J mice. IL-33 was administered i.p. daily. Cardiac allografts were harvested and immunohistochemical staining of cardiac tissue was performed. Further, splenic cells were isolated and examined by FACS. Alloantibody levels were determined by FACS-analysing donor splenocytes incubated with recipient serum for IgM and IgG loaded $CD3^+$ cells.

Results: Allogeneic transplanted controls showed progressive allograft rejection within 21.5 days after transplantation, whereas allograft survival in IL-33-treated animals was extended to more than 50 days. Prolonged allograft survival was accompanied by significant homeostatic changes of the lymphoid and myeloid compartment in both the cardiac allografts and the periphery. FACS analyses demonstrated a reduction of $CD19^+b220^+$ B cells following IL-33 therapy. Accordingly, IL-33-treated mice showed reduced IgM and IgG alloantibody levels in the serum. Further, as determined by immunohistochemistry, a diminished deposition of immunoglobulins in the allografts was observed after IL-33 therapy.

Conclusion: IL-33 treatment prolongs allograft survival after cardiac transplantation in mice. IL-33 therapy alters the composition of the lymphoid and myeloid compartment. Further, IL-33 administration reduces the antibody-mediated rejection against the allograft. Thus, IL-33 and its downstream effects need further evaluation as a possible therapeutic option for chronic allograft rejection.

P004 CD160IG FUSION PROTEIN TARGETS A NOVEL COSTIMULATORY PATHWAY AND PROLONGS ALLOGRAFT SURVIVAL

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CD160 is a cell surface molecule expressed by most murine NK cells and approximately 50% of CD8+ cytotoxic T lymphocytes. Engagement of CD160 by MHC class-I directly triggers cytokine production and cytotoxic function in NK cells and provides a costimulatory signal to TCR-induced proliferation, cytokine production and cytotoxic effector function in CD8+ T-cells. The role of CD160 in alloimmunity is unknown. Using a newly generated CD160 fusion protein (CD160Ig) we examined the role of the novel costimulatory molecule CD160 in mediating CD4+ or CD8+ T-cell driven CD28-dependent and independent allograft rejection. CD160Ig inhibits alloreactive CD8+ T-cell proliferation and IFN-g production *in vitro*, in particular in the absence of CD28 costimulation. CD160Ig prolongs fully mismatched murine cardiac allograft survival in CD4-/– and CD28–/– knockout and CTLA4Ig treated WT recipients, but not in WT or CD8–/– knockout recipients.The prolonged cardiac allograft survival in CD4–/– and CD28–/– knockout and CTLA4Ig treated WT recipients is associated with reduced alloreactive CD8+ T-cell proliferation, allospecific Th1 cytokine production and generation of effector/memory CD8+ T-cells. Thus, CD160 signaling is particularly important in CD28–independent effector/memory CD8+ alloreactive T-cell activation *in vivo* and may therefore serve as a novel target for prevention of allograft rejection.

P005 EFFECTS OF THE NOVEL IMMUNOSUPPRESSIVE PROTEIN KINASE C INHIBITOR SOTRASTAURIN ON THE REPLICATION CYCLE OF HEPATITIS B AND C VIRUSES

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Background: The pan-protein kinase C (PKC) inhibitor sotrastaurin (AEB071) is a novel immunosuppressant currently in phase II trials for immunosuppression after solid organ transplantation. Besides T-cell activation, PKC affects numerous cellular processes that are potentially important for the replication of hepatitis B virus (HBV) and hepatitis C virus (HCV), major blood-borne pathogens prevalent in solid organ transplant recipients.
Methods: This study uses state of the art virological assays to assess the direct, non-immune mediated effects of sotrastaurin on HBV and HCV. The influence of sotrastaurin on HCV replication was assessed in Huh-7.5 cells using cell culture-derived HCV. HCV RNA replication and infectivity was analysed by using luciferase assays.
Results: Sotrastaurin had a moderate inhibitory effect on HCV replication and a mild inhibitory effect on HBV replication. Specifically, the intracellular genome amplification stage of the viral replication cycle but not viral entry into uninfected cells seemed to be affected. Spread of HCV infection directly between adjacent cells, conceivably an important route of spread within an infected liver, was also unaffected. Notably, sotrastaurin exhibited anti-proliferative effects at concentrations only slightly above those needed to elicit anti-viral effects suggesting that the inhibition may be indirectly mediated through changes in the intracellular milieu rather than directly anti-viral. Importantly, no pro-viral effect was detected on either HBV or HCV.
Conclusion: These data supports the evaluation of sotrastaurin in HBV and/or HCV infected transplant recipients.

P007 HMGB1 TRANSLOCATION AND EXPRESSION IS CAUSED BY WARM ISCHEMIA–REPERFUSION INJURY, BUT NOT BY PARTIAL HEPATECTOMY IN RATS

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Mechanical injury or ischemia/reperfusion (I/R) injury induces HMGB1 translocation and release. However, the surgical procedure itself can initiate pathophysiologic processes causing damage to the respective organ. A liver resection, as an example, leads to portal hyperperfusion injury of the remnant liver. Therefore, we aimed to elucidate the impact of different hepatic surgical injury models on cellular localization and expression of HMGB1. Focal warm ischemia reperfusion (I/R) injury was induced by clamping the vascular blood supply to the median and left lateral liver lobes for 90 minutes followed by 0.5, 6 and 24 hours reperfusion, as reported previously. Liver injury by PH was induced by subjecting rats to 30%, 70% or 90% partial hepatectomy (PH) followed by a 24 hours observation period. Additional 12 rats were subjected to 90% PH and sacrificed at 1 and 6 hour to investigate the expression and release pattern of HMGB1. Elevation of serum liver enzymes indicating hepatic injury peaked at 6 hours and recovered thereafter in models, warm I/R injury and PH. Liver injury was confirmed by liver histology. HMGB1 was translocated from the nucleus to the cytoplasm in livers subjected to warm I/R; but not in livers subjected to PH. Both protein and mRNA expression of HMGB1 were significantly up-regulated in livers subjected to warm I/R. In contrast, neither 30% PH, 70% PH nor 90% PH caused an elevation of hepatic HMGB1 mRNA and protein expression. High serum levels of HMGB1 (30 ng/ml) were measured at 0.5 hour reperfusion period after warm I/R, much lower levels thereafter (<5 ng/ml). Similar low serum levels were measured at all time points after 90% PH. Subsequently expression levels of levels of TNF-α reached a peak (26-fold elevation) at 6 hour and decreased down to 5-fold at 24 hours after warm I/R. TNF-α expression levels after PH never exceeded a 5-fold elevation. In conclusion, HMGB1 translocation and expression depends on the type of liver injury as it is induced by ischemia, but not by liver resection/hyperperfusion. These results suggest that HMGB1 may be used as molecular marker to visualize ischemic damage. Mechanic injury in hepatic surgery is associated with focal warm ischemia, and thereby HMGB1 translocation reflects surgical quality in experimental PH. Expression of hepatic TNF-α follows the kinetic pattern of HMGB1, pointing to a muss less pronounced inflammatory response after successful PH compared to warm I/R injury.

P008 THE IMPORTANCE OF DEFINING EQUIVALENCES BETWEEN POTENTIALLY THERAPEUTIC IMMUNOREGULATORY CELLS FROM MICE AND MEN

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Although induction of allograft tolerance with immunoregulatory cells is a common method in experimental Immunology, its translation to the clinic has met many obstacles. One fundamental difficulty lies in defining precise equivalences between immunoregulatory cells studied in animals and their counterparts in man. Our work centers on the regulatory macrophage (M reg) as a means of attenuating responses against donor alloantigen. Mouse models prove convenient to test the combined effect of M reg treatment and conventional immunosuppression, but their clinical relevance hinges on the *degree of similarity* between mouse and human M regs. M regs of both species arise from monocytes under analogous conditions and express unique markers and T-cell-suppressive activities distinguishing them from monocytes, monocyte-derived DCs, and M0-, M1-, M2a-, M2b- and M2c-polarised macrophages. However, differences between mouse and human M regs exist, and weighing these differences against the likenesses is necessarily subjective. Therefore, gene expression profiling by microarray was adopted as an unbiased approach to evaluating their true similarity. In co-clustering analyses, M regs from the two species grouped together when the entire orthologous gene set was considered, but gene set enrichment analyses returned inconsistent results. Thus, human M regs are the closest counterpart of mouse M regs, but an unambiguous equivalence could not be demonstrated. This may reflect a biological limit to resolving interspecies homologies or show no closer equivalents exist. The imperfect correspondence of mouse and human M regs does not invalidate results from mice, but cautions against their direct extrapolation to the clinic.

P009 COMBINATION OF EVEROLIMUS OR TACROLIMUS WITH CLOPIDOGREL SUBSTANTIALLY REDUCES OBLITERATIVE BRONCHIOLITIS (OB) IN AN EXPERIMENTAL MOUSE MODEL

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Purpose: Obliterative Bronchiolitis is the primary limiting factor for long-term survival after lung transplantation. The aim of this study was to investigate if platelet inhibition with Clopidogrel in combination with Everolimus or Tacrolimus has an additional effect on the development of OB.
Methods: Fully major histocompatibility complex-mismatched C57BL/6 (H2(b)) donor tracheas were orthotopically transplanted into CBA (H2(k)) recipients. Mice received 1 mg/kg/day or 20 mg/kg/day Clopidogrel combined with 0.05 mg/kg/day Everolimus or 12 mg/kg/day Tacrolimus. Grafts were analysed by histology, morphometry and immunflourescence for CD4+ and CD8+ T-cells on day 30 and alloantibody production was analysed by FACS.
Results: Untreated controls showed substantial luminal obliteration on postoperative day 30. Animals treated with 1 mg/kg/d Clopidogrel and 0.05 mg/kg/d Everolimus showed less luminal obliteration in comparison to mice treated with 1 mg/kg/d Clopidogrel (33,97 8,96% (1 mg/kg/d Clopidogrel+0.05 mg/kg/d Everolimus) vs. 42,25 12,82% (1 mg/kg/d Clopidogrel) vs. 49,92 4,71% (untreated control), $n = 5$). Increased dose of 20 mg/kg/d Clopidogrel in combination with 0.05 mg/kg/d Everolimus resulted in an even lower amount of airway obliteration 24,72 3,63% (20 mg/kg/d Clopidogrel+ 0.05 mg/kg/d Everolimus) vs. 34,04 8,41% (20 mg/kg/d Clopidogrel) vs. 49,92 4,71% (untreated control), $n = 5$). Treatment with 1 mg/kg/d Clopidogrel and 12 mg/kg/d Tacrolimus also resulted in a reduction of luminal obliteration (30,95 0,71% (1 mg/kg/d Clopidogrel+ 12 mg/kg/d Tacrolimus) vs. 42,25 12,82% (1 mg/kg/d Clopidogrel) vs. 49,92 4,71% (untreated control), $n = 5$). Infiltration with CD4+ cells was reduced after treatment with Everolimus and Clopidogrel. Clopidogrel and Everolimus or Tacrolimus also decreased the amount of alloantibody production.
Conclusions: Clopidogrel in combination with Everolimus or Tacrolimus substantially reduces Obliterative Bronchiolitis (OB).

P010 CLOPIDOGREL REDUCES POST-TRANSPLANT OBLITERATIVE AIRWAY DISEASE (OAD)

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Purpose: Survival after lung transplantation is mainly limited by chronic rejection with its main feature OAD. The aim of this study was to investigate if platelet inhibition by Clopidogrel has an influence on the development of OAD.
Methods and materials: Fully major histocompatibility complex-mismatched C57BL/6 (H2(b)) donor tracheas were orthotopically transplanted into CBA (H2(k)) recipients. Mice received different doses of Clopidogrel postoperatively. Grafts were analysed by histology and immunoflourescence for CD4+

and CD8+ T-cells on postoperative days 15, 30 or 60. Alloantibody-production was analysed by FACS and cytokines were analysed by RT-PCR on postoperative day 14.

Results: Untreated mice showed significant amounts of luminal obliteration on postoperative days 15, 30 and 60. Mice treated with 1 mg/kg/d Clopidogrel for 30 days showed reduced tracheal obliteration [42.2% 12.8% (1 mg/kg Clopidogrel) vs. 45.8% 9.9% (control), $n = 5$]. Treatment with 20 mg/kg/d Clopidogrel resulted in stronger reduction of OAD [day 30: 34.4% 8.4% (20 mg/kg Clopidogrel) vs. 45.8% 9.9% (control) and day 60: 37.7% 14.0% (20 mg/kg Clopidogrel) vs. 41.8% 11.7% (1 mg/kg Clopidogrel) vs. 46.1% 13.1% (control), $n = 5$]. Platelet inhibition also resulted in reduced alloantibody production on postoperative days 15, 30 and 60 [day 15: 17992 6864 MFI (1 mg/kg Clopidogrel) vs. 25754 7201 MFI (control), $n = 5$] as well as significantly lower infiltration of CD4+ and CD8+ cells. We also found significantly lower expression of IL12, IL6, TNFa, PDGFb, MCP1, P-Selectin, L-Selectin, ICAM1 and CD40L in both treatment groups.

Conclusions: Here we can show for the first time that platelet inhibition with Clopidogrel significantly reduced the development of OAD.

P011 COMBINATION OF CLOPIDOGREL AND TACROLIMUS SIGNIFICANTLY REDUCED THE DEVELOPMENT OF TRANSPLANT ARTERIOSCLEROSIS

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Introduction: Our group has previously shown in an experimental mouse model that the formation of transplant arteriosclerosis (TxA) is reduced by platelet inhibition with clopidogrel and almost abolished by combined therapy with clopidogrel and the mTOR inhibitor everolimus. The aim of this study was to investigate whether a combined therapy with clopidogrel and the calcineurin inhibitor tacrolimus also has a beneficial effect on the development of TxA.

Methods: Fully allogeneic C57BL/6 ($H2^b$) donor aortas were transplanted into CBA ($H2^k$) recipients. Recipient mice were treated with clopidogrel (1 mg/kg/d) and tacrolimus (12 mg/kg/d) daily, single as well as in combination. Grafts were analysed by histology and morphometry on day 30 after transplantation.

Results: In mice treated with clopidogrel alone, TxA was significantly reduced as compared to untreated controls [intima proliferation 56%+/-11% vs. 81%+/-7% (control)/$n = 5$] and monotherapy with tacrolimus also demonstrated a beneficial effect on the development on TxA [intima proliferation: 49%+/-9% vs. 81%+/-7% (control), $n = 5$]. However, combination of clopidogrel and tacrolimus showed a substantial reduction of TxA [intima proliferation: 30%+/-5% vs. 81%+/-7% (control), $n = 5$].

Conclusion: These results demonstrate that combination of clopidogrel and tacrolimus can significantly reduce the development of TxA in a mouse aortic allograft model. These findings have important clinical implications as patients suffering from TxA after cardiac transplantation with contraindications for mTOR inhibitors may benefit from combined treatment with clopidogrel and tacrolimus.

P012 CYCLOSPORINE A IMPAIRS NOREPINEPHRINE INDUCED VASCULAR CONTRACTILITY AFTER EXPERIMENTAL RENAL TRANSPLANTATION

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Cyclosporine A (CsA) is used for immunosuppression after organ transplantation. Longterm usage of calcineurin inhibitors is often associated with nephrotoxicity, graft failure and development of hypertension. Aim of this study was to examine the effect of CsA on contractile properties of resistance arteries of rats after kidney transplantation.

Brown Norway rats (BN) serving as donors and Lewis rats (LEW) serving as recipients were used in the experimental renal transplantation model. Naive LEW rats (control) with and without cyclosporine A (CsA, 5 mg/kg) as well as unilaterally nephrectomized LEW rats (UNx) with and without CsA served as controls. The rats were sacrificed 28 days after transplantation and mesenteric resistance arteries were studied on a pressurized myograph. Contractile responses were assessed with cumulative dose-response-curves for norepinephrine and endothelin. Additionally mRNA expression of α1 (subtype a, b, d) and β (subtype 1, 2, 3) adrenergic receptors was quantified in mesenteric resistance arteries and aorta thoracica by qRT-PCR.

Contractile responses of mesenteric resistance arteries to norepinephrine were markedly reduced under treatment with CsA in control ($P < 0.05$) and unilateral nephrectomized rats (UNx) ($P < 0.05$ for maximal stimulation). In KTx rats there was a trend to reduced norepinephrine mediated contraction as compared to control. Contractile responses to endothelin were identical in all groups. Interestingly, the mRNA expressions of α1b, α1d, ß1, ß2 and β3 adrenergic receptors were significantly ($P < 0.05$) lower in all CsA treated rats (control+CsA, UNx+CsA, KTx+CsA). The aortic α1 a,b,d and β 1,2,3 adrenergic receptor expressions were not significantly influenced by CsA therapy.

Conclusion: Norepinephrine-induced, but not endothelin-induced, contractile responses of mesenteric resistance arteries are blunted in cyclosporine treated rats. A marked downregulation of adrenoceptors in mesenteric resistance arteries seems to be the underlying pathomechanism.

P013 INDUCTION OF CIRCULATING ENDOTHELIAL CELLS (CECS) AND CIRCULATING PROGENITOR CELLS (CPCS) AFTER POLYCLONAL ANTITHYMOCYTE GLOBULIN (ATG) THERAPY IN LIVER TRANSPLANTATION

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Background: Beside depletion of circulating lymphocytes there is a growing body of evidence suggesting that rabbit antithymocyte globulin (rATG) may play a pivotal role in modulating the immune system. As blood circulating endothelial cells (CECs) and circulating hematopoietic progenitor cells (CPCs) are thought to play important roles in tissue vascularisation, the study of both cell types is currently suggested as surrogate markers for vascular injury following kidney transplantation.

Methods: We identified viable CECs as CD31bright, CD34dim, CD45-, CD133- and viable CPCs as CD34bright, CD133+, CD45dim, CD31+cells in the peripheral blood of liver transplanted recipients ($n = 28$) until day 20 post transplantation.

Results: An induction of CECs was exclusively observed for rATG-treated patients ($n = 17$) increasing from 0.56%±0.98% pre transplantation to 1.83%±1.85% at day 1−2 post transplantation compared with control patients receiving standard immunosuppression ($n = 11$) ($P < 0.04$). The induction of CPCs was even more pronounced illustrating an increase in rATG treated patients from 0.20%±0.26% pre transplantation to 1.55%±1.75% at day 1−2 post transplantation ($P < 0.001$). A significant elevation of blood CPCs is still detectable at day 5 ($P = 0.0379$ compared with controls) and starts to decline at day 10 post transplantation.

Conclusion: We illustrated that rATG treatment results in a transient induction of CECs and CPCs allowing kinetic monitoring of these cell types post transplantation. As clinical correlations between these two populations and the effect of immunosuppressants has been already proven, validation of these cells as biomarkers in the setting of SOT remains to be determined.

P014 ORGAN-SPECIFIC MANIFESTATION OF CHRONIC GRAFT-VERSUS-HOST DISEASE IN A PRECLINICAL MOUSE MODEL

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Rationale: In chronic graft-versus host disease (cGVHD), the immunological basis for its organ-specific manifestation is not completely understood. Therefore, we aimed to determine the microenvironment in different organs during mild cGVHD in a preclinical mouse model. Besides the detection of anti-DNA antibodies at day 14 as cGVHD readout, we hypothesized that the microenvironment at the protein level is differentially affected in various organs such as kidney, liver, gut and spleen. Therefore, we determined the microenvironment in these organs at day 83 post application of allogeneic splenocytes at the protein level in order to define the most significant changes in an organ-specific manner.

Methods: For the induction of cGVHD, parental splenoctyes were injected into F1 offspring mice. The organ-specific microenvironment in control versus. cGVHD conditions was determined in protein lysates from the different organs. Tissue lysates, serum and urine of each mouse were analyzed by the multiplex technology quantifying 32 cytokines, chemokines, growth factors. Frozen tissue was analyzed by immune histochemistry for pathological evaluation of GVHD.

Results: The microenvironment is differentially affected by cGVHD in different organs, kidney versus. liver or gut, for instance. The comprehensive analysis of many parameters revealed a hierarchy in aGVHD-associated alterations which correlates with the pathological characterization.

Conclusion: The comparison of the microenvironment in different organs during cGVHD at the protein level represents a feasible approach for the definition of an organ-specific GVHD manifestation. This hierarchical contribution of several factors in different tissues may be useful for the search of GVHD biomarkers in an organ-specific manner.

P015 TRANSGENIC EXPRESSION OF HUMAN HEME OXYGENASE-1 PROTECTS PORCINE ENDOTHELIAL CELLS

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Introduction: Endothelial cell (EC) activation is one of the underlying pathomechanisms during acute xenograft rejection. Therefore, EC protection mechanisms are strongly required. Heme oxygenase-1 (HO-1) is proven to have anti-inflammatory, anti-oxidative and anti-apoptotic properties and is therefore an interesting cell protective enzyme for EC. Compared to low constitutive expression under physiological conditions, the enzyme is upregulated in response to various conditions such as oxidative stress, hypoxia and

inflammation. The aim of this study was to investigate if transgenic expression of human HO-1 in porcine EC might result in EC protection.

Methods: Humane HO-1 (hHO-1) was cloned into pharmacological inducible expression vectors and further transduced into immortalized porcine endothelial cells (PED). These cells were incubated with different concentrations of cytokines to induce EC activation. The effects were measured via expression of EC activation markes, such as E-Selectin by flow cytometry analyses.

Results: Transduction of PED with hHO-1 resulted in reduced EC activation after incubation with cytokines compared to wild-type endothelial cells. Transgenic porcine EC showed reduced levels of EC expression markers, namely decreased expression of E-Selectin in flow cytometry analyses.

Conclusion: In this study we analyzed the *in vitro* effects of transgenic hHO-1 expression in porcine endothelial cells. EC activation is reduced in HO-1 transgenic cells compared to wild type cells. The presented data underline the potential of HO-1 as a protective gene in xenotransplantation, further research is necessary to establish hHO-1 transgenic pigs as a potent source for xenotransplantation experiments.

P016 TELOCYTES AND MYOCARDIAL REMODELING IN HUMAN PATIENTS

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The classical definition of myocardial remodelling, 'genome expression resulting in molecular, cellular and interstitial changes', has been suggested by Cohn and coauthors in 2000. According to this definition, ECM remodelling is an important component of myocardial remodelling. In the last years we have intensively studied ECM in diverse cardiac diseases in human patients with pressure-overloaded hearts, cardiomyopathies and atrial fibrilation. These studies have demonstrated that ECM remodelling of diseased human hearts is associated with a specific matrix metalloproteinase/tissue inhibitor of metalloproteinase (MMP)/(TIMP) balance, and that each aetiology of cardiac disease possesses a specific MMP and TIMP portfolio. However, in addition to the different MMP/TIMP profiles and different changes of regulatory factors, the failing myocardium is characterized by discordant changes in collagen type I and III metabolism. This observation implies that multiple mechanisms acting alone or in concert are active in fibrosis development.

Recently we embarked on several studies aiming at clarifying the role of telocytes in ECM remodelling. Our data indicate that the number of telocytes/telopodes correlates positively with the amount of denaturated collagens, deposition of non-fibrillar collagens and with the grade of acute or chronic myocardial inflammation. On the other hand, severe interstitial fibrosis and increased amounts of fibrillar collagens, upon achieving a threshold, lead to telocyte cell death via apoptosis and shrinkage and shortening of telopodes.

Taken together, our studies suggest that telocytes/telopodes are very flexible cell/structures and respond promptly to any quantitative and qualitative changes in the ECM composition.

P017 PRESENCE OF ALLOANTIBODIES AGAINST DONOR-SPECIFIC RECEPTOR PROTEINS OF THE NATURAL KILLER GENE COMPLEX IS ASSOCIATED WITH REDUCED SURVIVAL OF MHC-INCOMPATIBLE GRAFTS IN A RAT SKIN TRANSPLANTATION MODEL

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Introduction: The Natural Killer Gene Complex (NKC) in rat is encompassing numerous polymorphic genes and is mapping on chromosome 4. LEW and LEW.TO-NKC represent a system of NKC-congenic rat strains sharing the genetic background of strain LEW but carrying different NKC-haplotypes. Immunogenicity of the respective NKC-haploytpes was shown by immunization studies. Using this strain system we have analysed herein whether preformed donor-NKC-antibodies might function as modulators of allograft survival (donor LEW.1U). To this end LEW.TO-NKC recipients were sensitized by two skin grafts from LEW (exclusively NKC disparity). Afterwards, sensitized animals received a skin graft from LEW.1U (combined MHC/NKC-disparity).

Methods: Skin transplantation (2×2cm) was performed without immunosuppression. Induction of donor-specific anti-NKC antibody formation in sera was assessed by flow cytometry.

Results: NKC-disparate skin grafts (LEW $\rightarrow$ LEW.TO-NKC, $n = 10$) were not rejected. Induction of donor-NKC-antibody formation was observed in four animals after second grafting. Donor-NKC-antibody positive animals ($n = 4$) rejected MHC/NKC-disparate skin grafts (LEW.1U) after 10.5 ± 0.57 days, whereas antibody negative/low animals ($n = 6$) rejected MHC/NKC-disparate skin grafts after 12.7 ± 0.81 days ($P = 0.0018$), comparable to unsensitized rats ($n = 20$), who rejected MHC/NKC-disparate skin grafts after 13.6 ± 1.54 days.

Conclusion: These data show that shortened survival of LEW.1U skin grafts is associated with the presence of donor-NKC-antibodies in sensitized LEW.TO-NKC recipients. Studies are on the way to define immune mechanisms induced by preformed anti-NKC antibodies leading to a faster rejection of genetic disparate skin grafts. These studies might be of importance for clinical organ transplantation.

P018 INHIBITION OF PROTEASE-ACTIVATED RECEPTOR-4 ATTENUATES PLATELET AND T CELL RECRUITMENT DURING HEPATIC ISCHEMIA-REPERFUSION

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Background and aim: Platelets play a critical role during hepatic ischemia-reperfusion (I/R). Anti-platelet strategies during liver transplantation are, however, limited because of the bleeding complications. Thrombin is activated during reperfusion and regulates platelet and endothelial cell function via the protease-activated receptor-4 (PAR-4). Interventions at the level of PAR-4, the main platelet receptor for thrombin, are suggested to attenuate the pro-inflammatory effects of thrombin without affecting blood coagulation. The aim of our study was to analyze the impact of PAR-4 blockade on platelet recruitment and microvascular injury during hepatic I/R.

Methods: Platelet- and neutrophil recruitment as well as sinusoidal perfusion were analyzed using intravital fluorescence microscopy in hepatic microvessels of sham-operated mice, mice after warm hepatic I/R (90/60 minute), and mice after I/R treated with a selective PAR-4 antagonist tcY-NH2 (30 μg/mice i.v. before the onset of reperfusion, $n = 7$ each group). Since platelets thought to modulate the activation of T-cells, we analyzed the recruitment of freshly isolated and fluorescence-labeled CD4+ T-cells in a separate set of experiments ($n = 3$ each).

Results: Hepatic I/R caused a significant increase in the number of platelets adherent in venules (606 ± 48 /mm^2) and sinusoids (13 ± 1/acinus) as compared to the sham-operated group (49 ± 10 /mm^2 and 3 ± 1/acinus, respectively). In contrast, platelet recruitment was significantly attenuated in mice treated with the PAR-4 antagonist (venules: 161 ± 28 /mm^2; sinusoids: 5 ± 1/acinus). Moreover, CD4+ T-cell adherence was markedly lower in the tcY-NH2-treated group as compared to the untreated controls. Neutrophil migration was not affected by the treatment with tcY-NH2. The postischemic sinusoidal perfusion failure was significantly improved upon PAR-4 blockade.

Summary: These *in vivo* data show that inhibition of PAR-4 attenuates platelet-endothelial cell interactions after hepatic I/R. This effect is associated with reduction of T-cell activation and amelioration of perfusion failure.

P022 RENAL TRANSPLANT RECIPIENTS LACK CIRCULATING CD19$^+$CD24^{HI}CD38^{HI} BREGS

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Background: Recent studies have demonstrated that CD19$^+$CD24^{hi}CD38^{hi} regulatory B cells (Breg) appear to suppress immune effector mechanisms by cell-contact dependent interactions and/or through the secretion of IL10. Studies of Bregs in humans are challenging since specific markers are lacking.

Methods: Here, we characterized peripherally circulating Bregs by flow cytometry in healthy subjects and renal transplant recipients receiving calcineurine inhibitor based immunosuppression.

Results: In healthy subjects the amount of CD19$^+$ B cells among lymphocytes was 11±3%, and of these co-expression of CD24 and CD38 was 54 ± 17%. A distinct subset of CD24^{hi}CD38^{hi} Breg cells was found to be ~8 ± 3%. In contrast, renal transplant recipients had 6 ± 4% CD19$^+$ B cells fully lacking co-expression of both molecules. Next, we evaluated whether these CD19$^+$CD24^{hi}CD38^{hi} B cells (Bregs) exhibit a distinct pattern in expression of chemokine receptor and/or costimulatory molecules. CD1d and CD40 is expressed on all CD19$^+$ B cells and therefore is not useful for further Breg characterization. Whereas the chemokine receptor CXCR4 was expressed on all CD19$^+$ B cells, this subset lacked expression of both CCR5 and CCR7 in any of the subjects. Interestingly, expression of CXCR3 was significantly higher on CD19$^+$CD24^{lo}CD38^{lo} cells (~20%) compared to the CD19$^+$CD24^{hi}CD38^{hi} Breg subset (~1−3%).

Conclusion: In this study, we consistently detected a CD19$^+$CD24^{hi}CD38^{hi} Breg subset in peripheral blood of healthy individuals but not in renal transplant recipients. These results may also suggest that presently known markers like CD24 or CD38 are not applicable in patients receiving calcineurine inhibitors.

P023 THE T503C POLYMORPHISM IN THE HUMAN KLRB1 (CD161) GENE AFFECTS THE FREQUENCY OF PERIPHERAL BLOOD NK CELLS

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Objectives: Genetic variability in immunologically relevant genes contributes to the individual patient's risk for rejection and/or infection. The T503C polymorphism in exon 5 of the human KLRB1 gene leads to an amino-acid

exchange (I168T) in the extracellular domain of the CD161 receptor. CD161 is expressed on subsets of T-cells and most NK cells. Enhanced levels of CD161 mRNA have recently been identified as being part of a pattern of tolerance in liver transplanted patients. In search of functional consequences of the T503C polymorphism we compared the expression patterns of CD161 molecules and the frequency of CD161 expressing T and NK cell subsets in healthy individuals displaying different CD161 genotypes.

Methods: Peripheral blood lymphocytes were isolated and frequencies of CD161 expressing $CD4^+$ and $CD8^+$ T-cells as well as NK cells were analyzed by flow cytometry. The CD161 genotype was determined using PCR based amplification of exon 5 of human KLRB1 followed by DNA sequencing.

Results: In a cohort of 103 healthy blood donors, 42 TT and 14 CC homozygous individuals as well as 47 CT heterozygous donors were identified, yielding allele frequencies of 0.36 and 0.64 for the C and T allele, respectively. Analysis of the expression density of CD161 molecules on $CD4^+$ and $CD8^+$ T-cells, and NK cells revealed no significant differences between TT, CC and CT individuals. This was also true for the frequency of $CD4^+CD161^+$ and $CD8^+CD161^+$ T-cells. However, a higher frequency of $CD56^+CD161^+$ NK cells was observed in TT homozygous individuals compared to CC/CT donors (34.8 ± 11.0% vs. 25.4 ± 8.5%; P = 0.018). Further analysis of NK cell subsets revealed that the expanded NK population in TT individuals was mainly due to an increased proportion of $CD56^{dim}CD161^+$ cells (33.7 ± 10.8% vs. 23.7 ± 8.1%; P = 0.011). In contrast, the frequency of the $CD56^{bright}CD161^+$ cells was reduced in TT individuals suggesting alterations in the composition of the NK subset. In an ongoing study we are genotyping a cohort of kidney grafted patients. Preliminary data indicate a certain accumulation of rejectors in the group of patients carrying the TT genotype.

Conclusion: The association of the CD161 genotype with the frequency of NK subsets in peripheral blood suggests that CD161 may play a role in the regulation of NK cell homeostasis. Studies are underway to address the question as to how immunological variations resulting from different CD161 genotypes may influence the outcome after transplantation.

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P024 BLOCKADE OF KCA3.1 AS POTENTIAL NEW THERAPEUTIC STRATEGY FOR THE PREVENTION OF CHRONIC ALLOGRAFT REJECTION

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Background: The calcium-activated potassium channel KCa3.1 is critically involved in the proliferation and migration of T-cells, macrophages, dedifferentiated vascular smooth muscle cells and fibroblasts by regulating calcium influx. Therefore, it may play an important role in pathogenesis of obliterative airway diseases (OAD), and might be considered as a potential therapeutic target.

Methods: Tracheas from CBA donors were transplanted into the greater omentum of C57Bl/6J mice. Recipients in the treatment group received TRAM-34 (120 mg/kg/d i.p.) for 5 days or 28 days. KCa3.1-/- mice were used as control.

Results: Both the genetic knock out and pharmacological blockade of KCa3.1 with TRAM-34 reduced luminal obliteration from 89 ± 21% to 53 ± 26% and 59 ± 33% (P = 0.010 and P = 0.032 vs. no medication group). In the no medication group, huge amount of CD3+ T-cells and F4/80+ marcophages were found in subepithelial area on POD5, resulting in completely destroyed physiological airway epithelia, while the knock out and TRAM-34 group showed significant less infiltration (P < 0.001). Elispots revealed significantly reduced IFNγ spot frequencies in the knock out (68 ± 16) and TRAM-34 (74 ± 62) groups compared to the untreated allogeneic group (124 ± 62) ($P \leq 0.015$). The qRT-PCR and immunohistochemistry revealed significant lower levels of the KCa3.1 channels in the TRAM-34 group compared to the no medication group (P = 0.008). Channel blockade showed significant inhibition on the recipient splenocytes above 100nM in proliferation assay *in vitro* ($P \leq 0.007$).

Conclusions: Our findings suggest that KCa3.1 channels are involved in the pathogenesis of OAD and that TRAM-34 holds promise to prevent OAD development.

P026 THE MITOCHONDRIA-K^+ CHANNEL AXIS IS INVOLVED IN THE DEVELOPMENT OF INTIMAL HYPERPLASIA

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Background: The objective of the study was to evaluate if the concept of mitochondrial modulation can be used to inhibit intimal hyperplasia, which occurs in different diseases, such as the development of transplant vasculopathy.

Methods: Abdominal aortic denudation in Lewis rats was performed to induce neointimal proliferation. In the treatment groups, the mitochondrial modulator Dichloroacetate (DCA) was given at different time points via drinking water (0.75 g/l). The development of intimal hyperplasia was analyzed by histopathology and confirmed by serial intravascular ultrasound measurements. Confocal immunofluorescence microscopy and 3-dimensional reconstruction was performed to identify cell proliferation, apoptosis, and inflammatory cell infiltration. The co-localization of Kv1.5 channel and smooth muscle cell (SMC) was accessed by 3-dimensional imaging. The mitochondrial-K^+ channel axis (PDH, Erk, Akt, PDK2, Kv1.5) was investigated using immunoblotting.

Results: Developed luminal obliteration in untreated animals on day 14 and 28 was 22.4 ± 15.5% and 41.5 ± 9.7%, respectively. DCA treatment for 28 days significantly reduced intimal hyperplasia (13.6 ± 9.3%, P < 0.001 vs. untreated 28-day control). Delayed DCA treatment starting on day 14 resulted in significantly reduced neointimal formation (6.0 ± 3.1%, P < 0.001 vs. untreated 28-day control). Interestingly, delayed treatment of DCA induced apoptosis resulting in shrinking hyperplasia area (P = 0.037 vs. untreated 14-day control). SMC proliferation was also inhibited by DCA. Immunoblotting revealed significant downregulation of the Kv1.5 channel due to vascular injury, and normalized channel expression after DCA treatment.

Conclusion: Mitochondria-K^+ channel axis is involved in the development of neointimal hyperplasia and might represent a new target for therapeutic strategies.

P028 A NEW REVERSIBLE MODEL OF FULMINANT HEPATIC FAILURE BASED ON TRANSIENT ISCHEMIA IN THE RAT

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Background: Fulminant hepatic failure (FHF) is one of the most dramatic entities in clinical medicine. Knowledge and treatment of the FHF have been limited by the lack of satisfactory animal models. We have developed a reversible model in the rat by *total portal vein clamping* and *side-to-side portocaval anastomosis* in microsurgical technique.

Methods: The study validated several standard operating procedures describing in detail the surgical method and intensive care monitoring (blood chemistry control, and cardiovascular monitoring). FHF was induced in LEWIS rats with a mean of 140−160 g. Two surgical methods were compared: group I (ligation of hepatic arteries with total portal vein clamping and bile duct ligation, n = 6) and group II (side-to-side portacaval shunt without bile duct ligation and without portal vein clamping, n = 6).

Results: During total portal vein clamping (60 minutes), the animals in the group I developed severe hypotension, splanchnic congestion and metabolic acidosis. All animals in the group I died between 1.5 and 2 hours (mean, 1.75 ± 0.1 hour) after portal vein declamping characterized by a progressive increase in liver enzymes, ammonia, total bilirubin, and coagulopathy. Histological features of the liver showed coagulative necrosis of hepatocytes with absence of nuclei and collapse of cell plates. Brain histology revealed hypoxic cell damage. This model therefore represents a multiorgan failure model rather than an isolated FHF model.

In the group II, none of these side effects were observed, while clinical, laboratory and histopathological signs of FHF were evident. Survival of the rats ranged from 780 to 1250 minutes (mean 1015 ± 23 minutes). The animals remained stable until a few hours before brain death, an event heralded by a final sharp increase of the serum ammonia level and by a well-evident decline of both arterial pressure and liver-dependent clotting factors.

Conclusions: We have developed a simple, reproducible model of FHF in rats that has a number of features comparable with clinical FHF patients and is well suited for testing experimental bioartificial liver systems and investigating the pathogenesis of FHF. Although progress has been made, research must continue in this area to establish an animal model with minimal disadvantages that would accurately reflect the clinical syndrome seen in humans. The anhepatic model is very useful for validating new supportive measures to bridge the period between the onset of fulminant hepatic failure and the time at which a suitable organ becomes available In conclusion, we have developed and characterized a novel model of FHF in rats that has a number of physiological and biochemical features seen clinically in FHF.

P029 GENE EXPRESSION ANALYSIS OF HUMAN HEPATOCYTES - IDENTIFICATION OF EXPRESSION DIFFERENCES BETWEEN SMALL- AND LARGE HEPATOCYTES

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The liver has the capability to regenerate. Under special circumstances small hepatocytes (SH) with progenitor capability proliferate and participate in the regeneration process. SH have only been investigated in murines and are still poorly characterized in humans. They might act as bipotential progenitors able to differentiate into mature hepatocytes and bile duct cells. Aim of the study was to identify gene expression differences between SH and large primary human hepatocytes (LH) in culture.

We performed microarray analysis with 41,000 reporters (Agilent Whole Human Genome Oligo Microarray platform). Human hepatocytes were isolated by a two step collagenase protocol and cultivated *in vitro*. Via Laser Microdissection and Pressure Catapulting (LMPC) both cell types (altogether eight samples from three donors consisting of approx. 80 cells each) were collected and the cDNA analyzed.

One hundred and eighteen reporters showed significant and at least 2-fold differences between the subsets. The gene with the strongest preference for LH was C14ORF65 (13-fold enriched). At the other end of the spectrum was TMEM199 with a 7-fold preference for SH. Validation of the differential expression of selected genes by qPCR is currently underway.

Our results so far point to the existence of a set of differentially regulated genes between SH and LH. This analysis will serve as a basis to further characterize human SH and facilitate their identification in a mixed culture system obtained from human liver cell isolations.

KIDNEY

P032 FETUIN-A PRETRANSPLANT SERUM LEVELS, KIDNEY ALLOGRAFT FUNCTION AND REJECTION EPISODES – A 3 YEAR POST TRANSPLANTATION FOLLOW-UP

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Background: Fetuin-A is a negative acute phase protein, which acts as a potent calcification inhibitor and an antagonist of transforming growth factor-β. Thus, Fetuin-A levels are influenced by chronic inflammation and actively affect fibrosis and calcification processes, respectively. Graft rejection, interstitial fibrosis and tubular atrophy, chronic inflammation and calcification are common causes for kidney allograft loss. This study evaluated whether pretransplant Fetuin-A levels predict long-term graft survival and rejection episodes in patients after kidney transplantation.

Methods: In 206 renal transplant recipients pre-transplant Fetuin-A levels were measured in serum by ELISA. During the 36 months active follow-up (median 1249 days) 13 patients died (94% patient survival) and renal allograft failure was reported in 18 patients (91% graft survival).

Results: Pretransplant Fetuin-A levels did not differ among patients with incident graft failures as compared to patients with functional graft after long-term follow-up or rejection episodes (Fetuin-A: 393.6 ± 46 vs. 384.4 ± 69 vs. 405 ± 27.4 μg/ml). In logistic regression analysis, pre-transplant Fetuin-A levels did not correlate with graft failure after 3 years follow-up ($P = 0.895$). In COX regression analysis, Fetuin-A levels were not associated with the time to graft loss. Moreover, Fetuin-A levels correlated neither with renal and metabolic parameters nor with cellular or humoral rejection episodes.

Conclusion: Pre-transplant levels of Fetuin-A are no predictor for renal allograft loss or rejection episodes after 36 months follow-up in transplant recipients.

P035 PRE-TRANSPLANT SENSITIZATION AGAINST ANGIOTENSIN II TYPE 1 RECEPTOR (AT_1R-ANTIBODY) IS A NOVEL INDEPENDENT RISK FACTOR OF ANTIBODY-MEDIATED REJECTION

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Background: The angiotensin II type 1 receptor (AT_1R) is an emerging target of functional non-HLA antibodies. We examined the impact of pre-sensitization against AT_1R as a risk factor for acute antibody-mediated rejection (AMR).

Methods: We studied 599 patients who consecutively received a kidney transplant between 1998 and 2007. Coded sera samples were measured blind for Anti-AT_1R antibodies by a quantitative solid-phase assay. Predictive cut-off was statistically determined and set at 19.7 U. Extended Cox model was employed to determine risk factors for the occurrence of the first acute rejection.

Results: Anti-AT_1R antibodies > 19.7 U were detected in 77 patients (12.8%) at the time of transplantation. Multivariate analysis shows that the risk of rejection increased 2.9-fold for patients with ≥ 4 HLA-A-B-DR incompatibilities ($P = 0.0084$) and 2.2-fold for patients with a Panel Reactive Antibody peak on T-cells ≥ 25% ($P = 0.0110$). The presence of anti-AT_1R-antibody > 19.7 U before transplantation was also a strong and independent risk factor for acute rejection in the first four months (HR = 2.97, $P = 0.001$). AMR occurred in (70%) of patients with acute rejection in whom pre-transplant anti-AT_1R antibodies were > 19.7 U. In contrast, AMR occurred in only 29% of those patients with anti-AT_1R antibodies < 19.7 U ($P < 0.05$). High AT_1R antibodies pre-transplant correlated with de-novo anti-donor-specific HLA antibodies at the time of rejection. Neither anti-hypertensive nor anti-rejection drug regimens were different in AMR and non-AMR patients with acute rejection.

Conclusion: High pre-transplant anti-AT_1R antibodies may identify patients with increased risk for AMR.

P036 EXPRESSION OF MATRIX METALLOPROTEINASE MMP-9 AND GRANZYME B IN PATIENTS WITH LONG TERM KIDNEY ALLOGRAFT SURVIVAL

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Background: On activation, mononuclear leukocytes secrete metalloproteinase MMP-9, a well-known proinflammatory mediator, which affects kidney allograft function. Increased MMP-9 facilitates tissue invasion by leukocytes. Increased MMP-9 levels are associated with progression of vascular disease and chronic kidney nephropathy. Furthermore, granzyme B, a cytotoxic protein secreted by mononuclear cells, has been associated with kidney allograft rejection. Data on the association of MMP-9 and Granzyme B with long term kidney allograft survival is sparse. In the present cohort study we investigated MMP-9 transcripts and granzyme B transcripts in patients with kidney allograft.

Methods: We determined MMP-9 and granzyme B transcripts in mononuclear leukocytes from 180 patients after kidney allograft transplantation using quantitative RT-PCR. Patients were grouped according to time after transplantation: less than 1 year, 47 patients, 1–5 years, 59 patients, 5–10 years, 35 patients, and more than 10 years, 39 patients. Data were analyzed using non-parametric Kruskal–Wallis test. A two-sided P-value less than 0.05 indicated a significant difference.

Results: One hundred and thirteen were males, 67 were females. Mean age was 46 ± 1 years (mean ± SEM). Mean GFR was 49 ± 2 ml/minutes/1.73 m. In mononuclear cells granzyme B transcripts were 35-fold higher compared to MMP-9 transcripts. We observed a significant decrease of MMP-9 transcripts and a significant increase of granzyme B transcripts with increasing time after transplantation (Figure).

For all patients mean MMP-9 transcripts were 0.0015 ± 0.0002. In patients with functioning kidney allograft for more than 10 years, the MMP-9 transcripts were 25% of the transcripts observed in patients with kidney allograft for less than 1 year. Higher MMP9 transcripts during the first year after transplantation indicate higher inflammatory response against the allograft.

For all patients mean granzyme B transcripts were 0.0528 ± 0.0044. In patients with kidney allograft for more than 10 years, the granzyme B transcripts were 183% of the transcripts observed in patients with kidney allograft for less than 1 year. Cytotoxicity was still observed even after more than 10 years after transplantation.

Conclusion: The present results indicated that patients with functioning allograft for more than 10 years have reduced proinflammatory activity, whereas cytotoxicity can be still observed. Hence, proinflammatory activity may be more important for allograft survival.

P037 THE REDUCTION OF THE SUPPRESSIVE ACTIVITY OF $CD4^+CD127^{LOW+/-}CD25^+$-TREGS ASCERTAINED FOR REJECTING TRANSPLANT PATIENTS CORRELATES WITH A DECLINE OF THE HLA-DR MFI OF THE $DR^+CD45RA^-$-TREG SUBSET

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Recent studies show that regulatory T-cells (Tregs) play an essential role in tolerance induction after organ transplantation.

In order to examine whether there are differences in the composition of the total Treg cell pool between stable transplant patients and patients with BPR, we compared the percentages and the functional activity of the different Treg cell subsets ($DR^{high+}CD45RA^-$-Tregs, $DR^{low+}CD45RA^-$-Tregs, $DR^-CD45RA^-$-Tregs, $DR^-CD45RA^+$-Tregs). All parameters were determined during the three different periods of time after transplantation (G2: 0–30 days, G3: 31–1000 days, G4: > 1000 days).

From 157 transplant patients, 38 patients suffered a biopsy proven rejection (BPR). Patients with BPR showed deficiencies concerning the functional activity of their Treg pool. Thereby, its composition was changed in the way that the $DR^{high+}CD45RA^-$ -Treg subset, which was shown to possess the highest suppressive activity, was decreased. There was a positive correlation ($r = 0.546$, $P < 0.001$) between the HLA-DR MFI of the $DR^+CD45RA^-$-Treg subset and the suppressive activity of the total Treg cell pool. In contrast, the $DR^-CD45RA^-$-Treg subset with lower suppressive capacity was increased in transplanted patients with acute rejection. Especially the determination of the HLA-DR MFI of the $DR^+CD45RA^-$-Treg subset allowed a significant discrimination between patients with acute graft rejection and those without rejection.

The clinical usefulness of the monitoring of these peripheral blood parameters after solid organ transplantation and its relation with clinical outcomes needs to be investigated in large prospective cohort studies of transplant patients.

P038 CELLULAR INFILTRATES AND NF-KB SUBUNIT SIGNALING IN KIDNEY ALLOGRAFTS BY PATIENTS WITH CLINICAL OPERATIONAL TOLERANCE

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NF-kB activation plays a central role in inducing and maintaining transplant tolerance, by controlling genes important in initiating, perpetuating but also terminating inflammation. NF-kB subunit c-Rel is involved not only in the resolution of inflammation but in T-reg differentiation as well. Our aim was to characterize the cellular infiltrates and the expression of NF-kB1, c-Rel and its upstream regulators phosphoinositide 3-kinase (PI3K)/RAC-alpha serine-threonine kinase (Akt1) in allograft biopsies from patients with spontaneous clinical operational tolerance (COT).

Methods: Paraffin fixed kidney allograft biopsies from 28 patients with COT ($n = 4$), acute interstitial rejection (IR) ($n = 12$) and borderline changes (BC) ($n = 12$) with eGFR (MDRD) > 40ml/min at biopsy and no significant loss of

allograft function at 1 year afterwards were used in the study. Cellular infiltrates and immunohistochemical expression of key proteins of the NF-kB pathway were evaluated using digital image analysis software. Results are given as percentual of area positively stained, positive cells/mm^2 or percentual of positive cells.

Results: Biopsies from subjects with COT exhibited comparable amount of cellular infiltrate to IR and BC (COT: 190 ± 161; IR 291 ± 230; BC 178 ± 161 cells/mm^2), but reduced c-Rel expression in the infiltrates (COT: 1.7 ± 1.4; IR: 8.7 ± 6.4; BC: 5.9 ± 5.8 cells/mm^2, $P = 0.03$). In contrast, FOXP3 positive cells in the infiltrates were markedly increased in COT as compared to IR and BC (COT: 13 ± 5.6%; IR: 3.2 ± 2.2%; BC: 3.4 ± 2% of positive cells, $P < 0.01$). This was paralleled by a significantly lower tubular PI3K and c-Rel expression by COT compared to IR and BC (PI3K: COT: 3.1 ± 0.27%; IR 7.5 ± 3.2% and BC 7.4 ± 3.2% of area positively stained, $P = 0.03$) and (c-Rel: COT: 1.4 ± 0.51%; IR 7.2 ± 4.3% and BC 5 ± 3%, $P = 0.009$). HLA-DR tubular expression was significantly lower in COT compared to IR (COT: 0.23 ± 0.1% and IR: 0.71 ± 0.54%, $P = 0.02$).

Conclusions: Though displaying significant cellular infiltrates, allografts from patients with COT show signs of low immunogenicity and low c-Rel but high FOXP3 expression.

P039 THE DEVELOPMENT OF BK VIREMIA AFTER RENAL TRANSPLANTATION IS ASSOCIATED WITH A REDUCED CD8 CELL IL-2 RESPONSE

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Polyomavirus-associated graft nephropathy (PAN) has emerged as a significant risk factor for kidney graft loss. Risk factors for the development of PAN are ill defined. We performed a randomized prospective single-center study in 105 renal transplant recipients who were randomized to receive cyclosporin A/mycophenolate mofetil (CsA/MMF; $n = 31$), tacrolimus/MMF (Tac/MMF; $n = 32$) and Tac/MMF with conversion to everolimus (TErl; $n = 32$), respectively. 10 patients were not randomized (contraindications against MMF). The impact of immunosuppressive therapy and pre- and post-transplant (4 months, 1 and 2 years) immune responses (intracellular cytokine responses and cytokine receptor expression using triple fluorescence flow cytometry) on the incidence of BK viremia and PAN was analyzed.

BK virus screening was performed by rt-PCR testing in serum and urine specimens obtained on days 0, 14, 30, 60, 90, 120, 180, 270, 360 and 720.

7/105 (6.7%) patients developed biopsy-proven PAN (CsA/MMF: $n = 1$, Tac/MMF: $n = 3$, TErl: $n = 2$, not randomized: $n = 1$), and four of these lost their grafts (Tac/MMF: $n = 1$, TErl: $n = 2$, not randomized: $n = 1$). 21/105 (20.0%) patients had documented BK viremia. BK viremia preceded PAN in all cases and was significantly associated with Tac/MMF immunosuppression [4/31 (12.9%) CsA/MMF, 11/32 (34.4%) Tac/MMF, 5/32 (15.6%) TErl, and 1/10 (10.0%) not randomized patients; $P = 0.034$]. Patients with BK viremia showed significantly diminished Il-2 producing CD8 cells after 90 days (14.2% vs. 20.4%, $P = 0.011$) and 1 year posttransplant (14.1% vs. 20.5%, $P = 0.014$) compared with patients without BK viremia.

Patients on a Tac- and MMF-based immunosuppression were at higher risk of developing BK viremia. Patients with BK viremia differed from those without in a more profoundly reduced Il-2 response of CD8 cells. Our data suggest that low CD8 cell Il-2 responses may predict the risk of developing BK viremia.

P040 LONG-TERM RESPONSE TO VACCINATION AGAINST PNEUMOCOCCAL ANTIGENS IN KIDNEY TRANSPLANT RECIPIENTS

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Introduction: Vaccination against *Streptococcus pneumoniae* is recommended by the German advisory board for vaccination (STIKO) in immunocompromized patients such as kidney transplant recipients. *S. pneumoniae* mainly infects the upper respiratory tract; it can cause lobar pneumonia or meningitis. Morbidity and mortality are both increased in immunocompromized patients. It was the aim of the present study to define the long-term efficiency of vaccination in clinically stable kidney transplant recipients.

Methods: Forty nine patients (21 female, 28 male, median age 55, range 29–74 years) were immunized using Pneumovax 23. Antibodies against 14 pneumococcal capsular polysaccharide antigens (serotypes) were determined prior to, one month and 15 months after vaccination by multiplexed bead assay (Luminex).

Results: One month after vaccination, patients displayed a significant increase ($P < 0.0001$) in the total antibody concentration against 14 pneumococcal serotypes from a median of 18.2 mg/l (range 2.9–55.5) prior to vaccination to 53.6 mg/l (range 4.5–132.4). Fifteen month after vaccination, the total antibody concentration was still significantly higher ($P < 0.0001$) than prior to vaccination (median 41.3, range 4.9–105.0 mg/l). In addition, the kidney transplant recipients showed a significant increase in the number of serotypes recognized from a median of 8 (range 0–13) to 13 (range 0–14, $P < 0.0001$) at month 1 and to 11 (range 0–14, $P = 0.006$) at month 15. Antibody responses after vaccination were slightly, but significantly ($P < 0.0001$, both at month 1 and 15) lower than in a published cohort of vaccinated, healthy controls [14 (3–14), Borgers et al., 2009)].

Conclusion: Our results demonstrate that kidney transplant recipients can produce almost normal concentrations of antibodies against pneumococcal polysaccharides. Fifteen month post vaccination, 77% of the initial antibody response (at month 1) remained detectable.

P041 SWEET-SYNDROME AFTER KIDNEY TRANSPLANTATION - A CASE REPORT

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Introduction: The most common causes of recurrent fever and leucocytosis after kidney transplantation are infections. The occurrence of a neutrophilic dermatosis as the underlying cause is unusual and extremely rarely reported.

Case report: A 49-year old patient with a history of 7 years dialysis-therapy due to polycystic kidney-disease and state after nephrectomy on both sides underwent deceased donor kidney transplantation in 2009. Under immunosuppression with basiliximab-induction as well as tacrolimus, mycophenolate and prednisolone the patient suffered from early vascular rejection, so that an intensified immunosuppressive regimen was required. Two years after organ transplantation with stable kidney function (eGFR 40–50 ml/min) the patient admitted to hospital with recurrent fever > 39 °C and diffuse, partly maculopapulated rash on his decollete. Despite increased CRP-value and left-shifted leukocytosis no evidence for infection was found. Detailed examination was also unsuggestive for autoimmune or malignant disease. With empirical antibiotic therapy and reduction of immunosuppression the patient improved for a short time. Due to recurrent symptoms a biopsy of skin rash was done. The biopsy revealed a severely neutrophilic infiltration of the dermis. The histological findings and clinical symptoms were consistent with Sweet-syndrome. The patient received highly-dosed prednisolone-therapy with 1 mg/kg and improved rapidly. Definable causes for Sweet-syndrome such as underlying malignant disease, specific medicaments or active infection were excluded in this patient.

Conclusion: The pathogenesis of Sweet-syndrome, a neutrophilic dermatosis, after solid organ transplantation is not well understood yet. The disease was rarely reported after allogenic stem cell transplantation. Immunosuppression with impaired T-cell-function may have an important role in this process.

P042 CMV VALGANCICLOVIR PROPHYLAXIS VERSUS PREEMPTIVE THERAPY AFTER RENAL TRANSPLANTATION: TWO YEAR RESULTS OF A RANDOMIZED CLINICAL TRIAL

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Background: Prophylaxis and preemptive therapy are competitive approaches to prevent cytomegalovirus (CMV) infection after renal transplantation. Several prospective randomized studies show that in high risk (D+/R-) patients prophylaxis is the best option for preventing CMV, and to a lesser extend this is also true for D+/R+ patients. Within the last few years, evidence is growing that not only CMV disease but also asymptomatic (subclinical) active CMV infection correlates with increased long term morbidity, graft loss, diabetes, atherosclerosis and mortality following solid organ transplantation.

Methods: We performed a randomized clinical trial to determine if renal transplant recipients with a positive CMV serostatus had a higher rate of active CMV infection and disease when treated preemptively for CMV infection, compared to recipients treated with primary prophylaxis; and whether this correlates with a higher rate of chronic graft alteration and long-term graft and patient survival. Prophylaxis consisted of 2 × 450 mg (900 mg) valganciclovir tablets/day adjusted for renal function for 100 days post-transplantation. Patients were monitored with a quantitative CMV PCR test (Cobas® Amplicor® CMV-Monitor) and positive patients (≥ 400 CMV DNA copies/ml) received four valganciclovir tablets 1800 mg/day adjusted for renal function followed by secondary prophylaxis with two valganciclovir tablets 900 mg/day for 28 days. Patients were to be followed for 5 years; 24-month-data are presented in this abstract.

Results: In January 2011, data of 201 patients were analyzed, 99 receiving prophylaxis and 101 pre-emptive therapy. At 24 months overall tolerability was good for both treatments. 141 episodes of acute graft rejection occurred in 82 patients: in 43 on prophylaxis and in 39 under a pre-emptive regimen. Active CMV infection was significantly higher with preemptive therapy (preemptive: 36.0% vs. 10.3%, $P < 0.0001$), and most CMV infection was seen for D+/R+ patients receiving preemptive therapy (51.3% vs. 14.4%, $P < 0.0001$). Similarly, D+/R+ patients with preemptive therapy experienced the highest rate of CMV disease (20.5% vs. 4.4%, $P = 0.0016$). Renal function was similar in both groups, but D+/R+ patients under a preemptive regimen with CMV infection vs. prophylactic treatment recipients had a slightly lower GFR at 24 months (pre-emptive: 57.95 ± 22.85 ml/minutes vs. prophylactic: 60.57 ± 22.83 ml/minutes). Graft loss occurred for more pre-emptive patients ($n = 8$, 5.3%) versus prophylaxis ($n = 4$, 2.7%, $P = 0.3782$).

Conclusion: At 2 years post-transplantation, valganciclovir prophylaxis was associated with a sustained and significant reduction of incidence of active CMV infection, especially for the D+/R+ recipients. The ongoing follow-up of

this study will determine if the observed numerical advantage for graft survival in R+ recipients exposed to valganciclovir therapy may develop into a robust clinical difference over time.

P044 TREATMENT WITH THE CALCIUM SENSING RECEPTOR AGONIST R-568 DOES NOT PROTECT FROM RENAL ISCHEMIA/REPERFUSION INJURY

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Introduction: Renal ischemia/reperfusion injury is mediated by a number of cytokines and intracellular messengers resulting in apoptosis/necrosis and inflammation. Calcium plays an important role as a second messanger and seems to regulate cellular processes also via calcium sensing receptors. The calcium receptor agonist (calcimimetic) R-568 protected podocytes from apoptosis and, furthermore, reduced also proteinuria in the puromycin induced nephropathy model (Oh J et al., Kidney Int, 2011). So far it is not clear whether the calcimimetic R-568 has protective effects after ischemia/reperfusion injury in the kidney.Here we asked whether R-568 influences renal ischemia/reperfusion injury as well as related apoptosis and apoptosis regulating factors.

Material and methods: SD rats were used throughout the experiments. Ischemia of the left kidney was induced by clamping of the left renal artery for 45 minutes. Animals were assigned to three treatment groups: R-568 LD (low dose 30 mg/kg/day; $n = 6$), R-568 HD (high dose: 100 mg/kg/day, $n = 6$), and VEH (vehicle; $n = 6$). The observation period ended 24 hours after induction of ischemia. Serum creatinine, serum BUN, renal histology as well as PCR of apoptosis related factors bcl-2 (anti-apoptotic) and bax (pro-apoptotic) were analyzed. Furthermore additional four animals of the groups R-568 HD and VEH were observed for three days after induction of renal ischemia for the analysis of serum creatinine and serum BUN levels.

Results: One day after induction of renal ischemia the animals of the experimental groups had no significant differences with respect to serum creatinine or serum BUN. Three days after induction of ischemia there were also no significant differences detectable between animals treated with high dose R-568 and controls, respectively.Histology demonstrated on H&E staining moderate to severe ischemia/reperfusion injury irrespective whether animals had been treated with R-568 or vehicle.The analysis of the mRNA levels of the apoptosis related factors bax and bcl-2 demonstrated no significant differences between the groups.

Conclusion: Treatment of renal ischemia/reperfusion injury with the calcimimetic R-568 did not demonstrate a protective effect in this experimental model. In the future the expression of the calcium sensing receptor as well as the time course of its renal expression during ischemia/reperfusion should be analyzed.

P047 RENAL FUNCTION AND PROTEINURIA ARE PREDICTORS FOR A SUCCESSFUL CONVERSION TO SIROLIMUS

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Objectives and methods: In order to analyze overall outcome after late initiation of the mTOR inhibitor sirolimus (SRL) in a large cohort of patients (pts) we investigated all renal allograft recipients converted to SRL between 11.1.2000 and 12.12.2008 from 10 german transplant (Tx) centers. All data were retrospectively entered into a large national database. The aim of this study was to define predictors for a favourable outcome.

Results: In total 726 pts (age at switch: 49.8 years ± 13.4; 64% male) started SRL therapy after a median of 55 (1−343) months after Tx with a median follow-up of 24.3 (1−110) months. Before conversion 61% had received cyclosporine, 26% tacrolimus, 31% azathioprine, 53% mycophenolate and 97% steroids. In 75% it was their first Tx, 10% had received a combined Tx and 33% had experienced at least one rejection episode before conversion. Reasons for conversion included 191 pts (26.3%) with CNI toxicity, 166 pts (22.9%) with malignancy, and 128 pts (17.6%) with chronic allograft nephropathy (CAN).

During the observation period 53 pts died, and 134 pts returned to dialysis. Overall patient and graft survival (including death) at 1, 3 and 5 years after conversion was 96%, 90.8%, 82.4% and 86.5%, 71.8%, and 57.7% respectively. In 32.6% renal function (GFR) improved and 39.7% were stable, while in 27.7% GFR deteriorated after conversion, resulting in overall stable GFR in the first year after conversion (40.4 ± 18.4 before vs. 41.2 ± 20.4 ml/minute after conversion; P = ns). Pts, who survived with a functioning graft had better GFR (42.8 ± 19.5 ml/minute vs. 27.7 ± 13.8 ml/minute; $P < 0.001$) and less frequent proteinuria > 400 mg/l (39.7% vs. 60.3%; $P < 0.001$) at the time of conversion. Age, gender, time after Tx and previous rejection episodes did not differ between survivors and non-survivors. Pts with proteinuria > 400 mg/l, poor (< 40 ml/minute) GFR, and CAN had significantly ($P < 0.001$) inferior 5 year graft survival (75.1% vs. 88.9%, 55.0% vs. 75.7%, and 38.9% vs. 62.9%, respectively). Pts with malignancy had better graft survival (89.1% vs 60.7%; $P < 0.001$), while pts with CNI toxicity had similar overall graft survival (64.7% vs. 68.4%; P = ns).

Conclusion: Conversion to SRL is a valid option for patients with malignancy and CNI toxicity, especially for those patients with adequate renal function and no severe proteinuria.

P048 CRANBERRY JUICE SUCCESSFULLY PREVENTS URINARY TRACT INFECTION AFTER RENAL TRANSPLANTATION

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Background: Urinary tract infections (UTIs) constitute the most common infectious complication after renal transplantation. Recurrent UTIs significantly increase the recipients' mortality and reduce graft survival. Attempts to prevent recurrent UTIs include urine acidification (cranberry juice, methionine) and antibiotics. Data on the efficiency of cranberry juice, however, are controversial in the general population and there are no data at all on renal transplant recipients.

Methods: We present a retrospective analysis of 104 renal transplant recipients, who received prophylaxis for recurrent UTI with cranberry juice (2 × 50 ml/day, $n = 39$, 37.5%), methionine (3 × 500 mg/day, $n = 25$, 24.0%), antibiotics ($n = 10$, 9.6%), vaccination (Strovac^R, $n = 7$, 6.7%), or combination of different approaches ($n = 23$, 22.1%). We analyzed symptoms (fever, alguria, polyuria, dysuria), leukocyturia/nitritiuria, and the number of documented UTI events one year before and after initiation of prophylaxis.

Results: In the cranberry group, 22 patients (56.4%) had symptoms before and 13 (33.3%) had symptoms after the initiation of prophylaxis ($\Delta = 9$, 23.1%, $P = 0.008$). Leukocyturia/Nitrituria decreased from 31 (79.5%) to 20 (51.3%) by 11 (28.2%, $P = 0.02$). The number of UTI episodes decreased highly significantly by 57.1% from 3.5 ± 1.5 to 1.5 ± 1.4/a ($P < 0.001$). Methionine reduced the number of UTI episodes by 48.7% from 3.9 ± 2.0 to 2.0 ± 1.3/a ($P < 0.001$), antibiotics from 4.3 ± 1.9 to 1.8 ± 2.0/a (Δ 2.5 ± 2.9/a, 58.1%, $P = 0.01$).

Conclusion: In this retrospective analysis cranberry juice, methionine, and antibiotics successfully reduced the incidence of UTI by about 50% after renal transplantation. The observed efficiency of cranberry juice in renal transplant recipients opposes a recent report in non-transplant patients with recurrent UTI.

P049 THE IMPACT OF CYCLOSPORINE AND TACROLIMUS ON ARTERIAL FUNCTION

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Background: Cardiovascular complications are the leading cause of death and one of the leading causes of graft failure in renal transplant recipients. Calcineurin inhibitors induce an acceleration of atherosclerotic processes in the arterial wall. There are conflicting data whether cyclosporine (CsA) and tacrolimus (Tac) differ in their deleterious effects on arterial stiffening. The present study combines several measurement techniques in order to provide a global and reliable assessment of the differential effects of calcineurin inhibitors on the gold-standard parameters of arterial function.

Methods: Pulse wave analysis was performed by the SphygmoCor (AtCor^R), HEM-9000AI (Omron^R), and CR-2000 device (Hypertension Diagnostics^R) in 56 stable renal transplant recipients (29 CsA, 27 Tac).

Results: Groups were homogeneous for age, gender, body mass index, time on dialysis prior to transplantation, and graft function. Whereas systolic and diastolic blood pressure, central aortic blood pressure, cardiac index, large and small artery compliance (C_1 and C_2), and pulse wave velocity did not significantly differ between CsA and Tac, augmentation index (AI_{75}) was significantly lower in patients treated with Tac. This finding was consistent as assessed by two different measurement systems ($P < 0.05$).

Conclusion: Compared to CsA, Tac has a favorable impact on augmentation index, a strong independent predictor for cardiovascular mortality.

P050 SENESCENCE-RELATED POLYMORPHISMS ARE ASSOCIATED WITH CARDIOVASCULAR MORTALITY

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Recipient death with functioning graft is the leading cause for renal allograft loss and cardiovascular mortality is the most important cause. Single nucleotide polymorphisms (SNPs) in a non-coding region close to the INK4/ARF senescence genes have been associated with higher cardiovascular morbidity.

We selected 2064 recipients, 688 of which with a known cardiovascular cause of death and 1376 matched controls, from the Collaborative Transplant Study DNA bank. DNA specimens were genotyped for three SNPs with known risk allele and one SNP without a risk allele. To explore a possible genotype-phenotype correlation, we genotyped 106 endothelial cells from human umbilical cords (HUVECs) and measured the expression of *INK4/ARF*-senescence-genes ($p14^{ARF}$, $p15^{INK4b}$, $p16^{INK4a}$) and methylthioadenosin-phosphorylase (MTAP) by Real-Time PCR.

Baseline characteristics of the two recipient groups were similar. In patients that died from a cardiovascular event the risk allele for three different SNPs was detected significantly more often compared to matched controls (table 1). There was no difference between groups for the SNP, for which no risk allele

had been identified. In those 19 HUVECs that were homozygous for the risk alleles of SNP #1, #2 and #3, we found a tendency towards higher mRNA-expression for $p14^{ARF}$, $p15^{INK4b}$ and MTAP.

Our results support data from large cohort studies in normal populations suggesting a higher risk for cardiovascular events in individuals carrying certain SNPs in senescence associated genes. Notably, this holds true in a population at high risk for cardiovascular events. The preliminary data points towards a role of the investigated SNPs for the expression profile in endothelial cells.

Table 1. Percentage of patients who are either homozygote or heterozygote for the risk or non-risk allele, respectively. Exact Fisher's test is used.

P051 RECIPIENT AND DONOR BODY MASS INDEX AS IMPORTANT RISK FACTORS FOR DELAYED KIDNEY GRAFT FUNCTION

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Introduction: Recipient obesity is associated with worse outcome after kidney transplantation, whilst the number of overweight patients on the waiting list is increasing. We investigated whether donor and/or recipient BMI correlate with the occurrence of delayed graft function (DGF) after kidney transplantation.

Methods: Retrospective analysis of 1132 consecutive cadaveric kidney transplants between 01/2000 and 12/2009. Recipients/donors were devided into four groups according to their BMI (< 20, 20–25, > 25–30, > 30). DGF was defined as the requirement for more than one dialysis within the first post-transplant week. Impact of recipient-, donor- and transplant-characteristics were analyzed using uni- and multivariate analyses.

Results: Overall DGF rate was 32.4%, mean BMI was 23.75 (SD ± 3.8) for all recipients and 24.68 (SD ± 3.6) for all donors (median age 44.0; 40.3% female). In univariate analyses DGF rate was 25.2%, 29.8%, 40.9% and 52.6% in recipients with a BMI < 20, 20–25, > 25–30 and > 30 respectively ($P < 0.0001$). Donor BMI < 20, 20–25, > 25–30 and > 30 resulted in a DGF rate of 22.5%, 31.0%, 37.3% and 51.2% ($P < 0.0001$) in univariate analyses. Acute rejection (AR) rate in the DGF-group was 24.9% vs. 9.7% ($P < 0.0001$). BMI in AR-group was 24.72 versus 22.4 ($P = 0.0001$). Multivariate analyses revealed overweight in the recipient as an independent risk factor for DGF.

Conclusion: Not only recipient but donor BMI as well closely correlates with the incidence of DGF after cadaveric kidney transplantation. Awareness thereof should have an impact on peri- and post transplant measures in order to avoid DGF and complications thereof in cadaveric renal transplant recipients.

P052 INCIDENCE AND CHARACTERISTICS OF CYTOPENIA IN RENAL ALLOGRAFT RECIPIENTS EXPOSED TO 200 VS. 100 DAYS OF VALGANCICLOVIR PROPHYLAXIS – A SUBANALYSIS OF THE IMPACT STUDY

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Background: Extending valganciclovir (VGCV) prophylaxis in kidney transplant recipients from 100 to 200 days significantly decreases the incidence of CMV disease and viremia. This subanalysis of the IMPACT study assesses clinically relevant cytopenic events.

Methods: In this international, randomized, prospective, double-blind study in 318 CMV D+/R- patients received VGCV prophylaxis of 900 mg/d to 200 days post transplant (200d), in comparison to 100 days followed by placebo to day 200 (100d). Adverse events (AE) and serious adverse events (SAE) for leukopenia/neutropenia were assessed according to clinical severity and temporal occurrence in association to VGCV exposure. Furthermore, a toxicity grading was applied to assess captured central laboratory values.

Results: In total, 97 (30.3%) and 46 recipients (14.4%) experienced leukopenia and neutropenia as an AE at least once during the defined assessment period, respectively (Table). Leukopenia was graded mild/moderate in 76.9% (30/39) and in 91.4% (53/58) cases cases in the 100- and 200-day treatment group, respectively. Neutropenia – affecting less than 15% of patients – was graded as SAE in 37.5% (9/24) and 45.5% (10/22) cases in the 100- and 200-day treatment groups, respectively.

The proportion of patients with treatment emergent grade 4 white blood cell count abnormalities was similar during the first (0.6% vs. 1.3%) and the second 100 days (0.7% vs. 1.4%) in the 100- and 200-day groups, respectively. For neutropenia, the proportion were 5.5% vs. 6.4% (first 100 days) and 4.0% vs. 2.1% (second 100 days) in the 100- and 200-day groups, respectively.

Conclusion: The overall occurrence of AE for leukopenia and neutropenia in the study was low, with a marked decrease in incidences during the second 100 days of VGCV treatment. Leukopenia and neutropenia continued to occur even after cessation of VGCV exposure in the 100-day treatment group, although at a lower rate.

P053 PROTECTIVE ROLE OF APOLIPOPROTEIN E IN AN EXPERIMENTAL MODEL OF ACUTE RENAL ALLOGRAFT REJECTION

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Objective: Apolipoprotein E (Apo E) is a multifunctional protein, originally described in the context of lipoprotein metabolism and cardiovascular disease. More recently, anti-inflammatory functions of ApoE have been documented. ApoE was studied in the context of several inflammatory disorders, but its role in the pathogenesis of acute organ rejection is unknown. In this study, we test the hypothesis that ApoE attenuates acute renal allograft rejection.

Methods: The Dark Agouti or Brown Norway to Lewis rat strain combination was used to investigate fatal acute rejection. In addition, Fischer 344 kidneys were transplanted to Lewis rats to study reversible acute rejection. Isograft recipients and untreated Lewis rats were used as controls. ApoE mRNA expression was tested in intravascular leukocytes accumulating in the blood vessels of renal grafts and in graft tissue. Apo E protein levels were assessed in graft tissue and in plasma.

Results: Intravascular graft leukocytes and renal tissue obtained from animals undergoing reversible acute rejection expressed increased levels of ApoE mRNA, whereas during fatal rejection, ApoE expression remained unchanged. On the protein level, no changes in ApoE were seen in graft tissue and in plasma. However, we do not know if local leukocytic ApoE expression results in increased ApoE concentrations inside graft blood vessels. To test the protective potential of ApoE, recipients of Brown Norway kidneys were treated with ApoE-mimetic peptide. Preliminary data suggest that this treatment can reverse fatal acute rejection.

Conclusions: ApoE may play a protective role in acute organ rejection and may have a therapeutic potential.

P055 ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION USING ANTIGEN-UNSPECIFIC IMMUNOADSORPTION

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Purpose: ABO-incompatible kidney transplantation accomplished by desensitization with antigen-specific immunoadsorption (IA) results in good outcomes. However, a unique adsorption device is needed creating high cost.

Methods: From August 2005 to August 2010, 19 patients were desensitized for ABO-incompatible living donor kidney transplantation. Six patients treated with antigen-specific IA and 12 patients treated with antigen-unspecific IA were analyzed. A protocol was established with several differences to the original Stockholm protocol: (1) a starting isoagglutinin titer of > 1:128 was accepted, (2) use of antigen-unspecific IA, (3) number of preoperative IA dependent on starting antibody titer, (4) target isoagglutinin titer at transplantation < 1:16, (5) no postoperative IA, (6) no intravenous immunoglobulins, and (7) basiliximab induction on days 0 and 4.

Results: Median starting isoagglutinin titer before desensitization in Coombs technique was 1:32 and 1:64 in patients with antigen-specific IA and antigen-unspecific IA, respectively. Six patients that received antigen-specific IA had a median of 5 IA treatments, 12 patients with antigen-unspecific IA had a median of 6 IA treatments. Median average titer drop in Coombs technique was 1.2 and 1.7 in antigen-specific IA and antigen-unspecific IA, respectively. In two patients (33%) and four patients (33%) with antigen-specific IA and antigen-unspecific IA, IA was not sufficient for recipient desensitization and a median of 8 and 2 additional plasmapheresis treatments were performed, respectively. One patient with a starting isoagglutinin titer of 1:1,024 (Coombs) had 6 treatments with antigen-specific IA and 12 plasmapheresis treatments but could not have been transplanted. The 18-month graft survival rate for the 17 ABO-incompatible living donor kidney transplants was 100%. One male recipient who was desensitized according to the Stockholm protocol died 44 months after ABO-incompatible living donor kidney transplantation from sudden cardiac death with a serum creatinine of 1.2 mg/dl. All other patients were alive with a functioning allograft at last visit. At last follow-up, median serum creatinine for 16 patients was 1.5 mg/dl, median MDRD-GFR 54 ml/minutes/1.73 m^2, and median urinary protein to creatinine ratio 0.1.

Conclusion: We present a simplified protocol for the desensitization for ABO-incompatible kidney transplantation that is also used for the desensitization of crossmatch-positive patients with only minor modifications.

P056 EXPRESSION OF TUBULAR WATER AND SODIUM CHANNELS IN HIGHLY SENSITIZED KIDNEY TRANSPLANT PATIENTS WITH ALLOGRAFT DYSFUNCTION

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Purpose: Kidney allograft dysfunction is often accompanied by disturbances of salt and water homeostasis. The aim of this study was to further clarify the role of the water channels, aquaporins (AQPs) and the epithelial sodium channel (ENaC) after kidney transplantation.

Methods: We studied the expression of AQP-1, AQP-2 and ENaC by immunohistochemistry using semiquantitative analysis. Time-zero biopsies (group 2), protocol biopsies (group 3) or indicational biopsies (group 4) taken from kidney transplants of 33 highly sensitized patients were compared to biopsies of the tissue bank of the Department of Pathology of the University of Heidelberg (control group 1). Biopsy specimens were evaluated according to the BANFF criteria and graded according to the BANFF '07 update of the BANFF '97 classification.

Results: We observed a down-regulation of the expression of AQP1 in the time-zero kidney biopsies (group 2) compared to the biopsies of the control group (group 1). AQP2 protein expression was significantly downregulated in patients with an indicational biopsy (group 4) as compared to the time-zero biopsies (group 2) and the protocol biopsies (group 3). For ENaC a lower expression in the group of protocol biopsies (group 3) and in the group of indicational biopsies (group 4) compared to the control group (group 1) and the time-zero-biopsies (group 2) were observed. Spearman rank correlation indicated that the expression of AQP2 correlated to a decline of the MDRD-glomerular filtration rate and to indicational biopsies ($P < 0.05$) whereas the histological finding of acute rejection did not correlate to any of the examined proteins.

Conclusion: These data suggest a significant correlation between a decline in kidney function and disturbances of salt and water homeostasis with a downregulation of AQP2. A relationship of the histological findings of acute rejection to the examined proteins AQP 1, AQP2 and ENaC was not established.

P057 REVERSAL OF REFRACTORY ANTIBODY-MEDIATED REJECTION IN ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION BY ECULIZUMAB

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Purpose: Antibody-mediated rejection occurs in 15% of patients after ABO-incompatible kidney transplantation and is associated with allograft loss.

Methods: We report on reversal of refractory antibody-mediated rejection by the complement C5 inhibitor eculizumab which prevents generation of the cytotoxic membrane-attack complex C5b-9.

Results: A 62-year-old patient was referred for ABO-incompatible living donor kidney transplantation from his spouse. The donor had blood group AB, the recipient blood group A with an anti-B isoagglutinin titer of 1:16 (Coombs). In addition, donor-specific antibody against HLA-A32 (4,014 MFI) was detected by Luminex testing. The patient was successfully desensitized after seven immunoadsorptions and 750 mg rituximab. The anti-B isoagglutinin titer was reduced to 1:1 and the donor-specific HLA antibody to 105 MFI. Kidney transplantation was performed and, simultaneously, the patient underwent radical prostatectomy for early stage cancer. The postoperative course was unremarkable, and serum creatinine declined to 1.5 mg/dl on postoperative day 7. However, on the same day the patient developed 40 °C fever due to a surgical site infection. The anti-B isoagglutinin titer rose to 1:512 on day 10. A biopsy obtained on day 9 showed no rejection. After nine additional immunoadsorption treatments, the anti-B isoagglutinin titer fell to 1:4, however, serum creatinine increased to 4.8 mg/dl and the patient became oligo-anuric. Allograft biopsy on day 16 revealed antibody-mediated rejection. The patient received 600 mg Eculizumab on day 20. On day 24, urine output had returned to 5.8 l/day. Serum creatinine rapidly declined to 1.9 mg/dl on postoperative day 37, and CH50 and C5b-9 levels returned to normal indicating sustained termination of complement activation even after Eculizumab was eliminated from the circulation. Impaired microvascular kidney allograft perfusion assessed by real-time contrast enhanced sonography recovered from 4.8 dB/s before the administration of Eculizumab to 11.7 dB/s on day 29. The most recent serum creatinine on postoperative day 110 was 1.9 mg/dl.

Conclusion: This is the first report on the successful use of Eculizumab for reversing refractory antibody-mediated kidney allograft rejection after ABO-incompatible transplantation. Our data suggest that transient inhibition of complement by Eculizumab may be a suitable strategy for preventing antibody-mediated rejection and establishing allograft accommodation after ABO-incompatible kidney transplantation.

P059 RETROSPECTIVE ANALYSIS OF RENAL CO-MORBIDITY IN PATIENTS AFTER LUNG- OR COMBINED HEART-LUNG-TRANSPLANTATION

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Purpose: After solid organ transplantation, a high percentage of patients develop chronic kidney disease (CKD). The constant decrease in renal function is a serious and unsolved problem in this group of patients. The purpose of this retrospective analysis of patients after lung or combined heart-lung transplantation was to identify risk factors that could influence progression of renal disease.

Methods: A retrospective analysis was performed in Hannover Medical School. All adult lung transplant recipients between 1.7.1993 and 31.12.2007 were included. Patients after re-do procedure and not followed in our outpatient clinic were excluded. Risk factors to develop CKD were analyzed using Log-Rank Test (Mantel-Cox) and grouped for patients reaching the endpoint CKD KDOQI-Stage 3 (GFR < 60 ml/minute) or Stage 4 (GFR < 30 ml/minute).

Results: A total of 569 (46% female) patients were included transplanted at Hannover Medical School. The median age was 45 ± 12 years, 75% were double-lung transplant recipients (DLTx), and 8% were heart-lung transplant recipients (HLTx). Underlying diagnosis was COPD in 34%, pulmonary fibrosis in 24% and cystic fibrosis in 20%. with a median follow-up of 36 months. Patients started with a median baseline GFR of 109 ± 49 ml/min. 6.3% of these patients went on to dialysis. We found no difference in the groups reaching CKD-Stage 3 or 4 when we checked for gender, transplant era (Tx < 2000, Tx between 2000−2003 or Tx > 2003), diabetes, or when we compared the groups for cyclosporine or tacrolimus based immunosuppression. Significantly more patients were reaching the endpoint CKD-Stage 3 and 4 if the mean systolic blood pressure was above 130 and the diastolic blood pressure was above 90 mmHg ($P < 0.001$ and 0.014 respectively). A BMI of over 18.5 and a hemoglobin-level below 10 g/dl reached were associated with earlier developmentof CKD Stage 3 and 4. There were no differences if patients had tacrolimus trough levels < 8, 8−15 or > 15 ng/ml and cyclosporine trough levels < 100, 100−300 or > 300 ng/ml when they presented at the outpatient clinics after 12, 24 or 36 months.

Conclusion: In conclusion we found in our patient cohort, that blood pressure, hemoglobin levels and BMI are important risk factors for the development of CKD in patients after lung or combined heart-lung transplantation. These data suggest a necessity for sufficient blood pressure control and supplementation of erythropoietin in these patients.

P061 EFFICACY AND SIDE EFFECTS OF H1N1 VACCINATION IN RENAL TRANSPLANT AND DIALYSIS PATIENTS

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Background: In 2009 H1N1, a new influenza A virus, was discovered. It was responsible for the first influenza pandemic in the last 41 years. Similar to seasonal flu immunocompromised patients are likely to be at higher risk of severe infection. Therefore we vaccinated 31 renal transplant patients of our outpatient clinic (mean age 58.6 years + 13.5, 35.5% females, on the average 11.6 years + 7.5 years after transplantation) with the recommended new pandemic vaccine Pandemrix in November 2009 and determined the efficacy of vaccination under immunosuppressive therapy (antibody response rate, measured by hemagglutination inhibition assay (HI)). Furthermore, we looked for potential adverse events on transplant function and acute rejection rate.

Methods: We compared the antibody response rate of renal transplant recipients with the efficacy of vaccination in 47 dialysis patients (mean age 58.9 + 16.8, 27.5% females). Immunoprotection was stated with an H1N1 titre > 40, which was achieved in the general population in 98−100% (16 healthy controls). Serum samples were taken at least 4 weeks after vaccination and after 6−9 months, serum creatinine was determined 1 week, 4 weeks and 6 months after vaccination. Acute rejection episodes were monitored. Immunosuppression consisted of dual or triple therapy including CsA or tacrolimus, MMF or azathioprin and steroids.

Results: Only 50% of the transplant patients responded sufficiently to vaccination. In contrast to 38 out of 47 (81%) of the dialysis patients, who behaved similarly to the general population. This difference in response was highly significant ($P < 0.0005$) for transplant recipients compared to dialysis patients. Serum creatinine and GFR were stable in transplant patients over the whole time period (s-creatinine 1.7 mg/dl, GFR 50 ml/min). Acute rejection episodes did not occur. Within the transplant population responders and non-responders were not significantly different with respect to age, gender, time after transplantation, immunosuppressive medication or CD4 cell counts.

Conclusion: In renal transplant patients booster vaccination seems to be necessary to improve protection against H1N1 infection. This should be recommended, especially as AR or impairment of renal function were not observed under single shot Pandemrix vaccination.

P062 EFFECT OF APHERESIS FOR HLA AND ABO DESENSITIZATION ON ANTI-MEASLES-ANTIBODY TITERS IN RENAL TRANSPLANTATION

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Background: Recent outbreaks of measles and an increasing prevalence of non-immunized patients in Germany have focussed attention on the risk for immunosuppressed patients after renal transplantations. As desensitization strategies in patients with ABO blood group incompatibility or preformed donor-specific HLA-antibodies using plasma exchange (PE) or specific immunoadsorption (IA) further decrease immunoglobulin levels, we aimed to determine the impact of apheresis on anti-measles-antibodies.
Patients and methods: We included 14 patients: three patients with donor-specific HLA-antibodies (all PE) and 11 patients with ABO blood group incompatibility (1x PE only, 8x IA only, 2x IA and PE). Patients received pre-treatment with rituximab, IVIG and standard immunosuppressive therapy. Serum anti-measles-antibodies were measured before rituximab treatment, the day before transplantation and during follow-up by ELISA. A titer >150 mU/ml was defined positive. In 3 patients anti-measles-antibodies were determined before and after a single PE session.
Results: All patients had detectable anti-measles-antibodies before desensitization (median 2900 mU/ml, range 680–8100). In two patients tested regularly before and after transplantation, antibodies were stable over 3 years (SD 9% and 13%). During desensitization blood group or HLA-antibodies were reduced effectively. A single PE reduced anti-measles-antibodies by 42%. In patients treated with PE, anti-measles-antibodies decreased overall by 35% after 3–6 PE sessions. In one patient anti-measles-antibodies were even lowered under the threshold value. In patients with IA only, anti-measles-antibodies also decreased, but all patients had protective levels at time of transplantation. During follow-up (median 64 days after transplantation) in all patients anti-measles-antibodies increased to baseline values (median 3900 mU/ml, range 530–7100).
Conclusion: Immunity against measles was detectable in all patients. Anti-measles-antibodies were temporarily reduced by apheresis, but remained detectable in all but one patient. Although desensitization strategies result in prolonged reduction of HLA and blood group antibodies protective immunity against measles was not permanently compromised.

P063 EFFICACY, SAFETY AND OPTIMISED DOSING IN TACROLIMUS PROLONGED RELEASE VS TACROLIMUS IMMEDIATE RELEASE-BASED THERAPY IN RENAL TRANSPLANTATION – THE OSAKA STUDY

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Aim: The efficacy and safety of tacrolimus (Tac) prolonged-release (QD) and Tac immediate-release (BID) were compared in this multicenter, open-label, four-arm, parallel-group Phase IIIb international study with adult kidney transplant recipients.
Methods: Patients (n = 1251) were randomized 1:1:1:1 to 0.2 mg/kg/day Tac BID (Arm1), 0.2 mg/kg/day Tac QD (Arm2), 0.3 mg/kg/day Tac QD (Arm3), all with MMF+corticosteroids (CS) for 24 weeks, or 0.2 mg/kg/day Tac QD + MMF + basiliximab + peri-operative CS in Arm4. The primary composite endpoint was efficacy failure rate (graft loss, biopsy-confirmed acute rejection [BCAR], graft dysfunction). Graft dysfunction was set at a glomerular filtration rate (eGFR) of <40 ml/min/1.73 m^2. The pre-specified non-inferiority margin was 12.5% (per-protocol-set [PPS]).
Results: Mean age of organ donors was 51.5 years and ~50% were extended criteria donors (> 60 years, or > 50 years with two other risk factors). Non-inferiority was established for Arm2 vs. Arm1 in the PPS (95%CI:-12.2, 9.0) and for Arm3 vs. Arm1 in the full analysis set (FAS) only (95%CI: –10.3; 7.1). Efficacy failure rates for the PPS were: 40.6% (Arm1), 42.2% (Arm2), 44.2% (Arm3), and 48.2% (Arm4). The main contributor for efficacy failure was eGFR <40 ml/min. Incidence of BCAR and severity of BCAR were comparable across treatment arms (Arm1: 13.6%; Arm2: 10.3%; Arm3: 16.1%; Arm4: 12.7%). In each arm, > 94% of recipients experienced adverse events, with the majority (~60%) mild or moderate.
Conclusions: Tac QD-based therapy is non-inferior to Tac BID-based therapy for efficacy in renal transplantation, with Tac QD (0.2 mg/kg/day) comparable to Tac BID (0.2 mg/kg/day). An increased starting dose (0.3 mg/kg/day) offered no additional advantages. Seemingly high efficacy failure rates may reflect the level of eGFR set for graft dysfunction and the high number of extended criteria donors.

P064 URINARY NGAL ALLOWS FOR DIFFERENTIAL DIAGNOSIS OF ACUTE KIDNEY INJURY IN RENAL ALLOGRAFT RECIPIENTS

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Rationale: Neutrophil gelatinase-associated lipocalin (NGAL) regulates growth and differentiation in renal epithelia and is a component of innate immunity to bacterial infection. In the absence of systemic inflammation (SIRS), urinary NGAL is of renal origin and an early and specific marker of acute kidney injury. Here, we demonstrate urinary NGAL at respective cut-off to accurately predict acute rejection among other causes of acute kidney injury in renal allograft recipients on maintenance immunosuppression.
Methods: Spot urine specimen were prospectively assessed in 182 consecutive renal allograft recipients on maintenance immunosuppression upon presentation at our outpatient clinic. Samples were blinded and NGAL concentrations determined by ELISA. Patient data were classed according to allograft function and AKIN criteria into stable allograft function or acute kidney injury (AKI) and according to underlying condition into control, chronic allograft nephropathy (IFTA), bacterial- or viral infection, allograft rejection or other.
Results: In stable allograft recipients, median urinary NGAL [interquartile range] was 6.9 [3–13] ng/ml, or 10.8 [5–27] μg/g creatinine. Overall correlation between absolute and normalized values was r_P 0.92. A moderate increase was seen in IFTA, CMV and BKV infection. Urinary tract infection was associated with a significant increase in urinary NGAL, yet highest values were observed in acute allograft rejection. With a cutoff at 30 ng/ml, urinary NGAL discerned stable allograft function from AKI (AUC-ROC 0.93, sensitivity 0.81, specificity 0.95). At cutoff 100 ng/ml, elevated urinary NGAL accurately predicted acute allograft rejection within our cohort (AUC-ROC 0.98, sensitivity 1.0, specificity 0.93), even in the presence of urinary tract infection.
Conclusion: Urinary NGAL, at respective cutoff, accurately predicted acute allograft rejection among all other causes of acute kidney injury in kidney transplant recipients. As a readily available parameter, urinary NGAL facilitates to quickly delineate the pathogenesis of renal functional deterioration in allograft recipients presenting with a rise in serum creatinine.

P065 TERMINAL COMPLEMENT BLOCKADE BY ECULIZUMAB EFFECTIVELY REVERSES RECURRENT ATYPICAL HEMOLYTIC UREMIC SYNDROME AFTER KIDNEY TRANSPLANTATION

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Rationale: Recurrence of atypical hemolytic uremic syndrome (aHUS) is frequent after kidney transplantation, limiting transplant options for these patients. The reported incidence of 15–90% is largely dependent upon the underlying dysfunction of the complement system. Plasmapheresis is current standard therapy, yet of limited efficacy. The humanized C5b-antibody eculizumab is a novel therapeutic option, blocking terminal complement activation. We report eculizumab to effectively reverse recurrent aHUS in kidney transplantation.
Clinical setting: A 43-year-old patient with a history of post-partal aHUS presented for second kidney transplantation. Mutation of complement factor H had been ruled out. On day 7 after transplantation, the patient developed severe recurrent aHUS with systemic hemolysis, thrombopenia and acute kidney injury under calcineurin inhibitor-free immunosuppression. Complement C5b-9 (membrane attack complex, MAC) was highly detectable.
Results: In consideration of therapeutic options, eculizumab were administered. Eculizumab was continued weekly, with subsequent prolongation of intervals. Hemolysis and thrombopenia ceased quickly and allograft function fully recovered. No unwanted side effects were observed. Under continuous monitoring of SC5b-9, dosing intervals of eculizumab were successively tapered to every other month, currently. 6 months after transplantation, allograft function is excellent with an eGFR of 41 ml/min/1.73 m (serum creatinine 1.4 mg/dl) and C5b-9 within reference range.
Conclusion: Complement C5b blockade by eculizumab is highly effective in reversing recurrent aHUS in kidney transplantation. Pharmacodynamic monitoring of C5b-9 may guide adaptation of eculizumab dose and interval. As novel treatment option, eculizumab may facilitate access to transplantation for patients with aHUS.

P066 DUAL KIDNEY TRANSPLANTATION – AN UNDERUSED OPTION TO IMPROVE PATIENT OUTCOME AND UTILISATION OF EXTENDED CRITERIA DONOR ORGANS

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Rationale: Donor demographics, organ shortage and waitlist mortality have necessitated the increased acceptance of extended criteria donor (ECD) organs in kidney transplantation. Successful transplantation of ECD organs requires tailored organ allocation, peritransplant management and immunosuppression. Despite organ shortage, up to 30% of ECD organs allocated are not transplanted both, in Eurotransplant and UNOS regions, often due to uncertainty regarding functional reserve of the organ. Dual kidney transplantation provides an option to use these organs otherwise discarded and to improve patient outcome.
Methods: In 2009 we established the Tübingen two-for one (241) kidney transplantation programme. Suitability of recipients and ECD kidneys is assessed in a standardized decision tree including histopathology of allocated

organs. Kidneys are transplanted separately to the left and right fossa iliaca. The 2 year experience and outcome of our 241 programme is presented.

Results: Since 2009, six patients routinely received dual kidney transplantation. Median donor age was 85.5 [68–86] years. No surgical complications were observed. Renal function upon discharge was (eGFR) 36.2 [25.9–47.0] ml/minute/1.73 m with a scintigraphic side ratio (left/right) of 47.5 / 52.5, and 27.1 [18.5–42.9] ml/minute/1.73 m at a median follow-up of 365 [133–594] days. One patient suffered acute cellular rejection, responsive to corticosteroids. Patient and allograft survival is 100%.

Conclusion: In specialized programmes, dual kidney transplantation may reduce waiting time and improve patient outcome and utilisation of ECD organs, compared to single kidney transplantation. Strict donor organ and recipient selection is a pre-requisite. We recommend development and implementation of validated scores for donor and recipient assessment in dual kidney transplantation.

P067 RENAL TRANSPLANTATION IN OBESE PATIENTS – WHERE ARE THE LIMITS?

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Background: The incidence of obesity among patients waiting for a renal transplant is continuing to increase. Depending from the age group it affects up to 70% of the patients. Obesity represents a significant health risk for ischaemic heart disease, hypertension, dyslipidaemia and insulin resistance. The impact of recipient obesity on patient and graft survival is controversial. In the general population, obesity significantly increases the risk of perioperative complications such as wound infections, delayed wound healing, hernias and deep vein thromboses.

Question: Are graft and patient outcome, post-operative course and renal function affected by obesity of the recipients? Shall we suspend obese patients from renal transplantation?

Material and methods: From January 1st to December 31st 2010 128 renal transplants were performed. Outcome in recipients with a BMI $\geq$ 30 was compared with recipients having a BMI < 30.

Results: Average BMI was 24.8 among all 128 recipients. 12 patients had a BMI over 30 (average 31.7). 22 patients (17.2%) suffered from a wound infection (superficial or deep with surgical revision). 5 patients with a BMI greater than 30 developed a wound infection (41.7%; $P < 0.01$). Outcome in these 5 patients was devastating. One patient died from sepsis, one patient had a prolonged clinical stay > 150 days. Average creatinine for the surviving 4 patients is 2.45 mg/dl compared with 1.71 mg/dl for the 116 patients with a BMI < 30 ($P < 0.01$). Creatinine among the obese patients without wound infection is 1.82 mg/dl ($P > 0.05$).

Conclusion: Obese recipients for a renal allograft show a significant elevated risk for a wound infection. If they suffer from an infected wound, the outcome is inferior to patients with a normal body weight. However without wound infection the outcome is similar to regular weight patients. Patients cannot be denied access to renal transplantation solely based on their body weight. Strategies for wound closure and post-operative management have to be adapted for obese patients to improve the results.

P068 SUCCESSFUL TRANSPLANTATION OF A SINGLE PAEDIATRIC KIDNEY INTO A STANDARD ADULT RECIPIENT

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Small paediatric grafts are usually transplanted into paediatric recipients. If offered to an adult recipient, uncertainty exists regarding the post-operative function and graft survival. Data on outcomes are scarce and grafts are often discarded due to this uncertainty.

We report on a successful kidney transplantation using a single right kidney from a 17-month-old male donor into a standard adult recipient. The child donor died after suffering anoxic brain damage as a result of a severe metabolic crisis. He had a history of metabolic disorder 'very long chain acyl-CoA dehydrogenase deficiency' (VLCAD), an autosomal recessive inborn error of metabolism characterized by impaired mitochondrial beta-oxidation of fatty acids resulting in recurrent hypoketotic hypoglycaemic conditions. Body mass index at time of hospital admission was 15 (10 kg, 83 cm height). The left kidney was successfully allocated to a paediatric recipient. The right kidney was allocated according to ET criteria to three adult recipients, all of whom declined to accept the graft because of the small graft size. Subsequently, allocation was switched to rescue allocation (i.e., the organ was offered in a centre-oriented way). Six regional transplant centres declined acceptance of the kidney for all compatible recipients for the same reason. The kidney was accepted for a 46 years old female recipient with chronic renal failure on the basis of chronic interstitial nephritis. Recipient bodymass index at time of transplant was 31 (75 kg, 155 cm). She was first admitted to dialysis in September 2009 and medical history revealed arterial hypertension, hypertensive cardiomyopathy and a hiatal hernia. Blood groups of the transplanted graft and recipient were identical and HLA mismatch was 2-1-1. Kidney anatomy was normal and reperfusion was performed after 13.5 h of cold ischaemia. The post-operative course was uneventful with immediate start of sufficient diuresis. A slow but constant decrease of retention parameters was observed. Serum creatinin was 6 mg/dl on the day of transplantation and decreased to 3.1 mg/dl, 2.5 mg/dl and 1.5 mg/dl on post-operative days 14, 28, and 46, respectively. The size of the kidney graft was 7 cm $\times$ 4.5 cm $\times$ 3 cm on the day of transplantation; a CT scan 2 month post-transplant revealed a size of 9 cm $\times$ 6 cm $\times$ 6 cm. The patient was discharged on post-operative day 10. At this time (3 month post-operatively) the patient is well without episodes of rejection or severe post-operative complications.

We conclude that single paediatric kidney grafts from donors younger than 5 years can be transplanted successfully into standard adult recipients. This is consistent with data recently published from US transplant centres. In this, transplantation of single kidneys from paediatric donors less than or equal to 10 kg into standard adult recipients was associated with an increased risk of delayed graft function without compromising 2-year graft survival or function.

P069 COMPARISON OF THE ''ANTERIOR APPROACH'' OPEN RETROPERITONEAL AND THE RETROPERITONISCOPIC HAND-ASSISTED (HARP) DONOR NEPHRECTOMY

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Introduction: Donor safety and comfort as well as graft function are the main criteria to choose of a live donor procedure. Minimal-invasive procedures have become increasingly popular due to advantages concerning donor comfort. The following analysis examines perioperative data of one center switching from an open retroperitoneal to a retroperitonoscopic approach.

Patients and methods: The analysis includes the last consecutive 30 open retroperitoneal donor nephrectomies (open) until June 2010 and the first 30 consecutive retroperitoniscopic donor nephrectomies (HARP) thereafter.

There were no differences in donor characteristics': gender female 19 (open) versus 22 (HARP), right kidney 6 (open) vs. 9 (HARP), donor age(years) 52.5 ± 9 (open) vs 52.3 ± 11 (HARP), BMI 28 ± 5 (open) vs 26 ± 4 (HARP), more than 1 artery 0 (open) vs 2 (HARP).

Results: There was no mortality and no major morbidity in all 60 donors. There was one case of superficial wound healing disturbance in each group. One patient in the HARP group developed a metamizol induced leucopenia, which revealed after discontinuation of metamizol. The pain score according to a visual analog scale was comparable in both groups, however the amount of medication needed was significantly higher in the open group including 12 vs 0 peridural catheters. The following parameters showed a significant difference: mean operative time (minutes) 135 (open) vs 149 (HARP), warm ischemia time (seconds) 23 (open) vs 158 (HARP), blood loss (ml) 327 (open) vs 76 (HARP), intraoperative breathing volume (l) 6.5 (open) versus 9.3 (HARP), maximum pCO2 (mmHg) 36 (open) vs 41 (HARP). There were no differences concerning the following parameters at any point of time: donor hemoglobin, donor creatinine, recipient creatinine.

Conclusions: HARP can safely replace the open retroperitoneal approach. Besides advantages in cosmetics, there is a significant reduction in pain. Operative time and warm ischemia are longer without negative implications for transplant function. CO_2 inflation causes a significant increase of intraoperative ventilation volume.

P071 PROCEDURAL ADVANTAGE OF ABO INCOMPATIBLE LIVING DONOR KIDNEY TRANSPLANTATION

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Background: AB0 incompatible living donor kidney transplantation (AB0i) has evolved to a routine procedure. The key components of recipients pre-treatment generally involve anti-CD 20 AB induction, immunosuppression with tacrolimus and MMF, in addition to plasmapheresis or immunoabsorption to decrease anti ABO titers down to 1:4–8.

Methods: We have reviewed our last 67 living related renal transplants performed from 1.1.2009 till 31. 5. 2011 (14 LD-ABOi and 53 LD AB0 compatible) for outcome relevant parameters (patient and graft survival, s-creatinine, biopsy-proven acute rejections, lymphoceles, CMV and urinary tract infections).

Results: There was no difference in patient and graft survival between ABOi and AB0c recipients of a living renal allograft. There were significant more rejection episodes in the AB0c group (11, 20.8%) compared with the AB0i group (2; 14.3%) ($P < 0.05$). Regarding post-operative complications lymphocele formation was significant more frequent in the ABOi (4; 28.6%) than in the AB0c (3; 5.7%) group ($P < 0.05$). No difference was seen in CMV or urinary tract infections.

Discussion: For the time being ABO incompatible living donor kidney transplantation seems to have a superior outcome as compared to regular LD kidney transplants. There are significant less rejection episodes. Graft function shows a trend towards better serum creatinine. However, lymphocele formation may be a specific complication of ABOi pre-treatment. The surgical procedure should be adapted to this problem. As the influence of B-cell activation for acute and chronic rejection becomes more apparent in the recent

years pre-treatment with anti CD 20 AB for recipients of an AB0c living renal graft should be discussed.

P072 RECURRENCE OF GOODPASTURE'S DISEASE 14 YEARS AFTER RENAL TRANSPLANTATION

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Introduction: Goodpasture's disease is characterized by circulating antibodies directed against the glomerular basement membrane (GBM) presenting with pulmonary hemorrhage and rapidly progressive glomerulonephritis often leading to end-stage renal failure.

Case report: We report the case of a 41-year old male who was admitted with rapidly worsening of the renal function accompanied by hemoptysis 14 years after renal transplantation due to Goodpasture's disease. The immunosuppressive therapy consisted of tacrolimus and mycophenolate mofetile. The baseline serum creatinine level amounted about 1.0 mg/dl the last years before. Laboratory investigations indicated acute renal failure with a serum creatinine of 5.6 mg/dl. Urin analysis was remarkable for proteinuria and hematuria with acanthocytes. Biopsy of the renal allograft was performed the same day. Histology stated extracapillary proliferative glomerulonephritis with crescents and blood tests were positive for anti-GBM antibodies (47.7 IU/ml) but negative for ANCA. X-ray of the chest showed bilateral interstitial perihilar shadowing. Recurrence of Goodpasture's disease was diagnosed and treatment was started with steroids and plasma exchange followed by repeated intravenous cylophosphamide. Thereby, hemoptysis disappeared and the patient improved clinically. Unfortunately, kidney transplant function did not recover and the patient needs further dialysis.

Discussion: In patients with Goodpasture's disease the incidence of recurrent IgG-staining in the renal allograft may be as high as 50%. However, most patients remain asymptomatic and graft loss due to recurrent disease is rare. Administration of immunosuppressive maintenance therapy may be one important reason. Usually recurrence occurs within a couple of years after transplantation. Recurrence more than ten years after transplantation has not been described yet. Treatment of recurrent disease includes steroids, cylophosphamide and plasma exchange, but as in native kidneys therapy sometimes cannot avoid the development of end-stage renal disease.

P073 MANAGEMENT AND OUTCOME OF INFECTIONS BY KLEBSIELLA PNEUMONIAE WITH DECREASED SUSCEPTIBILITY TO CARBAPENEMS IN RENAL TRANSPLANT PATIENTS

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Background: Up to 20% of urinary tract infections (UTI) in renal transplant recipients (RTR) are reported to be caused by ESBL-producing bacteria. Due to their high resistance to ESBL-mediated hydrolysis, carbapenems are the treatment of choice in these cases. The occurrence of infections with carbepenem-resistant *Klebsiella pneumoniae* (KPC) however is a life-threatening event. Especially since optimal treatment of infections with KPC is not well defined and nephrotoxic agents seem to be required to reduce mortality rates ranging up to 57%.

Methods: We report an outbreak of *K. pneumoniae* with resistance or decreased susceptibility to carbapenems in six RTR of our centre between 05/2010 and 01/2011. Patient age was 65.5 ± 2.9 years. Five patients were de novo transplant recipients (index cultures (IC) 3 to 6 weeks after Tx), one patient was 5 years post-transplantation. Immunosuppressive regimens consisted of Basiliximab, MMF, steroids and CsA or Tac according to the local standard protocol. All patients received perioperative antibacterial-prophylaxis with imipenem and longterm PCP-prophylaxis. Antibiotic susceptibilities of isolated *K. pneumoniae* were determined by Vitek2 and E-test. Genetic relatedness was investigated using Enterobacterial Repetitive Intergenic Consensus (ERIC) – and repetitive sequence based (REP)-PCR. Follow-up is 9.5 ± 2.6 months. All values given as mean ± SD.

Results: Ertapenem-resistant *K. pneumoniae* were isolated from rectal swabs, urine or BAL-fluid. All *K. pneumoniae* were resistant to penicillins, cephalosporins, ertapenem, fluoroquinolones and cotrimoxazol. Some exhibited elevated MICs for meropenem, doripenem or imipenem but remained susceptible. ERIC- and REP-PCR analysis of isolates from 5 of 6 patients showed an identical profile suggesting a clonal spread. One patient was only colonized, three presented with UTI. Two patients, having been treated recently for BPAR or relapsing ITP, were severely ill suffering from pneumonia and/or sepsis due to ertapenem-resistant *K. pneumoniae*. Antibiotic treatment consisted of high dose meropenem, gentamicin, and inhalative colistin in case of pneumonia, for 19 ± 9 days. In 4 of 5 patients with infection, MMF was transiently withdrawn. Trough-levels (ng/ml) for CsA [(203.3 ± 11.4) vs (194.1 ± 36.7) $P = 0.82$; $n = 3$)] and Tac [(12.5 ± 1.2) vs (8.1 ± 0.2) $P = 0.07$; $n = 2$)] did not differ significantly before and after IC were obtained. There is no difference in s-creatinine [mg/dl] at date of IC and at latest follow up [(1.85 ± 0.25) vs (2.1 ± 0.5) $P = 0.67$; $n = 6$). All patients are alive with functioning grafts.

Conclusion: In potentially life-threatening infections due to ertapenem resistant *K. pneumoniae* in RTR, excellent patient and graft survival can be achieved. Early onset combination therapy with gentamicin / high dose meropenem and transient withdrawal of MMF have proved save and efficacious in our cohort.

P074 INFECTED DONOR ORGANS AS A CAUSE FOR HIGH BK-VIRAL LOAD IN KIDNEY TRANSPLANTED PATIENTS?

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Introduction: BK-virus (BKV) infection may lead to polyomavirus BK-associated nephropathy (PVAN), which is a serious complication after renal transplantation (RTX) and may lead to allograft loss. The exact etiology is still unknown. Concerning BK-seropositivity of more than 90% in general population PVAN could either be caused by a new infection or reactivation of the virus from the recipient or by augmented replication of the persistent virus in the allograft. Aim of this retrospective single-center analysis was to detect the prevalence of BK viremia of kidney transplant recipients and search for characteristics of patients with very high viral load.

Methods: BKV-screening was made by serum-PCR in 309 patients (pts) who were transplanted during January 2005 and July 2009. Particular attention was given to pts who had at least once a viral load (VL) of more than 250.000 copies/ml (cop/ml).

Results: From 309 Caucasian kidney transplant recipients with at least one assessment of BK-PCR, PCR became positive (VL > 500 cop/ml) in 56 pts (18.1%). 10 pts (3.2%) had at least a VL of more than 250.000 cop/ml in one PCR. In this group the maximum values were between 253.106 cop/ml and 196.000.000 cop/ml. From all of the 10 pts allograft biopsy was made. In 4 (40%) preparations PVAN could be confirmed by immunohistochemistry using SV 40-antigen. Six pts (60%) showed no ''definitive PVAN'' by immunohistochemistry but 3 pts (30%) had epithelial cell necrosis or lesions of tubules in light microscopy which indicate a ''presumptive PVAN''. Neither significant increase of creatinine values nor previous elevations of drug levels of immunosuppressive-therapy were found. From the 10 pts with very high VL twice 2 pts have achieved the organ from the same donor. In one of these ''recipient-pairs'' a definitive PVAN could be confirmed by immunohistochemistry.

Conclusion: The prevalence of BK viremia in kidney transplanted patients in our centre is high (18.1%). In 40% of the patients with very high VL (> 250.000 cop/ml) a PVAN could be confirmed. From the 10 pts with the highest VL there were 4 pts who achieved their allograft from only two donors. In one of these ''recipient-pairs'' a PVAN could be confirmed in both allografts. This indicates that infected donor organs could be responsible for a high replication of BKV and also for PVAN. Prospective studies and monitoring of further ''recipient-pairs'' from shared allograft donors are necessary to confirm this assumption.

P075 ECULIZUMAB IMPROVES ATYPICAL HUS IN A PATIENT AFTER KIDNEY TRANSPLANTATION

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Atypical hemolytic uremic syndrome (aHUS) is a rare thrombotic microangiopathy leading to end stage renal disease in approximately 60% of patients. The hallmark of aHUS is the association with alternative complement pathway activation leading to deficient host cell protection and inappropriate complement activation on platelets and endothelial cells, particularly in the kidneys. In some cases, a complement factor H defect induces inappropriate activation of the membrane attack complex (MAC) C5b-9. Kidney transplantation in patients with *CFH* mutations is associated with high-recurrence rate and poor 1-year graft survival. Present therapeutic strategies include lifelong plasmapheresis. Kidney transplantation as well as combined liver-kidney transplantation are associated with a high rate of post-transplant recurrence of the underlying disease.

We report a 36-year-old woman with end stage renal disease since 1996 due to complement factor H mutation. In December 2010 she received a cadaveric kidney transplant. Both kidneys of a 16-month-old child were on bloc transplanted. The immunosuppressive regimen included induction with interleukin-2 receptor-antagonist basiliximab as well as a standard triple therapy consisting of cyclosporine A, mycophenolate mofetil, and steroids. Ureter complication needed revision 12 weeks after transplantation. Plamsapheresis started immediately after kidney transplantation was performed daily. When reduced to one session per week renal biopsy proven aHUS recurred 6 weeks after transplantation. Eculizumab was started once weekly (600 mg) for 4 weeks and thereafter 900 mg at monthly intervals. Eculizumab application has been extended to 8 weeks intervals half a year after kidney transplan-

tation. The function of the transplanted kidneys are excellent with a current creatinine value of 1.1 mg/dl. No signs of allograft rejection or recurrence of aHUS were detected after start of eculizumab therapy.

Eculizumab is a complement C5 blocking antibody which inhibits the formation of C5a and the complement membrane attack complex and has been approved for the treatment of PNH. We demonstrate in this patient that blocking complement activation in aHUS following renal transplantation may protect these patients from loss of renal function.

P076 RISK FACTORS FOR POST-TRANSPLANT DIABETES, RETROSPECTIVELY ANALYSED IN A COHORT OF THE HANOVER CONTROL BIOPSY PROGRAM

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Background and aim: The incidence of the so called ''post-transplantation-diabetes (PTD)'' after kidney transplantation was recently increasing. Some risk factors for PTD a sage or body mass index were already shown in recent trials. The increasing frequency of PTD-patients – however – was suggested to be associated with new immunosuppressive drugs. We aimed to identify the risk profile pf PTD in the cohort of our ''control biopsy program'' in Hanover concluding well established factors as well as co medication and immunosuppressive and explore the impact of PTD on transplant function over time.

Patients and methods: A total of 526 patients, transplanted and undertaking a control biopsy during October 2000 and November 2007, were included in the study. PTD was defined using repeated random glucose values as well as fastening glucose values as well as HbA1c. All data were statistically evaluated using appropriate models (Chi-Quadra *t*-test, Kruskal–Willis test, Mann–Whitney *U*-test).

Results: A total of 92 Patients were defined as PTD (17.5% of all patients). PTD was gender-independent. In 41% of the PTD-patients, PTD occurred within 90 days after tx. The man age of PTD-patients was higher as compared to non-PTD-patients (55.9 ± 3 vs. 47.9 ± 13.6 years, $P < 0.0001$). The body mass-index at tx was higher in PTD-patients (25.6 ± 3.5 vs. 23.7 ± 3.3) as well as serum phosphate levels (mM/l; 1.05 ± 0.23 vs. 0.98 ± 0.20; $P < 0.03$). The graft function (lowest GFR within the first 6 weeks after tx) was lower in PTD-patients (48.8 ± 23.2 vs. 52.67 ± 21.9 ml/min, $P < 0.05$) with a trend towards accelerated function loss one year after tx. Under initial immunosuppressive therapy using tacrolimus, the incidence of PTD was lower than in under cyclosporine initial therapy. After switching to tacrolimus during the maintenance phase in the 1st year after tx, PTD was more frequent (23.5% vs. 13.7%; $P < 0.04$). In this patient group, rejection episodes (0.67 ± 0.92 vs. 0.37 ± 0.64; $P < 0.0001$) and additional steroid bolus therapy were also more frequent (1.32 ± 0.95 vs. 1 ± 0.87; $P < 0.031$). Age-independently, in the PTD-patient group, the need for vitamin D substitution was higher (20.7% vs.12.7%; $P < 0.02$). The use of carvedilol (36% vs. 16.6%; $P < 0.013$) and central sympatholytic drugs (23.1% vs. 15.1%; $P < 0.029$) was also more frequent in PTD-patients.

Conclusions: Established risk factors for PTD as body mass index and age were confirmed in our control biopsy patient cohort. The more frequent use of distinct antihypertensive drugs as carvedilol and central sympatholytica votes for an increased co morbidity in PTD-patients as pre-disposing factor. The loss of vitamin D associated with PTD may be based upon a decreased insulin secretion rate, as suggested by recent results. We suggest that not the use of tacrolimus, but a higher rejection rate and need of steroid bolus therapy aggravate the occurrence of PTD in tacrolimus-switched kidney transplant recipients. In this risk group, also the trend to transplant function loss may be multifactorial.

P077 EVEROLIMUS AND MTORC1 SIGNALING IN ENERGY BALANCE AFTER DE NOVO KIDNEY TRANSPLANTATION

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Introduction: The mammalian target of rapamycin complex 1 (mTORC1) pathway regulates cellular responses to fuel/energy availability. Recent studies have demonstrated that mTORC1 represents an essential intracellular target for the actions of hormones and nutrients on food intake and body weight regulation. By being at the crossroads of a nutrient-hormonal signaling network, mTORC1 also controls important functions in peripheral organs, such as muscle oxidative metabolism, white adipose tissue differentiation and ß-cell-dependent insulin secretion. Everolimus (EVR), an mTOR inhibitor and immunosuppressant approved to prevent rejection of organ transplants affects solely the mTORC1 protein and not the mTORC2 protein. Here, the effect of an EVR based immunosuppressive regimen on evolution of body weight (BW) after kidney transplantation (KTx) is assessed.

Results: 662 *de novo* KTx recipients were randomized and prospectively exposed to either (1) l-EVR [C0 3–8 ng/ml] or (2) h-EVR [C0 6–12ng/ml] + reduced-CsA(RD-CsA) or (3) mycophenolate sodium(MPA) + standard-CsA(SD-CsA). The evolution of body weight (BW) after KTx was evaluated. Overall weight change Month(M) 1–12 was 5.6 kg for MPA, 4.9 kg and 4.8 kg for l-EVR and h-EVR, repsectively, (MPA vs. h-EVR, $P = 0.198$). BW analyses by baseline BMI categories showed: ≤ 20 kg/m^2 (3.0/4.6 kg); > 20–25kg/m^2 (5.7/5.8 kg); > 25–30 kg/m^2 (6.3/4.5 kg); >30kg/m^2 (5.6/4.1 kg) for MPA vs h-EVR, respectively (Fig 1). The same pattern was consistently observed in gender and age subgroup analyses. Multivariate/univariate risk factor analyses will be presented.

Conclusion: In *de novo* KTx recipients overall BW increase M1-12 was highest in MPA/CsA treated patients. However, a different pattern was observed when analyzed by BL BMI categories. Here, patients with a low and normal BMI had a higher BW increase when exposed to h-EVR, whereas patients with a high and very high BMI had greater increase when treated with MPA. Thus, mTOR treatment seems to not affect weight increase in low/normal BMI categories, but shows less increase in high/very high BMI categories. A possible explanation may be the effects of EVR on metabolic regulation and adipose tissue differentiation.

LIVER

P078 COMBINED HEPATOCELLULAR-CHOLANGIOCARCINOMA: AN INDICATION FOR LIVER TRANSPLANTATION?

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Background: Combined hepatocellular-cholangiocarcinoma (HCC-CC) is a rare hepatobiliary neoplasm combining hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC). Pre-operative diagnosis is important to determine the optimal therapy, but challenging. Only a few cases of liver transplantation in HCC-CC have been reported, yet.

Methods: In this retrospective observational study we report our single-center experience of 7 patients with HCC-CC initially diagnosed HCC, who underwent liver transplantation according to the Milan criteria. We compare these findings with a matched control group of 50 HCC regarding clinicopathological characteristics and outcome.

Results: HCC-CC patients were male in 86%, with a median age of 60 years and cirrhosis in 86%. Recurrence was observed in 57%, with a 3-year recurrence-free survival of 44.4% (confidence interval (CI) 0.167−1) and a 3-year overall survival of 34.3% (CI 0.112−1). Vascular invasion was observed in 43%, and lymph node metastasis in 14%. HCC patients were male in 82%, with a median age of 63 years and cirrhosis in 96%. Recurrence was observed in 20%, with a 3-year recurrence-free survival of 75.2% (CI 0.629-0.9) ($P = 0.0335$) and a 3-year overall survival of 58.4% (CI 0.458−0.744). Vascular invasion was observed in 32%, but no lymph node metastasis.

Conclusions: Although HCC-CC shared many features with HCC, the recurrence-free survival was significantly shorter than compared with HCC. From similar overall survival rates we conclude that liver transplantation in HCC-CC should be considered as therapeutic option. In advanced cases, an aggressive multimodal approach should be evaluated in further studies.

P079 JOINT IMPACT OF DONOR AND RECIPIENT PARAMETERS ON THE OUTCOME OF LIVER TRANSPLANTATION: THE GERMAN EXPERIENCE

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Introduction: The shortage of donor organs in Germany has led to the use of organs from donors with extended donor criteria (EDC). EDC have been defined on the basis of expert opinions, but their clinical relevance is controversial. This may lead to the loss of organs otherwise available for transplantation. We evaluated the impact of donor and recipient factors in liver transplants with EDC on patient and graft survival in a nationwide German multicenter analysis.

Material and methods: An anonymzed database was created from data on livers donated and transplanted in Germany between 2006 and 2008 as provided by Deutsche Stiftung Organtransplantation and BQS Institute. Cox regression (significance level 5%, risk ratio [95%-CI]) was used including only recipients with first liver transplants ($n = 2095$) for calculating impact on patient survival and all transplants ($n = 2175$) for impact on graft survival.

Results: Patient and graft survival were significantly affected only by donor age [1.012 (1.006−1.019) and 1.011 (1.005−1.017) per year], recipient age [1.019 (1.010−1.029) and 1.014 (1.006−1.022) per year], creatinine [1.248 (1.174−1.327) and 1.205 (1.136−1.278) per mg/dl], bilirubin [1.022 (1.014−1.030) and 1.023 (1.016−1.030) per mg/dl], and high urgency status [1.783 (1.312−2.423) and 1.809 (1.398−2.342)]. Inferior organ quality resulted in lower graft survival [1.243 (1.001−1.545)].

Conclusion: Multiple Cox regression revealed no significant impact of EDC or Donor-Risk-Index on patient and graft survival except for donor age, which is probably attributable to donor selection. Among recipient variables, only age, creatinine and bilirubin, and high urgency status were associated with poorer outcome.

P081 ACUTE CELLULAR REJECTION AFTER LIVER TRANSPLANTATION SHOWS INCREASED NUMBERS OF REGULATORY T CELLS

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Background and aims: Acute cellular rejection (ACR) occurs frequently after liver transplantation and is usually easily controlled. A lack of immunoregulation is suspected as a cause of such rejection episodes. In human various studies analysed regulatory T cells (Treg) in peripheral blood samples or intragraft FOXP3 expression after liver transplantation. Currently there are no investigations of intrahepatic immune responses during ACR.

Methods: We retrospectively analysed 151 patients (pat.) with graft hepatitis between 2004 and 2008, of whom 54 liver biopsies were stained with multicolour immunofluorescence (CD4, CD8, FOXP3, DAPI).

Results: In mild to moderate grades of ACR $CD4^+$ cells dominated the portal T cells infiltrates, while the continuous increase of $CD8^+$ cell infiltration dominated in severe ACR (BANFF ≥ 7). Furthermore CD8 and not CD4 infiltration is correlated with serologic markers of hepatocyte damage. Surprisingly the overall rise of portal effector T cells (Teff) density during ACR was accompanied by an up to 2 fold higher increase in portal Treg infiltraion. Neither portal Treg infiltration nor portal Treg/Teff ratio are influenced by immune suppressive regiment prior to biopsy. The continuous increase of intragraft Treg/Teff ratio may offer an explanation for the trend to a survival benefit of patients with more severe ACR (87.2% vs. 74.1%) and the low incidence of severe ACR (8%) in this study.

Conclusion: Intrahepatic immune response during ACR is not associated with a lack but rather an increase of Treg infiltration and thus may as well explain the low incidence as result in a survival benefit of higher grades of ACR after human liver transplantation.

P082 LIVER TRANSPLANTATION IN PATIENTS WITH ACTIVE PNEUMONIA

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Patients with chronic liver disease are at high risk for severe infection due to increased bacterial translocation and immune suppression associated with liver dysfunction. Furthermore patients presenting with severe pneumonia and acute decompensation of cirrhosis are generally not considered for liver transplantation because it is unknown if these patients can recover from infection while under immunosuppression.

We performed an observational study where patients with cirrhosis of the liver remained on the waiting list although suffering from active pneumonia.

The aim of this study is to review the survival rate of Patient with severe Pneumonia after lever transplantation.

Included patients had to fulfill common criteria for severe pneumonia with sepsis. Furthermore patients had to fulfill pre-defined criteria, which we considered necessary for an acceptable prognosis. After inclusion of a patient we commenced a goal-directed-therapy analogous to the treatment of sepsis and the patients were observed for 48 hours. When the patient's status deteriorated we did not perform transplantation.

Twelve patients on the waiting list for liver transplantation were screened for inclusion in the study because they suffered from severe pneumonia. Three patients were excluded because they did not fulfill the inclusion criteria and three patients were excluded because of deterioration under goal-directed therapy. The remaining six patients all underwent transplantation and all recovered quickly from infection and had acceptable graft function.

We therefore propose that in patients with chronic liver disease and active pneumonia transplantation is a treatment option that should not hastily be abandoned.

Our clinical experience suggests that this is not merely due to goal-directed therapy but also may be attributed to the restoration of liver function in these patients.

P083 LIVER TRANSPLANTATION IN PATIENTS WITH BUDD-CHIARI SYNDROME: AETIOLOGY, THERAPEUTIC STRATEGIES AND OUTCOME

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Background: Budd-Chiari-syndrome (BCS) is a relatively rare disease marked by an obstructed venous out-flow of the liver resulting in portal hypertension and hypoxia with consecutive liver cell necrosis which may end in acute liver failure. In some cases liver transplantation (LT) is the only therapeutic option. Aims of the study were to investigate the aetiology and the outcome after LT.

Patients and methods: We analyzed data of nine patients (7f) with BCS (7 acute, 2 subacute/chronic), who underwent LT between 06/1993 and 08/2010 at our center.

Results: The mean age of nine (2 m) patients with BCS at LT was 41 ± 12 years. The underlying thrombophilic disorders were: polycythaemia vera ($n = 3$), essential thrombozythaemia ($n = 3$) and Protein C-deficiency ($n = 2$). In one patient no causative disorder could be verified. 44.4% suffered from a thrombosis of the portal vein, six patients got an anticoagulant therapy ($n = 2$), and one patient a TIPS-implantation before LT. The mean waiting time from the primary diagnosis of BCS to LT amounted 5.6 months (0.1−36

months). Two patients required a re-transplantation due to thromboembolic complications 1 month and 7 years after LT. Mean follow-up was 1–113 months. Three (33.3%) patients died 2, 42 and 101 months after LT. All of the six surviving patients are in a good condition.

Conclusion: Despite of the thrombophilic disorders LT can be a successful therapeutic option in patients with BCS. In the context of an adaequate symptomatic therapy of the underlying disease a relapse could be prevented and the patients could obtain good survival rates.

P084 AUXILIARY LIVER TRANSPLANTATION IN 2 CHILDREN WITH ACUTE LIVER FAILURE

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Introduction: Acute liver failure (ALF) is a rare illness in children with a high mortality unless liver transplantation is performed. In some cases ALF resolves without need for transplantation and liver function normalizes with complete restitution of liver parenchyma. Therefore auxiliary partial orthotopic liver transplantation (APOLT) can be a good option for patients necessitating liver transplantation to bridge the acute situation and allow native liver to regenerate. This technique gives these patients the opportunity to withdraw immunosuppressive therapy.

Methods: We report on two children (2.25 and 9.5 years; one female, one male) who presented with fulminant ALF necessitating rapid liver transplantation. The cause of ALF was Non-A-to-E-Hepatitis in one case and a simultaneous infection with rotavirus and herpes virus type 6 in the other case. Both children received APOLT by a left lateral lobe of a deceased donor. The graft was placed in the position of the left lateral lobe after resection of the recipients liver segments I, II and III. Graft size was small enough to allow primary closure of the abdominal wall. In both cases standard immunosuppression with Basiliximab, Prednisolone and Cyclosporine was initiated. Graft function was monitored by laboratory testing, graft perfusion via Doppler ultrasound. Excretory liver function was screened by repeated HIDA scans.

Results: Both children survived and are doing well. In both cases graft function was good and rapidly stabilized the clinical situation, liver function tests gradually normalized. In the 2-year-old patient the native liver regenerated completely and immunosuppression could be withdrawn. In the older patient the native liver did not regenerate, graft function is good. This patient remains on immunosuppression.

Conclusion: These two cases emphasize that APOLT is a good option for patients with ALF necessitating transplantation. It gives the opportunity to withdraw immunosuppression if native liver regenerates.

P085 AUXILIARY LIVER TRANSPLANTATION IN A CHILD WITH CRIGLER-NAJJAR-SYNDROME TYPE I

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Introduction: Crigler-Najjar-Syndrome type I (CNS I) is a rare inherited disease caused by complete deficiency of bilirubin glucuronyltranferase activity. The disease presents with pronounced jaundice during the neonatal period and thereafter. The enzyme deficiency leads to markedly elevated values of unconjugated bilirubin with risk of neurologic complications. Acute treatment relies on daily phototherapy for multiple hours. Up to date the only effective long-term treatment is liver transplantation. Yet, someday genetic engineering may be a viable treatment. Therefore auxiliary partial orthotopic liver transplantation (APOLT) should be considered as a bridging option.

Case: We report on a 4-year-old girl who was transplanted because of CNS I. It was decided to perform APOLT because of above mentioned reasons. APOLT was performed by a left lateral lobe of a deceased donor. The graft was placed in position of the left lateral lobe after resection of the recipients liver segments I, II and III. Gaft size was small enough to allow primary closure of the abdominal wall. Immediately after transplantation bilirubin levels decreased to low normal values demonstrating good graft function. The remaining native liver demonstrated good perfusion at all times. Yet, portal venous perfusion of the graft was repeatedly compromised necessitating recurrent operations. In the end portal venous flow to the graft was stabilized by subtotal narrowing of the portal vein to native liver.

After a liver biopsy the child developed an arterio-portal fistula in the graft resulting in retrograde portal venous flow in the graft. Over the following weeks an increase of unconjugated bilirubin was evident giving suspicion of impaired graft function. Furthermore the graft decrease in size. It was therefore decided to embolize the arterio-portal fistula by interventional angiography. After embolization the portal venous flow in the graft recovered and remains orthograde up to date. Bilirubin levels returned to normal and graft size remains stable.

Summary: APOLT is a good option for correcting metabolic diseases mainly localized in the liver, giving patients the latter option of genetic engineering and withdrawal of immunosuppression. Surgically this technique is a challenging procedure. In our case the course was complicated by an arterio-portal fistula impairing graft function. After interventional embolization of the fistula graft perfusion and function recovered.

P087 ELEVATION OF LBP AFTER LIVER ISCHEMIA AND REPERFUSION INJURY IN RATS

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Background: LBP is an acute phase protein, which is mainly synthesized in the liver and up-regulated in inflammation and infection. The inflammatory response to LPS is mainly depending on the binding of LPS-LBP to CD14-MD2-TLR4 complex. During liver I/R, gut derived molecules, including endotoxin are released into the blood stream due to the congestion of intestinal wall. We were interested whether the expression of LBP was increased after liver ischemia and reperfusion injury in rats and was associated with the severity of the inflammatory response.

Methods: Rats were subjected to for 90 minutes warm ischemia followed by 0.5 hour, 6 hour and 24 hour reperfusion (n = 6/group), six rats were subjected to liver transplantation (LTx) and sacrificed 24 hour postoperatively. Another set of six rats were included as normal controls. Serum LBP levels were determined by ELISA method. The expression of mRNA of LBP, CD14, MD2, TLR4, TNF-α and IL-6 were measured by quantitative PCR.

Results: Following 90 minutes of warm ischemia, LBP mRNA expression was up-regulated to sevenfolds as early as 6 hours after reperfusion and further increased to 12-folds with reperfusion time up to 24 hours. LTx also increased the expression of LBP mRNA up to ninefold 24 hours postoperatively. LBP-protein was released into peripheral circulation with maximum to 43 ng/ml at 6 hours after warm I/R. The mRNA expression of CD14, TLR4 were increased up to 3–4 folds after warm I/R and LTx. Hepatic TNF-a and IL-6 mRNA were upregulated to 20-folds and 100-folds 6 hours after warm I/R, respectively.

Conclusion: Hepatic LBP mRNA expression was increased and serum LBP released into systemic circulation, and was associated with an increase of hepatic TNF-a and IL-6 mRNA expression. These findings suggest that the elevated LBP may mediate LPS-induced liver damage after I/R. Blocking experiments, planned as a next step, will help to elucidate its role as potential molecular target for preventing IR-injury.

P088 BENEFIT OF NEEDLE BIOPSY BEFORE LIVER TRANSPLANTATION IN HEPATOCELLULAR CARCINOMA

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Introduction: According to our treatment protocol patients with hepatocellular carcinoma (HCC) are repeatedly treated by transarterial chemoembolisation (TACE) before liver transplantation (LT). Needle core biopsies (NCB) were performed before including the patients into the protocol. As angioinvasion and grading are prognostic factors of tumor recurrence after LT and response to TACE may depend on grading, NCB is compared to histology of the surgical specimen (HSS) in order to access its accuracy.

Patients and methods: A total of 118 patients were included in this study. Grading in NCB was available in 79 patients before TACE (median 5 cycles) followed by LT. In 14 of these patients comparison of NCB and HSS was not possible due to total tumor necrosis after TACE. Grading of NCB was compared to HSS and correlated to tumor progression during TACE.

Results: Rate of identical grading in NCB when compared to HSS was 66%. False positive rate (overestimation of tumor grading) was 6% and false negative rate (underrating of tumor grading 28%. NCB was incapable of identifying angioinvasion. Tumor progression during TACE could not be predicted by grading (NCB: *P*:0.309, HSS: *P*:0.196).

Conclusion: The benefit of NCB in predicting definitive grading was questionable. The rate of identical grading in NCB and HSS reached only 66%. The extremely low sensitivity of NCB in recognizing poorly differentiated tumors is of major concern. Downgrading by TACE pre-treatment before LT could not be predicted by grading. Response to TACE is obviously controlled by factors different from grading.

P089 COGNITIVE FUNCTIONING IN CHILDREN WITH BILIARY ATRESIA AFTER LIVER TRANSPLANTATION

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Purpose: Because different primary diseases might bias cognitive functioning outcomes of liver transplanted children, we exclusively examined children with biliary atresia after transplantation (Ltx). We hypothesized that children are below the population mean.

Method: The sample consisted of 70 liver transplanted children (age at assessment: 8;4 ± 3;5 years, range: 5;0−15;2; age at Ltx: 1;5 ± 2;3 years, range: 0;1−14;8 years). 49% had received a living related donation (LRD). Assessment of cognitive functioning included: K-ABC (5−7 years, $n = 27$) and WISC-III (8−17 years, $n = 43$).

Results: Children scored within the normal range (100 ± 15) but significantly below the population mean in several subscales: K-ABC-Achievement-Scale (AS): 90.5 ± 16.2, t(25) = −3.0, $P = 0.006$; WISC-III-Performance-IQ (PIQ): 86.2 ± 16.0, t(42) = −5.7, $P = 0.000$; WISC-Full-Scale-IQ (FIQ): 91.5 ± 17.4; t(42) = −3.2, $P = 0.003$. Between 18.5% (SES) and 51.2% (PIQ) of the children scored below the normal range. Regarding all WISC-III-subscales, children with a LRD performed significantly better than children with a postmortem donation (PMD): LRD-VIQ: 102.4 ± 18.4 vs. PMD-VIQ: 89.8 ± 13.3, t(41) = −2.5, $P = 0.014$; LRD-PIQ: 92.2 ± 17.6 vs. PMD-PIQ: 80.0 ± 11.4, t(41) = −2.7, $P = 0.010$; LRD-FIQ: 97.4 ± 18.6 vs. PMD-FIQ: 85.3 ± 14.0, t(41) = −2.4, $P = 0.021$). LRD was associated with higher maternal educational level (r = 0.28, $P = 0.019$). High correlations between the height percentile pre-Ltx and intelligence subscales (r = 0.44, $P = 0.023$ to r = 0.57, $P = 0.003$) were obtained.

Conclusion: By assessing exclusively children with biliary atresia, we eliminated confounding due to various primary diseases. We confirmed our main hypothesis: Liver transplanted children with biliary atresia revealed more cognitive restraints compared to the norm. Children's preoperative decelerated body height could be regarded as an index of children's decelerated brain growth, which might be relevant to explain the interrelation between height percentile (pre-Ltx) and cognitive functioning after transplantation. However, family socioeconomic status seems to have a mediating role. Our results emphasize the urgent need of routinely performed psychological diagnostics and support to liver transplanted children.

P090 BEHAVIOUR AND SOCIAL FUNCTIONING AFTER PAEDIATRIC LIVER TRANSPLANTATION

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Purpose: Liver transplanted children have an increased risk to develop serious developmental problems. Based on previous research, we hypothesized that liver transplanted children show more behavioural problems and poorer social functioning compared to the norm.

Method: The sample consists of 117 children (53% girls, aged 10.3 ± 3.7 years) that completed a behavioural questionnaire late postoperatively (i.e., 8.8 ± 4.3 years after transplantation). The mean age at transplantation (Ltx) was 41.0 ± 46.1 months. Assessment included: behaviour (SDQ, self- and proxy-report) and intelligence (WISC, K-ABC).

Results: Regarding behaviour 77−91% of the assessed children scored within the normal range. In the subscales (proxy-report) hyperactivity (t = 5.0, $p < 0.001$), problems with peers (t = 8.7, $p < 0.001$), and total sum score (t = 3.0, $p = 0.004$) results were significantly below the population mean. In the self-report only problems with peers (t = 10.4, $p < 0.001$) was significantly below population mean. Here 22.7% of the children scored within the borderline or abnormal range compared to 13.3% in the norm population. Moreover, problems with peers was highly correlated with all subscales of the K-ABC (r = −0.37, $p = 0.04$ to r = −0.54, $p = 0.002$) and total IQ-score of the WISC (r = −24, $p = 0.04$). In semi-structured interviews, parents with children that experience more problems with peers reported more social problems at school (r = 0.49, $p < 0.001$), more problems with teachers (r = 0.38, $p = 0.004$) and more problems with subject materials (r = 0.38, $p = 0.001$).

Conclusion: The results corroborate our hypotheses in parts. Regarding social adjustment, our results provide evidence suggesting that liver transplanted children might be at risk of interpersonal difficulties, i.e. peer problems. Further research is needed to understand the origin of these problems. Interrelations between behaviour and intelligence support the previous findings that comprehensive psychological diagnostics and psychosocial support are necessary to ensure children's social integration.

P091 LIVER TRANSPLANTATION IN PATIENTS WITH ICU-STAY ASSOCIATED SECONDARY SCLEROSING CHOLANGITIS

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Background: Secondary sclerosing cholangitis (SSC) is a rare chronic hepatobiliary disorder characterized by inflammation of the intrahepatic and/or extrahepatic ducts, leading to narrowing or obliteration accompanied by local periductular fibrosis.

In the past few years, SSC was diagnosed in a growing number of patients who received aggressive treatment in an intensive care unit (ICU).

ICU-associated SSC is characterized by very rapid progression to biliary cirrhosis and/or acute liver failure.

Thus, early orthotopic liver transplantation (OLT) might be the therapy of choice in these patients.

Methods: Four patients (mean age 54 years, range 42−65, mean MELD pre-OLT:21, range 14−33) with ICU-associated SSC underwent OLT between 2007 and 2011. Diagnosis was done via ERCP/MRCP and verified by histology. Three patients had polytrauma, one patient developed SSC after respiratory failure due to Wegener's granulomatosis. No patient had pre-existent liver disease. Following parameters were investigated: duration of ICU stay, artificial breathing, haemodialysis, time to diagnosis, time to OLT, and post operation mortality.

Results: ICU stay was 82 days (mean; range 47−164) including ventilation for 31 days (mean; range 13−60) and haemodialysis for 42 days (mean; range 39−44). Time between initial event and OLT was 487 days (mean; range 148−987). Two patients were accepted as high urgency cases due to acute biliary liver failure. Explant histopathology confirmed pretransplant diagnosis in all cases. Mean post-transplant follow-up is currently 3 month (range: 1−5). Three of them are still alive.

Conclusions: Since survival rate of patients who do not underwent OLT is disastrous, OLT seems to be the therapy of choice in selected patients suffering from ICU associated SSC.

P092 CHANGES IN GLOBAL HAEMOSTASIS IN PEDIATRIC LIVER TRANSPLANTATION − A SINGLE CENTER EXPERIENCE

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Background: Liver transplantation means a reset of the hemostatic system, because most coagulation and anticoagulation factors are synthesised by the liver. Little systematic data exist for pediatric liver transplantation regarding this fundamental event.

Material and methods: This retrospective study analyses the first seven days following liver transplantation in 35 pediatric patients, 2008−2010, in a single center. Mean age was 5.8 years, 14 patients were transplanted due to biliary atresia (after Kasai procedure), three for progressive familial intrahepatic cholestasis, two for Alagille syndrome, three due to cystic fibrosis, three for acute liver failure, and five for unspecified reasons. There were five re-transplantations during this period.

Routinely tested parameters of coagulation (PT, aPTT, TT and fibrinogen) and anticoagulation (AT), red blood count, and the use of blood products during the first seven days post-transplantation were captured and analysed.

Results: Global tests just before tranplantation were minimally compromised. PT dropped more than 10% during the first 12 hours, slowly recovering over the next 36 hours. The aPTT was clearly prolonged the first 12 hours post-tranplantation, recovering after 4 days.

AT dropped 20% post-transplantation and could only be stabilised the first 4 days with direct substitution of AT-concentrate.

The use of Fresh Frozen Plasma was necessary only in a few patients, due to losses of ascites. Blood loss was minimal. The use of thrombozyte-concentrates was necessary in seven patients. We had one Heparin-induced-thrombocytopenia.

Discussion: Today liver transplantation is a standard procedure for surgeons and hepatologists. But from the perspective of a hemostaseologist the first days after liver transplantation are still poorly understood. The routinely tested parameters of global hemostasis in our center give only a very limited view. Systematic prospective studies are warranted to collect more comprehensive data.

P093 REDUCTION OF HEPATIC ARTERIAL PERFUSION BY INHIBITION OF NITRIC OXIDE PRODUCTION IMPAIRS THE RECOVERY FROM FOCAL HEPATIC VENOUS OUTFLOW OBSTRUCTION IN LIVER RESECTED RATS

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Background: Extended partial hepatectomy (PH) is leading to portal hyperperfusion but reduced hepatic arterial inflow (HAI) (1) and is invariably causing focal hepatic outflow obstruction (FHOO). We observed that FHOO caused confluent parenchymal necrosis interspersed with viable portal tracts in the obstructed territory and large sinusoidal vascular canals in the border zone after rat PH and right-median-hepatic-vein-ligation (RMHV-L) (2). Lack of hepatic arterial perfusion impaired spontaneous course of recovery in terms of enlarged parenchymal necrosis, delayed regeneration and the absence of draining vascular canals. We hypothesized that restoration of the reduced HAI in PH-rats via application of the NO-donor Molsidomine would reverse the impairment of the spontaneous course.

Methods: Lewis rats were subjected to 70% PH with RMHV-L. Either Molsidomine (10 mg/kg) or of L-Name (NO-synthase inhibitor, 100 mg/kg) applied daily (POD1_to_POD7) was used to increase respectively decrease hepatic arterial inflow. Hepatic damage, microcirculation, regeneration and vascular remodeling were evaluated at POD1, 2 and 7.

Results: As expected, significant increase of portal venous inflow with a concomitant decrease in HAI was observed in all groups after PH. Molsidomine-treatment did neither affect hepatic hemodynamics nor the spontaneous course. In contrast, L-NAME-treatment further decreased HAI which impaired hepatic microcirculation (reduced mean sinusoidal diameter and a reduced functional capillary density), aggravated parenchymal dam-

age, decelerated recovery, and impaired the formation of sinusoidal canals.
Conclusion: Reduction of HAI via inhibition of nitric oxide production worsened the recovery from FHOO. Drugs increasing HAI need to be evaluated to reverse the hyperperfusion induced impairment of the spontaneous course after FHOO.

P094 A THERAPEUTIC EXPLORATORY STUDY TO DETERMINE THE EFFICACY AND SAFETY OF CALCINEURIN-INHIBITOR-FREE DE-NOVO IMMUNOSUPPRESSION AFTER LIVER TRANSPLANTATION (CILT): THE INTERIM ANALYSIS

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Introduction: Acute renal dysfunction has been observed in up to 50% of all patients after orthotopic liver transplantation (OLT). More than 90% of patients receive calcineurin inhibitors (CNI) for immunosuppression after OLT, and nephrotoxicity of CNI contributes to renal impairment. Early renal dysfunction significantly increases the risk of chronic renal failure and subsequently the risk of premature death. Multiple trials investigated the effect of delayed CNI and reduced-dose CNI regimens or early withdrawal of CNI. Generally, avoidance of CNIs improves kidney function and does not result in higher rate of rejection when an adequate level of immunosuppression is maintained, e. g. by use of alternative, non-nephrotoxic agents such as mTOR- inhibitors and mycophenolate, or concomitant interleukin-2 receptor blockade by induction with anti- CD25 antibodies.
Methods: Based on the aforementioned data we conducted a prospective, non-controlled, test-of-concept study to evaluate the efficacy and safety of CNI-free de-novo immunosuppression after OLT. Here we report the interim analysis after the allocation of nine patients. All patients transplanted within the same time frame but not allocated to this study because of not fulfilling the inclusion or exclusion criteria served as control group.
Results: Nine patients were allocated to the study group and were compared to 61 patients in the control group; three patients were excluded because they received combined kidney-liver transplant or no measurements could be obtained. We did observe one rejection in the study group. Furthermore the safety profile was comparable except for a higher incidence of wound healing disturbances in the study group. Liver function tests were not significantly different but patients in the study group had a better recovery of kidney function. Graft and patient survival were not different in this small group of patients.
Conclusion: This interim analysis shows that the new therapeutic regimen has an acceptable safety profile. Furthermore there are indications for an improvement of kidney function in patients with hepatorenal syndrome. We will continue with the ongoing study until the allocation of 29 patients as planned.

P095 MELD IS DETERIORATING POST-TRANSPLANT RESULTS IN HCV POSITIVE LIVER RECIPIENTS

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Background: Since December 2006 liver graft allocation is carried out according to MELD-score, which primarily assesses the impairment of liver function and secondary reflecting the extent of kidney damage. While struggling against common post-transplant HCV-related issues, the worsening of the outcome made us suspect recent innovations in the procedure of liver allocation to be responsible for negative the experiences in routine postoperative care after transplantation for HCV-induced liver disease.
Methods: We compared 377 grafts received by HCV-positive patients before the introduction of MELD-score to 78 grafts transplanted within MELD-era ($n = 410$) based on histological and biochemical analysis. Hereby, we statistically (SPSS, version 15) assessed the overall graft survival, rates of re-transplantation, fibrosis progression and antiviral treatment response within first 60 post-transplant months before and after the introduction of MELD-system.
Results: Significantly lower probability of survival ($P = 0.004$), shorter mean survival time ($P = 0.027$) and higher rates of re-transplantation were detected after MELD-introduction compared to previous eras. Furthermore, lower response rates to predominantly interferon-based antiviral therapy ($P = 0.025$) and faster development of advanced fibrosis stages ($P < 0.001$) in MELD-group was found to be significant.
Conclusion: Our everyday success in the management of HCV-related graft disease depends on formal aspects of organ allocation, which may even result in significant disadvantages. The introduction of MELD-score, as an apparently reasonable attempt to improve the procedure of organ allocation, may need some disease specific adjustment in order to restore and to improve pre-MELD-results in post-transplant HCV-management.

P096 POSTOPERATIVE OUTCOME OF DONORS IN RIGHT LOBE LIVING DONOR LIVER TRANSPLANTATION IN ADULTS – A SINGLE CENTER EXPERIENCE

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Background: Because of the persistent organ shortage and growing numbers of patients on the waiting lists, living liver donation appears to be of increasing importance. Maintaining the well-being of the donor is the greatest challenge in living donor liver transplantation (LDLT). Our retrospective analysis reports the postoperative outcomes of donors for right lobe LDLT in a single center.
Patients and methods: From July 2004 to April 2011, we performed 50 consecutive right lobe LDLTs in adults. This accounts for 14.1% of all LTs performed during this period. We reviewed the patient demographics, operative details, postoperative complications and the follow-up. For outcome analysis the validated Clavien five-tier grading (described in 2004) and a quality of life (QOL) questionnaire were used.
Results: The mean age of donors was 42.1 years (range 22–63 years). The donors were predominantly female (66%; $n = 33$). 24 complications occurred in 23 donors. 15 were Grade III complications. Biliary leak ($n = 7$) in the early postoperative period and incisional hernia ($n = 4$) in the long-term follow up were the most common. There were no donor mortality and no grade IV morbidity.
Conclusion: The morbidity rate in our study was comparable with the results of other Western series. These data support the importance of a standardized short-term and long-term follow-up protocol in order to estimate the potential risk for the donor.

P098 REDUCTION OF IMMUNOSUPPRESSIVE LOAD PROMOTES SURVIVAL AFTER LIVER TRANSPLANTATION IN HIGH MELD PATIENTS

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Background: It has been recently demonstrated that liver transplantation (LT) in patients with high MELD decompensated end-stage liver disease (ESLD) is associated with 1-year survival below 50%. This disastrous outcome result is mainly related to a high postoperative rate of septical complications.

The aim of this trial was to analyze the potential role of early posttransplant immunosuppressive therapy for improving outcome in this special subset of high-risk liver transplant candidates.

Patients: Between January 2007 and May 2010, a total of 26 liver transplant patients with end-stage liver disease and a minimum MELD of 30 were included in this trial. Immunosuppressive therapy consisted of a triple regimen (Tac, initial intended trough level 10–15 ng/ml, MMF, Prednisone) until 2010 (initial period) and dual regimen (Tac, initial intended trough level 2–5 ng/ml, Prednisone), thereafter (late period). The impact of both different regimens and other relevant clinical parameters on early post-transplant outcome was analyzed.
Results: Mean recipients' age at LT was 56.5 years (range: 32–68). Mean MELD score at LT was 37.2 (range: 30–40). Overall 3- and 12-months survival was 84.4% and 56%, respectively. Higher recipients' age, pretransplant ventilation, dialysis pre-LT, need for catecholamine therapy at LT and peak Tac level post-transplantation had a negative impact on outcome (log rank < 0.05).

Actuarial overall survival 3 and 12 months post-LT was 44% and 40% in the initial (high Tac-level; $n = 16$) and 100% and 100% in the late (low Tac level, $n = 10$) period, respectively ($P = 0.01$).

Both subpoulations differed in early post-LT peak Tac trough levels (mean: 18.3 ng/ml vs. 9.3 ng/ml; $P = 0.02$) and recipients' age (mean 59.6 years vs. 51.6 years, $P = 0.04$). Recipients' risk profile at LT (ventilation, dialysis, catecholamine, ICU stay) and donor risk index were comparable between the groups. Early post-transplant mortality was mainly related to septical complications.
Conclusion: Our results clearly demonstrate that, apart from adequate patient selection and optimized interdisciplinary management, reduction of immunosuppressive load plays a crucial role for improving outcome in this high risk population.

P104 HEPATIC PORTAL VENOUS GAS AFTER PEDIATRIC LIVER TRANSPLANTATION – A SERIES OF FOUR CASES

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The detection of hepatic portal venous gas (HPVG) is often associated with severe ileocolitis and a poor prognosis. An exception is the early phase after

liver transplantation (LTX), during which this event is reported in up to 18% of cases without implications for regular unfavorable prognosis. However, questions remain concerning underlying causes and indications for interventions.

The patients with HPVG after pediatric LTX ($n = 4$ of 67) identified during postoperative serial ultrasound studies were analysed retrospectively regarding time after surgery, potential risk factors, outcome and differences compared to patients with unremarkable ultrasound.

The median age of the four patients with HPVG (0.8 years, range 0.4−1.3) was not different from that of unremarkable controls (0.95 years, range 0.2−14.5, $P = 0.45$). HPVG was detected at a median time of 40 hours (range 39−58) after transplantation and persisted for 23.5 hours (range 15−31). All patients had dilated intestinal loops, only one patient underwent surgical revision because of associated increasing infection parameters, without abnormal intra-operative findings. After bowel decompression, HPVG was no longer detectable postoperatively. In the other patients no cause of HPVG was found, and HPVG subsided without specific therapy. All four patients showed a further unremarkable course. Systematic analysis of liver perfusion, ABG (including lactate), infection parameters and liver function tests showed no differences compared to the unremarkable controls.

This case series confirms the mostly benign nature of HPVG in the early phase after liver transplantation. The possible causes requiring intervention (infection, ileus) still warrant close surveillance and relaparotomy in case of signs of acute abdomen.

P106 DOES THE PRE-OPERATIVE BODY MASS INDEX (BMI) INFLUENCE THE OUTCOME OF LIVER TRANSPLANTATION?

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Introduction: The lack of a sufficient number of organs for transplantation and the increasing number of death on the waiting list obligate us to a thorough evaluation of pre-operative risk factors. The BMI is a simple established parameter of the nutritive status of a patient. However, does the pre-operative BMI influence the outcome of liver transplantation? And if yes, which BMI is to classify at risk?

Method: Retrospectively the data of the patients' liver transplanted in the period between January 1992 and December 2004 were evaluated regarding the BMI and the survival. The number of liver transplantation was 202 in 173 patients, due to re-transplantations. Therefore the effective number of evaluated transplantations is 173. The period under consideration is from the date of transplantation until December 2006 or the date of death.

Results: The transplantation was performed in 74 (43%) female and 99 (57%) male patients. In the group (1) of the underweighted were 22 patients with an median age of 57 years and a BMI of 18.7 ± 0.9. In the group (2) with normal weight were 73 patients with an median age of 51 years and a BMI of 22.5 ± 1.2. In the group (3) of slightly over weighted were 63 patients with an median age of 54 years and a BMI of 27.1 ± 1.3. In the obese group (4) were 15 patients with an median age of 54 years and a BMI of 31.7 ± 1.9. The time span of days on the intensive care unit were in group 1 = 29.2 days; group 2 = 18.8 days; group 3 = 14.9 days; group 4 = 20.1 days. The length of stay in the hospital were in group 1 = 62.1 days; group 2 = 46.5 days; group 3 = 48; group 4 = 57.5 days. The survival after 30 days was in all groups similar, at the time points 1 and 2 years after transplantation the survival rate was higher in group 1 and 4, after 5 and 10 years the difference among the patients survival is not significant.

Conclusion: In comparison to patients with a normal BMI the direct post-operative period is in patients with a BMI < 20 or > 30 is combined with a higher number of complications and the number of days on ICU and overall days in hospital is extended. However, the long term outcome is not significant different due to the BMI.

P107 LIVER TRANSPLANTATION IN THE MELD ERA: THE HAMBURG'S DATA

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Survival benefit of a liver transplantation might depend on the disease severity as calculated by MELD in candidate. Since December 2006 organs in the EUROTRANSPLANT region are allocated based on the MELD system, however, the gap between potential recipients and donors available is growing. This study aims to evaluate the outcome of liver transplantation since the implementation of MELD in allocation mechanistic from a single center-based data.

Methods: During December 2006 to December 2010, 277 consecutive adult transplantations were performed (89 female, 188 male). The outcome or survival benefit of patients after liver transplantation were analyzed.

Results: Of 228 whole and 45 split livers (16 living related) were transplanted. The number of recipients with a MELD over 30 increased continuously and reached 44% in 2010 with average one-year survival of 76.3%. The recipient survival significantly correlated with MELD that is strongly associated with mortality at 6 months. There is no difference in terms of recipient survival 6 months after transplantation. Donor risk index (DRI) could not predict the outcome of a liver transplantation in our data setting. Overall average DRI in each year increased continuously. The low- or high-risk organs were equally distributed in each MELD subgroups. However, DRI was significantly lower in recipients with meld over 30 that had higher mortality at 6 months as compared to those MELD under 30. In addition, D-MELD increased significantly and reached to a plateau in 2009 and 2010. There is no add-on effect by using D-MELD in the prediction of outcome.

Conclusion: Using low-DRI organs could not improve the overall outcome of a liver transplantation in patients with MELD greater than 30. Given that survival benefit of a liver transplantation is justified to those with MELD over 30, high-DRI organs should be considered for the transplantation.

P108 EXPRESSION OF GOOSECOID, AN EPITHELIAL MESENCHYMAL TRANSITION (EMT)-ASSOCIATED TRANSCRIPTION FACTOR, IS ELEVATED IN HUMAN HEPATOCELLULAR CARCINOMA (HCC)

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More than one million new cases of hepatocellular carcinoma (HCC) are reported per year. Furthermore HCC is the fifth common cancer in the world, while it is the third common cause of death in cancers. The aim of our work was to investigate wether EMT and in particular transcription factors involved in EMT processes play a role in hepatocancerogenesis. The transcription factors Goosecoid, TWIST and SNAIL have been described to be associated with EMT and were increased in carcinomas with a high metastatic potency. Therefore we analyzed expression of Goosecoid, Twist and SNAIL in human HCC. Total RNA was isolated from biopsies of human HCC and corresponding adjacent normal liver tissue. Human liver biopsies were evaluated by a pathologist. mRNA expression levels of EMT related transcription factors SNAIL, TWIST and Goosecoid (GSC) were analyzed by qRT-PCR. Analyzing 53 paired samples of HCC and adjacent normal liver tissue, we found a significantly increased expression of GSC in HCC compared to corresponding normal liver ($P < 0.001$). The mRNA expression of GSC was approximately 7-fold higher in the HCC than in normal liver tissue. TWIST expression was also found to be elevated in HCC compared to normal liver tissue. Furthermore, we observed a positive correlation of GSC with TWIST expression. On the other hand SNAIL was expressed at almost identical levels in HCC and normal tissue. In addition we performed correlation studies of mRNA expression levels with histopathological and clinical parameters. We found high GSC expression levels in normal liver tissue positively correlated to AFP-levels and a high number of intrahepatical metastasis. Additionally TWIST expression was significantly enhanced in G1/2 tumors compared to G3 tumors (pathological grading). In conclusion, transcription factors known to be involved in the EMT process such as TWIST are enhanced in HCC samples compared to non involved liver tissue. Additionally, we found Goosecoid, a recently discovered important mediator of EMT to be enhanced in HCC. Therefore, GSC is an interesting potential biomarker and therapeutic target.

P110 PREDICITION OF HCC RECURRENCE AFTER LIVER TRANSPLANTATION BY DETECTION OF AFP MRNA

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Background: One therapeutic option for hepatocellular carcinoma (HCC) without extra hepatic metastases is the orthotopic liver transplantation (OLT). A major cause of death is the recurrence of HCC in the recipient. At the moment there are established and potential novel tumor markers helping to screen, diagnose and monitor the response to therapy, for recurrence after curative treatment (resection or OLT) as well as to estimate the prognosis. In this study there were detected alpha-fetoprotein (AFP) mRNA levels in the tissue of the liver as well as in the venous blood of patients undergoing orthotopic liver transplantation (OLT). The aim of the study was to compare the quantity of AFP mRNA levels in the different samples gained during OLT and to make a prognosis concerning recurrence of HCC.

Methods: Between Mai 2008 and November 2010, 97 patients at our center in Mainz underwent OLT. In 42 cases indication for transplantation was HCC, in 23 patients there was used IBSA (intraoperative blood salvage autotransfusion). In 20 patients there was used transarterial chemoembolisation (TACE) before for down staging/bridging to OLT. During OLT AFP mRNA levels were detected by real-time reverse transcriptase-polymerase chain reactions (RT-PCR) in healthy liver tissue, the tumor itself, the venous blood and the blood salvaged by IBSA. As control albumin mRNA-levels were detected in the same assays.

Results and conclusion: In 20 out of 23 cases AFP mRNA in the tumor itself was lower than in the normal liver tissue. There needs to be more investigation what's the cause for this result. (TACE before OLT?, which cells are producing AFP mRNA in case of HCC?, etc.)

So far only two of the patients suffered from recurrence of HCC. Although there need to be a higher amount of patients included in this investigation and a longer follow up, AFP mRNA measurement in the liver tissue (healthy/tumor) and the peripheral blood, does not seem to be a valuable factor for a prognosis of HCC recurrence (so far no statistical significance) at the point of OLT.

P111 NONALCOHOLIC STEATOHEPATITIS AN INCREASING INDICATION FOR LIVER TRANSPLANTATION

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in western countries and considered to be a hepatic manifestation of the metabolic syndrome. The clinicopathologic spectrum ranges from simple steatosis through steatohepatitis (NASH) to end-stage liver disease (cirrhosis) and hepatocellular carcinoma. NASH cirrhosis is a consistently increasing indication for transplantation. This study aims to report our experience with patients who underwent liver transplantation due to NASH-related liver cirrhosis.

Methods: We retrospectively studied 432 consecutive liver transplants between October 2007 and January 2011. Forty transplants were initially performed due to NASH. These patients' peri-operative course, short- and long-term outcomes were analyzed.

Results: Forty cases were clinically and pathologically identified as NASH cirrhosis. There were 16 women and 24 men, ranging in age from 25 to 68 years (mean and median, 53.97 years and 54.65 years, respectively). The median MELD score was 23.8. The transplanted BMI ranged from 23.7 to 46.1 (mean and median, 31.06 and 29.9, respectively). 26 of the initial 40 patients are still alive. Nine patients who died had remarkably increased BMI scores in mean of over 32.

Conclusion: First, a significant number of liver transplantations in our center were due to NASH. Second, liver transplantation in NASH patients is associated with a high mortality and post-operative complications, most likely due to associated obesity and metabolic syndrome. Improvement of obesity prior to LTx might lead to a better outcome in NASH patients with end stage liver disease.

PANCREAS

P112 OBESITY IMPAIRS THE RESULTS OF PANCREAS TRANSPLANTATION

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Obesity increases postoperative morbidity and the incidence for delayed graft function (DGF) in kidney transplantation alone. It was investigated whether this also holds true for transplantation of the pancreas.

We analyzed the postoperative morbidity and graft function of pancreas transplantation with regards to the recipients BMI.

From 1/04 to 1/11 50 pancreas transplantations were performed, 49 simultaneous pancreas/kidney transplantations and 1 pancreas after kidney transplantation.

The patients' mean age was 42.4 ± 7.7 years, mean duration of diabetes was 27.0 ± 8.2 years and duration of dialysis was 24.2 ± 28.6 months. The mean BMI was 24.4 ± 4.7 kg/m^2, there were 31 (62.0%) patients with normal weight (BMI 18.5–24.9 kg/m^2), 11 (22.0%) overweight (BMI 25–29.9 kg/m^2) and 8 (16.0%) obese (BMI 30–40 kg/m^2).

The incidence of kidney DGF was not significantly different in the 3 groups (16.1% vs. 20.0% vs. 16.7%). The risk for long term kidney graft loss was significantly higher among obese patients (7.1% vs. 25%, $P = 0.017$). Pancreas graft survival was significantly lower with BMI > 30 kg/m^2 (85.3% vs. 50.0%, $P = 0.05$). The need for pancreas graft related relaparotomy was significantly more frequent in the obese group (14.7% vs. 50.0%, $P = 0.05$). Patient survival was not significantly different, 3/50 patients died 21–107 days after transplantation.

Despite the low number of patients we showed a correlation of obesity and an increase of pancreas graft related complications. Pancreas and kidney graft survival for obese patients is reduced. The BMI should be considered for evaluation of pancreas graft recipients.

P113 MODIFIED RELEASE TACROLIMUS IN DE NOVO IMMUNOSUPPRESSION AFTER SIMULTANEOUS PANCREAS KIDNEY TRANSPLANTATION

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Backround: Modified release tacrolimus is a once-daily oral formulation of the established immunosuppressive agent tacrolimus. Little is known about de novo immunosuppression after simultaneous pancreas-kidney (SPK) transplantation with regard to using modified release tacrolimus.

Methods: To test the feasibility of de novo modified release tacrolimus in SPK, we conducted a prospective study of 53 consecutive transplants using modified release tacrolimus (Advagraf, ADV), mycophenolate mofetil and low-dose corticosteroids as the initial immunosuppressive regimen. All patients received an antibody induction therapy using thymoglobulin. Mean recipient age was 45.0 ± 7.8 years. Patient and graft survival, the rates of acute rejection, graft function as well as ADV dosages and trough-levels (Cmin) were investigated after a mean follow-up time of 21.9 ± 11.8 months.

Results: Patient, kidney, and pancreas graft survival were 96%, 94%, and 84%, respectively. Two patients died with functioning grafts (intracerebral bleeding $n = 1$, myocardial infarction $n = 1$). Six pancreas grafts were lost due to study period (thrombosis $n = 2$, pancreatitis $n = 2$, death $n = 2$) and two patients developed insulin dependent pancreas graft dysfunction. Biopsy-proven acute rejection rate at 12 months was 38%. ADV was well tolerated in the majority of patients. In seven cases, ADV was stopped because of neurotoxicity ($n = 2$), pancreas graft loss/dysfunction ($n = 2$), rejection ($n = 1$), insufficient trough-levels ($n = 1$) and alopecia ($n = 1$). In weeks 2 and 3 post-transplant, a significant adjustment in the ADV-dosage was necessary, although mean ADV trough levels did not reach the pre-specified targets.

Conclusions: Our data demonstrate that patients after SPK can be safely treated with modified release tacrolimus. In consequence of insufficient trough levels, initial ADV-dose should be slightly higher (e.g. 0.25 mg/kg) than with Prograf. In our experience, conversion to ADV in SPK patients after a post-operative period of 4–6 weeks is more advantageous. Further studies are needed to investigate pharmacokinetic profiles of modified release tacrolimus after SPK.

P114 PANCREAS RETRANSPLANTATION AFTER PRIMARY PTX: LONG-TERM RESULTS OF 70 CASES AT A SINGLE INSTITUTION

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Background: Technical failure, graft thrombosis and chronic rejection are the most common causes of early and late pancreatic graft loss after primary PTA, SPK and PAK. They have led to an increased number of potential candidates for pancreas retransplantation (PRT). Early PRT has been considered a risk factor for both post-operative complications and diminished graft survival. The aim of this study was to determine short- and long-term results after elective PRT. This study presents one of the largest series of PRT at a single institution.

Patients and methods: More than 500 pancreatic transplantations (PTx) were performed at our institution. We retrospectively studied a subgroup of 60 patients, who underwent 70 PRT-procedures between 1982 and 2009. Morbidity, Mortality and long-term results were analyzed.

Results: The mean age of pts was 35.8 and 41.3 years at the time of primary PTx and PRT, respectively, with a sex distribution 1:1.7 (f/m). The median follow-up of pts and the median interval between primary PTx and PRT was 14.9 years and 5.5 years, respectively. During our study period all 60 pts received a total of 224 organs (including kidney retransplants). 51 pts underwent one, and 9 pts underwent more than one (and up to 3) PRT. Re-SPK was performed in 34 and Re-PAK in 36 cases. The main immunosuppressant after primary PRT was cyclosporine in 23 and tacrolimus in 37 patients. Indications for primary PRT were graft-thrombosis (23), chronic (12) and acute (5) rejection, bleeding or leakage (5), infectious complications (5), severe graft pancreatitis (3), pancreatic cancer (1) and others (6). Post-operative complications after PRT were not significantly different to those after primary PTx ($P = 0.12$) and post-operative in-hospital mortality was low (1.6%). 1-year patient- and graft survival were 95% and 62%, and mean graft survival was reduced after PRT (4.85 years) compared to primary PAK at the same institution ($P > 0.05$). Six pts died during follow-up, due to cardiac and metabolic disorders and suicide. At the end of our study 51 of 60 pts were alive, and 22 of 51 pts had a good functioning pancreatic allograft.

Conclusions: PRT can be performed safely without an increase in surgical complications and post-operative mortality. Graft survival after PRT, however, is significantly inferior to that after primary PTx, probably for both immunologic and non-immunologic reasons. PRT and Re-SPK should be offered to suitable candidates, since it often results in long-term graft function.

THORACIC ORGANS

P117 EFFECTIVENESS OF ORAL VALGANCICLOVIR PROPHYLAXIS FOR CYTOMEGALOVIRUS INFECTION IN HEART TRANSPLANT PATIENTS

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Background: Cytomegalovirus infection is a serious complication following heart transplantation (HTx).

Patients and methods: This study retrospectively assessed the effectiveness of oral valganciclovir (vGCV) prophylaxis in adult heart transplant recipients during the first year after HTx. In patients with normal renal function vGCV was applied 900 mg bid for 14 days and thereafter 450 mg bid for 6 months post-HTx. In case of renal insufficiency, vGCV was adjusted according to manufacturer's recommendations (Roche Pharma AG, Grenzach-Wyhlen, Germany). Effectiveness was evaluated by monthly antigenemia testing (pp65 antigen) and PCR to document exposure. From 2003 until 2010 146 patients (74.0% men) with a mean age at HTx of 50.7 ± 10.3 years were included.

Results: A total of 16 patients (11.0% of total, 75.0% male) had a positive pp65 testing result during the first year post HTx; 4 of these after pausing the vGCV prophylaxis within the first 6 months post HTx (leucopenia $n = 4$, 2 of these patients developed a CMV colitis). Two further patients developed a CMV pneumonia during vGCV prophylaxis. Eight patients had a positive pp65 testing throughout months 6–12 post HTx (P = NS), 1 of these patients developed a CMV pneumonia.

Of 146 patients, 37 (25.3%) were CMV D+/R− and 7 (18.9% of subgroup) of these patients had a positive pp65 testing result. In D+/R− patients the risk of positive pp65 testing was significantly elevated ($P = 0.0226$).

Conclusions: CMV prophylaxis with oral vGCV for 6 months post HTx is effective and safe. No significantly elevated rate of positive antigenemia testing or CMV infections after 6 months of vGCV prophylaxis could be observed. However, in case of vGCV discontinuation close monitoring of CMV antigenemia appears warranted. In line with previous studies D+/R− patients have a significantly elevated risk for a positive CMV testing result.

P118 CONVERSION TO EVEROLIMUS IN PERSISTENT CYTOMEGALOVIRUS VIREMIA FOLLOWING THORACIC ORGAN TRANSPLANTATION

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Purpose: Despite well established regimens for prophylaxis and treatment of Cytomegalovirus (CMV) infection, CMV viremia remains a major cause of post-transplant morbidity. Modulation of maintenance immunosuppression might lower the incidence of CMV: there are first data in favour of PSI/mTor inhibitors regarding the risk for CMV and other virus infections. However no data are available regarding conversion to PSI to revert refractory viremia.

Methods and materials: We present a case series of 9 pts (7 m, 2 f, mean age 49.7 (range 21–70 years; 8 heart transplants, 1 heart lung transplant) with recur rent and/or persistent viremia despite antiviral prophylaxis and treatment. Maintenance immunosuppression consisted of the combination of tacrolimus, mycofenolate mofitil (MMF) and steroids in 5 pts and cyclosporine, MMF and steroids in 4 pts. The viremia occurred within the 1st post-transplant year in all pts. Fivepts had had≥ 2 relapses of CMV viremia despite preemptive treatment using valganciclovir (VGCV) and i.v. ganciclovir (GCV), four pts had persistent CMV viremia.

Results: These pts were intentionally converted to everolimus to restrain persistent CMV viremia. MMF was discontinued. This modulation of maintenance immunosuppression was arranged 3–15 months post-transplant. All pts presented seronegative within 6 weeks. Antiviral treatment was discontinued, and all pts maintained seronegative for a follow up of 12–69 months. One patient died 33 months after conversion due to hepatocellular carcinoma, one patient was switched to an immunsosuppressive regimen with everolimus and MMF due to worsening renal function. The other patients had an uneventful clinical course.

Conclusions: This case series indicates that the conversion to everolimus replacing MMF might be a safe and effective option in persistent cytomegalovirus viremia following thoracic organ transplantation.

P119 OVER 2-YEAR DISEASE-FREE SURVIVAL AFTER SEQUENTIAL THERAPY OF A PRIMARY CARDIAC LYMPHOMA

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Objective: We describe the follow-up of 2 years in a young woman with fulminant heart failure due to primary cardiac T-cell lymphoma.

Methods: The patient underwent cardiectomy, BVAD implantation, and finally heart transplantation.

Results: The patient was admitted in cardiogenic shock and a va-ECMO was implanted under the suspicion of fulminant myocarditis. When complete cardiac arrest occurred, emergency cardiectomy with implantation of a custom-made total artificial heart became necessary. As immunohistological examination revealed an anaplastic T-cell carcinoma chemotherapy was started. Twelve days later heart transplantation was performed. Polychemotherapy was continued with 5 courses of the CHOP regime with a 50% dose reduction. Immunosuppression was kept in a sub-therapeutic range and based on cyclosporine, mycophenolate mofetil (MMF), and steroids. The first myocardial biopsy showed single tumor cells within the graft, but all consecutive biopsies confirmed complete resolution of T-cell infiltration. Six months after transplantation endomyocardial biopsies revealed mild rejection, which was successfully treated with bolus steroids. The 1-year heart catheterization showed no transplant vasculopathy. After 10 months, the patient developed severe facial actinomycosis infection necessitating high dose antibiotic treatment. Actual immunosuppression consists of MMF, sirolimus and tacrolimus in a subtherapeutic range. Follow-up has currently exceeded 2 years and the patient is at home with an excellent overall condition.

Conclusion: Patients with primary cardiac T-cell lymphoma can successfully undergo heart chemotherapy. Whether heart transplantation is a therapeutic option is yet to be determined. Hence, our patient's success may lead to new treatment strategies to overcome this dreadful disease.

P120 INCREASING AGE IN THORACIC ORGAN DONATION

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Introduction: Because of the continuing shortage of organs and the general increasing age of organ donors in Germany and throughout the Eurotransplant area, more and more thoracic organs are offered from older donors and are transplanted. The DSO – East Region has investigated the realized heart and lung donations in their region during the years 2006 to 4/2011 from donors over 60 years.

Material and methods: We examined reports from 2006 to 4/2011 of donors older than 60 years, the resulting explantations, especially the thoracic donations. We reviewed the results of this study, laboratory parameters, catecholamine doses, causes of death and survival rates.

Results: During the considered period we counted 882 donors in our region reports, including 337 (38%) over 60 years. This number consists of 119 single organ – and 218 multi-organ explantations. There were 38 reports of explanted hearts: 10 were withdrawn for medical reasons, 12 could not be allocated, two were not transplanted. 14 heart donations (4.2%) were performed. For lungs were 39 reports, of which 10 were withdrawn for medical reasons, 13 were not allocated, 1 was not transplanted, 15 lung donations (4.5%) were performed. Of these, five were combined heart-lung donations.

Causes of death in donors were with predominant number cerebral bleeding (61%), followed by trauma (17%), infarction (13%) and reanimation (8.5%). The average age was 64.8 years, 14 female and 10 male donors.

Findings in transplanted organs: In the 14 transplanted hearts in all cases were found bland results without significant pre-existing diseases and with low norepinephrine doses. In lung donations the laboratory parameters showed a good oxygen uptake and no abnormalities in the X-ray and bronchoscopy examination.

On demand in the transplantation centers eight patients survived the heart transplantation, from the lungs 12 are alive after transplantation.

Conclusions: Because of the existing shortage of organ transplant centers accept an increasing number of thoracic organs from older donors. Here, a careful screening and an intensive donor conditioning is needed to optimize the function of organs. In good overall constellation, these organs can be transplanted successfully. This helps acutely to relieve waiting lists. The long-term course needs to be carefully evaluated.

P121 EVEROLIMUS-BASED IMMUNOSUPPRESSION TO REVERSE CALCINEURIN-INHIBITOR-ASSOCIATED MYOPATHIC SYMPTOMS IN HEART TRANSPLANT RECIPIENTS

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Background: Post-transplantation myopathic discomfort has been attributed to calcineurin inhibitor (CNI) based immunosuppression. Mammalian target of rapamycin inhibitors might allow the modification of maintenance immunosuppression to avoid CNI-associated myopathy.

Methods: In a single-center study, 17 male heart transplant recipients (56 ± 8 years) were converted to everolimus-based immunosuppression (EBI) because of severe myopathy considered to be CNI-associated. The conversion strategy was either CNI discontinuation ($n = 9$) or dose reduction ($n = 8$). Changes in myopathic symptoms as perceived by the patient were assessed on a 3-point scale at 1, 2, and 3 months.

Results: Symptomatic improvement ($n = 9$) or absence of symptoms ($n = 5$) were observed in 14 patients (82%) at 1 month; of the former 9 patients, 7 improved further such that a total of 12 patients were symptom-free at

3 months. Three patients had remained nonresponsive by 3 months. (Figure 1) Symptomatic response was more frequent in patients converted early (≤ 1 year) after transplantation than in patients converted late (100% vs. 57%, $P = 0.0515$). Over a median follow-up of 37 months, 4 patients experienced single Grade 2R acute rejections; all resolved after steroid bolus therapy.

Conclusions: The conversion, particularly early after transplantation, from CNI-based immunosuppression to EBI is a viable therapeutic option to relieve myopathic symptoms in heart transplant recipients. CNI-associated myopathy might be an underestimated problem following solid organ transplantation, which could be perceived more frequently with knowledge of a feasible treatment option.

P122 EVEROLIMUS-INDUCED DRUG FEVER IN 3 HEART TRANSPLANT RECIPIENTS

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The occurrence of drug fever caused by immunosuppressive therapy is rare. We report drug-induced fever in 3 non-rejecting patients (pts) in association with Everolimus (Eve) after cardiac transplantation (Tx).

Case 1: In a 71-y.o. pt, 4 years post HTx, Eve to alleviate CNI-related renal dysfunction had been started 24 mos. before the onset of fever. The pt reported recurrent fever, chills and progressive exhaustion. Excessive diagnostic work-up excluded an infectious disease. BAL, colonoscopy and PET-CT showed negative findings. Resolution of fever occurred within a few days of discontinuing Eve; Eve was replaced by cyclosporine (CsA).

Case 2: A 69-y.o. pt, 2 years post HTx, commenced Eve 12 months prior to mitigate CNI-related nephropathy. Hospitalization was required due to progressive fever, weight loss and exhaustion. After microbiologic diagnostics proved negative results, Eve was withdrawn. Within the next days the fever and chills vanished, and the pt was discharged home on CSA, with no relapse of fever.

Case 3: In a 58-y.o. pt, Eve was started according to a de novo multicentre study protocol (RAD2310). Fever and CRP manifested as early as 14 days after Eve commencement with no infectious focus diagnosed. Within the next 3 months fever, night sweats and chills recurred 3 times. Extensive diagnostics ruled out viral, bacterial or fungal infections via cultures, serology, PCR, radiographic studies and PET-CT. Co-medication was sequentially interrupted. Instantaneous cessation of fever occurred 2 days after withdrawal of Eve.

Although uncommon, Eve may induce fever and should be considered when infection and rejection are ruled out.

P123 SEVERE SUPERINFECTION BASED ON INFLUENZA A (H1N1) PNEUMONIA IN A HEART–LUNG TRANSPLANT RECIPIENT

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Background: Influenza A (H1N1) is a severe risk factor for patients after solid organ transplantation and particularly lung transplant recipients are at increased risk for grave complications because of direct graft exposure. Immunosuppressive treatment reduces the inflammatory response and can lead to less severe or uncommon symptoms or even to asymptomatic influenza infections.

Case presentation: A 47-year-old female heart-lung transplant recipient presented with upper airway infection to our out-patient transplant clinic. The laboratory results were in normal range. After precautionary hospitalization, H1N1 nose and throat swabs were performed. Initially rt-PCR essay for H1N1 virus was negative. Because of undetermined results broncheoalveolar lavage (BAL) was performed and showed positive result for Influenza A (H1N1) infection. An antiviral therapy with oral oseltamivir was initiated. Due to a superinfection with *Pseudomonas aeruginosa* and *Aspergillus terreus*, her clinical condition worsened and she required ICU care. Under antibiotic and antifungal therapy and intermittent continuous positive airway pressure mask ventilation, the clinical condition of the patient slowly improved. She could be discharged after 5 weeks in good clinical condition.

Conclusion: Routine nose and throat swabs should be collected in recipients with infections of the upper airways and screened for H1N1. In patients with lower airway infection, we advocate to perform BAL due to 20% of negative swab results for Influenza A in those patients. If there is any suspicion of super infection, antibiotic and antifungal therapy should be initiated as soon as possible to avoid severe complications.

P124 MECHANICALLY-INDUCED VENTRICULAR TACHYCARDIA BY THE HEARTWARE LVAD

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Background: The HeartWare LVAD is used in an increasing number of patients with acute or chronic heart failure. In cases of myocarditis, HeartWare has been used successfully as a bridge to recovery.

Case presentation: A 40-year-old male patient with cardiogenic shock was referred to our center. Recent history of flue-like symptoms and rapid deterioration were suggestive of myocarditis. Echocardiography showed left ventricular (LV) ejection fraction of 8% and massive left ventricular dilatation. Emergent ECMO implantation was performed as a bridge-to-bridge and HeartWare LVAD implantation was performed electively the next day. The patient quickly recovered and had preventive ICD implantation before discharge. During follow-up, echocardiography showed gradual improvements of LV function. Five month after LVAD implantation, the patient reported palpitations and episodes of dizziness, especially while bending forwards. Interrogation of the ICD memory showed frequent occurrence of self-terminating ventricular tachycardias (VTs). 12 lead ECG revealed the inferior/septal LV wall as origin of the VTs. Due to further improved LV function, we decided to explant the HeartWare system. No further VTs occurred during the 1-year follow-up after LVAD explantation and the patient is doing well with stable LV function.

Conclusion: We suspect mechanical irritation by impingement of the HeartWare inflow against the septum in the remodeled LV with normalized diameters to have triggered the VTs. We therefore recommend routine ICD implantation in HeartWare LVAD patients before discharge to terminate VTs caused by the primary disease or the LVAD system itself.

P125 SUCCESSFUL ECMO WEANING AFTER CRT-ICD IMPLANTATION IN A PATIENT WITH END-STAGE HEART FAILURE

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Background: CRT is successfully used in patients with end-stage heart failure, ventricular dys-synchrony and left bundle branch block to improve left ventricular (LV) ejection fraction and ventricular synchrony.

Case presentation: We report the case of a 46-years-old male patient with end stage heart failure due to ethyltoxic cardiomyopathy. Heart transplantation was contraindicated due to continuing alcohol abuse and LVAD implantation was refused by the patient. Echo findings revealed poor LV ejection fraction of 15% and ventricular dys-synchrony. 12-lead ECG showed left bundle branch block (QRS 140 ms) so we decided to conduct CRT-ICD implantation.

In general, anesthesia for the CRT-ICD implantation cardiac function worsened. Left ventricular ejection fraction dropped to 8% and the patient required high doses of catecholamines and mechanical ventilation with 100% oxygen. Due to deteriorating hemodynamics, the CRT-ICD implantation was aborted and emergent veno-arterial ECMO implantation was performed. During the following five days of ICU stay, weaning from ECMO was not possible. We decided to proceed with CRT-ICD implantation while still on ECMO support. With biventricular stimulation, cardiac function improved and the patient could be weaned from ECMO on the same day. He was discharged from hospital after four weeks. Now, three month after CRT-ICD implantation, LV ejection fraction is 22% and the patient is in functional NYHA class II.

Conclusion: In this case, CRT implantation showed improvement of LV function and made successful ECMO weaning possible.

P126 COMBINED HEART-, LUNG- AND LIVER-TRANSPLANTATION IN AN ADULT

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Purpose: Combined transplantation of the liver and thoracic organs presents a therapeutically option in highly selected patients. ISHLT registry contains 32 documented cases of a combined Heart-, Lung- and Liver-Transplantation most of them in children.

Case Report: We report the case of a 62-year-old patient with dilatative cardiomyopathy and consecutive fixed pulmonary hypertension and cirrhoses cardiac. On June 1st 2010 the simultaneous combined Heart-, Lung- and Liver-Transplantation was performed without any complications. After the implantation of the Heart-Lung package the transplantation of the liver was performed during reperfusion after aortic clamp removal (95 minutes). Duration of operation was 378 minutes. The organs adjusted optimal function immediately after weaning from bypass. The patient was sent to ICU in stable conditions with mild inotropic support. During the further postoperative course he acquired nosocomial pneumonia. The treatment required long term intubation and tracheotomy. The patient was discharged to rehabilitation on 55th postoperative day.

Conclusion: In highly selected patients a combined Heart-, Lung- and Liver-Transplantation may be an option.

P127 APICAL HYPERTROPHIC CARDIOMYOPATHY SEVEN YEARS AFTER ORTHOTOPIC HEART TRANSPLANTATION

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Background: Apical hypertrophic cardiomyopathy is an uncommon form of hypertrophic cardiomyopathy (HCM) characterized by isolated myocardial hypertrophy in the apical region. It is known as a phenotypic expression of HCM more unique to Japanese patients. Typical findings are spade-shaped configurations of the LV cavity at end-diastole and giant T-wave negativity ($\geq$ 1 mV) in the ECG. Despite a generally benign prognosis, adverse events such as sudden death, embolic stroke, arrhythmias and apical infarctions have been described.
Case: We report a case of a symptomatic apical hypertrophic cardiomyopathy in a patient 7 years after orthotopic heart transplantation. The patient is a 68 years old female presenting with progressive dyspnea in our transplant clinic. On echo she showed apical left ventricular hypokinesia, diastolic dysfunction and pulmonary hypertension. There was no coronary artery disease but cardiac allograft vasculopathy Stanford I. Contrast echocardiography then revealed the pathognomonic ace-of-spades sign. There is no additional information available on the donor. The patient is currently treated with a calcium antagonist and is stable at NYHA class II.
Conclusion: This is the first description of apical hypertrophic cardiomyopathy in a heart transplant recipient. Since it is already rare in non-transplanted patients with mainly loco-regional occurences, it may be speculated that the donor heart carried the propensity to develop this rare form of cardiomyopathy and it then became symptomatic in the recipient.

P128 BILATERAL LOWER LOBE LUNG TRANSPLANTATION IN A SMALL RECIPIENT – A CASE REPORT

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Introduction: We report on a 41-year-old female patient with global respiratory failure who underwent bilateral lower lobe transplantation.
Aims: Calculated TLC for this patient was 4.9 L. She was placed on the high urgency waiting list for lung transplantation due to terminal respiratory failure from pulmonary fibrosis. Her forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were only 0.81 L and 0.76 L, respectively. Due to her small body size (1.60 m) and rapidly deteriorating condition, it was not possible to get a size-matched donor organ in time. Larger donor lungs were accepted (TLC 8.0 L) and bilateral lower lobe transplantation was performed through a bilateral thoracosternotomy using cardiopulmonary bypass. Excellent size match was observed after blood circulation and inflation. The patient recovered quickly from surgery. After 7 days, she was ambulatory and free from oxygen support. The patient was discharged from hospital after 2 weeks in excellent respiratory condition. At 1-month follow-up, her FVC and FEV1 were 1.94 L and 1.46 L, respectively. (Figure: (a) pre-LTX, (b) post-LTX)
Conclusion: Lobar lung transplantation can safely be performed for smaller recipients, especially those with extremely small thoracic cavities due to restrictive pulmonary disease.

IMMUNOSUPPRESSION

P129 THE INTRA-OPERATIVE HIGH-DOSE INDUCTION WITH ATG-FRESENIUS IN ELDERLY KIDNEY GRAFT RECIPIENTS IS AS EFFICIENT AND SAFE AS IN YOUNGER RECIPIENTS

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Aim: One important point of the European Senior Program is the allocation of older donor kidneys without HLA-matching to shorten cold ischemia time (CIT). But with respect to the immunosuppressive treatment of the recipients no recommendations are available or generally accepted for this age group. In our centre we developed an effective kidney protecting therapy which combines the highly efficacious intra-operative high-dose induction (HDI) with ATG-Fresenius (ATG-F, single dose of 9 mg/kg body weight) with a markedly reduced cyclosporine (CS) dose (trough level 100 ng/ml) during the first post-operative week. The results of a retrospective analysis comparing the long-term results in elderly and in younger recipients are presented here.

Material and methods: To assess the efficacy and safety of this immunosuppressive regimen depending on recipient age, two patient cohorts were analysed: cohort 1: $n = 40$ recipients ≥ 60 years; cohort 2: $n = 205$ recipients between ≥ 20 and 40 years. All recipients received the immunosuppression with CS, azathioprine and steroids (TDT) and an intra-operative HDI with ATG-F. There were no systematic differences in donor characteristics with respect to age, sex, CIT and HLA-mismatches. The recipients' characteristics also did not differ except for age (63 ± 2.7 vs. 31.6 ± 5.3 years, mean ± SD).

Results: As expected long-term patient survival (censoring at 120 months) was significantly better in younger recipients than in elderly ones (mean: 109.6 vs. 87.4 months; 95% confidence interval: 105.4–113.8 vs. 72.2–102.6 months; $P < 0.001$), but long-term graft survival did not differ significantly ($P < 0.067$). Importantly, the graft loss within 10 years was lower in the elderly recipients (4/40 vs. 56/205, $P < 0.02$), but a higher portion of death with function (11/40 vs. 11/205) was seen. The rates of biopsy confirmed rejections of any type were comparable (10/40 vs. 69/205), as were the rates of malignancies (0/40 vs. 1/205), PTLD (0/40 vs. 1/205), CMV diseases (9/40 vs. 40/205) and viral pneumonias (2/40 vs. 2/205). Only bacterial pneumonias were more frequent in elderly recipients (3/40 vs. 2/205, $P < 0.008$).

Conclusions: In transplant recipients aged 60 and above the intra-operative high-dose induction with ATG-F, combined with a reduced CS dose during the first post-transplant week, is associated with a comparable graft survival and safety profile as in younger recipients and can be therefore recommended.

P131 LONG-TERM EFFECTS OF ATG INDUCTION THERAPY ON THE HUMORAL IMMUNE RESPONSE

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Background and methods: We showed previously that rabbit ATG induction therapy induces a strong decrease of CD4+ T cells together with impaired in-vitro IL-2 secretion up to 1-year post-transplant. To further characterize long-term immunological effects of ATG induction 2 and 5 years post-transplant, we used sensitive intracellular cytokine analysis in the same prospective study of 84 renal transplant recipients (with ATG, $n = 44$).

Results: Five-year kidney graft outcome was not significantly different between the low-risk non-ATG group and the immunological risk ATG group. This favorable clinical result of ATG-treated patients coincided with comparable levels of the immune parameters sCD30 and neopterin (Neo/Cr) at 1 year, which were associated with immune-mediated graft deterioration or loss within 5 years (logistic regression: $P < 0.006$, sCD30; $P < 0.064$, Neo/Cr). Long-term humoral effects of ATG induction included a profoundly downregulated production of the B-cell growth and differentiation factor IL-10 by CD4 cells at 2 years post-transplant ($P < 0.004$; logistic regression: $P < 0.054$) and a persistent decrease of CD4+ T helper cell counts in peripheral blood even at 5 years post-transplant ($P < 0.0005$ vs. non-ATG treatment) which was associated with suppressed T-cell dependent B cell responses ($P < 0.026$) but not with suppressed CD4 cell functions. In contrast to non-ATG patients, ATG patients showed no rise in CD19+ B cell counts between 2 and 5 years post-transplant (non-ATG: 192 ± 35 vs. 96 ± 13 /ul, $P < 0.001$; ATG: 119 ± 25 vs. 84 ± 13 /ul, $P < 0.101$).

Conclusion: Long-term suppression of humoral responses by ATG induction (profoundly diminished CD4 cell IL-10 production at 2 years, persisting low CD4 cell counts associated with suppressed T-dependent B cell responses even at 5 years, and the absence of a rise in B cells between 2 and 5 years) may provide longterm graft-protective effects which may affect HLA antibody formation.

P132 LONG-TERM IMMUNOLOGICAL EFFECTS OF ATG INDUCTION ASSOCIATED WITH AN INCREASED RISK OF INFECTION

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Background and methods: We showed previously that rabbit ATG induction therapy induces a strong decrease of CD4+ T cells together with impaired *in-vitro* IL-2 secretion up to 1 year post-transplant. To analyze long-term immunological effects of ATG induction at the 2- and 5-year post-transplant time points, we used sensitive intracellular cytokine analysis in the same prospective study of 84 renal transplant recipients (wit ATG, $n = 44$).

Results: Five-year kidney graft outcome was not significantly different between the low-risk non-ATG group and the immunological risk ATG group. However, ATG induction was associated with an increased frequency of severe infectious disease within 2 years (20/44 (46%) vs. 9/40 (23%) patients, $P = 0.027$) but not beyond (2–5 years post-transplant: 11/40 (28%) vs. 11/38 (29%); $P = 0.887$). This increased risk of infection coincided with suppressed T cell functions (T cell proliferation (CD69 expression), $P = 0.011$; intracellular CD4 cell IL-2 and IL-10 responses, $P = 0.036$ and $P = 0.004$, respectively) at 2 years which were no longer detected at 5 years post-transplant. A persistent decrease of CD4 cell counts was evident even 5 years post-transplant in ATG compared with non-ATG patients ($P < 0.0005$), however, this was not associated with impaired CD4 cell functions (CD4 helper activity and cytokine production) nor with an increased risk of severe infectious disease or malignancy.

Conclusion: A strongly increased risk of severe infectious disease within 2 years after ATG induction coincided with an impairment of T cell functions within this timeframe. Profoundly decreased CD4 cell counts persisted even 5 years after ATG treatment but were not associated with suppression of CD4 cell functions nor with an increased risk of severe infectious disease following year 2.

P136 IS CYTOMEGALOVIRUS PROPHYLAXIS DISPENSABLE IN PATIENTS RECEIVING A MTOR-INHIBITOR BASED THERAPY?

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Cytomegalovirus (CMV) infection remains to have significant morbidity and mortality after transplantation. Basically there are two principal strategies to cope with CMV, – preemptive and prophylactic therapy, associated with significant side effects and costs. Still the principal question remains whether the amount of immunosuppression or the class of immunosuppressive agent influences CMV replication. Recent studies show that mTOR inhibitors may have anti-CMV properties while providing effective immunosuppression. Data from the SMART study, show that with an equal CMV profile in the SRL (D+R– 14.5%; $n = 71$) and the CsA +MMF + ST (D+R– 14.1%; $n = 69$) arm, there was a significantly higher incidence of CMV infection in the CsA arm over an observation period of 36 months after kidney transplantation; 7.3% vs. 29.6% ($P < 0.01$). A meta-analysis of randomized trials, comparing a SRL-based with CNI-based immunosuppressive therapies, showed a clear trend towards lower rates of CMV infections in mTOR-inhibitor based regimens. This data suggests that CMV prophylaxis, which is cost-intensive and associated with considerable side effects, may be dispensable in patients receiving an mTOR-inhibitor based therapy.

P137 REDUCTION OF CARDIAC ALLOGRAFT VASCULOPATHY WITH EVEROLIMUS OVER MYCOPHENOLATE MOFETIL: INTRAVASCULAR ULTRASOUND RESULTS OF A RANDOMIZED MULTICENTER TRIAL

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Purpose: Cardiac allograft vasculopathy (CAV) is a major cause of long-term mortality following heart transplantation (HTx). In an earlier study, the mTOR inhibitor everolimus (EVR) was associated with lower incidence of CAV by 1-year intravascular ultrasound (IVUS) vs azathioprine. Here we investigated whether EVR provides CAV benefit by IVUS vs mycophenolate mofetil (MMF).

Methods: A2310 is a 24-month (M), randomized, multicenter, open-label, study of efficacy and renal function comparing 2 starting doses of EVR (1.5 [$n = 282$] or 3 mg/day [$n = 168$]) with reduced cyclosporine (CsA) exposure vs. MMF3 g/day ($n = 271$) with standard CsA exposure in de novo HTx recipients (HTxR). Enrollment in the EVR 3mg arm was prematurely terminated due to higher mortality. The IVUS sub-study assessed the change

in average maximum intimal thickness [MIT], the incidence of de novo coronary disease (0.5 mm increase in MIT at sites with MIT < 0.5 mm at baseline) and incidence of CAV (0.5 mm increase in MIT) from baseline (BL) to M12.

Results: Of 88 EVR and 101 MMF HTxR had evaluable IVUS data (minimum of 11 matched slices) at BL and M12. Change in average MIT from BL to M12 was significantly smaller in EVR vs. MMF. Incidence of de novo coronary disease at M12 was lower in EVR vs. MMF. Incidence of CAV at M12 was also significantly lower in EVR vs. MMF. Other IVUS parameters also significantly favored EVR vs. MMF. A subpopulation analysis including diabetics, gender and age > 50 years demonstrated consistent CAV benefit of EVR vs. MMF group.

Conclusions: EVR demonstrated significant benefit in IVUS measures of CAV (including important subpopulations) vs. MMF.

P138 COMPARISON OF WOUND HEALING EVENTS AND EFFUSIONS IN DE NOVO HEART TRANSPLANT RECIPIENTS TREATED WITH EVEROLIMUS VS. MMF BASED REGIMENS

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Purpose: This analysis compares prospectively collected information on wound healing events (WHE) and effusions between everolimus (EVR) and mycophenolate mofetil (MMF) based immunosuppressive regimens in de novo heart transplant recipients (HTxR).

Methods: A total of 721 de novo HTxR were randomized in Study A2310, a 24-month (M), multi-center, open-label study to receive within 72 h post-Tx first dose of EVR 1.5 or 3 mg/day (target C0 3–8 ng/ml or C0 6–12 ng/ml) with reduced cyclosporine (CsA) or 3.0 g/day MMF with standard CsA and steroids. Induction therapy was center-specific (basiliximab/thymoglobulin/no induction). Comparison of event rates at M12 is only presented for EVR 1.5 mg ($n = 279$) and MMF ($n = 268$), due to the early termination of enrollment in the EVR 3 mg arm. Detailed information on symptoms, diagnosis and intervention was prospectively collected for WHEs and effusions.

Results: A total of 34.1% and 29.1% of HTxR in the EVR 1.5 mg and MMF group experienced any WHE (P = NS). Non-sternal WHE incidence was 13.3% and 13.1% for EVR 1.5 mg and MMF (P = NS). Incidence of sternal WHE was 24.4% with EVR 1.5 mg vs. 19.4% with MMF (P = NS). Incidence of mediastinitis was 1.8% (5/279) with EVR 1.5 mg vs. 0.7% (2/268) in the MMF group (P = NS). Comparisons of treatment of sternal WHE were all non-significant. Pericardial effusion events occurred more often in EVR 1.5 mg vs. MMF (43.4% vs. 28.4%, $P < 0.001$), along with a higher incidence of symptoms of hemodynamic compromise ($P = 0.001$) and echo signs of imminent cardiac tamponade (P = NS). No patient died due to cardiac tamponade. Incidence of pleural effusion was comparable for EVR 1.5 mg vs. MMF (28.0% vs. 23.1%; P = NS).

Conclusion: The incidence of WHEs and pleural effusions in de novo HTxR was comparable between EVR 1.5 mg and MMF. Frequent echocardiographic monitoring of pericardial effusions is recommended for HTxR receiving EVR during the early post-operative course.

P140 THREE YEARS FOLLOW-UP OF THE ZEUS TRIAL: MAINTAINED BETTER 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 on renal function, efficacy and safety after a conversion to an Everolimus/Enteric-Coated Mycophenolate Sodium (EC-MPS) regimen after Cyclosporine (CsA) withdrawal in de novo kidney allograft recipients at month (Mo) 36 post-transplantation (tx).

Methods: In this prospective, open-label, controlled, multi-center study renal allograft recipients were randomized to a regimen consisting of either Everolimus/EC-MPS or CsA/EC-MPS at Mo 4.5 after tx. After completion of the core study at Mo 12, patients (pts) were included in an observational 24-Mo Follow-up.

Results: A total of 300 pts were randomized to either Everolimus/EC-MPS ($n = 155$) or CsA/EC-MPS ($n = 145$), 242 (80.7%) pts completed Mo 36. At Mo 36 fewer pts in the Everolimus group had a decline of GFR compared to renal function at randomization (Nankivell: 26.6% vs. 41.8%; $P = 0.0034$) compared with CsA. Renal function expressed as cGFR (Nankivell) was similar in both groups at randomization (4.5 Mo post tx) with an improvement by 7.27 ml/minute/1.73 m in favor of the Everolimus/EC-MPS regimen ($P = 0.0098$) at Mo 36 (60.6 ± 16.4 vs. 67.9 ± 21.6) compared to 10.6 ml/minute/1.73 m at Mo 12. The difference in GFR from randomization to Mo 24 was +4.8 [+0.4, +9.3] for Everolimus/EC-MPS and −2.7 [−7.3, +2.0] ml/minute/1.73 m for CsA/EC-MPS pts. Three deaths and 2 graft losses were observed in the CsA group, one death and one graft loss in the Everolimus group. BPAR was reported in 20 (13.0%) Everolimus vs. 7 (4.8%) CsA treated pts between randomization and Mo 36. The number of pts with infections (31 (20.0%) Everolimus pts. vs. 29 (20.0%) CsA pts) and hospitalization (48 (31.0%) Everolimus pts vs. 41(28.3%) CsA pts) in the follow-up period (Mo 24–Mo 36) was comparable. After 12 months two additional BPAR occurred in the Everolimus vs. 5 additional BPAR in the CsA group.

Conclusions: The conversion to Everolimus/EC-MPS in de novo renal transplant patients after CNI withdrawal early after transplantation reflects a novel therapeutic approach which significantly maintains renal function over a period of 36 months without compromising efficacy and safety.

P141 TWO YEAR DATA OF THE APOLLO TRIAL: RENAL FUNCTION OF AN EVEROLIMUS BASED THERAPY AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN MAINTENANCE RENAL TRANSPLANT RECIPIENTS

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Objective: Assessment of renal function, safety and efficacy of an Everolimus regimen after Calcineurin-Inhibitor (CNI) withdrawal in maintenance kidney allograft recipients.

Methods: In an open-label, randomized, controlled, multi-center study 93 patients on a stable immunosuppressive therapy consisting of CNI, Enteric-Coated Mycophenolate Sodium (EC-MPS) with or without corticosteroids were randomized to either continue CNI treatment and EC-MPS or convert to an Everolimus/EC-MPS based regimen. After completion of the core study at Mo 12, patients were included in an observational 12-Mo follow-up study.

Results: Ninety-three pts with a mean time since the most recent transplantation of 6.4 years were randomized to either Everolimus/EC-MPS ($n = 46$) or CNI/EC-MPS ($n = 47$), 79 (84.9%) pts completed the 24 month visit. The median trough level was 103.0 ng/ml in CsA, 5.4 ng/ml in Tacrolimus and 6.4 ng/ml in Everolimus treated pts. Two deaths and one graft loss were observed in the CNI/EC-MPS group, one death in the Everolimus/EC-MPS group and no BPAR was observed in either group. The number of patients with infections was 13 pts (28.3%) in the Everolimus vs. 9 pts (19.1%) in the CsA group. Four (10.6%) malignancies occurred in the CNI group compared to one (2.2%) case in the Everolimus group. At Month 24 after randomization mean calculated GFR (Nankivell) was higher by 5.0 ml/min/1.73 m^2 for the Everolimus compared to the CNI group (63.9 ± 19.9 vs. 58.9 ± 15.9 ml/min/1.73 m^2; P = ns). The observed GFR slope from conversion to month 24 was +2.6 [−1.2, +6.3] for Everolimus/EC-MPS and −2.0 [−6.1, +2.1] ml/minute/1.73 m for CNI/EC-MPS pts.

Conclusions: The late conversion to an Everolimus/EC-MPS treatment in maintenance renal transplant patients after CNI withdrawal leads to a better renal function.

P142 RENAL FUNCTION IN EVEROLIMUS/ENTERIC-COATED MYCOPHENOLATE SODIUM TREATED *DE NOVO* LIVING RENAL TRANSPLANT RECIPIENTS AFTER CALCINEURIN INHIBITOR WITHDRAWAL: SUBGROUP ANALYSIS OF THE ZEUS STUDY

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Objective: Assessment of renal function after Cyclosporine (CsA) withdrawal in a subgroup of de novo living donor at month 12 post-transplantation.

Methods: In this prospective, open-label, controlled, multi-center study (ZEUS study) renal allograft recipients were randomized to an immunosuppressive regimen consisting of either Everolimus/EC-MPS or CsA/EC-MPS at Mo 4.5 after transplantation. Renal function was assessed by the calculated Glomerular Filtration Rate (cGFR).

Results: A total of 300 pts were randomized to either CsA/EC-MPS ($n = 145$) or Everolimus/EC-MPS ($n = 155$). In total 80 pts (26.7%) received

a living donor (38 (26.2%) in the CsA and 42 (27.1%) in the Everolimus group). Renal function expressed as calculated GFR (Nankivell method) was similar in both groups at baseline (randomization 4.5 Mo post tx) with an improvement by 13.5 ml/minute/1.73 m in favor of the Everolimus/EC-MPS regimen ($P = 0.008$) at Mo 12 (61.6 ± 15.9 vs. 75.1 ± 13.5 ml/minute/1.73 m; unadjusted mean). The observed GFR slope from randomization to Mo 12 was +9.98 [+6.22, +13.73] for Everolimus/EC-MPS and −0.62 [−4.75, +3.50] ml/minute/1.73 m for CsA/EC-MPS pts ($P < 0.0001$). BPAR was reported in 6(14.6%) Everolimus vs. 1(2.6%) CsA treated pts between randomization and Mo 12. One death occurred in the the CsA group. No graft loss was observed in either group. Proteinuria was reported in 21% of Everolimus and 13% of CsA treated pts with no differences at Baseline (545 ± 946 mg/day vs. 434 ± 337 mg/day). At month 12 proteinuria increased in Everolimus/EC-MPS (328 ± 214 mg/day) and decreased in CsA/EC-MPS pts (156 ± 94 mg/day).

Conclusion: Conversion to an Everolimus based therapy 4.5 month after transplantation in de novo living renal transplant recipients significantly improves renal function.

P143 BELATACEPT COMPARED WITH CYCLOSPORINE IN RENAL ALLOGRAFT RECIPIENTS OF EXTENDED CRITERIA DONOR KIDNEYS: 3-YEAR OUTCOMES FROM THE PHASE III BENEFIT-EXT TRIAL

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Background: Recipients of extended criteria donor (ECD) kidneys have poor long-term outcomes compared to recipients of standard criteria donor kidneys. The efficacy and safety of belatacept in recipients of ECD kidneys were evaluated at 3 years to characterize longer-term outcomes and durability of treatment effect.

Methods: BENEFIT-EXT was a 3-year, phase III study in recipients of *de novo* ECD kidneys ($n = 543$) who were randomized 1:1:1 to a more intensive (MI) or less intensive (LI) belatacept regimen or cyclosporine (CsA).

Results: At 3 years, 323 patients remained on therapy ($n = 109$ MI, $n = 114$ LI, $n = 100$ CsA). The proportion of patients surviving with a functioning graft was comparable between groups (80%-MI, 82%-LI, 80%-CsA). Mean calculated GFR at 3 years was 11 ml/min higher among belatacept-treated patients compared to CsA treated patients (42.7-MI, 42.2-LI, vs. 31.5 ml/min-CsA). Belatacept-treated patients showed less decline of renal function over time (ml/min/year), with slopes of -0.9 (MI), -0.6 (LI), and -1.9 (CsA). More CsA-treated patients (44%) progressed to GFR<30 ml/min (CKD stage 4 or 5) vs. those receiving belatacept (27−30%). Acute rejection (AR) occurred in 1 additional patient in each group after year 2; most AR occurred by month 6. PTLD risk was highest in the first 18 months (2 in MI, 3 in LI groups), with 2 additional cases (1 each LI and CsA) occurring after month 36. Tuberculosis was reported in 2 (MI), 4 (LI), and 0 (CsA) patients. A risk-prediction model suggested treatment with belatacept would extend graft half-life by 22 months, from 8 years to 10 years.

Conclusions: Among recipients of ECD kidneys, treatment with belatacept resulted in comparable patient and graft survival, similar rates of AR, with better renal function compared with CsA at 3 years after transplantation. No new safety issues were observed at 3 years.

P144 3-YEAR SAFETY PROFILE OF BELATACEPT IN KIDNEY TRANSPLANT RECIPIENTS FROM THE BENEFIT AND BENEFIT-EXT STUDIES

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Background: Belatacept, a selective co-stimulation blocker, is associated with better renal function and similar patient/graft survival vs cyclosporine (CsA) in kidney transplant recipients in the BENEFIT and BENEFIT-EXT studies at 3 years. The current analysis focuses on pooled safety data for belatacept vs CsA through Year 3 of BENEFIT and BENEFIT-EXT.

Methods: Patients were randomized to a more intensive (MI) or less intensive (LI) regimen of belatacept, or CsA.

Results: The pooled analysis included 1209 intent-to-treat patients (MI = 403; LI = 401; CsA = 405).

Infection was the most common cause of death in each study. More TB cases were observed in belatacept patients; most cases occurred in countries where TB is endemic. Although the frequency of PTLD, particularly CNS PTLD, was increased for belatacept patients regardless of EBV status at baseline, the highest risk remained in patients who were EBV(-). All but 1 case of PTLD in the belatacept patients occurred within the first 18 months post-transplant; 2 cases occurred after month 18 ($n = 1$ LI; $n = 1$ CsA). The incidence rate of all malignancies remained stable over time in each study, while the incidence rate of PTLD and most infections appeared to decrease over time.

Conclusions: Belatacept LI was associated with fewer deaths and serious infections vs MI or CsA. The primary risks with belatacept are PTLD, particularly CNS PTLD, and PML. The risk of PTLD is highest in EBV (-) patients, and appears to decrease after 18 months. There were no new safety signals through Year 3.

P145 THREE YEAR OUTCOMES BY DONOR TYPE IN PHASE III STUDIES OF BELATACEPT VS CYCLOSPORINE IN KIDNEY TRANSPLANTATION (BENEFIT AND BENEFIT-EXT)

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Introduction: Belatacept was associated with better renal function, similar pt/graftsurvival, higher rate (BENEFIT)/grade ofacute rejection (AR), and higher risk of PTLD vs. CsA through 3 years in kidney transplant pts. Here we report outcomes by donor type.

Methods: In BENEFIT patients received living (LD) or standard criteria deceased donors (DD) kidneys; In BENEFIT-EXT donors met protocol specified ECD. Each was a 3 year, randomized, Phase III study of belatacept in more intensive (MI) and less intensive (LI) regimen vs CsA. All received basiliximab, MMF, and corticosteroids. Pt/graft survival, GFR (MDRD) and AR through 3 years are reported.

Results: In BENEFIT, 385 and 281 received LD and DD kidneys respectively. In BENEFIT-EXT 384 received UNOS ECD and 55 received DCD kidneys. Among LD and DD recipients treated with belatacept, pt/graft survival and GFR were consistent and were comparable to the ITT population.

No new cases of AR occurred in belatacept treated patients after year 2; 1 new case occurred in CsA in BENEFIT. In UNOS ECD and DCD kidney recipients, pt/graft survival with belatacept was comparable to CsA and consistent with ITT; 1 new pt in each treatment arm had AR after year 2. The differential benefit in GFR of 20 ml/minute (SCD) and > 10 ml/minute (ECD) seen with belatacept in the ITT p was at least preserved across donor types. Safety of belatacept across donor types was consistent with ITT.

Conclusions: Belatacept demonstrated better renal function and comparable pt/graft survival compared CsA regardless of SCD or ECD donor type through 3 years. After year 2, new cases of AR were infrequent.

P146 ELEMINATION OF NOROVIRUS IN A CHRONICAL CARRIER UNDER IMMUNOSUPRESSION – EFFECT OF EVEROLIMUS

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Introduction: Immunosupression is a valuable tool to enable transplantation of solid organs, but it is also strongly connected to infectious problems. Susceptibility to bacterial and viral infections, amongst others norovirus, is significantly higher under immunosupression. Norovirus can be life-threatening in patients after heart transplantation and is difficult to treat, particularly in chronic carriers.

Case report: A 24-year-old woman was initially admitted to our department in December 2010, when severe post-partal cardiomyopathy was diagnosed. End of January 2009, a left-ventricular assist-device had to be implanted as bridging to transplant, there were no signs of recovery of LV-function under full CHF-medication. She underwent heterotopic heart transplantation in April 2010. FK506, mycophenolatmofetil (MMF) and prednisolone were used as immunosupression. End of December 2010, the patient suffered from an acute norovirus infection with diarrhoea and vomiting. During the following weeks, the patient continiously suffered from recurrent diarrhoea, and PCR for norovirus was continiously positive, thus she was chronic carrier for norovirus. End of Feruary 2011, immunosupression was switched to everolimus + MMF + prednisolone due to significant decease of renal function. Under this medication, diarrhoea stopped. 8 weeks after switching of to everolimus, PCR for norovirus became negative in several consecutive measurements. Renal function significantly improved within few weeks. Everolimus was well tolerated by the patient.

Conclusion: Spontaneous elimination of norovirus rarely occurs in chronic carrier, particularly in patients under immunosupression after heart transplantation – to the best of our knowledge – no case is reported. The mechanism of elimination under everolimus is probably identical with the mechanism leading to lower rates of cytomegalovirus-infections under everolimus. The exact mechanism is still not known. Switching immunosupression to everolimus can

be an option in selected patients with infectious problems after heart tranaplantation.

P147 EXPECTED MEDIAN GRAFT SURVIVAL PREDICTION FOR BELATACEPT PHASE III TRIAL OUTCOMES IN KIDNEY TRANSPLANTATION

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Introduction: The primary objective of kidney transplantation is long-term survival with graft function (all cause graft survival). Novel immunosuppressants are being developed to advance this goal. However, the endpoints of clinical trials of these agents are short-term because long-term trials are expensive and difficult to manage and would delay availability of new agents to the general patient population. We describe the development and validation of a statistical tool designed to predict long-term graft survival after the third-year following kidney transplantation. Long-term graft survival is predicted for recent international multi-center trials of belatacept.

Methods: The survival expectation models, from 3-years post-transplant through 14-years post-transplant, were fit independently for SCD and ECD recipients as defined by the trials with multivariate Cox regression using adult renal transplant recipient data from the United States UNOS registry database. Covariates were drawn from the graft survival prediction model developed by the UNOS Kidney and Kidney Allocation Review Committees. Additional covariates included recipient and donor race, first year acute rejection, and one- and 3-year post-transplant estimated glomerular filtration rate (eGFR) derived using the abbreviated Modification of Diet in Renal Disease (MDRD) equation. Clinical trial data was derived from the international multicenter belatacept Phase III trials for SCD (BENEFIT) and ECD (BENEFIT-EXT) in kidney transplant recipients. Expected median survival was calculated using observed 3-year survival and the effects of eGFR and acute rejection at 3-years post-transplant on subsequent survival. Models based on data available from the trials at 1-year post-transplant were used to validate the techniques. All calculations were made following an intention to treat design.

Results: Significant correlates with graft survival were as expected and included typical recipient, donor, and transplant characteristics, eGFR and acute rejection. Median expected survival with graft function in the SCD (008) trial was 12 years 10 months for patients treated with belatacept compared to 10 years 11 months for cyclosporine treated controls, a 23 month advantage to belatacept. Median expected survival with graft function in the ECD (027) trial was 9 years 9 months for patients treated with belatacept compared to 7 years 11 months for cyclosporine treated controls, a 22 month advantage to belatacept. Validation showed excellent prediction of actual two and three year survival in the trials and when applied to survival in the US and international data in the PORT registry.

Conclusions: Long-term graft survival effects are seldom known at the time new regimens are adopted. The statistical model presented here performed well in independent validation and in prediction of two and three year graft survival differences given first year data. The model predicts that the benefits to belatacept treated patients compared to controls will increase with additional follow-up and approximately a 2 year increase median survival with graft function attributable to belatacept treatment compared with controls.

P148 IMMUNOLOGICAL CONSEQUENCES AND TRAFFICKING OF HUMAN REGULATORY MACROPHAGES ADMINISTERED TO RENAL TRANSPLANT RECIPIENTS

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The human *regulatory macrophage* is a novel type of suppressor macrophage which may be a particularly suitable cell type for promoting transplantation tolerance in the clinical setting because of its robust nature, stable phenotype and potent T cell-suppressive activity. Building on evidence from pre-clinical transplant studies that preoperative treatment with donor-derived M regs promotes allograft acceptance, M regs were administered to two living-donor renal transplant recipients. Both patients were minimised to low-dose tacrolimus monotherapy within 24 weeks of transplantation and subsequently maintained excellent graft function. To assess the fate of M regs following infusion, the cells were labelled with Indium-111-oxine and tracked in serial whole-body SPECT studies. After central venous administration, most M regs remained viable and were seen to traffic from the pulmonary vasculature via the blood to liver, spleen and bone marrow. By 1 year post-transplantation, both patients displayed patterns of peripheral blood gene expression converging upon the *Indices of Tolerance* (IOT-RISET) tolerance signature (Sagoo 2010). Furthermore, both patients maintained levels of peripheral blood FoxP3, Tolerance-associated gene 1 (Toag-1) and alpha-mannosidase mRNA expression within the range consistent with non-rejection. It is concluded that M regs warrant further study as a potential immune-conditioning therapy for use in solid organ transplantation. The results of this work are being used to inform the design of *The ONE Study*, a multinational clinical trial of immunomodulatory cell therapy in renal transplant recipients funded by the 7th Framework Programme of the European Union.

Reference: Sagoo P. *et al.* JCI 2010; **120**(6): 1848–61.

P149 TACROLIMUS AFTER HEART TRANSPLANTATION – NON-INTERVENTIONAL TRIAL IN CHRONIC STABLE PATIENTS

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Background: The use of tacrolimus in heart transplant recipients has increased over the last years. Data from registries and smaller clinical trials suggest an advantage for a tacrolimus based immunosuppression in terms of rejection frequency, renal function and lipid profile. Therefore we evaluated effects of a switch from a cyclosporine A to a tacrolimus based regimen in chronic stable patients after heart transplantation (HTX).

Patients and methods: Ten chronic stable patients (nine male, one female) with a mean age of 50.2 ± 15.8 years were included in this study. Mean time post-HTX was 4.6 ± 2.5 years. All patients received a dual immunosuppressive regimen consisting of mycophenolate mofetile in nine patients and azathioprine in one patient. Tacrolimus was discontinued in one patient due to deterioration of renal function (switched to a calcineurin inhibitor free immunosuppressive regimen). Therefore nine patients were available for statistical analysis. 6 months after switch to a tacrolimus containing regimen, a significant reduction in LDL values (122.0 ± 33.11 mg/dl vs. 98.3 ± 24.1 mg/dl, $P = 0.04$) and a trend towards lower total cholesterol values was observed (195.1 ± 46.21mg/dl vs. 166.3 ± 24.0 mg/dl, $P = 0.06$). Whereas no significant change of renal function parameters was seen (baseline 1.4 ± 0.4 mg/dl vs. 6 months 1.4 ± 0.4 mg/dl, P = ns). No rejection episodes or newly diagnosed malignancies were documented during the course of study.

Results: In summary, switch from a cyclosporine A to a tacrolimus based immunosuppressive regimen is clinically feasible and safe. Thus, the switch of selected maintenance patients to a tacrolimus based regimen may be advantageous in the presence of risk factors for certain post-transplant complications, e.g. lipid abnormalities or malignant rejection profile.

P150 TACROLIMUS AFTER HEART TRANSPLANTATION - NON-INTERVENTIONAL TRIAL IN *DE NOVO* PATIENTS

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Background: The use of tacrolimus in heart transplant recipients has increased over the last years. Data from registries and smaller clinical trials suggest an advantage for a tacrolimus based immunosuppression in terms of rejection frequency, renal function and lipid profile. Therefore we evaluated effects of a tacrolimus based regimen for 1 year after heart transplantation (HTX) in de novo patients.

Patients and methods: A total of 60 patients (44 male, 16 female) with a mean age of 52.2 ± 9.3 years were included in the present study. According to our HTX center's clinical routine all patients received a primary immunosuppressive regimen consisting of tacrolimus and mycophenolate mofetile. 49 patients were available for statistical analysis as tacrolimus was discontinued in 5 patients due to deterioration of renal function ($n = 2$, switched to a calcineurin inhibitor free immunosuppressive regimen) and gastrointestinal disturbances ($n = 3$, switched to cyclosporine A). Six patients died during follow-up.

Results: 12 months after heart transplantation mean daily tacrolimus dose was 4.8 ± 4.1 mg compared to 7.1 ± 3.3 mg one month after HTX and mean daily tacrolimus levels were 8.5 ± 3.5 mg/l vs 13.9 ± 2.9 mg/l (both $P < 0.05$). A trend towards improved creatinine levels was observed 12 months after HTX (12 months: 1.3 ± 0.5mg/dl vs. 1 month: 1.5 ± 0.9mg/dl, $P = 0.15$). A significant reduction in LDL values (12 months: 83.5 ± 25.6mg/dl vs. 1 month: 99.2 ± 35.2 mg/dl, $P = 0.0017$) and total cholesterol values was seen (12 months: 158.8 ± 44.2 mg/dl vs. 1 month: 187.8 ± 51.5 mg/dl, P <ISHLT 2R were documented during the course of study. After 1 year, 39 patients (79.6% of total) had a normal left ventricular function and 10 patients (20.4% of total) a mildly decreased left ventricular function. 15 patients (30.6 percent of total) developed any form of transplant vasculopathy 1 year after HTX as assessed by coronary angiography.

A tacrolimus based immunosuppression after HTX is effective and well tolerated given the number of rejection episodes and side-effects in this cohort of patients early after HTX. Thus, a tacrolimus based regimen may be advantageous in the presence of risk factors for certain post-transplant complications, e.g. lipid abnormalities or malignant rejection profile.

P151 MISOT – MESENCHYMAL STEM CELLS FOR IMMUNOMODULATION AFTER LIVER TRANSPLANTATION

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Liver transplantation is the succesful treatment for many end-stage diseases. However, life-long immunosuppression that is needed to prevent graft rejection causes clinically significant side effects. In fact, the overall and long-term success of liver transplantation as a curative therapy often depends on the occurrence and management of drug-related side effects. Mesenchymal stem cells (MSC) can be used as an adjunct to standard-of-care immunosuppressive pharmacotherapy.

The MISOT study group has brought European investigators together and a variety of protocols to complement immunosuppressive pharmacotherapy with MSC have been suggested. To decide if patients undergoing organ transplantation can be safely treated with MSC and if these therapies yield a benefit for patient and graft survival, careful consideration of all available pre-clinical and clinical data has to be carried out. The Regensburg team of MISOT has succesfully implemented a phase I study to evaluate the safety and immunologcial efficacy of infusing MSC after liver transplantation and the study design and regulatory network of this study will be presented.

P152 CLINICAL OUTCOME OF CALCINEURIN INHIBITOR FREE THERAPY WITH EVEROLIMUS AND MYCOPHENOLIC ACID DERIVATES IN MAINTENANCE HEART TRANSPLANT RECIPIENTS WITH CHRONIC RENAL FAILURE: A SIX MONTH FOLLOW-UP

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Background: The calcineurin inhibitor cyclosporine A and tacrolimus (CSA, TAC) have nephrotoxic side effects. The calcineurin-inhibitor-free therapie with Everolimus and Mycophenolic-acid (MPA) seems to be an efficient option to avoid renal failure in Cardiac Transplant Recipients with Chronic Kidney Disease.

Methods: During 01.01.2010 and 31.10.2010 we switched 13 maintenance cardiac transplant (CTx) recipients with chronic kidney disease (CKD) stages 3–4 from dose-reduced calcineurin-inhibitor-therapy + everolimus to everolimus (EVL) + Mycophenolic-acid (MPA) such as Cellcept, Myfortic. Kidney function, lipid metabolism, and cardiac function were investigated.

Results: A cardiac rejection (proved by echocardiography) did not occur during six month follow up. Three patients developed herpes zoster infection (thoracic) and one patient developed pneumonia. One patient died during 6 month follow-up due to sepsis induced by pneumonic infection. Creatinine decreased from 2.3 (1.7–2.5) mg/dl to 1.7 (1.0–2.2) mg/dl ($P < 0.05$), urea decreased from 108.8 mg/dl to 79.5 mg/dl and GFR increased from 28.9 (19–40) ml/minutes to 43.4 (30–70) ml/minutes ($P < 0.05$). Leucocytes, Hb and thrombocytes were stable during the 6-month follow-up.

Conclusion: EVL combined with MPA has moderate beneficial effects on kidney function in CTx patients with CKD stages 3–4. The combination of everolimus and MPA was safe with respect to rejection and adverse events. One 71-year-old male patient with end stage renal insufficiency died 91 month post-htx during the 6-month follow-up because of sepsis due to pneumonic infection.

P153 SPECIFIC EFFECTS OF VERY LOW DOSE CNI-REDUCED THERAPY WITH EVEROLIMUS COMPARED TO THE STANDARD DOSE

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Introduction: The influence of a very low dosage everolimus (EVL) therapy in combination with calcineurin – inhibitors (reduced) in heart transplant recipients with chronic renal failure on the development of renal function and safety is currently not known.

Methods: A total number of 81 patients after heart transplantation with chronic renal failure were included in our data analysis. Initial immunosuppressive regime was Cyclosporine A and prednisolon. 56 patients undergoing conversion to EVL between 1.1.2004 and 31.12.2004 received regular dose (RD) with EVL (level: 5–8 μg/l). 25 patients undergoing conversion to EVL between 1.1.2005 and 30.06.2006 received a very low dose (VLD) therapy with EVL (level: 3–3.5 μg/l), which were selected as a very low dose subgroup from 51 patients with low dose EVL. Follow up: 1 year.

Results: Leukocytes, erythrocytes, thrombocytes, cholesterin (incl. HDL, LDL) and triglycerides did not differ significantly during the follow up ($P > 0.05$). Course of creatinine during follow-up (VLD): Creatinine before conversion (t_0) 2.2 mg/dl; 12 months after conversion (t_{12}):2.1 mg/dl. (RD): Creatinine t_0: 2.1 mg/dl; t_{12}: 2.3 mg/dl. Rejection rate: VLD: 16%; RD: 8.9%. Drop-out-rate: VLD: 20%; RD: 32%. Adverse events VLD 8%; RD: 53.6% ($P < 0.05$). Infections: VLD: 16%; RD 3.6%. The clinical aspect of the infections (fever, lassitude, no microbiological findings) in the VLD was suspect for viral genesis but CMV DNA PCR as well as markers for fungal infection were negative in the 81 patients.

Conclusion: We conclude that the clinical results such as laboratory and safety in the VLD and RD are comparable. Nevertheless in the very low dose group more infections occurred without positive proof of CMV DNA. On the other hand in the regular dose group more adverse events such as dyspnea, edema and cytopenia occurred.

P154 URINARY TRACT INFECTIONS AFTER TWO DOSES OF MYCOPHENOLATE MOFETIL IN RENAL TRANSPLANT PATIENTS RECEIVING STEROIDS AND TACROLIMUS

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Introduction: Urinary tract infections (UTI) are the most common form of bacterial infection after kidney transplantation and can affect long-term graft function. The purpose of this study was to compare the incidence of UTI between high and low dose of mycophenolate mofetil based immunosuppressive regimens with steroids and tacrolimus.

Methods: This is a retrospective cohort study, which assessed the incidence of UTI among kidney transplant recipients receiving the same immunosuppression apart from two dosages of mycophenolate mofetil (1 g/day vs. 2 g/day) with a total of 300 recipients (2004–2009) and a follow-up of 12 months.

Results: A total of 130 patients received initially 2 g/day mycophenolate mofetil (high dose, HD), 170 patients received 1 g/day (low dose, LD). In both groups, immunosuppression was reduced in steps. Groups did not differ in terms of demographics, delayed graft function, rejection rates and graft function (creatinine after 12 months: mean 1.8 mg/dl HD vs. 1.7 mg/dl LD).

Urinary tract infections were observed in 34.6% (HD) and 14.7% (LD) in the first month, 42.3% (HD) and 28.3% (LD) in the first 3 months, 50.8% (HD) and 36.9% (LD) in the first 6 months, and 54.6% (HD) vs. 39.4% (LD) in the first 12 months (each time $P < 0.05$). The most common were Gram-negative bacteria (59.3% HD vs. 65.2% LD) with predominance of *E. coli* (44% HD vs. 47.8% LD). Gram-positive bacteria were detected in 39% (HD) and 30.4% (LD) of positive cultures.

Conclusions: Infections are highly prevalent in the first year following transplantation. Initial dosage of 2 g/day leads to a significantly higher rate of urinary tract infections despite comparable rejection rate and transplant function.

P155 CORRELATION OF RECIPIENT FACTORS WITH THE COURSE OF LYMPHOCYTES AFTER ALEMTUZUMAB INDUCTION IN RENAL TRANSPLANTATION

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Introduction: The recovery of lymphocytes after alemtuzumab induction has been investigated in a number of trials, however, the clinical course after renal transplantation (KTx) has not been correlated with lymphocyte recovery. Herein, we correlate the outcome as well as recipient factors with lymphocyte recovery after alemtuzumab.

Methods: Retrospective analysis of 225 KTx between 01/2004 and 12/2010 which received 30 mg alemtuzumab as induction agent. Patients were devided into four groups according to lymphocyte recovery at four points of time (pre-Tx, 1–3 weeks post-Tx, 3 weeks-3 months post-Tx and 3–6 months after KTx). The relevance of recipient-characteristics was analyzed. Delayed kidney graft function (DGF) was defined as requirement for more than one dialysis within the first week after KTx. Statistical analysis of variance for repeated measurements with measurement time as within-subject factor and with age, CMV status, DGF status as between subject factors were performed

Results: Among all factors analyzed, DGF, CMV status and age showed a significant correlation with lymphocyte counts. The lymphocyte-counts in the DGF-group were higher, 10.7% vs. 13.13% ($P = 0.036$) in the first 3 weeks post-Tx. CMV-status of the recipient influences the quantity of lymphocytes pre-Tx significantly ($P = 0.009$). Age showed an influence on lymphocyte count 3 months post-Tx ($P = 0.032$).

Conclusion: CMV-status and age have a significant impact on lymphocyte recovery after alemtuzumab induction. Lymphocyte counts early after transplantation represent a prognostic factor for kidney function early after transplantation. A detailed analysis of phenotype and function of lymphocytes after alemtuzumab induction together with a correlation with the clinical course is warranted.

P158 MYCOPHENOLATE ACID ASSOCIATED ACUTE INFLAMMATORY SYNDROME IN A PATIENT AFTER PANCREAS TRANSPLANTATION

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Background: Mycophenolate acid (MPA) in its different forms (MMF or EC-MPA) represents a standardized immunosuppressive agent in different kinds of transplantation. Its main side effects are: infections, gastrointestinal disorder, myelosuppression, CMV-recurrence and wound healing disorder.

Aim: To report about a case of acute inflammatory syndrome (AIS) not associated to any kind of infection, in a patient who underwent pancreas after kidney (PAK) transplantation (Tx).

To our knowledge this is the only case reported up to now worldwide.

Case report: A 54-year-old man with IDDM Type 1 underwent PAK-Tx 11.2010 (KTx 2004).

Operation: Modified Boggi's technique with endocrine drainage in IVC (CIT 7 hours; WIT 16 minutes).

Immunsuppression: Induction with IL2rAb; Maintenance with Steroids (early withdrawal), Tacrolimus (itrough aim level 10 ng/ml) and MMF (2x1 g).

Postoperative course: Good primary graft's function without technical problems.

At POD 10 AIS occurred i.e. recurrent fever attacks (≥ 39 °C) with shivering and diffuse upper limb and shoulder girdle myalgias, Leukocytosis, CRP up to 13.41 mg/ml. All infectivological investigations of all body's fluids as well total body imaging revealed no infective origin of AIS. Notwithstanding, we reduced IS by stopping administration of MPA.

Within 36 hours the AIS disappeared. 1 week later AZA replaced MPA. Six months later the patient is doing well with god graft function of both kidney and pancreas with no rejection.

Conclusion: In case of AIS unclear origin in a transplant patient an association with MPA should be considered; MPA should be stopped and eventually replaced with AZA.

P159 EARLY POST-TRANSPLANT BLOOD LEVELS IN *DE NOVO* RENAL RECIPIENTS ON TACROLIMUS PROLONGED RELEASE (TACQD) VERSUS TACROLIMUS IMMEDIATE RELEASE (TACBD) IN A PHASE III DOUBLE-BLIND DOUBLE-DUMMY STUDY

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Introduction: Achieving consistent tacrolimus blood levels within therapeutic range early after transplantation prevents rejection and toxicity in renal recipients. This analysis of an international Phase III multicenter study compares Tac whole blood trough levels (TL) for 28 days (d) post-transplant with TacQD or BD.

Methods: Patients were randomised to TacQD ($n = 331$) or BD ($n = 336$) at 0.2 mg/kg/day in combination with MMF (2 g/day to d14 then 1 g/day) and a 3-month steroid taper (20 to 0−5 mg/day, d2-85) without antibody-induction. The first doses of TacQD/BD and MMF were given pre-transplant. Target levels for Tac were 10−15 ng/ml (d1-28), 5−15 ng/ml (d29-168) and 5−10 ng/ml afterwards. Tac TL, time to target and number of dose changes to reach target levels were determined.

Results: More patients on TacQD were in target range and in 5−15 ng/ml throughout the study. Median time to target (2 consecutive TL in target ± 10% or 3 if period between TL ≤ 2d) was 9 days for both TacQD and BD; mean number of dose changes were 3.0 (3.0) and 3.5 (3.0), respectively. Fewer patients (%) on TacQD than BD were exposed to TL ≥ 20 ng/ml: d1 (10.2 vs. 28.6), 4 (16.9 vs. 26.4), 7 (4.5 vs. 11.3), 14 (6.5 vs. 9.3), 21 (5.1 vs. 12.7), and 28 (7.4 vs. 5.8). A minority of patients had TL < 5 ng/ml or TL < 3 ng/ml. Mean dose during week 4 was higher with TacQD (0.21 mg/kg/day vs. 0.17 mg/kg/day), TL similar (12.3 ng/ml vs. 12.8 ng/ml), and biopsy-proven acute-rejection-free rate similar (0.83 vs. 0.87 at d28; 0.79 vs. 0.83 at d365: ns).

Conclusions: Initiating therapy with TacQD preoperatively resulted in more patients attaining the target range early post-transplant than with TacBD. Time to target and number dose changes in the initial phase after transplantation were not different to TacBD. Fewer patients on TacQD were exposed to potentially toxic blood levels, especially in week 1.

P160 GASTROPARESIS AS A SEVERE SIDE EFFECT OF TACROLIMUS

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Purpose: The combination of Tacrolimus, Mycophenolate Mofetil (MMF) and steroids is the first choice immunosuppressive therapy after Lung Transplantation. Mild gastro-intestinal side effects are frequently found in these patients and are mostly attributed to MMF activation. Tacrolimus related gastropareses has never been described.

Case Report: We report a case of a 52-year-old female lung transplant recipient that showed 2 days after transplantation severe nausea and vomiting several times a day. The onset of these symptoms coincided with the commencement of the immunosuppressive therapy with Tacrolimus, MMF and steroid directly after transplantation. The switch from MMF to Everolimus, with the idea to evoid the well known gastro-intestinal side effects of MMF was not effective. Upper gastrointestinal x-ray series demonstrated a severe gastroparesis. An infectious, central or ischemic etiology could be excluded. Finally, with lack of none options we converted the immunosuppressive therapy from Tacrolimus to Cyclosporine. After conversion, nausea and vomiting immediately resolved, the patient recovered and remained in a stable condition. Actually oral alimentation is fully estabiched.

Conclusion: Our case suggests that tacrolimus may cause gastropareses in lung transplant patients. This gatropareses is a new side effect that has not been described before and was quickly reversible after stopping Tacrolimus therapy.

P162 IMMUNOSUPPRESSION WITHDRAWAL AFTER PAEDIATRIC LD-LT PREVIOUS HAEMATOPOIETIC STEM-CELL TRANSPLANTATION

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Complete immunosuppression (IS) withdrawal and tolerance induction still represent a dream in the world of paediatric liver transplantation

Aim: Report of a case of child who became IS free after LD-LT because of acute liver failure after GVHD secondary to haploidentical haematopoietic stem cell transplantation because of inborn complex immunodeficiency.

Case Report: A 10-month-old child with complicated immunodeficiency (T-/B- and NK-SCID) underwent haploidentical haematopoietic stem cell transplantation from his mother without pre-conditioning. Two weeks later he developed a GVHD at skin and intestinal level with additional adenovirus related fulminant liver failure.

He underwent a LD-LTx (left lateral lobe) from his mother as donor again.

Immunosuppression consisted of high dose Tacrolimus, steroids and MMF.

The course of LT was uneventful the whole time.

GHVD-associated skin lesions persisted for 7 months treated finally with UVB.

14 months after LT FACS analysis showed complete CD 3 chimerism and IS was completely withdrawn.

30 months after LTx the actually 4-year-old child is doing well and free of IS.

Conclusion: Induction of chimerism and consequent tolerance induction by means of stemcell transplantation from same donor before LD-LT may be represent a strategy of IS free LT.

P163 EFFECTS OF IMMUNOSUPPRESSION ON A AND B CELL RENEWAL WITHIN TRANSPLANTED MOUSE ISLETS

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Background: The antiproliferative effects of immunosuppressive drugs such as sirolimus and tacrolimus used in human islet transplantation interfere with the capacity of β cells to balance cell renewal and cell loss. This feature may be an important contributor to progressive graft dysfunction in islet transplant recipients over time. We analyzed the influence of different immunosuppressants on α and β cell proliferation and transplant outcome following syngeneic β cell transplantation in mice.

Methods: Syngeneic islets (300 IP) were injected into the right liver lobes of C57BL/6 diabetic recipients. Osmotic pumps filled with bromodeoxyuridine (BrdU) (control) or BrdU and an immunosuppressant [tacrolimus, sirolimus, everolimus, or mycophenolate mofetil (MMF)] were implanted. Glycemic control was assessed using glucose tolerance tests. After four weeks, proliferation of α and β cells was detected by BrdU incorporation. In addition, fractional β cell area and average β cell size was determined by morphometric analysis.

Results: The average blood glucose levels were significantly higher in all treatment groups compared to controls. Glucose tolerance was improved only in control animals ($P = 0.009$). The fractional β cell area and β cell proliferation in MMF-treated mice were comparable to control mice ($P = 0.66$). In contrast, treatment with everolimus and sirolimus led to a significant reduction in β cell proliferation and fractional β cell area. While transplanted β cells from animals treated with tacrolimus also presented a reduced replication rate ($P = 0.023$), the fractional β cell area was not affected compared to untreated controls ($P = 0.72$).

Conclusions: Our results demonstrate that the β cells of transplanted islets have a strong capacity for self-renewal when not affected by immunosuppression or immune assault. In contrast to other immunosuppressants, MMF does not affect β cell replication and fractional β cell area; therefore, its use may lead to improved long-term results in islet transplantation.

VARIA

P164 REVERSIBLE SEVERE IMMUNOLOGICAL AND INFECTIOUS COMPLICATIONS IN INTESTINE TRANSPLANT: A CASE REPORT

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Background: Clinical outcome after severe acute intestine graft rejection treated by maximized immunosuppression, followed by CMV graft enteritis and fungal pneumonia.
Methods: A 29-year-old female patient with chronic intestinal pseoudoobstruction of unclear etiology underwent intestinal transplantation in December 2009. Basic immunosupression consisted of pulsed thymoglobuline induction, infliximab, tacrolimus and steroids. After good initial function with normal graft biopsy, a severe acute rejection with biopsy proven complete epithelial destruction occurred on post-operative day 26 following a preceeding one-time low tacrolimus level (9 ng/ml). After immediate high dosed antirejective treatment with totally 6.5 g methylprednisolone, thymoglobuline for 10 days and tacrolimus trough level about 20 ng/ml, no histological improvement was found until day 32. Mucosal regeneration was noted on day 40, progressing to regeneration of normal epithelial after day 44, allowing cautious taping of steroids doses. Azathioprine was added and temporarily discontinued due to a severe leucopenia, as was sirolimus for the same reason. On day 46, a CMV histologically proven graft enteritis was successfully treated by anti-CMV hyperimmunoglobuline + gancyclovir with cautiously reduced immunosuppression. A bilateral *Aspergillus* pneumonia occurred at month 6.
Results: Within a consequent systemic and topic therapy of liposomal Amphotericin B, followed by systemic Voriconazol and reduction of immunosuppression (obtained tacrolimus trough level 10 ng/ml) and low dosed prednisolone (10 mg), the pneumonia was regredient until month 9. At the end of the 1st year, the patient is in good general condition with a stable graft function (biopsy proven), sufficient oral alimentation, stable body weight, normal leucocyte count and reversible infections (cystitis, lid abscess, enoral herpes).
Conclusion: A stable graft function and quality of life was achieved after severe acute rejection, CMV-graft enteritis and pulmonary aspergillosis by cautiously adapting immunosuppression and a consequent antimicrobial treatment.

P166 HANDS-ON TRAINING OF SURGICAL TRAINEES HAS NO SIGNIFICANT IMPACT ON SURGICAL QUALITY PARAMETERS OF KIDNEY TRANSPLANT PROCEDURES

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Introduction: Attractive surgical training programs are needed to recruit young surgeons to the field of transplantation. However, learning curves in transplant surgery may be associated with an unacceptable patients' risk. We have reviewed our transplant training program for objective indicators of surgical quality, which would suggest surgical training-based inferior outcomes.
Methods: Eighty-one consecutive kidney transplant procedures from postmortal donation in 2010 were analyzed. Operative procedures were divided in cases where the trainee (HANDS-ON) had the active operative role, assisted by a senior transplant surgeon and cases were the trainee was helping the senior surgeon to do the case (WATCH). Quality parameters assessed included the total operative time, warm ischemia time, post-operative surgical complication rates. The decision on who was the operating surgeon was made on a case-to-case basis left to the discretion of the senior surgeon.
Results: There were no significant differences objective quality parameters between the operative procedures performed by the trainee or by the senior transplant surgeon. The total operative time was 151 ± 4.7 (minutes), the warm ischemic time was 34 ± 3.1 (minutes) in the HANDS-ON group 155 ± 5.9 (minutes) and 38 ± 2.0 (minutes) in the WATCH group, respectively. There were seven postoperative complications, which needed a surgical revision in the HANDS-ON and five in the WATCH group.
Discussion: Appropriate case selection adapted to the individual surgical skills and a complaisant intra-operative teaching attitude allows safe hands-on training of surgical trainees in transplant surgery.

P168 EN BLOC PANCREAS-LIVER TRANSPLANTATION DUE TO BILIARY CIRRHOSIS AND DIABETES MELLITUS FOLLOWING DUODENOHEMIPANKREATEKTOMIE

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Background: Fifteen months after uneventful duodenohemipancreatectomie for suspected but not confirmed tumor of the head of pancreas a 50-year-old patient presented with insulin-dependend diabetes mellitus (IDDM). An operative revision of a stenosis of the hepatico-Jejunostomie could not prevent the development of cirrhosis which was seen in liver biopsyprior to listing the patient for combined Pancreas-liver-Tx.
Methods: After 3 months a 45-year-old donor was accepted. The recipients antrum (stomach) as well as spleen had to be removed due to severe adhesions; pancreatectomy and hepatectomy was followed by preparing an aorto-iliac-graft-bypass. The en-bloctransplantation included Pancreas, liver, spleen, antrum and prox. Jejunum.(KIT 6:39 hour, WIZ 40 minutes). Immunosuppresssiveregimen consisted of ATG-Induction, FK506, MMF and steroids.
Result: Early postoperative course was uneventful apart from a relaparotomy due to perihepatic hematoma. Perfusion of all organs was excellent and patient was insulin-free immediately after transplantation. Oral intake was delayed but fully possible after 3 week, patient was discharged after 38 days. However severe repetitive cholangitis caused repeated readmissions in the following months.
Conclusion: Combined Pancreas−liver Transplantation following Duodenohemipancreatectomy is a feasible option in patients suffering from IDDM and liver cirrhosis. Whether total pancreatectomie, splenectomie and (partial) gastrectomy has to be carried out and needs extension of transplant procedure by stomach, spleen and prox. jejunum depends on individual circumstances.

P169 UREA-FORMULATED PROTEINS: A NOVEL TOOL FOR IMPROVED MONITORING OF VIRUS SPECIFIC CELL MEDIATED IMMUNE RESPONSES IN TRANSPLANT PATIENTS

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Background: Immunosuppressive therapy of transplant recipients is indispensible to prevent graft rejection or GvHD. However, inadequate suppression of cell-mediated immunity (CMI) may cause reactivation of herpes viruses and related clinical complications. Thus, accurate quantification and monitoring of CMI in the course of immunosuppressive therapy may not only help to predict the onset of these complications, but also to adjust antiviral and immunosuppressive therapy.
Methods: Urea-formulated antigens combined with the sensitive interferon-γ (IFN-γ) ELISpot have been applied to quantify and monitor CMI. EBV/CMV load was monitored by qPCR. Results of immunologic and virologic monitoring were correlated with clinical parameters.
Results: We have evaluated a diagnostic tool for the analysis and monitoring of CMI utilizing urea-formulated stimulator antigens and the IFN-g ELISpot. UREA-formulated proteins are processed by cross-presentation, enabling the simultaneous reactivation of a broad spectrum of antigen-reactive leukocytes including T-helper and cytotoxic T-cells, but also NK- and NKT-cells. Established assays allow a robust detection and monitoring of functional CMV/EBV protein-reactive leukocytes in > 90% of seropositive healthy individuals regardless of genetic factors. Moreover, studies in renal and allogeneic stem cell transplant recipients revealed the suitability of these assays to assess functional impairment of CMI in immunocompromized individuals. In addition, initial results emphasise correlations between the numbers of virus-reactive leukocytes and clinical complications.
Conclusion: Monitoring clinically relevant blood leukocytes by applying urea-formulated herpesvirus-derived stimulator antigens and the ELISpot technology may represent an interesting strategy to assess the immune status of immunosuppressed transplant recipients and to guide pre-emptive therapeutic choices in appropriate high-risk populations.

MALIGNANCY AFTER TRANSPLANTATION

P171 PAEDIATRIC EPSTEIN-BARR VIRUS-ASSOCIATED POST-TRANSPLANT SMOOTH MUSCLE TUMORS

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Patients after solid organ transplantation are at higher risk to acquire Epstein-Barr-virus (EBV)-associated diseases. While post-transplant lymphoproliferative disorder (PTLD) may occur in up to 10% of patients, development of post-transplant smooth muscle tumors (PTSMT) represents a very rare complication.

We present the characteristics of three PTSMT and demonstrate smooth muscle phenotype and EBV infection of the tumor cells. The three young female patients (mean age at transplantation 8 years) developed PTSMT after a mean interval of 46 months following liver or heart transplantation. PTSMT manifested either in the liver transplant, pharynx or the lung. By applying short *tandem repeat PCR analysis ("molecular fingerprinting")* we could demonstrate the donor origin of one PTSMT in the liver graft. In addition to PTSMT, all three patients showed PTLD: two early lesions and one monomorphic PTLD.

We also re-evaluated data on PTSMT of the last 20 years from an additional 28 cases: approximately two-third of EBV^+ solid tumors were of low malignant potential and did not significantly limit the survival of patients while post-transplant leiomyosarcomas could be shown to have a poorer 5-year survival rate ($P = 0.0398$).

P173 SMOKING-RELATED CARCINOMA AFTER LIVER TRANSPLANTATION

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Introduction: Liver transplantation (LT) is a well-established therapeutic option of acute and chronic liver diseases. The incidence of malignomas after LT has a great impact on long-term survival. Smoking is associated with bronchial, oropharyngeal and urogenital carcinomas compromising survival of patients after LT. In view of sparse available data, patients with and without history of smoking transplanted at our center were compared.

Material and method: All data were taken from our prospectively maintained database. Patients ($n = 547$) transplanted between September 1997 and May 2011 were included. Survival, total number of solid tumors as well as smoking related carcinomas (SRC) were evaluated. For the assessment of long-term survival patients with post-operative (90 days) mortality were excluded. Recurrence of hepatocellular carcinoma and cutaneous tumors were not considered in the evaluation.

Results: In 433/547 patients after LT smoking history was verified: 165 had never smoked, 155 quitted smoking before LT whereas 113 were smokers before and after LT. The 5-year survival-rate in non-smokers was 70%, in smokers 63%. Peri-operative mortality was identical in both groups ($P = 0.703$). Excluding perioperative mortality, 146 non-smokers and 240 smokers were left. After exclusion of the 90-day-mortality, 5-year survival was 77% and 68% ($P = 0.029$). The number of solid carcinomas (including non SRCs) was similar (8/146 in non-smokers, 22/240 in smokers; $P = 0.240$). SRCs did not occur in non-smokers, but 11 times among the smoking patients ($P = 0.008$). Dividing in non-smokers, former smokers and current smokers there were 0, 3 and 8 cases of SRCs found ($P = 0.001$). The survival of these patients was significantly compromised when compared to patients without SRC ($P = 0.025$).

Conclusion: Smoking leads to a significant increase of bronchial, oropharyngeal and urogenital carcinomas. Patients should quit smoking before transplantation. Outcome of LT is substantially impaired by smoking history or ongoing smoking.

P174 TUMOR MARKERS AS A SCREENING METHOD FOR DE-NOVO NEOPLASMS AFTER HEART TRANSPLANTATION

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Backround: Previously the major limitations for long-term graft survival were necrosis and allograft rejection however with recent success in immune suppression and improvement in aseptic surgery complications such as the development of de-novo malignancies are becoming more clinically relevant. At the moment we do not have a satisfying method to detect neoplasms in an early stage so patients could benefit from sufficient time treatment. Therefore the utilization of tumor markers as a screening method is taken into consideration.

Methods: The study included 561 patients, which underwent a cardiac transplantation between 2002 and 2010 at the General Hospital Vienna. Average age was 63.68 (± 12.61) years, the mean follow-up was 123 (± 66) months. We analyzed beta 2-microglobulin (ß 2-MG), carcinoembryonic antigen (CEA), carbohydrate antigen 15-3 (CA 15-3), carbohydrate antigen 19-9 (CA 19-9), carbohydrate antigen 125 (CA 125).

Results: For ß-2MG a sensitivity of 80% and a specificity of 38% was calculated. The *P*-value was $P = 0.243$. CEA resulted in a sensitivity of 38%, a specificity of 70%, whereas the *P*-value was $P = 0.418$. CA125 made up a sensitivity of 25%, a specificity of 82% and a *P*-value of $P = 0.965$. Thus no significant *P*-value was calculated for any of these markers.

CA 19-9 resulted in a sensitivity of 75% and a specificity of 88%. The *P*-value was $P < 0.001$ and therefore significant.

Conclusion: Based upon the results of our current study we conclude that CA 19-9 is a significant marker to monitor the possibility of occurring malignancies post-transplantation.

P175 POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE IN CHILDREN WITH INTESTINAL TRANSPLANTATION – CASE REPORT AND REVIEW OF LITERATURE

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Background: Post-transplant lymphoproliferative disease (PTLD) in children with intestinal transplantation (ITx) is a recognized condition, however data with respect to incidence, localisation, relapse rate, treatment and prognosis are rare.

Methods: Case report and review of the literature using PubMed.

Case report: The girl was transplanted for total aganglionosis and received small bowel and colon graft. The induction of immunsuppression (IS) was performed with IL-2 receptor antibodies, steroids and tacrolimus. The recipient was negative and donor was positive for EBV. Seven months after transplantation PTLD was found in stomach, pharynx and lungs of the recipient. Treatment with anti-CD20 antibodies (rituximab) and lowering of IS were successful. Nineteen months after ITx a relapse of PTLD was found in the jejunum (graft). Another extended cycle of rituximab was given and rapamycin (sirolimus) was added to IS allowing further reduction of tacrolimus. Up to now this treatment was effective, the child is in excellent clinical condition.

Review of literature: PTLD is the most frequent malignant complication in solid organ transplantation with highest rates in visceral Tx up to 27% (Grant, 2005). Children and especially those with multivisceral transplantation are more often affected than adults (Reyes, 1996 and Abu-Elmagd, 2009). Identified risk factors for PTLD apart from EBV status and kind of transplantation are age, type of induction and extent of IS, time post-transplant, frequency of rejections and splenectomy. Treatment strategies are based on IS modification, antiviral therapy, anti CD20 antibodies, chemotherapy. New immunosuppressive drugs, e.g. mTOR inhibitors, are important to achieve modification of treatment and reduce risk of PTLD recurrence. A new approach consists of therapy with EBV antigen specific T-cells (Haque, 2002). No representative data were found for PTLD localisation sites and the relapse rate in children. Allover mortality rates in solid organ Tx are reported to range between 25 and 80% but there is insufficient data predicting the outcome of PTLD in children with ITx.

Conclusion: In contrast to adults and other organ transplantations children with ITx are on the highest risk for EBV associated PTLD. Further studies are required concerning PTLD localisation, relapse rate and long term outcome in children using modified immunosuppressive regimes. As in other cases of PTLD in solid organ recipients, anti CD20 therapy is the cornerstone of treatment in the majority of cases in ITx patients.

PHARMACOGENETICS/BIOMARKER

P176 C-TERMINAL AGRIN FRAGMENT (CAF) – A PROMISING NEW BIOMARKER FOR KIDNEY FUNCTION?

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Background: Agrin is the major proteoglycane of the renal glomerular base membrane. One of its cleaving products is a C-terminal 22-kDa fragment (CAF), which has been found in brain, muscle and kidney. While serum levels of CAF have been observed to be altered in sarcopenia, the influence of kidney function on CAF is not yet described.

Patients and methods: Blood samples were obtained from 263 individuals (169 healthy individuals and 94 patients with end-stage-renal-disease (ESRD) of whom 66 underwent renal transplantation). CAF-levels were measured in all 263 subjects by ELISA and Western blotting. Moreover, CAF-levels were measured in the 66 patients before and several times after transplantation and compared to creatinine and urea levels.

Results: CAF-levels were significantly lower in healthy subjects than compared to ESRD patients (6.4 ± 2.2 vs. 65.0 ± 42.9 ng/ml, $P < 0.001$). CAF-levels pre- and post-dialysis CAF did not differ (57.1 ± 6.6 vs. 57.1 ± 8.8 ng/ml). CAF-levels were 68.5 ± 41.7 ng/ml before transplantation and decreased to 14.7 ± 15.2, 9.0 ± 10.8 and 6.5 ± 4.6 ng/ml at week 1, month 1 and month 2 after transplantation respectively. Compared to creatinine levels, the within-subject-correlation was r = 0.8 ($P < 0.01$), the between-subject-correlation was r = 0.5 ($P < 0.001$). The correlation to urea was r = 0.22 ($P = 0.01$) and r = 0.29, ($P = 0.028$). When adjusted for age, weight and sex, only patient's gender had an impact on between-subject-correlation (r = 0.55 ($P < 0.001$) for CAF-creatinine).

Conclusions: This is the first study ever to show that CAF might be a promising biomarker to evaluate kidney function. Further studies have to investigate whether CAF predict changes in renal function more accurately than serum creatinine.

P177 *IN VITRO* ENDOTHELIAL CELL ACTIVATION AFTER HEART TRANSPLANTATION AS A BIOMARKER FOR THE DEVELOPMENT OF CHRONIC REJECTION

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Purpose: Endothelial cell (EC) activation is observed in allograft vasculopathy after heart transplantation (HTX). The disease is characterized by increased cell adhesion molecular expression. The aim was to determine whether serum from HTX patients possesses a net proinflammatory bioactivity to activate cultured EC.

Methods and materials: Serum was obtained from patients before and during the 1st year post-HTX. Net proinflammatory bioactivity of serum was investigated by monitoring the surface expression of E-selectin, VCAM-1 and ICAM-1 in cultured human umbilical vein EC (HUVEC) following incubation with serum from 34 HTX patients and 38 healthy volunteers. Tumor necrosis factor (TNF) was used as a reference.

Results: Serum from volunteers was used to determine a threshold for the basal expression of adhesion molecules (mean + 2x standard deviation, E-selectin, 3.9; VCAM-1, 4.6; ICAM-1, 3.2; % of TNF-stimulated cells). Serum samples from patients did not stimulate the expression of E-selectin. Serum from six patients induced a small increase in VCAM-1 (factor 1.5 ± 0.6 over threshold, $P < 0.05$). ICAM-1 stimulation: Serum from four patients did not activate HUVEC. The remaining samples significantly increased the expression of ICAM-1 (factor 2.7 ± 1.2 over threshold, $P < 0.05$). The time course of the ICAM-1 expression over 1 year showed a steady increase (12 patients, factor 4.9 ± 2.2 over threshold), 1–5 peak values (11 patients, factor 4–10 over threshold), or high pre-HTX values (five patients, factor 5–8 over threshold) reaching threshold values after 2 weeks of HTX. There was no correlation with biopsy-proven rejection or clinical infection.

Conclusions: During the 1st year post-HTX patients released pro-inflammatory parameters which induced ICAM-1 on the surface of EC. Close monitoring over a long-term period might bring us information about the sensitivity and reliability of this biomarker to predict rejection episodes in the vascular system of the allograft.

P178 IMMUNE MONITORING WITH UREA-FORMULATED EBV PROTEINS FOR PREDICTION OF EBV REACTIVATION IN RENAL TRANSPLANT PATIENTS

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Background: In immunocompetent individuals, Epstein-Barr virus (EBV) infections are effectively controlled by cell-mediated immunity (CMI). However, impairment of CMI by immunosuppressive therapy after transplantation occasionally causes EBV reactivation and viremia, which can be associated with the occurrence of post-transplant lymphoproliferative disorders (PTLD).

Method: We performed a 2 year prospective non interventional study in a cohort of 83 renal transplant recipients (20 female, 63 male, median age was 51 years) to evaluate the use of urea-formulated (u)BZLF1 in combination with an IFN-γ ELISpot for monitoring of functional EBV protein-reactive blood leukocytes. EBV viral load was determined in serum samples using quantitative PCR.

Results: Monitoring (u)BZLF1-specific functional blood leukocytes was feasible in 68 of 73 EBV seropositive patients (93.2%). These patients showed substantial numbers of (u)BZLF1-reactive leukocytes prior to immunosuppression, which rapidly decreased within the first three weeks post-transplantation and remained low during the next month. However, BZLF1-functional leukocytes recovered in month 6 to 18 post-transplantation reaching in part maximum levels exceeding that observed prior to immunosuppressive treatment. Weak EBV reactivation (10^2–10^3 viral copies/ml) occurred in 34.9% of the seropositive patients whereas transient EBV reactivation with $> 10^3$ EBV DNA copies/ml was observed in only 7.2% of transplant recipients. None of the patients developed PTLD. Importantly, patients showing no EBV-reactivation revealed significantly higher numbers of BZLF1-reactive blood leukocytes, suggesting a protective effect of BZLF1-specific CMI.

Conclusion: Monitoring of functional EBV protein-reactive blood leukocytes may be an important new strategy to assess the risk for EBV reactivation and related complications and to guide immunosuppressive as well as antiviral treatment.

P179 CXCL13 AS A NOVEL MARKER FOR DIAGNOSIS AND DISEASE MONITORING IN PEDIATRIC PTLD

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Purpose: Post-transplant lymphoproliferative disorders represent the most common malignancies in pediatric solid organ transplant recipients. Depending on the type of organ graft and the type and potency of immunosuppressive therapy incidences range from 1–2% in renal and liver transplant patients to up to 20% in thoracic organ and intestinal transplantation. Diagnosing PTLD often imposes a challenge on the treating physician. Symptoms are unspecific, and PTLD is diagnosed in heavily pre-treated patients. Therefore, in many cases PTLD is diagnosed only at later stages. The identification of a simple monitoring marker for the diagnosis of PTLD is from great clinical interest and was the purpose of this trial.

Methods: To test whether CXCL13 is elevated in pediatric PTLD patients, serum samples of 21 solid organ transplant patients with PTLD of different histology's were analyzed by quantitative determination of serum CXCL13 with a Quantikine kit (R&D Systems, Minneapolis, MN, Catalog Number DCX130). Patient samples were analyzed according to their availability. The samples were measured by personnel blinded to the clinical patient data. For statistical analysis Graphpad prism software was employed. The median CXCL13 levels were compared using two-sided Mann–Whitney test.

Results: CXCL13 was significantly higher in PTLD patients (median 151.6 pg/ml; $P < 0.0001$) than in healthy pediatric controls (61.5 pg/ml). In 15 of 21 PTLD patients CXCL13 was above 113.1 pg/ml (mean + 2x standard deviation). PTLD patients had higher values than pediatric solid organ recipients with EBV reactivation (76.1 pg/ml; $P = 0.0006$), the latter group not differing significantly from healthy children. For 12 patients serum from both EBV reactivation and PTLD diagnosis were available. Except for one patient CXCL13 increased from EBV reactivation to PTLD diagnosis. Where available, we tested serial serum samples in the course from organ transplantation to PTLD and after PTLD for CXCL13. Of note, we found in two representative patients with Hodgkin's disease resp. Burkitt's lymphoma elevated CXCL13 levels up to 2 years prior to diagnosis of PTLD. Following cytotoxic treatment both patients went into continuous complete remission and S-CXCL13 normalized Since CXCL13 is in sepsis patients closely associated with CrP we correlated CXCL13 with CrP values. No association was detected rendering a mere infection-associated elevation in PTLD patients unlikely.

Conclusion: Taken together, CXCL13 might be a readily available surrogate marker for diagnosis and disease monitoring in pediatric PTLD. However, our study is limited by its retrospective nature. The definitive value of CXCL13 in serum has to be evaluated in prospective clinical trials. Thus, CXCL13 levels will now be incorporated into the pediatric PTLD register.

P180 IMPACT OF ATP-BINDING CASSETTE TRANSPORTER POLYMORPHISMS ON FIBROSIS PROGRESSION IN RECURRENT HEPATITIS C AFTER LIVER TRANSPLANTATION

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Background: ATP-binding cassette (ABC) transporters are transmembrane proteins that mediate the translocation of various substrates across cell

membranes. Hepatic insulin resistance increases expression of ABC transporters G8/G5 implicated in the regulation of cholesterol metabolism and in severity of hepatitis C virus (HCV) infection. Expression of ABCB1 has been shown to be increased in activated hepatic stellate cells in chronic liver diseases.

Aim: To assess the influence of ABC-transporter polymorphisms on graft survival and HCV recurrence after liver transplantation (LT).

Methods: We prospectively genotyped ABCG5 (C1810G), ABCG8 (C1199A and C1895T), ABCB1 (C1236T, G2677T, C3435T) in 174 LT recipients (49 with recurrent hepatitis C after LT, 125 controls transplanted for other liver diseases) by PCR-restriction fragment length polymorphism assay.

Results: Analyses of single nucleotide polymorphisms (SNPs) of the above mentioned genes revealed no differences regarding the distribution of the genotypes between HCV and non-HCV patients. Graft survival in HCV patients was significantly lower compared to controls (45.2% vs. 74.5% at 10 years, $P = 0.01$). None of the ABCG5/8 or ABCB1 genotypes influenced graft survival following LT. In the univariate analysis, presence of ABCG8-1895C/C, ABCB1-3435T/T, and ABCB1-2677G/T+T/T alleles, and treatment with bolus corticosteroids for acute rejection episodes were identified as predictors of fibrosis stages 3–4 in recurrent hepatitis C. Independent predictors of severe HCV recurrence included presence of ABCG8-1895C/C and ABCB1-3435T/T alleles, and bolus administration of corticosteroids.

Conclusions: HCV LT recipients with ABCG8 and ABCB1 polymorphisms have a significantly higher prevalence of advanced fibrosis.

P181 SINGLE POINT MEASUREMENT OF IMPDH ACTIVITY IN ERYTHROCYTES REFLECTS MPA EXPOSURE OVER TIME

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Introduction: Mycophenolic acid (MPA) inhibits inosine monophosphate dehydrogenase (IMPDH), resulting in decreased GTP concentrations and thereby inhibition of lymphocyte proliferation. Ongoing MPA therapy has been associated with increased IMPDH activity in whole blood and erythrocytes but not in lymphocytes. Elevated IMPDH activity described in erythrocytes does not translate into reduced efficacy of MPA but the consequences of the observed higher IMPDH activity in erythrocytes are unclear.

Methods: In the present study, we investigated IMPDH activity in erythrocytes (eryIMPDH) in a large cohort of patients ($n = 160$) during the first year after renal transplantation. Results were compared with patients, who experienced significant dose reductions, cessation of MPA therapy, or who did not receive MPA after transplantation.

Results: IMPDH activity in erythrocytes is significantly lower than in MNC before initiation of MPA treatment ($n = 77$:12.99 ± 10.8 vs. 102.45 ± 32.3 XMP /μmolAMP*s). Beginning with the first week after initiation of MPA therapy we observed a gradual increase of eryIMPDH over the first 3 months after transplantation resulting in a more than 20-fold higher eryIMPDH at month 3 ($n = 44$: 305.5 ± 230.0 XMP /μmolAMP*s). In contrast we did not observe any differences in eryIMPDH activity between pre-transplant and 3 months post-transplant in 18 patients treated without MPA. In patients with significant MPA dose reductions early after transplantation (< 3 months after Tx) no further increase of eryIMPDH was observed after dose reduction (35.5 ± 22.2 vs. 34.6 ± 13.9). Interestingly late (> 3 months after Tx) dose reductions resulted in a significant decrease of eryIMPDH (240.5 ± 98.2 vs. 122.3 ± 97.2, $P = 0.03$).

Conclusions: The role of eryIMPDH as surrogate maker for long-term MPA exposure and association with outcome should be further investigated.

IMMUNOLOGY

P182 ESTABLISHMENT OF A FLOW CYTOMETRY PANEL FOR LEUCOCYTE SUBTYPING IN SOLID ORGAN TRANSPLANT RECIPIENTS

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A series of recent high-profile publications have identified immunological parameters indicative of chronic rejection or stable, drug-free transplant acceptance in renal (Sagoo, P. *et al.* JCI, 2010; Newell, K. *et al.* JCI, 2010) and liver (Martínez-Llordella, M. *et al.* JCI, 2008) transplant recipients. Although individual management of immunosuppression according to immune monitoring tests remains a distant prospect, marker profiles of tolerance and rejection of transplanted organs are of increasing interest to academic transplant centers as short-term measures of outcome in clinical trials. Here, we report the establishment of technical procedures to evaluate the frequency of peripheral blood leucocyte subsets previously found to be associated with transplant outcomes. Specifically, this flow cytometry panel quantifies: B cell subsets, including $CD38^{++}$ $CD24^{+}$ transitional B cells; T cell subsets, including $CD27^{-}$ $CD57^{+}$ activated T cells, $CD45RA^{+}$ $CCR7^{+}$ $CD62L^{+}$ central and $CD62L^{-}$ effector memory T cells, and suppressor T cell populations, including $CD4^{+}$ $CD25^{+}$ $CD127^{low}$ T reg and $CD8^{+}$ $CD28^{-}$ Ts; and, NK cell, monocyte and dendritic cell subsets. Reagents were selected to give optimal performance on an 8-colour Canto-II cytometer (Becton Dickinson). Standardised sample preparation conditions are described and procedures for quality control are given. Reference ranges for each leucocyte subset are quoted for a population of normal, healthy volunteers. This flow cytometry panel is now being used, in conjunction with analysis of peripheral blood gene expression by quantitative PCR and functional assays, to assess the immunological status of patients recruited to the *Bottom-Up Study* (Clinicaltrials.gov: NCT01023542) being conducted at University Hospital of Regensburg.

P183 DETECTION OF ANTIBODIES IN ELUATES OF IMMUNOADSORPTION (IA) CAUSING HUMORAL REJECTION IN PATIENTS AFTER SOLID ORGANS TRANSPLANTATION

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Patients on the waiting list are periodically screened only for the presence of HLA-antibodies and a positive crossmatch is a contraindication to transplantation. Non-HLA antibodies were not tested before and after transplantation.

Material and methods: We studied 13 patients, who were treated with 69 immunoadsorptions (median 3–13 treatments, 1.3 l plasma volume exchanged) after humoral rejection (nine kidney Tx and four heart Tx). Antibodies in the 69 Eluates were tested using (1) the complement-dependent lymphocytotoxicity (LCT), (2) the solid-phase enzyme-linked immunosorbent assay (ELISA) and (3) Luminex technology. We investigated antibodies against HLA-I/-II, non-HLA Glycoprotein (GP) IIb/IIIa, Ib/IX, Ia/IIa, AT1/2-receptor and ETAR-receptor. We parallely investigated the antibody titer in the patient sera before and after IAs

Results: The antibody titer against HLA-antigens was 1:4–1:256, the antibodies against GP IIb/IIIa 1:1–1:32, GP Ib/IX negative to 1:32, GP Ia/IIa 1:1–1:16. We detected in 78% (44/57) antibodies against AT1/2-receptor and in 82.46% (47/57) antibodies against ETAR-receptor.

By the course of decreasing the antibody titer we can show the effectivity of the elimination of bound antibodies on the transplant. We could not find antibodies against GP IIb/IIIa, GPIb/IX, GPIa/IIa in the sera before and after IAs. By using LCT before IA HLA-antibodies were detected in sera with 32% vs. 53% in eluate and 50% vs. 100% by using ELISA and Luminex. In more than 50% of the examined probes we could not find HLA-antibodies in sera before IA. Patients with antibodies against AT1/2-and ETAR-receptors (38%) and other non-HLA-AB showed periodically rejection in comparison to HLA-AB.

Conclusion: In the antibody mediated humoral rejection the AB are bound on the transplant (HLA and non-HLA). The main problem is to detect these AB in the sera before and after IA. The sensitivity of the LCT in this situation is too low. We should screen the sera of all patients on the waiting list for non-HLA-antibodies.

P184 THE KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTOR GENOTYPE CORRELATES WITH ACUTE KIDNEY FAILURE IN THE EARLY POST-LIVER TRANSPLANTATION PERIOD

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Acute kidney injury (ARI) and acute renal failure (ARF) are major complications following liver transplantation (LT) leading up to chronic end-stage renal disease. The etiology of post-LT impairment is multi-factorial but it is suggested that e.g. during ischemia initial insults provoke morphological and functional changes within the vascular endothelium and tubular epithelium. As it has been demonstrated, that ischemic ARF can occur in the absence of classical T cell function and that Natural Killer (NK) cells can kill syngeneic tubular epithelial cell (TEC) *in vitro*, we aimed to elucidate the role of NK cells and their receptors in the context of early post-liver transplant ARI and ARF more precisely. For instance, patients with impaired kidney function (serum creatinine levels > 1.2 mg/dl, $n = 13$) illustrated heightened peripheral NK cell frequencies prior LT compared with patients showing stable renal function ($n = 9$) (17.22 ± 10.56% vs. 12.98 ± 9.09%). We further retrospectively tested 89 liver transplant recipients for their killer-cell immunoglobulin-like receptor (KIR) genotype and the risk of ARI and ARF. During the first week post liver-transplantation ARI occurred in 12% and ARF in 22% of the patients, respectively. ARI was a significant risk factor for acute rejection ($P = 0.0009$) and ARF led to elevated serum creatinine levels (> 1.2 mg/dl) at the time of hospital discharge ($P = 0.008$). Interestingly, significantly less patients having a homozygous KIR haplotype A/A (characterized by the presence of only one activating KIR gene) displayed a stable early postoperative kidney function, compared to patients with a KIR haplotype B/x (more than one activating receptor) ($P = 0.025$, odds ratio 2.3, CI = 1.3–3.9). Moreover, the absence of KIR2DL2/DS2 genes significantly influenced the risk of acute renal failure ($P = 0.05$). A multivariate regression model of both clinical and genomic risk factors for acute kidney injury/failure confirmed a link between the KIR haplotype A/A and post-LT acute renal failure ($P = 0.04$). In summary, we observed a higher percentage of NK cells prior to LT in patients with impaired renal function and identified the KIR haplotype A/A as an independent genetic risk factor for ARF within the first postoperative week. Our data therefore provide new aspects of an innate immune response within the setting of post-LT kidney injury and failure.

P185 REFINED DEFINITION OF CMV INFECTION STATUS BY DETERMINATION OF CMV SPECIFIC T-CELL IMMUNITY IN INDIVIDUALS WITH PASSIVE ANTIBODY TITERS

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Background: In pediatric transplantation, the correct identification of CMV donor and recipient serostatus in children less than 18 months of age is complicated by the variable persistence of maternal CMV antibodies. Similarly, uncertainty in correctly assigning the actual CMV status exists in adults that have received plasma preparations. As adaptive cellular immunity is not passively transferred, this study was carried out to assess whether the presence of CMV specific T cells may represent a more correct parameter to assign the individual CMV status.

Methods: A total of 151 children below the age of 18 years were analysed. Among those, 47 were < 18 months of age including 28 cord blood samples. In addition, 41 mothers and one transplant candidate before and after receiving hyperimmunoglobulins were recruited. Humoral immunity was determined using standard ELISA, CMV specific CD4 T cells were assessed by flow-cytometry after specific stimulation with CMV lysate. Specific T cells were identified based on intracellular accumulation of IFNg, IL2, and TNFa. CMV-negative control lysates and staphylococcal superantigen SEB served as negative and positive controls, respectively.

Results: In general, specific cells produced IFNg, whereas immunity in cord blood and newborns was dominated by IL2 and TNFa. In children above 18 months of age (10.6 ± 4.7 years), 20/104 (19.2%) were CMV seropositive and had detectable CMV specific T-cell frequencies (median 0.39%, IQR 1.88%), and agreement between both tests was perfect (K = 1.0). Cord-blood samples had the same serostatus as the mothers (17 negative, 11 positive, K = 1.0), while CMV specific T cells were not detectable. Among the 18 children < 18 months of age, 11 were negative in both tests. Conversely, both tests were positive in six children indicating true positive infection status. Interestingly, only one child was seropositive and T-cell negative indicating presence of maternal antibodies. Likewise, while the transplant candidate was negative in both tests before infusion of plasma products, the sample was seropositive but remained T-cell negative thereafter.

Conclusion: In conclusion, the additional determination of CMV specific T cells may allow a refined definition of the true CMV infection status in children and adults where serological testing is limited by the presence of maternal or passively administered antibodies. In newborns, analysis of IL2 and TNFa may help to increase diagnostic accuracy of T-cell analysis.

P186 VENTRICULAR ASSIST DEVICES ARE RISK FACTORS FOR HLA-SENSITIZATION PRIOR HEART TRANSPLANTATION

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Objective: Patients who were bridged to heart transplantation (HTx) with ventricular assist device (VAD) have a higher incidence for the development of antibodies directed against human leukocyte (HLA) or against non-HLA anti-

gens like major histocompatibility complex class I-related chain A (MICA). HLA and MICA antibodies have been associated with acute and chronic rejection leading to decreased survival after HTx. We monitored these clinical relevant antibodies to evaluate sensitization during the first year after VAD implantation.

Methods: Sera of 28 patients who underwent VAD implantation were analyzed by Luminex technology for anti-HLA and anti-MICA antibodies. Blood transfusion history, gender, age and panel reactive antibody (PRA) level before VAD implantation were reviewed.

Results: Mean age was 53.3 ± 13.7 years and the group consists of 25 men and 3 women. 42.9% (n = 12) of VAD-implanted patients showed HLA and/or MICA antibodies within the first year after VAD implantation, whereas 28.6% (n = 8) with HLA-class I antibodies, 21.4% (n = 6) with HLA-class II antibodies and 14.2% (n = 4) with MICA antibodies were identified. Of these patients 10.7% possessed HLA antibodies in combination with MICA antibodies. An accumulation of antibodies with specificities against HLA-A or HLA-DR antigens was observed. In particular, antibodies against the specificities HLA-A68, -DR4 or -DR9 occurred in more than 14% of VAD implanted patients within the first year after implantation.

Conclusions: Patients with VADs prior to transplantation have a higher risk to develop HLA-A and HLA-DR antigens. Future studies will show the impact of alloreactive antibodies in these patients on the outcome post-HTx.

P187 HLA-SPECIFIC ANTIBODIES, PROTEINURIA, AND TRANSPLANT GLOMERULOPATHY; A RETROSPECTIVE ANALYSIS OF 364 PATIENTS OF THE FRANKFURT TRANSPLANT CENTER

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Here we present an extension of our retrospective analysis investigating the significance of HLA specific- and/or cytotoxic antibodies (AB) for kidney graft survival performed in the transplant center of the University Clinic Frankfurt/Main. Proteinuria was investigated in a collective of transplant recipients without and with HLA specific- and/or cytotoxic AB. In 26 patients (pts) with HLA specific- and/or cytotoxic AB the kidney biopsies were performed for indication and were evaluated.

Methods: AB were monitored by ELISA in combination with the lymphocyte cytotoxicity assay (LCT). To avoid hyperacute rejection of the transplanted kidney, no transplantation was performed against donor specific historic or acute HLA-class-I AB. Immunosuppression of HLA-class-I AB negative transplant recipients was performed with cyclosporine A, mycophenolate mofetil, and steroids. Pts with HLA-class-I AB received intensified immunosuppression with tacrolimus, mycophenolate mofetil, antithymocyte globuline, and steroids. Proteinuria was determined by dipstick analysis. Biopsies were performed in case of impairment of renal function and /or proteinuria.

Results: Kaplan–Meier estimates revealed a significantly reduced long term kidney graft survival in pts immunized with both pre-transplant HLA-class-I AB and HLA-class-II AB detected by ELISA (91% vs. 65%; P = 0.008). A significantly reduced long term graft survival was found in pts with a positive LCT prior to transplantation (91% vs. 67%, P = 0.004). Immunization with HLA class I or HLA class II AB detected by ELISA or immunisation with cytotoxic AB detected by LCT was associated with a significantly increased proteinuria. Transplant glomerulitis or glomerulopathy was found in 43% of pts positive in the LCT, 42% and 50% pts positive for HLA class I or II AB in the ELISA, respectively, and in 62% of pts positive for HLA class I and II AB. Interstitial fibrosis was found in 25% of pts positive in the LCT, 37% and 42% of pts positive for HLA class I or II AB in the ELISA, respectively, and in 38% of pts positive for HLA class I and II AB. C4d was found in 25% of all biopsies, and in 50% of pts positive for HLA class I and II AB. Almost all pts displayed tubular damage. 6/26 pts biopsied lost their transplants, at 21.5 ± 15.2 months after transplantation. In 4/6 graft losses transplant glomerulitis or glomerulopathy was diagnosed. The other two graft losses were due to combined vascular and interstitial rejection at an earlier time point.

Conclusion: Our results confirm the significance of HLA-class-I and -II AB for graft loss both detected in the ELISA and LCT. The increased proteinuria in immunized pts and the predominance of transplant glomerulopathy and glomerulitis as well as C4d staining in the biopsies support the immunological aetiology of chronic transplant failure in this cohort.

P188 T-CELL FUNCTION SHOWS MARKED CIRCADIAN VARIATION THAT IS DIFFERENTIALLY MODULATED BY IMMUNOSUPPRESSIVE DRUGS

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Introduction: Endogenous steroids are subject to circadian rhythm with high and low plasma levels in the morning and at midnight, respectively. Transplant recipients usually take steroids in the morning and other immunosuppressants twice daily. As this may influence T-cell function, we investigated potential circadian variations in the number and activation of T-lymphocytes in immunocompetent and immunosuppressed individuals.

Groups and methods: We examined two groups of patients after kidney transplantation (7 short-term and 13 long-term). Blood was taken at 8 am 12 am, 8 pm, 12 pm and the following day 8 am. Differential blood counts and drug levels were measured. In addition, functional activity of lymphocytes was flow cytometrically quantified on the basis of cytokine production after stimulation with staphylococcus enterotoxin B (SEB). The control group consisted of six healthy subjects.

Results: Cell distribution and cell function showed a pronounced circadian rhythm that differed in the two groups. In controls, the number of SEB-reactive T-cells increased by 25.6 ± 9.6% at midnight, indicating that T-cell function mainly depended on endogenous cortisol levels. In transplant recipients SEB-reactive T-cells decreased after intake of the morning medication. The extent of the variation in cell numbers and cell reactivity was primarily determined by the type and dose of immunosuppressive agents. Interestingly, calcineurin inhibitors had a differential effect; while patients with cyclosporin A showed a significant decrease in T-cell reactivity 4 hours after drug intake (remaining reactivity 62.8 ± 31.3%), T-cell reactivity was unaffected 4 hours after the intake of tacrolimus (remaining reactivity 101.8 ± 18.0%).

Conclusions: T-cell numbers and reactivity show a marked circadian rhythm in healthy as well as in immunocompromised individuals. However, while healthy controls show a tight association with endogenous cortisol production, circadian changes in immunosuppressed individuals are essentially determined by dose and type of immunosuppressive drugs, showing striking differences between cyclosporin A and tacrolimus.

P190 SMICA IN PATIENTS AWAITING TRANSPLANTATION

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Introduction: Soluble MICA transcripts, which may have immunomodulatory effects on NK-cells and gamma/delta T-cells, have been reported to be shed by various tumor entities. MICA expression has been shown to be increased under cell-stress conditions. In organ transplantation, the activation of endothilium may lead to MICA expression and may result in a humoral MICA antibody response. Thus, the immunomodulatory potential of sMICA may also be relevant in this clinical setting. The aim of our study was to determine sMICA expression in patients awaiting transplantation in correlation with clinical parameters.

Materials and methods: We developed a highly sensitive Luminex bead array assay for detection of sMICA in serum samples. 1259 patients awaiting transplantation were measured. sMICA levels were correlated with presence/absence of MICA*008 genotype (predicted upon linkage disequilibrium between MICA and HLA-B), number of previous transplantations, HLA-immunization (PRA) and gender.

Results: Presence of MICA*008 genotypes (A5.1 alleles) showed strong correlation with sMICA levels (P < 0.001). Patients with at least one previous transplantation had higher serum levels of sMICA (P = 0.01). Immunized patients (PRA > 5) had higher sMICA levels (P < 0.001). Gender had no influence on sMICA levels (P = 0.21).

Discussion: The MICA*008 phenotypes (A5.1), which are characterized by a premature stop codon in exon 5 and defective membrane anchorage, tend to shed sMICA transcripts. Other immunological factors such as previous transplantations and HLA-sensitization also influenced the presence of sMICA in serum samples. The latter observations may be a secondary effect due to an underlying immunological condition, however at present a causal connection between HLA-sensitization and MICA expression cannot be definitely excluded.

P191 NON-HLA ANTIBODIES TARGETING G-PROTEIN COUPLED RECEPTORS INDUCE MTOR SIGNALLING IN HUMAN MICROVASCULAR ENDOTHELIUM

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Aim: Functional allo- and autoantibodies targeting G protein-coupled receptors (GPCR) Angiotensin II Type 1 receptor (AT1R) and Endothelin-1 Type A receptor (ETAR) are implicated in pathogenesis of renal and cardiac transplant vasculopathy. Both non-HLA antibodies activate canonic G-protein related Extracellular signal-regulated kinases 1/2 (ERK 1/2). While ERK-signaling may represent general cellular response to agonist stimulation, the molecular link between receptor stimulation and development of vascular obliterative lesion has not been fully established yet. The aim of our research was to investigate the role of the PI3K/Akt downstream signalling target mammalian target of rapamycin mTOR) and assess the relative importance of the two different signalling complexes mTORC1 and mTORC 2 in the pathogenesis of vasculopathy.

Methods: Human microvascular endothelial cells (hMEC) with reliable expression of target antigens were stimulated with AT1R-Ab and ETAR-Ab containing IgG from patients with obliterative vasculopathy. Protein chemistry by means of phospho-specific antibodies directed against mTOR downstream targets was used to assess activation of mTORC1 (pp70S6K at Thr ^{389}) and mTORC2 (pAkt at Ser ^{473}).

Results: Signalling activity of both, mTORC1 and mTORC2, was increased after short-term treatment with patient IgG compared to cells treated with IgG from healthy controls. This effect could be inhibited by preincubating the cells with specific blockers of the AT1R (Valsartan) and ETAR (Sitaxentan). Phosphorylation of p70S6K was completely abolished by the mTOR inhibitor Rapamycin. Additional preliminary experiments demonstrated an ERK 1/2 independent activation of both mTOR complexes indicating a direct activation via PI3K/Akt.

Conclusion: We provide evidence that functional non-HLA antibodies targeting AT1R and ETAR induce mTORC1 and mTORC2 signalling which is independent of canonic ERK 1/2 activation in human microvascular endothelium. Our data may provide a translational rationale for therapeutic mTOR inhibition in patients with non-HLA antibodies.

P192 INTERLEUKIN-10 RECEPTOR DEFICIENT ENTEROCOLITIS – SWIFT REMISSION AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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Introduction: Interleukin-10 (IL-10), a pleiotropic cytokine and its receptor (IL-10R) are secreted and expressed by a wide variety of hematopoetic cells. The IL-10/IL-10R signalling pathway regulates the antigen triggered inflammation. Inactivating mutations of the IL-10R induce enterocolitis with an onset in infancy, recurrent inflammation of the skin and bacterial infections resistant to conventional therapeutic approaches.

Case report: Two-years-old female who presents postnatal with an extended dermatitis resistant to topical steroids and bacterial superinfections, febrile respiratory infections, otitis and mastoiditis. At the age of 10 months she develops profuse chronic watery and bloody diarrhea with an increasing failure to thrive. Endoscopically she presented with a chronic inflammation of the upper and lower intestines resembling Crohn's disease refractory to any immunosuppressive, immunmodulatory and antibiotic regimen. A molecular analysis of the IL-10R gene revealed a yet unpublished compound heterozygous mutation with an indication for allogeneic stem cell transplantation. She was transplanted successfully from a matched unrelated donor using a myeloablative conditioning and achieved after an uncomplicated post-transplant recovery a remarkable improvement of her intestinal symptoms and healing of a prominent recto-vaginal fistula, followed by an impressive catch-up growth and development.

Conclusion: In early-onset severe enterocolitis refractory to conventional therapeutic regimens an IL-10R defect must be considered. Allogeneic stem cell transplantation is the only curative option leading to a swift recovery.

ORGAN DONATION, ORGAN PROCUREMENT, -PRESERVATION

P194 HYPOTHERMIC RECONDITIONING AFTER STATIC LIVER PRESERVATION REDUCES INNATE IMMUNOREACTIVITY AND IMPROVES SURVIVAL AFTER TRANSPLANTATION

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Early graft dysfunction due to preservation/reperfusion injury represents a dramatic event after liver transplantation affecting long term prognosis of graft viability and patient outcome.

Any improvement in the preservation of grafts, especially those experiencing extended times of preservation represent a valuable advance to enlarge the total number of viable donor organs and to circumvent the need of retransplantation.

Here we provide in vivo evidence for the efficacy of the previously developed end-ischemic gaseous oxygen persufflation technique to resuscitate liver grafts after extended storage times.

Porcine livers (n = 6/group) were harvested according to standard multiorgan procurement protocol and subjected to species specific extended cold storage (CS) at 4 °C. Hypothermic reconditioning (HR) was performed in some livers by gaseous oxygen persufflation via the caval vein for 2 hours prior to transplantation. Viability was assessed by orthotopic liver transplantation and one week follow-up. HR significantly improved pretransplant energy charge and initial graft function after transplantation.

HR also promoted improved early functional resumption of sinusoidal endothelium as disclosed by a significant reduction of hyaluronic acid accumulation immediately after liver transplantation.

One week survival after CS was 0% while five of six pigs (83%) survived in the HR group. At that time coagulation parameters were in the normal range and histological analysis disclosed healthy liver tissue with normal trabecular architecture in the treated grafts. Molecular analyses identify the prevention of ischemia induced decline of cellular autophagy and mitigation of innate immune machinery (HMGB-1, IFN-beta) as operative mechanisms among the protective effects provided by HR.

P196 IS STUDENT PERFUSION SERVICE FOR ORGAN HARVESTING A ''PATHFINDER'' TO AN OCCUPATIONAL CAREER IN CARDIAC SURGERY?

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Purpose: Perfusionists are an essential part of the explantation teams. In some departments the perfusion service is performed by medical students. As for many departments of cardiac surgery it is very difficult to find young medical professionals. A student based perfusion service may be a reasonable instrument to arouse interest for this special discipline. We analyzed all occupational careers of medical students who have participated in the student perfusion service in our centre.

Methods: Since 1999 thoracic organs have been transplanted in Jena. Next to the explanting surgeons one perfusionist is part of the explantation team. Besides the preparation of the equipment, the perfusionist is the communicator with the implanting team. In our centre the perfusion service is currently organized and being performed solely by medical students.

Results: During the last 12 years, 25 medical students have participated in the student perfusion service in Jena. After finishing medical school 14 of them decided to pursue a career in cardiac surgery in four different departments. Five chose another medical field and six are currently still active in the perfusion service and attending medical school.

Conclusion: Involving students in organ harvesting activities as perfusionists in explantation teams results in a high rate of recruitment for our field. Thus, a student based perfusion service is a highly effective recruiting instrument in cardiac surgery.

ETHICS, PSYCHOSOMATICS AND QUALITY OF LIFE

P201 ARE THERE SEX DIFFERENCES IN HEALTH – RELATED QUALITY OF LIFE AND EXERCISE CAPACITY FOLLOWING LUNG TRANSPLANTATION?

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Background: The purpose of this study was to investigate sex differences in exercise capacity (EC) and health-related quality of life (HRQoL) in lung transplant recipients (LTR) and to compare patients ratings with the age-matched general German population.

Methods: A total of 152 consecutive LTR of our lung transplantation (LTx) follow-up program (80 female, 95 double-LTx, 4.5 ± 3.2 years after LTx, age 50 ± 11.9 years, 14 female BOS stage ≥ 1 and 14 male BOS stage ≥ 1) were evaluated using the 6-minute walk test and were interviewed with the standardized global SF-36 questionnaire, the "St. Georges' Respiratory Questionnaire" (SGRQ) as well as the "Quality of Life Profile for Chronic Diseases Questionnaire" (PLC).

Results: Both groups were statistically indistinguishable in terms of clinical data. There were no gender specific EC differences (female 460 ± 94 m vs. male 462 ± 146 m; $P = 0.97$). Female recipients demonstrated a higher social health (PLC; $P = 0.041$) and fewer disease specific respiratory symptoms (SGRQ; $P = 0.023$) in comparison to male LTR. Compared to the age-matched norm population, HRQoL self-ratings were significant reduced in six of eight SF-36 subscales for male, and in five of eight for female patients ($P \leq 0.01$). "Mental health" values were equivalent to the norm regardless of gender ($P > 0.05$). Female LTS even experienced significantly less "pain" than the standard population ($P = 0.03$).

Conclusion: Our data suggest that there are relevant gender specific differences regarding HRQoL after LTX. These findings need to be considered for future studies assessing outcome of LTX by HRQoL questionnaires.