Parallel Sessions 19–36

Session 19. Experimental & clinical islet transplantation

O-170 IN VITRO XENOGENIC IBMIR IS MORE AGGRESSIVE THAN ALLOGENIC AND IS MEDIATED BY CLASSICAL AND ALTERNATIVE COMPLEMENT PATHWAYS

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Purpose: Intropartally transplanted islets provoke an instant blood-mediated inflammatory reaction (IBMIR), rejection indicator. In an in vitro model, we compared the mechanisms of IBMIR induced by human (allogeneic, A) and pig (xenogeneic, X) islets.

Methods/Materials: A549EOQ were incubated with fresh non-anticoagulated human blood in petri dishes in an incubator-shaker (37°C, 100rpm). Clotting times were recorded. Levels of complement activation products (iC3b for common pathway and Bb for alternative pathway) and C-peptide were compared to dishes with blood alone at 5, 30 and 60min. After clotting times were reproducible, islets were added in increasing amounts. IL-1ra and assayed for cellular integrity by flow cytometry and gene expression for the classical pathway of complement activation.

Results: Exposure of human and pig islets to human blood induced equally rapid clotting (A: 3.13±1.31min vs. X: 3.54±1.42, P<0.05), compared to blood alone: 46.25±16.23min, P<0.0001. In X, average porcine C-peptide levels increased from 118 to 627ng/mL between 5 and 60min, indicating islet cell lysis. In A, human C-peptide only increased from 41 to 64ng/mL (P<0.05). IgM and IgG binding were observed on pig but not on human islets. In X, supernatants released by clots at 30min contained 50% more C3b and 77% more Bb than controls. These increases were only 7% and 38% in A (P<0.05). Dextran sulfate completely prevented clotting and reduced iC3b and Bb to control levels. However, in X, it did not prevent antibody binding and porcine C-peptide release. Conclusion: Xenogeneic IBMIR is more aggressive than allogeneic IBMIR in an in vitro model of islets exposed to human blood. Dextran sulfate prevented complement activation through the alternative pathway, but did not prevent antibody binding to pig islets and C-peptide release, suggesting an important role for the classical pathway of complement activation.

Aims/Hypothesis: Pro-inflammatory cytokines (PIC) impair islet viability and function by activating inflammatory pathways that induce both necrosis and apoptosis. The aim of this study was to utilize an in vitro rat islet model with exogenous PIC treatment to evaluate the efficacy of a clinically approved IL-1b receptor antagonist (Anakinra) in blocking PIC induced islet impairment.

Methods: Isolated rat islets were cultured ± IL-1b, IFNγ, and TNFa and ± IL-1ra and assayed for cellular integrity by flow cytometry and gene expression by RT-PCR. Nitric oxide (NO) release into the culture media was measured by Griess reaction. Islet functional potency was tested by glucose stimulated insulin secretion (GSIS) and by transplantation into streptozotocin-induced diabetic NOD-SCID mice.

Results: IL-1ra completely abrogated the effects of PIC with respect to NO production, necrosis, apoptosis, mitochondrial dysfunction, GSIS, and in vivo potency. Rat islets cultured alone, with IL-1ra alone, or the combination of PIC and IL-1ra were indistinguishable and showed high viability, low apoptosis and equivalent glucose-induced insulin secretion. PIC treated rat islets showed strong induction of iNOS and NO release into the culture media. IL-1ra treatment abrogated PIC induced iNOS gene expression and NO production. Indicators of mitochondrial integrity (JC-1 staining and glucose induced metabolic flux) were diminished by PIC treatment, but prevented by co-culture with IL-1ra. The recipients of untreated, IL-1ra alone, and PIC + IL-1ra showed rapid restoration of normoglycemia and stable blood glucose control over the duration of the experiment (28 days).

Conclusion: These data demonstrate that Anakinra is an effective agent to inhibit the activation of IL-1b dependent inflammatory pathways in cultured rat islets and support the extension of its application to human islets in vitro and potentially as a therapy for islet transplant recipients.

O-172 QUANTIFICATION OF TRANSPLANTED IRON OXIDE-LABELLED ISLET CELLS BY 3-DIMENSIONAL 3T MAGNETIC RESONANCE IMAGING (MRI)

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Background: Monitoring mass and function of islet grafts is vital for the improvement of results of islet transplantation in type 1 diabetes. MRI provides non-invasive imaging for iron-labelled islets. Quantification of the engrafted islet mass has not yet been reported.

Methods: Syngeneic Resovist-labelled islets were transplanted into the portal vein of SD rats. Increasing islet numbers were transplanted (0, 500, 1000, 2000, 4000). Imaging was carried out on a clinical 3T MRI scanner. Scanning was performed 1 day, and 1, 2 and 8 weeks after surgery. Respiratory triggering was performed with a trigger delay of 150ms. Images obtained with a novel 3-dimensional Ultrashort-Echo-Time (UTE) technique were compared with conventional 2-dimensional acquisition sequences. Quantitative assessment included measurement of the number of iron-related pixels (over all liver slices) and correlation with number of transplanted islets.

Results: The isotropic 3-dimensional images can be viewed with the same resolution in all three orientations. When imaging at day 1, surgical disturbance makes visualization of clusters difficult. At 1, 2 and 8 weeks, cell visualisation and quantification is more defined with isolated enhanced spots within an uniform background. UTE images show a good correlation between the number of counted pixels and the number of transplanted islets, and this is reproducible over time.

A rapid increase of the signal was observed in rats transplanted with xenogeneic human islets. The novel technique also offers an improved signal-to-noise ratio, due to lower background and better signal detection due to control of motion artifacts.

Conclusion: This novel MR imaging technique offers reproducible quantification of transplanted islet grafts. Development of the technique on a clinical MRI scanner makes its application in a human clinical study promising.
Islet Transplant Registry (CITR), currently the most comprehensive collection of human-to-human islet transplant data.

**Methods:** CITR collects and monitors comprehensive data on allogeneic islet transplantation in North America, Europe and Australia since 1999.

**Results:** As of February 2009, the CITR registry comprised 396 adult recipients of 796 islet infusions derived from 937 donors. At three years post first infusion, 27% of islet-alone recipients were insulin independent (II, >2 years); 30% were insulin using with detectable C-peptide, 26% had lost function, and 18% had missing data. 70% of IA recipients achieved sustained II at least once, of whom 70% were still II 1 year later and 50% at 2 years. Higher numbers of infusions, greater number of total IEQs infused, lower pre-transplant HbA1c levels, processing centers related to the transplant center; and larger islet size are factors that favor the primary outcomes. Protocols with daclizumab or etanercept during induction had higher rates of II and lower rates of function loss, respectively, which endorse the current approaches. Infusion-related AE incidence was 0.7 events/person-year (E PY) in year 1, while immunosuppression-related AE incidence was 0.9 E PY, both declining to less than 0.07 E PY thereafter.

**Conclusions:** Clinical islet transplantation needs to be evaluated using the most clinically relevant endpoints such as glucose stabilization and severe hypoglycemia-free episodes. The cumulative results of the Registry confirm the inarguably positive impact of islet transplantation on metabolic control in T1D.

**O-174 DIFFERENCES IN BASELINE LYMPHOCYTE COUNTS AND AUEROREACTIVITY ARE ASSOCIATED WITH DIFFERENCES IN OUTCOME OF ISLET CELL TRANSPANTATION IN TYPE 1 DIABETIC PATIENTS**

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**Purpose:** The metabolic outcome of islet cell transplants in type 1 diabetic patients is variable. This retrospective analysis examines whether differences in recipient characteristics at the time of transplantation are correlated with inadequate graft function.

**Methods:** Thirty non-uremic C-peptide negative type 1 diabetic patients had received an intraportal islet cell graft of comparable size under an ATG-mycophenolate mofetil regimen during more than one year of follow-up. Baseline patient characteristics were compared with outcome parameters during the first 6 posttransplant (PT) months, i.e plasma C-peptide, glycan variability and gain of insulin independence. Correlations in univariate analysis were further examined in a multivariate model.

**Results:** Patients that did not become insulin-independent exhibited significantly higher counts of B-lymphocytes, as well as a T-cell autoreactivity against multiple antigens in univariate model. Correlations in univariate analysis were further examined in a multivariate model. Higher total and B-lymphocyte counts and presence of T-cell autoreactivity were associated with lower graft function.

**Conclusion:** Higher total and B-lymphocyte counts and presence of T-cell autoreactivity are factors that favor the primary outcomes. Protocols with daclizumab or etanercept during induction had higher rates of II and lower rates of function loss, respectively, which endorse the current approaches. Infusion-related AE incidence was 0.7 events/person-year (E PY) in year 1, while immunosuppression-related AE incidence was 0.9 E PY, both declining to less than 0.07 E PY thereafter.

**O-176 TETRAHYDROBIOPTERIN OFFSETS MICROVASCULAR RENAL ISCHEMIA REPERFUSION INJURY VIA SUSTAINMENT OF NO HOMEOSTASIS**

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**Purpose:** Tetrathiydrobipterin (BH4) is an essential cofactor for nitric oxide synthases (NOS) and thus a critical determinant of NO production. BH4 depletion during cold ischemia leads to uncoupling of NOS and contributes to reperfusion injury (IRI) due to increased superoxide formation. The role of BH4 during warm ischemia, however, is still largely unknown.

**Material and methods:** Ischemic renal injury was induced by clamping the left renal artery for 45 min in male Lewis rats immediately after right-side nephrectomy. Reperfusion was studied at R0 (no reperfusion), 15min (R1), 2hours (R2) and 7days (R3). BK-80 animals received either BH4 (20mg/kg/BW) prior to ischemia significantly improved renal function at all time points studied following reperfusion (p<0.001). Furthermore, BH4 reduced ischemia induced histologic damage (increased inflammation, interstitial edema, hemorrhage, tubular atrophy and focal areas of necrosis) and diminished peroxynitrite formation and nitrotyrosine staining (R1-R3). Subsequently, microcirculatory changes correlated with kidney peroxynitrite generation, and improved considerably through BH4 treatment.

**Conclusion:** BH4 treatment significantly improves post-ischemic renal function as well as histologic and microcirculatory function and might be a promising novel therapeutic strategy in attenuating IRI via maintenance of NO homeostasis.

Session 20. Ischemia/reperfusion injury & intervention

**O-175 THE ROLE OF COMPLEMENT REGULATORY PROTEINS IN CARDIOPROTECTION OF ISCHEMIC POSTCONDITIONING IN HEART ISCHEMIC-REPERFUSION INJURY**

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Complement activation during ischemic-reperfusion injury (IRI) is an important reason that impacts cardiovascular and myocardium after heart transplantation. Recent evidence showed that ischemic postconditioning (Pos-con) may obviously lessen injury on the heart after IRI. We explored the hypothesis that the Pos-con protects heart, at least in part, through up-regulation of decay-accelerating factor (DAF), CD59, and/or membrane cofactor protein (MCP).

Isolated working rat hearts were subjected to a global total ischemia, followed by reperfusion. The results of the Regimen revealed that lower graft function in the Pos-con group was significantly higher counts of B-lymphocytes, as well as a T-cell autoreactivity against multiple antigens in univariate model.

Correlations in univariate analysis were further examined in a multivariate model. Higher total and B-lymphocyte counts and presence of T-cell autoreactivity were associated with lower graft function.
Ex vivo pharmacodynamic analysis demonstrated a significant increase in V and highly elevated in P. Significance was found for IDO, urine flow at 30 minutes after reperfusion.

Kidneys from 9 donor animals (n=18; german landrace pigs, mean body weight 41.5 kg) were perfused with cold solution (HTK, 72 ml/kg BW). Group V (vehicle, n=10 kidneys) included 10 μM CBS-3830; group P ( placebo, n=8 kidneys) only vehicle. Cold storage time was mean 26.1 hrs. for V and 25.8 hrs. for P (n.s.). Organs were reperfused with preserved and heparinized pig blood from the same donor including CBS-3830.

The test compound (dose: 1 mg/kg) was administered i.v. starting 70 minutes prior organ removal. Simultaneously we determined CBS-3830 plasma concentration and tissue distribution. Neutrophil accumulation was determined by a myeloperoxidase (MPO) assay

We demonstrated that an significant increase of C3 and C5aR in the aged liver, which results in more severe tissue damage, earlier graft dysfunction and poorer outcome, therefore the utilization of aged liver is limited in clinic. Complement activation is a critical factor in liver I/R injury. We hypothesized that knock down of complement pathway may prevent liver injury.

Methods: Liver I/R injury. We hypothesized that knock down of complement pathway may prevent liver injury. We investigated the effect of the new MAPKinase-Inhibitor CBS-3830, (c-a-i-r biosciences, Germany) in an isolated ex-vivo kidney hypoperfusion system on ischemia and reperfusion injury (I/R) and the activated intra-cellular signaling pathways. CBS-3830 is a highly potent inhibitor of p38 MAPK, JNK-2 and -3 and showed no further interactions with any of the 380 kinases.

Results: HMGBl-1, LDH,MDA as well as peroxynitrite formation (p<0.0001). In contrast, pre-treatment of donor animals with VitC did not improve recipient survival. H4B attenuates I/R related graft pancreatitis and significantly improves recipient survival rate and might therefore be a novel promising agent preventing graft pancreatitis.

Ischemia-reperfusion injury (I/R) is a major cause for the occurrence of graft pancreatitis following pancreas transplantation. Recent findings showed significantly reduced early parenchymal damages following murine pancreas transplantation if donors were pre-treated with tetrahydrobiopterin (H4B), an essential cofactor of nitric oxide synthases and strong antioxidant. In this study we analyzed if H4B supplementation was also able to prolong recipient survival, since occurrence of graft pancreatitis in this model showed to be lethal. Male syngenic C57BL6 (H-2b) mice were used as size-matched donor and recipient pairs. Murine cervical pancreas transplantation was performed with a modified no-touch technique. To induce graft pancreatitis grafts were subjected to 16h prolonged cold ischemia time (CTI) as well as to 45min warm ischemia time (WIT).

Conclusion: The activation of p38 MAPKinase through I/R can be treated with CBS-3830. There is evidence that the damage and the activation of innate immune response is decreased. This experiment is limited by time.

Results: Neutrophil accumulation was determined by myeloperoxidase (MPO) assay and immunohistochemical staining. Lipid peroxidation was assessed by malondialdehyde (MDA) levels. Quantitative PCR was used to test gene silencing efficacy in vitro and in vivo.

Conclusions: We demonstrated that an significant increase of C3 and C5aR genes. 12-month old BALB/c mice were treated with siRNA by hydrodynamic injection. Liver I/R injury was induced by interrupting blood supply to one or both left lateral and median lobes of the liver for 60 min following by reperfusion 6 hrs. I/R injury was evaluated using liver histopathology, as well as levels of serum alanine transamerase (ALT) and aspartate transaminase (AST). Neutrophil accumulation was determined by a myeloperoxidase (MPO) assay and immunohistochemical staining. Lipid peroxidation was assessed by malondialdehyde (MDA) levels. Quantitative PCR was used to test gene silencing efficacy in vitro and in vivo.

Conclusions: The activation of p38 MAPKinase through I/R can be treated with CBS-3830. There is evidence that the damage and the activation of innate immune response is decreased. This experiment is limited by time.

Conclusions: This is the first demonstration that I/R injury in aged livers can be effectively prevented through gene silencing of complement genes, high-lighting the potential of siRNA-based therapy in clinical liver transplantation when the aged organs are used.

Methods: Following kidney transplantation, chronic allograft nephropathy represents a major cause of allograft dysfunction and loss. Efficient therapeutic strategies to counter disease progression are missing. As ischemia-reperfusion injury is determined in a murine model of renal ischemia-reperfusion injury. Renal dysfunction was evaluated using 99mTc-MAG3 scintigraphy, laser Doppler perfusion measurement, and histological analysis.

Results: Prolonged ischemia was associated with a significant rise in pressure. Compared to baseline, a 7-fold increase was found 60 min following reperfusion. Pressure values continued to be unphysiologically high until returning to base-line by 48 h post ischemia. Strikingly, we found a de novo increase in pressure in long-term follow-up examination.

Conclusion: We showed that increased pressure within the renal compartment may play a major role in the development of chronic allograft dysfunction via microcirculatory impairment, increased inflammatory response, and direct cell damage. Our data support the notion of a renal compartment syndrome as a significant trigger to a pathophysiological cascade of immunological and inflammatory events.

Session 20. Ischemia/reperfusion injury & intervention Tuesday, 1 September 2009 47
Session 21. Long term complications in kidney transplantation

O-181 AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE: RISK FACTOR FOR NON-MELANOMA SKIN CANCER FOLLOWING KIDNEY TRANSPLANTATION
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Introduction: Non-melanoma skin cancers (NMSC) are the most common malignant tumors following solid organ transplantation. Risk factors for NMSC mainly include immunosuppression, age, sun exposure and patient phenotype. Recent findings have suggested that autosomal dominant polycystic kidney disease (ADPKD) may increase the risk of developing NMSC.

Patients and methods: We performed a monocenter retrospective study including all kidney recipients between 1985 and 2006 (n=1019). We studied the incidence of NMSC, solid cancers and post transplantation lymphoproliferative disease (PTLD) and analyzed the following parameters: age, gender, phenotype, time on dialysis, graft rank, immunosuppressive regimen at 3 months post-transplantation, history of cancer and kidney disease (ADPKD vs others). Results: ADPKD was the cause of renal failure in 156 patients (15.3%). A second kidney graft was performed in 10.5% of all patients and a third graft in 0.9%. Median follow-up was 5.5 years (range 0.02-20.6, 79,838 patient-years).

The cumulated incidence of NMSC ten years after transplantation was 12.7% (9.3% for solid cancers and 3.5% for PTLD). ADPKD and age were risk factors for NMSC (HR 2.63; p=0.0001 and HR 2.21; p=0.0001) after adjusting for age, gender and phototype using multivariate analysis. The association between ADPKD and NMSC remained significant after adjustments for age, gender and phenotype using multivariate analysis. The association between ADPKD and NMSC remained significant after adjustments for age, gender and phototype using multivariate analysis. The association between ADPKD and NMSC remained significant after adjustments for age, gender and phototype using multivariate analysis.

Conclusion: Our findings confirm that ADPKD is an independent risk factor for non-melanoma skin cancer following kidney transplantation.

O-182 MEASURING TOTAL BLOOD CALCIUM DISPLAYS A LOW SENSITIVITY FOR THE DIAGNOSIS OF HYPERCALCEMIA IN RENAL TRANSPLANT RECIPIENTS
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Background: Hypercalcaemia is a common complication in renal transplant recipients and has been associated with nephrocalcinosis and poor graft outcome. The Kidney Disease: Improving Global Outcomes (KDIGO) position states that the measurement of ionized Ca (iCa) is preferred and that if total Ca (tCa) concentration is used instead, then it should be adjusted in the setting of hypoalbuminemia.

Methods: We compared the ability of noncorrected and albumin-corrected iCa concentration to identify low, normal, or high iCa concentration in an unselected cohort of 268 renal transplant recipients (RTxRs). Patients were studied 3 and 12 months after successful engraftment (male 61%, age 53.8±13.4 yrs).

Results: Hypercalcaemia, defined as iCa >1.29 mmol/L, was present in 58.6 and 44.8% of the patients at month 3 and 12, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Month 3</th>
<th>Month 12</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.92±0.40</td>
<td>1.36±0.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>iCa (mg/dL)</td>
<td>9.7±0.6</td>
<td>9.7±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>hypercalcaemia (tCa, %)</td>
<td>13.1</td>
<td>13.1</td>
<td>NS</td>
</tr>
<tr>
<td>iCa (mmol/L)</td>
<td>1.32±0.09</td>
<td>1.29±0.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hypercalcaemia (Ca, %)</td>
<td>56.6</td>
<td>44.8</td>
<td>0.0002</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>22.2±2.6</td>
<td>22.9±2.6</td>
<td>0.0002</td>
</tr>
<tr>
<td>creatinine &lt;22 mmol/L</td>
<td>48.1</td>
<td>37.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>43.5±3.4</td>
<td>44.1±2.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Albumin &lt;35 g/L</td>
<td>3.0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>2.8±0.6</td>
<td>3.0±0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hypophosphatemia (%)</td>
<td>31.4</td>
<td>11.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Noncorrected and albumin-corrected iCa concentrations > 10.3 mg/dL, respectively, were observed in 13.1% of the patients only. Measuring iCa had a low sensitivity (20.3 and 23.5% at month 3 and 12, respectively) for the diagnosis of hypercalcaemia. The agreement (k coefficient [95%CI]) between noncorrected iCa concentrations and Ca was poor (Month 3: 0.11 [0.05-0.17]; Month 12: 0.20 [0.11-0.30]). Albumin-corrected iCa does not predict Ca better than noncorrected iCa. The risk for underestimating iCa was increased by a low total bicarbonate concentration.

Conclusion: Hypercalcaemia is commonly associated with successful engraftment. The high prevalence of metabolic acidosis requires the measurement of iCa for the accurate assessment of blood calcium levels in renal transplant recipients, at least in the early posttransplant period.

O-183 LOW SERUM MANNOSE BINDING LECTIN AS A RISK FACTOR FOR NEW ONSET DIABETES MELLITUS AFTER RENAL TRANSPLANTATION
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Introduction: In renal transplant recipients, infections and new onset diabetes mellitus after transplantation (NODAT) are frequent complications. Alterations in innate immune function may contribute to both infection and type 2 diabetes susceptibility whether the immune, liver-synthesized protein mannose binding lectin (MBL), which circulates at high concentrations in plasma, was associated with NODAT and infection.

Patients and methods: Between March 2005 and October 2006 consecutive non-diabetic renal transplant recipients were recruited. MBL, soluble tumor necrosis factor receptor 2 (sTNFR2) and neutrophil gelatinase associated lipocalin (NGAL) (as markers of chronic inflammation) were determined before transplant, and at 1 and 3 months following transplant. An oral glucose tolerance test was also performed at 3 months.

Results: A total of 125 patients were recruited and 111 patients had a functioning graft at 3 months. MBL levels remained unchanged following transplantation. Subjects with low MBL (lower tertile) had higher pretransplant sTNFR2 levels and a higher prevalence of NODAT at 3 months (44.4 vs 22.6%, p=0.01). Multivariate analysis confirmed that MBL was a risk factor for NODAT (relative risk 3.3, 95% confidence interval 1.3-8.3; p=0.013) after adjusting for relevant age and BMI.

Conclusion: Pretransplant MBL constitutes a new risk factor for NODAT development in addition to infection susceptibility.

O-184 THE USE OF ORAL GLUCOSE TOLERANCE TEST TO STRATIFY THE RISK OF DIABETES MELLITUS AFTER KIDNEY TRANSPLANTATION
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Background: Impairment in glucose metabolism after kidney transplantation
Session 22. Pediatric liver transplantation

Tuesday, 1 September 2009

49

Session 22. Pediatric liver transplantation

O-185 POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS IN RENAL ALLOGRAFT PATIENTS: FRENCH REGISTRY UPDATE AT 10 YEARS
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PTLD is a well-recognized complication occurring in transplant patients. In order to evaluate the incidence, identify clinical and histopathological features, and assess patients outcome, a French Registry of PTLD occurring after renal transplantation was set up.

Methods: We prospectively identified 378 new cases of PTLD between 1/1/1998 and 31/12/2007.

Results: Patients (256 M, 122 F) ranged from 18 to 75 years (mean: 46±13 years). The median time between grafting and PTLD was 83 months (1 m to 28 y) with 21% of early-onset PTLD. 12% of patients were EBV negative. Lymphoma involved a single site in 69% of cases and multiple sites in 31% of cases. Locations were graft in 61 cases, brain in 48 cases, gastrointestinal tract in 92 cases and lymph nodes in 80 cases. Only 20 PTLD expressed markers of T lineage, others were of B lineage. 2/3 of tumors were EBV positive, 50% of tumors were polyomavirus and 70% monoclonal. Most patients were treated with immunosuppression reduction. Rituximab was used alone in 73 patients and associated with chemotherapy in 96 patients. Chemotherapy alone was chosen in 99 patients. 88 patients were treated with surgery and 24 with radiotherapy. 155 patients died, mostly in the first year, half of them before PTLD progression. The median survival was 98 months. Patient survival was 73% at 1 year and 56% at 10 years. Factors positively influencing survival were: recipient's age above 50 years (p=0.001), early-onset occurrence (p=0.006), single vs multiple location (p=0.013), creatininemia < 150 μmol/l (p=0.04) and PTLD localized in the graft (p=0.02).

Conclusion: This ongoing registry is designed to better understanding epidemiology, risk and prognostic factors of PTLD occurring after renal transplantation in order to further establish consensus on treatment modalities.

O-186 PREVALENCE OF VITAMIN D DEFICIENCY IN RENAL TRANSPLANT RECIPIENTS. EFFECTS OF 25OHD SUPPLEMENTS
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Background: The KDQCI clinical practice guidelines in chronic kidney disease (CKD) give some recommendations about diagnosis and treatment of vitamin D deficiency. These guidelines may also be applied to renal transplant patients. However, there are few studies about 25-hydroxyvitamin D (25OHD) deficiency in renal transplant recipients. The aim of the present study was to assess the vitamin D status and the effects of vitamin D3 supplements in a cohort of kidney graft recipients.

Patients and methods: 320 renal transplant recipients non treated with vita-

min D supplements and with a follow-up of over 12 months were included in a retrospective cross-sectional study.

Results: Serum creatinine was 1.7±0.7 mg/dl and estimated GFR 46±17 ml/min/1.73m². The 25OHD levels were 10.2±6.6 ng/ml, 25OHD concentrations 19.9±11.3 ng/ml and 36.1±21.3 pg/ml respectively. When stratified according to 25OHD levels: 39.1% had 25OHD deficiency (< 16 ng/ml), 47.5% had 25OHD insufficiency (16 < < 30 mg/ml) and 13.4% had normal 25OHD levels (<30 ng/ml). Moreover, 25OHD concentrations correlated with several other variables such as age, gender, time of follow-up, graft function, total CO2 concentrations, iPTH, 1,25OHD concentrations, treatment with ACEI/ARB and season of 25OHD determination. On multivariate analysis: gender, length of follow-up, iPTH and 1,25OHD concentrations, season of 25OHD determination and treatment with ACEI/ARB were the variables that remained in the model. Twenty patients were treated with 25OHD2 supplements (400 IU/day). At 6 months iPTH and 1,25OHD levels showed no change but 25OHD levels increased (14.2±6.7 vs 21.6±10.2 ng/ml; p=0.045).

Conclusions: 25OHD deficiency or insufficiency is frequent in renal transplantation even in sunny regions. Low 25OHD concentrations may be, at least in part, the cause of persistent hyperparathyroidism. Treatment with 25OHD supplements improved vitamin D status without any effect on iPTH levels.

O-187 BENEFICIAL USAGE OF ANATOMICAL FULL-LEFT SPLIT LIVER GRAFT FOR MIDDLEWEIGHT PEDIATRIC RECIPIENT UNDER HIGH URGENCY STATUS
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Background: Commonly used left lateral segmental split grafts (LLGs) are normally not suitable for middleweight pediatric recipients of liver transplantation (LTx) especially under high urgent (HU) status. Therefore anatomical full-left segmental split grafts (FLGs) are sometimes selected for such recipients.

Objective: To assess the impact of LTx using FLGs on middleweight recipients under HU status.

Patients: We analyzed the data of 73 middleweight (15-35kg) pediatric recipients who received deceased organ graft among 1205 LTx between 1998 and 2007. All split grafts were made in ex situ splitting.

Results: Sixteen cases (21.3%) were under HU status and 57 (78.7%) cases were not. Fulminant hepatitis and Re-transplantation were major indications in the HU group. Biliary disease and metabolic disease were common in non-HU group. FLGs were used more frequently in HU group (37.5% in HU group vs. 8.3% in non-HU group, p<0.05). Fewer LLGs were adopted in HU group (31.3% in HU group vs. 48.3% in non-HU group, p<0.05). One and 5 year patient survival was 80.0% and 80.0% in HU group and 94.2% and 92.0% in non-HU group (P=0.09), respectively. One and 5 year graft survival was 73.3% and 73.3% in HU group and 87.0% and 83.0% in non-HU group (P=0.16), respectively. In HU group, 2 of 5 LLGs and 1 of 6 FLGs were lost but no graft loss was occurred in HU group. Eight remnant full-right grafts were used in our institute to non-HU recipient and only one graft was lost.

Conclusion: With the view of severe shortage of organ graft, FLGs are a comparable option to LLGs even for the pediatric recipients under HU status.

O-188 EFFICACY OF VALGANCICLOVIR AS PREEMPTIVE THERAPY OF INFECTION DUE TO EBSTEIN BARR VIRUS IN PEDIATRIC LIVER TRANSPLANTATION
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Aim: To analyze the response to preemptive therapy with valganciclovir (VCV) in children with liver transplantation and high quantitative EBV-PCR

From June 2005- Dec. 2007, we have tested 979 EBV-PCR in 80 children. 2180 PCR were tested from the date of transplant, 59/80 belonged to the historical cohort. Patients were divided in 2 groups depending if they received VGC treatment (n=22) or not (n=16). The response to VGC was considered complete if PCR was negative at 30 and 60 days of treatment, partial if PCR decreased at least 50%, and no response if PCR remained the same or increased. The clinical response was defined as: 41% partial, 23% no response. In non-treated group it was: 6%, 25% and 68% respectively (p<0.01). We did not find differences in episodes treated during 60 days. No patients reached recommended GAN therapeutic levels at...
2 hour (6 mg/L). However, mean PCR was lower when the GAN levels were higher than 4 mg/L. The PCR in blood was negative in 3/8 biopsied patients with presence of EBV (EBER). Four patients were long-term treated because of persistent high viral load, 1/4 developed PTLD 2 months after stopping therapy.

Conclusion: There is a response in the EBV viral load to VGC after 30 days of treatment. There is a high interpatient variability of GAN serum concentrations suggesting the need of pharmacokinetic monitoring to optimize treatment. There was a relationship between GAN levels and the EBV viral load. Presence of EBV in tissue can occur with negative EBV-PCR.

O-189 LONG-TERM EVOLUTION OF DE NOVO HEPATITIS C INFECTION (HCV) AFTER PEDIATRIC LIVER TRANSPLANTATION

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To establish HCV characteristics in long term follow-up of de novo HCV infection in children with LTx. Between 1985-2005, 157 pts. underwent 218 LTx. 10/57 pts developed de novo HCV, 1 pt also was infected with HBV. 8 pts were transplanted before HCV screening. Mean age at LTx: 8.4 yrs (1: 1.8-16 years). 1 pt. was liver-kidney graft.

Diagnosis: HCV antibodies (Ab), HCV-RNA by PCR. Mean age at time of tx: 8.2 yr (r: 1.9-16 yr). Patients were divided in 2 groups depending if they received antiviral treatment with Peg IFN and ribavirin.

Results: All patients are alive. Mean time of HCV diagnosis after LTx: 10.8 yr; 2 m-yr to 6/10. HCV diagnosis was by screening, 4/10 because of liver dysfunction. 9/10 had HCV+ Ab. HCV genotypes (G): G 1a:1; G 1b:7; G 3:1. 1 pt had spontaneous virus clearance. Liver biopsy was performed in 7/10 pts. 3/7: Active chronic hepatitis with F2, F4; 4/7: Minimal changes, 1 of those with concomitant chronic rejection. Treatment group n=4: (1 G1a-b; 3 G1b). In all, virus clearance occurred with sustained virological response and none of the pts developed chronic rejection solved on TAC and MMF. No treatment group n=6: (4 G1b, 1 G3 and 1 YHC Ab + RNA- 2 pts (1 G1b, 1 HCV Ab+) had spontaneous viral clearance, 2 pts (G1b) are RNA+ normal liver function, and the remaining 2 pts (1 G3/VHB, 1 G1b hepatopat) are RNA+ with liver dysfunction.

The behaviour of de novo HCV after pediatric liver tx is better than in adults. After treatment, the sustained viral response of HCV G1b is good; There is a risk for chronic rejection development.Spontaneous viral clearance may occur.

O-190 CENTRAL SINUSOIDAL FIBROSIS (CSF) AS AN INDICATOR OF INADEQUATE IMMUNOSUPPRESSION IN CHILDREN WITH TACROLIMUS WITHDRAWAL AFTER LDLT

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We have withdrawn tacrolimus (Tac) in 191 of 675 children undergoing LDLT. Initially, Tac was decreased slowly and finally discontinued as far as liver function tests (LFT) were normal without liver biopsy, and was discontinued in 103. We began follow-up biopsy for all children in 2004 and found CSF in considerable number of children with normal LFT after discontinuing Tac. Hence, we hypothesized that CSF is an indicator of inadequate immunosuppression and began a prospective study of Tac resumption.

Methods: Patients with CSF and normal LFT after Tac withdrawal were enrolled and patients with ongoing rejection, biliary or vascular complications were excluded.

Results: Tac was resumed in 25 children (age: 0.7-9 years at LDLT, 6-24 years at resumption) because of CSF. Tac had been discontinued in 12, administered every month in 4, every 2 weeks in 2, every 1 week in 3, every 3 days in 2 and every other day in 1. 1 pt. suspected to have diabetes and resumed finally to Tac. Duration of immunosuppression ranged from 6 to 98 months (median: 23). CSF was graded from stage 0 to IV according to Dixon’s criteria. The CSF was graded in IV in 1, III in 1, II in 3, I in 7, and C4d deposition was endothelial/stromal in 10, endothelial in 6, and no in 7, and not available in 2 at resumption. CSF was subsequently improved in 5 (follow-up: 12-48 months), not progressed in 12 (6-16 months), progressed in 2, and not assessed because of short follow-up in 6. C4d deposition turned to negative in 4 and decreased in 1 among the 5 CSF-improved children.

Conclusion: CSF might be an indicator for inadequate immunosuppression and the mechanism was possibly humoral immunity.

O-191 PREDICTORS OF OPERATIONAL TOLERANCE AFTER PEDIATRIC LIVING DONOR LIVER TRANSPLANTATION: THE ABSENCE OF EARLY REJECTION, THE PRESENCE OF IN-FETUS HLA-MISMATCH, LLAGN, AND THE MECHANISM

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Background: After our pediatric living-donor liver transplantation (Tx), a substantial number of the patients achieved complete cessation of IS (operational tolerance), which had been long exceptional in clinical Tx. On the other hand, some patients encountered rejection while they were undergoing weaning from IS. Nonetheless, reliable predictors of operational tolerance are not available yet.

Method: The study group consisted of group-tolerance (Gr-Tol) in which 50 patients were successfully weaned off from IS 10 years post-Tx and group-intolerance (Gr-Intol) in which 13 patients were still on maintenance IS due to the experience of rejection during or after weaning IS at identical time point. Two groups were compared with respect to following clinical parameters to identify predictors of operational tolerance: donorrecipient age and gender, HLA mismatch, HLA-DR antigen, early (< 1 month) rejection episode, initial tacrolimus trough level (< 1 week).

Results: Among clinical parameters tested, multiple logistic regression identified the presence of HLA-B mismatch (Gr-Tol vs Gr-Intol; 93% and 80%, OR [odds ratio] =33.36, p=0.05) and the absence of early rejection episode (Gr-Tol vs Gr-Intol; 90% and 31%, OR=34.48 p=0.002) as independent predictors of successful IS withdrawal. Univariate analysis revealed that female donor was associated with successful IS cessation (Gr-Tol vs Gr-Intol; 37% and 38%, p<0.01). By multiple logistic regression, OR of female donor reached as high as 5.32, although it was not a statistically significant predictor of successful IS cessation (p=0.16). The other parameters were not associated with operational tolerance.

Conclusion: This is the first report showing the possibility that the presence of donor-recipient HLA-B mismatch, the absence of early rejection and female donor could be useful as predictors of operational tolerance after pediatric living-donor liver transplantation.

O-192 THREE HUNDRED LIVING DONOR LIVER TRANSPLANTATIONS IN ADULTS AND CHILDREN: TECHNICAL EVOLUTIONS AND LESSONS LEARNED

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Purpose: Living donor liver transplantation (LDLT) has become a legitimate and accepted alternative in such countries where cadaveric organs are scarce. In this study, we present our technical evolutions and critically evaluate the result of our consecutive 300 LDLTs to identify risk factors for poor outcome.

Methods: The selection criteria for grafts are based on graft-to-standard liver volume ratio (GV/SV). Left lobe (LL) grafts are the primary choice for all patients, however, right lobe (RL) grafts are considered if calculated GV/SV<35%. Graft types included left lobe (LL) grafts (n=187), right lobe (RL) grafts (n=93), left lateral segment (LLS) grafts (n=19) and dual grafts (n=1). Grafts were defined as extra-small (ES, GV/SV<30%, n=18), small (S, 30-40%, n=94), medium (M, 40-50%, n=114) and large (L, >50%, n=74) grafts according to the size.

Results: The mean GV/SV of the LL, RL and LLS grafts were 40.0%, 48.6% and 81.3%, respectively. The mean MELD score in adult patients was 14.5 (range 1-54). Several technical evolutions were phased in including introduction of RL grafts (n=93), duct-to-duodenum biliary reconstruction (n=205), planned temporary portal-vein occlusion (n=71), auxiliary surgery for recipient liver dysfunction (n=2), temporary hepaticocaval shunt (n=2), and dual-graft LDLT (n=1). Overall 1, 5 and 10-year patient survival rates were 85.1%, 75.6% and 69.9%, respectively. On multivariate analysis, high MELD score (<20) was the only significant factor for poor prognosis while other factors graft type and size were not.

Conclusion: Our left lobe-centered policy can offer acceptable results and should be a viable option in LDLT. However, indications for LDLT in patients with a high MELD score should be limited due to poor prognosis.

Session 22. Pediatric liver transplantation

Tuesday, 1 September 2009
Session 23. Mechanisms & evolution of chronic allograft nephropathy

O-193 THE AUTOANTIBODY LEVEL OF CITRATE SYNTHASE IS CORRELATED WITH C4D DEPOSITION IN RATS WITH CHRONIC ALLOGRAFT NEPHROPATHY

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Purpose: This study is to investigate the relationship between C4d deposition and autoantibodies of citrate synthase (CS) in rats with chronic allograft nephropathy (CAN).

Methods: Fisher344 rat renal grafts were orthotopically transplanted into Lewis rats following the procedure of Karmada with our modification. All the recipients were given CsA 10mg/kg d\(^{-1}\) x10d and then divided into three groups: (1) Vehicle; (2) CsA: 6mg/kg d\(^{-1}\); (3) MMF: 20mg/kg d\(^{-1}\). At 4w, 8w, 12w after transplanted, the renal allografts were harvested and the sera was collected. The SCR was measured and the pathological changes were assessed according to the Banff 97 criteria. The IgM and IgG isotype of CS antibodies in all the recipients were detected by indirect enzyme-linked immunosorbent assay (ELISA). The deposition of C4d was detected by immunofluorescence and analyzed by Integrated Optical Density (IOD).

Results: The level of IgM of CS autoantibodies was more obviously stable and higher than IgG isotype in all blood samples. With the progression of CAN from 4w, there was a strong positive correlation between the level of IgG isotype of CS and C4d deposition (r=0.973, p<0.001) in vehicle group. CsA didn’t prevent the formation of IgG isotype of CS (r=0.75) and the deposition of C4d (r=0.906) at 8w and 12w. The differences of IgG and deposition of C4d between MMF and other two groups were all statistically significant (p=0.001) at 12w.

Conclusions: This study shows that there was a strong positive correlation between the level of IgG isotype of CS and C4d deposition. The CS autoantibodies may contribute to progression of CAN. MMF may inhibit the progression of CAN by decreasing the formation of IgG isotype of CS and the deposition of C4d. CsA has no these effects.

O-194 INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY (IFTA) AFTER RENAL TRANSPLANTATION: TIME COURSE AND FACTORS INFLUENCING SCARRING

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A subgroup of 86 patients had a 12 months protocol biopsy. Ordinal logistic regression was used to look at possible factors influencing the degree of scarring at that time. Variables included patient: age, gender, BMI, time on dialysis, diabetes, smoking, HLA mismatches, peak PRA, donor: gender, status, age, serum creatinine, and; cold ischaemia time, delayed graft function, GFR at 3 months, ACE inhibitor or IL2 antagonist induction, ATN on biopsy during 1st week, number of borderline or acute rejections. With the progression of CAN from 4w, there was a strong positive correlation between the level of IgG isotype of CS and C4d deposition (r=0.973, p<0.001) in vehicle group. CsA didn’t prevent the formation of IgG isotype of CS (r=0.75) and the deposition of C4d (r=0.906) at 8w and 12w. The differences of IgG and deposition of C4d between MMF and other two groups were all statistically significant (p=0.001) at 12w.

Conclusions: This study shows that there was a strong positive correlation between the level of IgG isotype of CS and C4d deposition. The CS autoantibodies may contribute to progression of CAN. MMF may inhibit the progression of CAN by decreasing the formation of IgG isotype of CS and the deposition of C4d. CsA has no these effects.

O-195 INDIRECT ALLOGRECOGNITION OF HLA ‘PUBLIC’ T-CELL EPITOPES IS ASSOCIATED WITH CHRONIC ALLOGRAFT DYSFUNCTION

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Introduction: We previously reported on human CD4+ T lymphocyte responses to peptide epitopes derived from HLA class I that exhibit little polymorphism, some that are identical to self and many of which bind promiscuously to MHC class II ('public T cell epitopes' PTE). This contrasts with other studies in which there is an assumption that epitopes arise from highly polymorphic sequences. If responses to PTE’s are representative of indirect alloimmunity in general, then they may allow the development of a standardised assay of cellular immunity for chronic allograft dysfunction (CAD).

Methods & results: Immune response to a restricted set of HLA PTEs was assessed by PBMC γ-interferon production using ELISPOT in 110 kidney transplant recipients under long-term follow-up at our centre. The relationship between responses and two indicators of (CAD): late transplant biopsy (LTB) for clinical indication after the 1st post-transplant year and deteriorating renal function in the previous 3 years (DRF) was assessed. 30 patients underwent LTB and an interacting group of 30 defined as having DRF. In both groups, 22 patients made a significant response in the ELISPOT (ER). This was significantly higher than in patients in whom there was no LTB or DRF (22/30 vs 32/80, 73.3% vs 40%; p=0.002 in both). In multivariate analysis ER was the variable most strongly associated with LTB and the only variable associated with DRF. Of 54/110 transplant recipients with an ER, 22/54 had undergone LTB compared to 8/56 non-responders.

Conclusion: These data suggest that responses to PTE’s are significantly associated with a diagnosis of CAD in a population unscreened for HLA type. Although limited by the retrospective nature of the analysis, the strength of these associations suggest that this is a viable biomarker of ‘chronic rejection’ meriting further investigation.

O-196 DIFFERENTIAL EVOLUTION OF STRUCTURAL AND FUNCTIONAL CHANGES DURING THE FIRST YEAR OF KIDNEY TRANSPLANTATION (KT)

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The usefulness of measured GFR during the first year post KT is not well established. In this study, we determined if there was a correlation between the mGFR impairment and BANFF lesions worsening during the first year post KT.

Conclusion: Interventions to prevent the development of IFTA need to be instigated early. The use of IL2 antagonist induction, ACE inhibitors, and early recognition of compliance problems might prove beneficial.
In conclusion, using GRP78 as a surrogate marker of ER stress, immunohistochemical allograft nephropathy score (IF/TA score, 0.4 ± 0.1 vs 0.2 ± 0.1, respectively), tubular atrophy score (ct score, 0.5 ± 0.05 vs 0.3 ± 0.05, respectively), and arteriosclerosis lesions worsening (37% vs 41%, respectively), and cg lesions progression (7.9% vs 4.1%, respectively). Both 3-months and 1-year mGFR were strongly predictive of allograft survival. This study suggests the absence of paraneoplastic disease and a marginal decrease of mGFR but a much higher histological worsening during the first year post KT. Consequently, the GFR variation between 3 months and 1 year cannot be used as a decision criterion in performing a screening biopsy at 1 year, but might be useful to predict long-term graft outcome.

Results: Twenty-four months after HSCT, CKD, defined as glomerular filtration rate (GFR) <60 mL/min/1.73 m², was noted in 49 patients (40%). Age ≥ 45 years, early acute renal failure and a baseline GFR <60 mL/min/1.73 m² predicted the occurrence of CKD. The drop in GFR occurred in the first two years after HSCT, with a slight and transient improvement in GFR but retained CKD and ten patients (22%) had a sustained improvement of GFR. Among 62 patients without CKD at 24 months after HSCT, 3 (5%) developed CKD during follow-up. Inclusion: HSCT-related CKD probably includes two subsets: a frequent early-onset CKD occurring during the first two years after HSCT, mainly as a consequence of ARF in older patients with pre-existing renal impairment and a rare late-onset CKD occurring after 12 months (p<0.0001) and probably related to radiation nephropathy and calcineurin inhibitors toxicity.

Session 24. Clinical immunosuppression: news

O-199 SOTRASTAUIN: PHARMACOKINETICS AND EXPOSURE-EFFICACY RELATIONSHIP IN RENAL TRANSPLANT RECIPIENTS


Pharmacokinetics of sotraastaurin, a novel, selective inhibitor of protein kinase C, and relationship between normalized average trough blood levels and biopsy-proven acute rejection (BPAR) were assessed in 216 de novo renal transplant recipients.

Methods: Recipients were randomized to a control regimen of mycophenolic acid (MPA) with standard-exposure tacrolimus (n=74), or sotraastaurin 200mg bid with either standard (n=76) or reduced-exposure tacrolimus (n=66). At month 3, tacrolimus was replaced with MPA in the sotraastaurin arms. All study arms used basiliximab and corticosteroids.

Results: By week 2 sotraastaurin trough levels remained stable at 615±419 ng/ml till month 6. Sotraastaurin levels were neither affected by combination with standard- nor reduced-exposure tacrolimus. In patients whose tacrolimus was replaced by MPA (n=11), Sotraastaurin Cmax and AUC, pooled over treatments and across visits till month 6, were 1603±636 ng/ml and 12222±4179 ng.h/ml, respectively. Pooled AUC intersubject variability was moderate (27%) and not influenced by age, weight, or sex (p=0.86, p=0.37, respectively). Sotraastaurin levels significantly correlated with AUC (R=0.62, p<0.001). From month 1 to month 3, four BPARs occurred in both so-traastaurin arms, whereas post-conversion from tacrolimus to MPA (months 3 to 6) 15 BPARs occurred. Recipients were classified based on their average so-traastaurin levels (<400, 400-600, >600ng/ml) and the freedom (%) from BPAR was determined in each group (Table): fixed-dose sotraastaurin+ tacrolimus showed good efficacy in contrast to sotraastaurin+MPA, especially for the lower sotraastaurin exposure.

Conclusions: Sotraastaurin exposure was stable from week 1 onwards regardless of the combined immunosuppressant. Sotraastaurin intersubject pharmacokinetic variability was similar to that of other immunosuppressants. In combination with MPA, higher sotraastaurin exposure may be needed for equivalent efficacy to sotraastaurin+tacrolimus. Future studies are necessary to ascertain the best regimen for sotraastaurin.

O-200 COMPARISON OF COMBINATION PLASMAPHERESIS/ IVIG/ANTI-CD20 VERSUS HIGH-DOSE IVIG IN THE TREATMENT OF ANTIBODY-MEDIATED REJECTION

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Different strategies appear to improve the success in treatment of antibody-mediated rejection (AMR), although no one best method has yet emerged. The objective of this study was to compare the efficacy of the combination of
Plasmapheresis/IVIg/anti-CD20 based regimes versus high-dose IVIg alone in the treatment of AMR. Group A (12 patients) was treated with high-dose IVIg between 01/2000 and 12/2003; Group B (12 patients) was treated by Plasmapheresis/IVIg/anti-CD20 between 01/2004 and 12/2005. The evaluation of response was based on graft survival at 36 months and histologic and serologic data (detection of DSA by ELISA and Luminex SA) gathered at the time of AMR diagnosis and 3 months post-rejection. Graft survival at 36 months was 91.7% in Group B versus 50% in Group A (p=0.02). DSA levels detected 3 months post-rejection are significantly lower in Group B than in Group A: DSA ELISA score 6-8 (p=0.02), DSA MIFmax 2671 633 vs 9010 1651 (p=0.05) and DSA mean MFI 1030 498 vs 4437 1534 (p=0.004). The persistence of elevated DSA levels post-treatment is more frequent in patients with graft loss as compared to those with preserved renal function: score 6-8 on ELISA (p=0.04); mean MFI (p=0.0001); and MIFmax (p=0.008). The presence of a MIFmax post-AMR > 5000 is associated with graft loss with a sensitivity of 100% and a specificity of 77.8% (ROC curve with AUC of 0.68, p=0.004). DSA levels at the time of rejection were weakly predictive for graft loss (ROC curve with AUC of 0.74, p=0.08). We conclude that high dose IVIg alone is inferior to Plasmapheresis/IVIg/anti-CD20 as therapy for AMR and II DSA post-rejection can be quantified using solid phase assays, stressing that 3 months after AMR DSA levels are higher in patients with graft loss.

O-201 EVEROLIMUS WITH LOW OR VERY LOW EXPOSURE OF TACROLIMUS IN DE NOVO RENAL TRANSPLANT RECIPIENTS: THE ASSET STUDY
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Everolimus, a potent immunosuppressant and proliferation signal inhibitor (PSI) has been developed for the prevention of renal and heart allograft rejections and is used in combination with calcineurin inhibitors (CNIs). Renal function is impacted negatively by CNIs and early reduction may allow for better outcome. The ASSET study investigates whether everolimus with either low dose or very low dose tacrolimus can preserve renal function whilst maintaining efficacy in de novo renal transplant recipients (RTxR).

Methods: ASSET, a 12-month, randomized, multicenter, open-label study enrolled de novo RTxR with deceased or living donors, cold ischemia time <30h and donor age 10–65 years. Within 24 hours after transplantation, 229 patients were randomized (1:1) to the low dose (LDTac) or very low dose (VLDTac) tacrolimus arm. For the first 3 months all patients received everolimus (1.5 mg bid, Co-h; 3 < Co< h 8 ng/mL) and tacrolimus (0.1 mg/kg/day, Co-h 4-7 ng/mL) therapy. The LD Tac group continued the initial regimen unchanged whereas the VLDTac group received tacrolimus targeted to Co-h 1.5-3ng/mL until month 12. All patients received basiliximab and steroids.

Results: The study is ongoing at 36 centers worldwide and data will be available in May 2009. The primary objective is to compare renal function (cGFR [MDRD]) at month 12 between the treatment groups.

Conclusion: ASSET is a pivotal study to evaluate the efficacy of everolimus with low or very low-exposure of tacrolimus to prevent allograft rejection and provide beneficial renal function in RTxR.

O-202 EFFICACY AND SAFETY OF AN EVEROLIMUS/ENTERIC-COADED MYCOPHENOLEN SODIUM REGIMEN AFTER CALCINEURIN INHIBITOR WITHDRAWAL IN DE NOVO RENAL TRANSPLANT PATIENTS: RESULTS OF THE ZEUS TRAIL
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Aim of the study: To investigate safety and efficacy of an everolimus/enteric-coated mycophenolate sodium (EC-MPS) regimen after Cyclosporine (CsA) withdrawal in de novo kidney allograft recipients at month 12 post transplantation.

Methods: In this 1-year, prospective, open-label, controlled, multi-center study 300 renal allograft recipients were randomized to an immunosuppressive regimen consisting of either Everolimus/EC-MPS or CsA/EC-MPS. All patients (pts) received induction therapy with Basiliximab and were treated with CsA, Everolimus/EC-MPS and micrcestoridoids for the first 4.5 months. Subsequently patients were randomized 1:1 to either a) continue CsA/EC-MPS (n=145) or b) convert to Everolimus/EC-MPS (n=155). CsA and Everolimus trough levels were 100-150ng/mL and 6-10ng/mL, respectively. Dosing for CS-MPS was 720mg BID.

Results: BPAR was reported in 23/155 (14.8%) Everolimus/EC-MPS-treated vs 22/145 (15.2%) CsA/EC-MPS patients during the first year after transplantation. One death was observed in the CsA/EC-MPS group and no graft loss was reported in both groups. The number and proportion of patients withdrawn due to adverse events during 12 months were 37/155 (23.9%) in the Everolimus/EC-MPS and 28/145 (19.3%) in CsA/EC-MPS group. The table shows laboratory parameters and adverse events which are of interest after conversion from CsA to Everolimus.

Table 1

<table>
<thead>
<tr>
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<th>CsA/EC-MPS</th>
<th>Everolimus/EC-MPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalisations (%)</td>
<td>18.2</td>
<td>15.8</td>
</tr>
<tr>
<td>Biopsies (%)</td>
<td>12.1</td>
<td>11.9</td>
</tr>
<tr>
<td>Rejection (%)</td>
<td>2.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Death (%)</td>
<td>0.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Graft loss (%)</td>
<td>0.7</td>
<td>0.0</td>
</tr>
<tr>
<td>SFV (%)</td>
<td>3.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Serious infections (%)</td>
<td>1.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Renal function expressed as calculated GFR (Nankivell method) improved from randomization to month 12 by 10.4 mL/min/1.73m² in favor of the Everolimus/EC-MPS regimen (p<0.001).

Conclusions: The introduction of Everolimus/EC-MPS in de novo renal transplant patients after CNI withdrawal reflects a novel therapeutic approach which significantly improves renal function without compromising efficacy and safety.

O-203 INITIATION OF EVEROLIMUS WITH CALCINEURIN INHIBITOR (CNI) REDUCTION IN THORACIC TRANSPLANT PATIENTS WITH IMPAIRED RENAL FUNCTION: A MULTICENTER, RANDOMIZED STUDY
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Purpose: CNI therapy is a major contributor to deteriorating renal function following thoracic transplantation. Initiating everolimus and reducing CNI exposure may improve renal function. The Nordic Certican Trial in Heart and Lung Transplantation (NOCTET) is the first randomized, comparator study to assess this strategy in thoracic transplantation.

Methods: A 12-month, open-label, multicenter study was undertaken in patients receiving a heart or lung transplant –12 months previously who had GFR 20-90mL/min/1.73m². Patients were randomized to continue their current
CNI-based immunosuppressive regimen unchanged or start everolimus with CNI reductie targeting cyclosporine C0 75 ng/mL or tacrolimus C0 4 ng/mL. Primary endpoint was change in measured GFR from baseline to month 12.

Results: 283 patients (191 heart, 92 lung) were randomized: everolimus 141, controls 142. Baseline characteristics were similar between groups except for recipient age (everolimus 59±10 years, controls 56±11 years; p=0.03) and time post-transplant (everolimus 62±45 months, controls 75±45 months; p=0.02). Based on preliminary data from 169 patients (77 everolimus, 92 controls), mean cyclosporine C0 at randomization (n=244, 86.2%) was 136±50 ng/mL and 138±62 ng/mL in the everolimus and control groups, respectively, decreasing to <90 ng/mL from week 4 onwards in the everolimus group. Mean tacrolimus C0 (n=39, 13.8%) was similar at randomization (everolimus 10.3±2.7 ng/mL, controls 9.6±2.7 ng/mL), remaining in the range 4.4–5.6 ng/mL after week 4.

Conclusion: Study results will be presented comprising the primary endpoint, secondary endpoints (including left ventricular function in heart patients and incidences of bronchiolitis obliterans syndrome in lung patients), and safety data. In contrast to other recent trials, the study protocol was followed closely, resulting in the lowest cyclosporine levels reported in transplantation.

**O-204** TACROLIMUS REDUCES THE RISK FOR BRONCHIOLITIS OBLITERANS SYNDROME 3 YEARS AFTER LUNG-TRANSPLANTATION BY 50% IN COMPARISON TO CYCLOSPORINE IN A PROSPECTIVE RANDOMIZED INTERNATIONAL TRIAL OF 248 PATIENTS

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**Objective:** We performed a prospective randomized study comparing the efficacy and safety of two immunosuppressive regimens (Tac, MMF, Steroids vs. CsA, MMF, Steroids) after Lung Transplantation. Primary objective was the incidence of bronchiolitis obliterans syndrome (BOS). Secondary objectives were incidence of acute rejection and infection, survival and adverse events. 248 patients with a complete 3 year follow-up were included in this analysis.

**Methods:** Patients were randomized to treatment group A: Tac (0.01-0.03 mg/kg/d iv – 0.05-0.3 mg/kg/d po) or B: CsA (1.3 mg/kg/d iv – 2.8 mg/kg/d po). MMF dose was 1-4 mg/d. No induction therapy was given. Patients were stratified for cystic fibrosis. Intention to treat analysis was performed in switched patient groups.

**Results:** 3 of 123 Tac patients and 41 of 125 CsA patients were switched to another immunosuppressive regimen. Groups showed no difference in demographic data. Kaplan-Meier analysis revealed significantly less BOS in Tac treated patients (p=0.003, log rank test, pooled over strata). Cox regression showed a 50% lower risk for BOS in the Tac group. Incidence of acute rejection was 67.5% (Tac) and 75.2% (CsA) (p=0.583). 1- and 3-year survival rates were not different (85.4% Tac vs. 88.8% CsA, and 80.5% Tac vs. 83.2% CsA, p=0.583).

**Conclusion:** Tac significantly reduced the risk for BOS after 3 years. Both regimens have a good immunosuppressive potential and offer a similar safety profile with excellent one and three year survival rates. Acute rejection rates were similar in both groups. Incidence of infections and renal failure showed no difference.

**Session 25. Novel insights in perfusion & preservation**

**O-205** FOXP3+ REGULATORY T-CELLS PLAY A DOMINANT ROLE OVER RORγt+ T17-CELLS IN DECEASED DONOR KIDNEYS

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Th17 cells and regulatory T-cells (Tregs) have an interactive relationship mediated by IL-6 and TGF-β. Both cell types are involved in the reaction against alloantigens and in response to tissue injury. Here, we studied the Th17-Treg network in zero biopsies of kidneys from living donors with relatively short ischemia-reperfusion times and from deceased donors with brain death related inflammation and prolonged cold ischemia-reperfusion times.

**Results:** Biopsies from deceased donors (n=14, 1-14 h cold ischemia) and living donors (n=14, <2 h cold ischemia) were studied at the end of cold storage and after 20-30 min reperfusion. In deceased donor kidneys higher mRNA expression levels of CD40, CD43, CD59, IL-15, IL-17, IL-21, and TGF-β were measured compared to living donor kidneys demonstrating that more Th17-cells had infiltrated these allografts (p<0.04). Moreover, in deceased donor kidneys the expression levels of TGF-β, which acts as a differentiation factor for Foxp3+ regulatory T-cells, was abundant present (p=0.005) compared to living donor kidneys. Likewise, Foxp3 mRNA was detected at significantly higher levels in deceased than in living donor kidneys (p<0.001). In contrast, biopsies from deceased donor kidneys the mRNA levels of RORγt, the transcription factor that directs IL-17 transcription, was significantly lower than in samples from living donors (p<0.001). Concurrently, no induction of the RORγt inducing cytokines (IL-6, TNF-α, IL-21) and IL-17 was measured.

**Conclusion:** The high mRNA expression levels of CD40, TGF-β and Foxp3 in deceased vs living donor kidneys suggests that regulatory T-cells, but not Th17 cells play a role in controlling tissue injury resulting from the pathophysiological events inherent to deceased donation.

**O-206** PERITONEAL COOLING MAY PROVIDE ADDITIONAL ISCHAEMIC PROTECTION FOR UNCONTROLLED NON HEART BEATING KIDNEY DONORS

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Purpose: Uncontrolled NHBD renal transplantation is made possible by interventions which ameliorate the effects of warm ischaemia. In most centres these techniques are focused on the timely introduction of cold in-situ perfusion to provide sufficient renal cooling and preservation. However, at laparotomy in our category II donors we typically see temperatures of 29-30°C. In order to assess any potential benefit for contemporary uncontrolled NHBD programs we have developed a porcine model of the uncontrolled NHBD, comparing current in-situ perfusion (ISP) protocols with additional peritoneal cooling.

**Materials and methods:** Ten 30 kg pigs were used; the in-situ perfusion (ISP) group modelled our current protocol with the addition of peritoneal cooling (PC) group modelled current protocols with the addition of peritoneal cooling.

**Results:** In the ISP group only 1/4 cases reached a mean renal temperature of 25°C. In the PC group the mean time taken to reach 25°C was 14.5 minutes. The final temperature after 90 minutes was 26.3±1.46°C in the ISP group versus 16.9±1.17°C in the PC group (p<0.0001).

Renal parenchymal microdissection permitted measurement of biochemical markers of anaerobic metabolism (lactate) and cell injury/death (glycerol). At 4h
120 minutes significantly superior results were seen in the PC vs ISP group; peak lactate 3.78±0.6 versus 6.23±0.26 mmol/l (p = 0.0003), and peak glyc- erol 284±5.49 versus 554±7.43 mmol/l (p = 0.0008).

Conclusions: This study has demonstrated superior renal cooling, and bio- chemical microdialysis evidence of improved ischaemic protection, with supplemental peritoneal cooling for uncontrolled NHBDs.

O-207 "TWO LAYER PREPARATION METHOD" (TLM) IMPROVES POST-TRANSPLANT SURVIVAL AND EARLY KIDNEY FUNCTION FOLLOWING PROLONGED COLD ISCHAEMIA
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Purpose: Oxygen solubility in perfluorocarbon (PFC) is about 25 times higher than in conventional solutions. TLM based on oxygenated PFC overlaid with UW solution has been successfully used especially in islet transplantation. The aim of the present study was to see whether TLM prevents tissue damage and improves early kidney function in a rat model following prolonged cold ischemia time.

Methods: Brown-Norway syngeneic rats were used as kidney donors and re- cipient. Ten rats were harvested and stored for 24 hours either in cold ischemia (Group 1, n=16), with TLM (Group 2, n=16) or transplanted immediately (Group 3, n=12). Kidney transplantation was performed after bilateral nephrectomy. In half of the animals in each group (8, 8 and 6, with random assignment) only survival was followed. In the other animals the grafts were procured for histo- logical analysis 24 hours after transplantation. For tissue damage grading a blinded scoring method was applied. Apoptosis was assessed by using a TUNEL assay.

Results: One-month survival in groups 1, 2 and 3 was 12.5%, 62.5% and 100%, respectively. There was significant difference in survival time (Group 1 vs 2, p = 0.01). Median creatinine levels 24 hours after transplantation were 381 (292-443), 299 (255-374) and 121 (102-138) μmol/l, respectively Group 1 vs 2, p = 0.02. Histological scoring showed more severe tissue damage in Group 1 than in Group 2 (p = 0.01). Apoptosis was not the main cause of tissue injury because of its rather low rate in all 3 groups. It was detected mainly in tubular cells and was more frequent in Group 1 than Group 2 (p = 0.01).

Conclusion: Conservation with TLM significantly improves the outcome of kidney transplantation in a rat model and should be further studied in larger ani- mals.

Supported by Grant NR/9083-3 from IGA MZ CR

O-208 ENESICHEMIC HYPOTERMIC RECONDITIONING REVERSES PRESERVATION INDUCED LIVER INJURY BY MITOCHONDRIAL PROTECTION PRIOR TO REPERFUSION
Thomas Minor, Judith Stegemann. Surgical Research Division, University Hospital, Bonn, Germany

Background: Although the quality of cold-stored livers slowly declines beyond approximately 12 hours of ischemia and the risk of primary dys- or non-function steadily increases, up to 20% of all liver transplantations are done after cold ischemic time of more than 12 hours.WI induces mitochondrial DNA damage, mitochondrial dysfunction and subsequent tissue injury. Moreover, mitochondrial dysfunction and subsequent tissue injury.

Methods: Livers were harvested from male Wistar rats, flushed with 40 ml of Histidine Tryptophan Ketoglutarate (HTK) solution and cold-stored for 22h (CS22). Some grafts were subsequently subjected to 90 min of hypothermic reconditioning by venous systemic oxygen perfusion (VSOP) or oxygenated hypothermic machine perfusion (HMP) with HTK. Livers stored for only 6h (CS6) served as reference. Viability of all grafts was assessed thereafter by warm reperfusion in vitro.

Results: VSOP and HMP significantly increased endischemic tissue energy change, and abrogated cellular enzyme loss upon reperfusion even signifi- cantly below control values. Ammonia clearance and bile production were more rapid in livers that were harvested and stored to CS6. Hypothermic UW solution by both techniques induced mitochondrial chaperone expression (HSP70 family) and abrogated activation of caspase 9 and 3.

Conclusion: Viability of long preserved liver grafts can be augmented by tran- sient hypothermic reconditioning using either machine perfusion or gaseous oxygen perfusion, both preventing initial mitochondrial dysfunction and sub- sequent tissue injury. Owing to the simplicity and ease of application, gaseous oxygen perfusion recommends itself as feasible alternative to the more cumbersome and cost-intensive perfusion protocol.

O-209 MITOCHONDRIAL PROTECTION BY OXYGENATED PERFUSION AFTER WARM ISCHAEMIA
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Introduction: We have previously shown that oxygenated perfusion at physi- ological temperature resuscitates non-heart-beating-donor (NHBD) livers after warm ischaemic injury. We have examined mitochondrial functional changes during ischaemia-reperfusion in NHBD livers and the relationship of these with hepatocellular injury.

Methods: Porcine livers were retrieved after cardiac arrest and divided into three groups: Group 1 (Control, n=5) no warm ischaemic injury; Group 2 (n= 5) 60 minutes of warm ischaemia; Group 3 (n= 5) 60 minutes of warm is- chaemia followed by in-situ oxygenated perfusion. All livers were then cooled (60 minutes) during the bench work and then connected to an oxygenated reservoir through an oxygenated perfusion circuit for 24 hours, followed by isolated from sequential liver biopsies and analysed for ATP content; mito- chondrial function (respiratory control ratio (RCR)); cytochrome c release and caspase activation. The perfusate was analysed for serum transaminase and base deficit.

Results: Group 1 livers maintained normal mitochondrial function during cold preservation. In subsequent reperfusion, Group 2 livers, cellular ATP levels significantly (p < 0.01), with minimal change in mitochondrial function. However, subsequent cold preserva- tion produced a significant decline in mitochondrial function (RCR 3.5±0.4 vs. 2.2±0.2, p=0.001) with parallel decline in mitochondrial ATP (p=0.001) Mitochondrial injury was associated with increased hepatocellular injury as evidenced by raised transaminase release (p<0.05). In Group 3 livers, in situ oxygenated perfusion improved mitochondrial RCR (p<0.005) and ATP levels significantly (p<0.01) with greater functional recovery and bile production (p<0.05) compared to Group 2 livers.

Conclusions: These data suggest that mitochondria sustain progressive dam- age during sequential warm and cold ischaemia followed by reperfusion, leading to cell death in NHBD livers. In-situ oxygenated perfusion immedi- ately following warm ischaemia confers mitochondrial resilience to ischaemia-reperfusion injury and may have therapeutic benefits in NHBD transplantation.

O-210 RELEASE OF AST AND LFABP FROM ISCHEMICALLY DAMAGED LIVERS DURING MACHINE PERFUSION: A NEW TOOL TO PREDICT VIABILITY AND PRIMARY-NON-FUNCTION
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Introduction: Increasing use of donor livers exposed to Warm Ischemia (WI) demands the development of criteria to assess viability before Transplanta- tion (Tx). In analogy with the kidney, we hypothesized that analysis of specific biomarkers in perfusate of livers during Hypothermic Machine Perfusion (HMP) may provide viability criteria to predict risk of Primary-Non-Function (PNF).

Aim: To determine whether the cumulative release of Aspartate- aminotransferase (AST) and Liver-Fatty-Acid-Binding-Protein (L-FABP) in perfusate of ischemic livers during HMP correlates with liver viability and PNF.

Methods: Porcine livers (n=6/group) were exposed to incremental WI periods (0, 15, 30, 45, 60, 120), procured and HMP preserved. We reported earlier (TX2005) a PNF risk of 0% if WI<15, 25% for WI=30, 45, 100% if WI=60. We hypothesized that: AST and L-FABP release in perfusate was monitored during 240'. In individual livers, AST release could be represented by a logarithmic equation [AST= ln((10^βAST+βAST)*1/60)] during the initial 60' HMP, R^2=0.95-0.99. If PNF was predicted, it could be represented by a linear equation (L-FABP=βFABP+βFABP * time) during the initial 30' HMP, R^2=0.85-0.99. In addition, the different WI groups were clustered according to the aforementioned risk of PNF. We analyzed whether β-constant could discriminate the various WI groups and PNF clusters.

Results: β-constant was different among the 6 WI groups (p=0.0006 for ASTM.018 for L-FABP), and for the 3 functional clusters (p=0.0001 for AST;0.0002 for L-FABP). There was a linear correlation between βFABP and βFABP (R^2=0.61, p=0.0001); βAST and βFABP reflect WI damage and predict the risk of PNF.

Conclusion: β-constants calculated from initial AST or L-FABP release dur- ing HMP are promising clinical tools to predict viability of ischemic livers and subsequent risk of PNF. Based on our observations, Tx of HMP preserved porcine livers with a βAST <0.006 or βFABP < 0.004 is safe.
Hypothermic in situ machine perfusion with UW during deceased cardiac death donation improves early renal function

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In situ hypothermic machine perfusion (HMP) may optimize the quality of deceased cardiac death (DCD) donor organs during donation by restoring circulation after cardiac arrest using extracorporeal membrane oxygenation techniques under hypothermic conditions.

Methods: Conventional organ procurement through hypothermic single flush with the HTK or UW solutions was compared with HMP perfusion applying the Extra Corporal Organ Procurement System (ECOPS) in pig DCD donation (n=5 per group). After 20h cold storage, kidneys were transplanted in recipients followed by 4h blood reperfusion. In the donor, kidney temperature was monitored. After transplantation, urine production in the recipient during reperfusion was measured. Samples of blood, urine, perfusate and biopsies taken for histological evaluation and RT-PCR.

Results: At the end of the DCD donor procedure, mean temperature of the kidney using ECOPS was 15.3±0.6 °C (HTK-E) and 16.3±2.5 °C (UW-E) respectively compared to standard washout 23.8±6.4 °C (UW-C) (p<0.05). Diuresis after transplantation was 29.7±6.4 ml (HTK-E) and 221±41 ml (UW-E) in experimental groups compared to 14±6.9 ml (HTK-C) and 43±7.6 ml (UW-C) in control groups (p<0.05). GFR was highest in the UW-E group (maximum 10 ml/min at t=3hrs) compared to almost absent in HTK-E groups (maximum 0.2±0.2 ml/min). Renal function was restored earlier with use of UW in the ECOPS system as if you were reading it naturally.

Renal resistance (RR) during machine-perfusion (MP) are used to discard kidneys likely to fail post-Tx but threshold RR (above which kidneys are discarded) have been determined empirically.

Aims: We studied the prognostic value of RR on Delayed-Graft-Function (DGF) and Risk of Non-Function (PNF) graft-survival.

Methods: An international/prospective trial (NEJM-2009) including kidney pairs 336 consecutive Heart-Beating (HB) &Non-Heart-Beating (NHB) donors shows that MP leads to less DGF & prolonged graft-survival vs Cold Storage (CS). In this trial, recipient centres were blinded to preservation method (MP/CS) and to MP parameters. Surgeon decision to accept/discard kidneys was solely based on traditional donor data. In MP arm, the RR (mmHg/ml/min-Real Time) on LifePort Kidney-Transporter was recorded at (30/11/19±0.4/Vmp end). Univariate/multivariate analyses were done to determine impact of RR on DGF/PNF graft-survival.

Results: Higher RR at different time-points resulted in higher %DGF (17.3% poorer graft survival vs 4.1% among kidneys with RR<3). RR≥4 was associated with increased Odds Ratio for DGF (OR 2.69; p=0.03 for RR at MP end) independent of donor-type (HB vs NHB), -age, cold-ischemia-time, reTx or first-Tx. Highest RR groups showed higher serum creatinine up to 3 months post-Tx (p<0.001). RR of 10 ml/min at MP end resulted in 17% poorer graft survival vs immediately functioning grafts. Only 7 MP kidneys (2% of total) were discarded after MP/CS based on RR>10 ml/min at MP end.

Conclusion: This study demonstrates (for the first time) the exact prognostic value of RR during MP. RR correlates with DGF/PNF graft survival, not PNF. Many kidneys with elevated RR were probably erroneously discarded in the past. RR is an additional tool to increase the kidney pool. Pre-Rx knowledge of the risk of DGF may help clinicians to select recipients and/or adjust immunosuppression (nephron-sparing protocol in high risk grafts for DGF).

Renal resistance during machine perfusion is a risk factor for delayed graft function and poorer graft survival

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Renal Resistances (RR) during Machine-Perfusion (MP) are used to discard kidneys likely to fail post-Tx but threshold RR (above which kidneys are discarded) have been determined empirically.

Aims: We studied the prognostic value of RR on Delayed-Graft-Function (DGF) and Risk of Non-Function (PNF) graft-survival.

Methods: An international/prospective trial (NEJM-2009) including kidney pairs 336 consecutive Heart-Beating (HB) & Non-Heart-Beating (NHB) donors shows that MP leads to less DGF & prolonged graft-survival vs Cold Storage (CS). In this trial, recipient centres were blinded to preservation method (MP/CS) and to MP parameters. Surgeon decision to accept/discard kidneys was solely based on traditional donor data. In MP arm, the RR (mmHg/ml/min-Real Time) on LifePort Kidney-Transporter was recorded at (30/11/19±0.4/Vmp end). Univariate/multivariate analyses were done to determine impact of RR on DGF/PNF graft-survival.

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Igl-1 solution protects against liver ischemia-reperfusion injury by inhibition of endoplasmic reticulum-stress

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Injury due to cold ischemia-reperfusion (IR) represents a major cause of primary graft non-function following liver transplantation. We postulated that IR-induced cellular damage may cause alterations of the secretory pathway, particularly at the level of endoplasmic reticulum (ER) function. Under these circumstances, the ER triggers an adaptive response named the “unfolded protein response.” Here, we investigate the involvement of ER-stress in organ preservation, comparing cold storage in UW and in IGL-1 solution.

Spirague-Dawley Rats were flushed and preserved in UW solution for 8h at 4°C and then endotoxic liver transplantation was performed according to the Kamada’s cuff technique. In an additional experimental group, the same surgical procedures as described for group UW was carried out, but livers were flushed with a simple saline solution and preserved in IGL-1 solution.

Blood and liver samples were obtained 24h after liver transplantation. Hepatic injury was assessed by determination of AST/ALT. Mitochondrial damage and ATP depletion in IGL-1 and UW livers were carried out to evaluate reperfusion stress when livers were preserved in UW and IGL-1 solution. The following markers of stress were evaluated: GRP78, ATF6, eIF2α and CHOP.

IGL-1 solution reduces liver injury and mitochondrial damage. Thus, at 24h of transplantation transaminases levels and GLODH were reduced significantly when livers were preserved in IGL-1 solution compared with liver preserved in UW solution. Here, we delineate a role for endoplasmic stress during preservation/reperfusion of pre-damaged liver grafts, which is aggravated by the use of UW solution, and IGL-1 solution protect from RE stress, and attenuate significantly the expression of GRP78, CHOP, eIF2α and ATF6 when compared with liver preserved in UW solution. Our results show that IGL-1 solution may be a useful means to circumvent excessive endoplasmic stress reactions associated with liver transplantation.

Analysis of machine perfusion parameters of kidneys procured from expanded criteria donors

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Using expanded criteria donor (ECD) organs is one of the strategies for making more transplants available. Machine perfusion has been proven to offer advantages in kidney storage. Parameters of machine perfusion can be used as a predictors of kidney function after transplantation.

The aim of this study was to analyse the differences in perfusion parameters of kidneys depending on donor criteria.

Patients and methods: One hundred and seventy two patients received cadaveric renal transplants between January 1, 2006 and August 31, 2008. Data on donors, recipients and preservation were collected. 88 kidneys were stored by machine perfusion. Parameters of perfusion such as renal flow, resistance, lactate dehydrogenase and lactates measured in the fourth hour of perfusion were analysed. The ER triggers an adaptive response named the ‘unfolded protein response’. Here, we investigat
Session 26. Impact of donor risk factors on kidney graft survival

Conclusions: Selected perfusion parameters correlate with kidney donor criteria, with expanded criteria kidneys presenting poorer perfusion parameters and, possibly, inferior post-transplant function.

O-215 PREVENTION OF OXIDATIVE STRESS INDUCED ORGAN DAMAGE IN A PORCINE BRAIN DEAD DONOR MODEL.
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Introduction: The "autonomic storm" initiated after brain death induces a cascade of chemokine and cytokine release which causes cell damage and diminishes organ quality. Recently published data on donor pre-treatment showed limited success. This study aimed to evaluate the impact of an antioxidative treatment after brain death induction on organ quality in a pig model.

Methods: Brain death was induced in 16 pigs by trepanation of the skull and increasing intracranial pressure until brain stem herniation occurred. 10 hours after brain death diagnosis, the pigs were randomized in two groups (n=8). Group 1 was infused 500 ml of a solution containing alpha-ketoglutaric acid and 5-MMF over 4 hours whereas group 2 received 500 ml NaCl. Interleukins and markers for oxidative stress were determined using FlowCytomic. 24 hours after brain death multiorgan donation was performed and tissue samples were taken immediately after organ retrieval. Histology and immunohistochemistry as well as PCR analysis were performed.

Results: Markers of oxidative stress as well as the concentration of the interleukins analysed were highest in all animals 8 hours after brain death induction. It was feasible to lower CP and MDA levels as well as chemokine and cytokine concentrations significantly in the experimental group 1. Histology and immunohistochemistry revealed significantly lower apoptotic cells as well as lower anti-nitrotyrosine positive cells in group 1 when compared to group 2 immediately after explanation and after CIT. ATP levels were highest in the control group, but significantly higher in group 1 when compared to group 2 at any time point.

Discussion: We could diminish oxidative stress induced cell damage and prevent the detrimental effects of the "autonomic storm" by applying a solution containing alpha-ketoglutaric acid and therefore achieved better organ quality after multiorgan donation in a pig brain death model.

Session 26. Impact of donor risk factors on kidney graft survival

O-216 THE CUMULATIVE NEGATIVE IMPACT OF DONOR RISK FACTORS ON KIDNEY GRAFT HISTOLOGY AND FUNCTION
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With increasing number of patients waiting for kidney transplantation, there is a worldwide tendency to accept donors with comorbidities and older age. High donor age, hypertension and reduced GFR are known risk factors for post transplant graft function. The aim of our study was to analyse the cumulative influence of these and other donor and donation associated risk factors on donor kidney histology and transplant outcome.

Baseline biopsies of kidneys from 482 deceased donors and donor risk factors were examined. All biopsies were re-evaluated by one pathologist. Graft function and survival of the 635 renal transplantations from these donors were analysed.

The most frequent donor risk factors were cerebrovascular cause of death, smoking age over 50 years, hypertension and unstable hemodynamics after brain death. Less common were resuscitation, arteriosclerosis, ischaemic heart disease and alcohol abuse. We found a significant association between the cumulative number of concurrent risk factors and histological lesions, measured by % glomerulosclerosis, arteriolar hyalinosis, arteriolar hyalinosis, tubular atrophy and the CADI score (Figs 1, 2).

The increase of concurrent risk factors from 0 to 5-8 resulted an increase of DGF rate from 7.4% to 49.3% (Chi-square, p<0.0001) and a reduction of 3 year GFR (Cockroft-Gault) from 92.9 ml/min to 64.6 ml/min (ANOVA, p<0.0001). Transplantations from donors with >4 risk factors (N=67) had significantly decreased graft survival compared to those with ≤4 risk factors;

Conclusion: A thorough perusal of donor medical history can yield valuable information and predict donor kidney histology as well as post transplant graft function and survival.

O-217 ACCEPTABLE LONG-TERM RESULTS WITH KIDNEYS FROM OLD DONORS GIVEN TO OLD RECIPIENTS
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Purpose: To retrospectively assess long-term safety and efficacy of patients that received a cadaveric kidney transplant within the ET Senior program with special regard to recipients 70 years and older.

Methods/Materials: From 5/1999 to 12/2009 a total of 84 patients with a mean age of 67.8 (65-80) years, among them 21 recipients over 70 years received a kidney from a cadaveric donor over 65. The mean number of mismatches in ABO/DR was 2.8±1.4, cold ischemia time 13:41 (05:04 – 24:54) hours. Initial CNI-free immunosuppression consisted of an IL-II-blocker (n=81), Campath or Beclatcept (according to study protocols). Cyclosporine/Tacrolimus was started after stabilization of renal function at day 6.3/4.2. Observation time was 50.4 (1-116) months.

Results: Patient/grant survival of the entire cohort was 97.5%94% at year one, and 83%73% at year five. Mean serum creatinine levels at year 1/5 were 1.61±0.8 mg/dL. In the >70 years old population the patient/grant survival at year one was 89% each, at year five 66%50%. Two rejections occurred, controlled with steroid boluses. The causes of death were cardiac failure (n=4), pneumonia (n=2), sepsis (n=1), cerebrovascular accident (n=1). Six out of them died with a functioning graft. Major complications were cancer in six patients, congestive heart failure (n=3), an adverse reactions requiring pacemaker implantation (n=2), valve replacement (n=1), multiple bone fractures (n=1), perforation of sigmoid colon (n=1). The mean serum creatinine levels at year 1/5 were 1.41±1.8 mg/dL.

Conclusion: Transplantation of kidneys from old cadaveric donors given to old recipients produces excellent short term and acceptable long-term results. Most complications are caused by underlying age-associated comorbidities.
Session 26. Impact of donor risk factors on kidney graft survival

equate treatment of patients with end-stage renal disease. Donation after cardiac death (DCD) has been shown to increase the number of kidneys available for transplantation. However, the long-term outcome of DCD kidney transplantation remains to be established.

Methods: This observational cohort study included all DCD kidney transplantations recovered in our procurement area from 01/01/1981 until 12/31/2005 (N=857). Patients were followed until the earliest of death or 12/31/2006. Clinical outcomes were compared to matched kidney transplantations from brain dead donors (DBD, N=594), using multivariable regression models to adjust for potential confounders.

Results: DCD activity resulted in a 44% increase in the number of deceased donor kidneys from our organ procurement area. After adjustment for potential confounders, the odds of primary non-function and delayed graft function were 69% (95% CI: 3.5-11.8, p=0.001) and 35% (95% CI: 1.2-10.9, p=0.02) greater, respectively, for DCD kidneys compared to DBD kidneys. Recipients of DCD kidneys had a 6.2 mL/min (95% CI: 3.0-9.4, p<0.001) lower glomerular filtration rate at 1 year after transplantation but a similar rate of subsequent decline in kidney function (p=0.87) as recipients of DBD kidneys. The hazard of death-censored graft loss (restricted to viable grafts) and of recipient death were similar for DCD and DBD kidney transplantations (HR=1.22, 95% CI: 0.79-1.86, p=0.37 and HR=1.16, 95% CI: 0.87-1.54, p=0.32, respectively).

Conclusions: The satisfactory long-term prognosis of viable DCD kidneys and their recipients highlight the need for more widespread use of DCD kidneys.

O-221 DECREASED AFTER CARDIAC DEATH DONATION: LONG TERM RESULTS IN A MATCHED SINGLE-CENTER STUDY

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Purpose: The gap between performed kidney transplantations and active organ needs has resulted in alternative ways to increase the donor pool such as deceased after cardiac death (DCD) donation, gaining more importance due to decreasing donor numbers within the last decade. We performed a matched single-center study of kidney grafts from DCD-donors compared to grafts from heart-beating donors over a period of 25 years.

Material and methods: Between January 1984 and December 2008 4177 kidney transplantations have been performed at the transplant center in Vienna. Long-term outcomes were compared to 88 grafts obtained from non-DCD-program. Data were collected prospectively in our database and recipients were matched on a one-to-one basis according to sex, donor age, cold ischemic time (CIT), number and year of transplantation.

Prognostic significance of cold ischemic time (CIT), first warm ischemic time (WIT), delayed graft function (DGF), donor age, HLA-mismatch and acute rejection were calculated by a Cox-model, graft survival being the endpoint.

Results: Despite we noted a significantly higher rate of DGF in the cohort that received their graft from DCD-donors (71.6% versus 35%) long-term outcomes concerning graft and patient survival were similar in both groups.

At 15 years graft survival for recipients from heart-beating donors was 53.6% compared to 53.3% in other group.

Univariate analyses revealed donor age (p=0.03), DGF (p=0.0001) and CIT (p=0.0001) as risk factors, whereas only DGF (p=0.01) showed significance in multivariate analyses.

Conclusions: Despite a high DGF rate, outcomes from kidneys obtained from DCD-donors are similar to grafts from heart-beating donors. Due to a lack of large, matched studies our experience proves that grafts obtained from DCD-donors can be used successfully to increase the donor pool and offer good long-term results.

O-222 DUAL KIDNEY TRANSPLANTATION FROM ELDERLY DONORS: LONG TERM OUTCOME AND HISTOLOGICAL FEATURES

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Introduction: Dual kidney transplantation (DKT) has widened the use of organs from very elderly donors, however one of the major concerns about these grafts is their long term outcome. In this light, we have analyzed the histological changes in our DKT population who underwent a protocol biopsy and we have evaluated the renal function of those who have completed a 5 year follow up.

Patients and methods: Since 2000 to present, 130 DKT have been performed at our center. Mean donor age was 72±6 years. Mean recipient age was 61±5 years. Immunosuppression therapy was based either on CNI + MMF and steroids or PSI + MMF and steroids. 23 patients with T0 donor biopsies
Session 26. Impact of donor risk factors on kidney graft survival

underwent a protocol biopsy (T1) at a mean time of 22±16 months after transplant.

Inherited arteriolar hyalinosis and intimal fibrosis inherited from donor is a risk factor for long-term graft dysfunction

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Background: Older and marginal donors, who often have a vascular cause of death, are increasingly used in order to limit the continuously growing waiting time for organ transplantation. There is evidence that inherited vasculopathy frequently found in these donors' kidneys is a risk factor that determine short and long-term graft outcome. The aim of this study is to evaluate arteriolar hyalinosis and arterial intimal fibrosis as an independent histological factor of long-term graft dysfunction.

Method: All kidney recipients between January 2003 and June 2006, who had a graft biopsy at the time of transplantation, were retrospectively included. In the present study, grafts derived from brain-dead donors show inferior transplant outcome compared to living donors. We hypothesized that during cold ischemia and preservation of the kidney, the complement system is highly activated, contributing to the renal damage. The final endpoints were death censored graft survival and death censored graft survival after 6 months of follow-up in the two groups of transplant recipients.

Results: Median age of donor and recipient was respectively 59.6 (31-82) and 59.0 (31-82) years. Glomerulosclerosis and interstitial fibrosis, but not arteriolar hyalinosis (p>0.05), were significantly correlated with arterial intimal fibrosis at 1 year (p>0.05). The progression of glomerulosclerosis, interstitial fibrosis and arteriolar hyalinosis in the two groups was analysed by Real-time PCR in kidney biopsies obtained at donation, after cold preservation and 45' after reperfusion.

Conclusion: The presence of significant histological worsening, the good graft survival and the satisfactory renal function at 5 years suggest that kidney grafts from elderly donors are compatible with an optimal long-term outcome.

O-226 MEDIUM-TERM FOLLOW-UP OF RENAL TRANSPLANT RECIPIENTS FROM A RANDOMISED CONTROLLED TRIAL OF LAPAROSCOPIC VERSUS OPEN LIVE DONOR NEPHRECTOMY

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Background: Laparoscopic live donor nephrectomy continues to gain in popularity but there are still some concerns that this technique reduces morbidity in the donor at the expense of increased morbidity in the recipient. The aim of this study was to evaluate recipient outcome at a median follow up of 6 years following a randomised controlled trial of laparoscopic versus open incision open donor nephrectomy.

Methods: Eighty-four live kidney donors were randomised in a 2:1 ratio to laparoscopic (LDN n=56) or open incision open donor nephrectomy without rib resection (ODN n=28). The aim of this study was to identify the factors to protect renal function in actual live kidney donors who have undergone live donor nephrectomy at Tokyo Women's University Hospital between the years 2004 and 2005.

Results: The ratio of glomerular sclerosis (n=0.34, p=0.004), systolic blood pressure (n=0.31, p=0.01) and diastolic blood pressure (n=0.28, p=0.02) were positively correlated with donor age in simple regression analysis. Detection of renal function after donor nephrectomy was negatively correlated with BMI (r=-0.31, p=0.009) and positively correlated with severity of arteriosclerosis in interlobular artery (r=0.23, p=0.05). In a multiple regression analysis model respecting severity of arteriosclerosis in interlobular artery the influence of BMI at pre-operation and at 3 months of follow-up, uric acid at pre-operation and several other clinical parameters were assessed as risk factors. The severity of arteriosclerosis in renal arteries from the back table biopsy were semi-quantitatively evaluated and classified into four grades. The grade of glomerular sclerosis of the 0 hr biopsy specimen were also determined. Impairment of the renal function after surgery was expressed by the difference of serum creatinine at pre-operation and at 3 months follow-up.

Conclusions: Preventing progression of arteriosclerosis and selecting the optimal BMI before donor nephrectomy will help avoid the impairment of renal function in live kidney donors who have undergone live donor nephrectomy.
Conclusions: Laparoscopic nephrectomy does not lead to an increase in ureteric complications. Despite subjecting the donated kidney to a prolonged pneumoperitoneum and longer first warm ischaemic time, laparoscopic donor nephrectomy does not compromise long-term recipient renal function.

Session 27. Surgical challenges in liver transplantation

O-227 INTENTIONAL PORTAL PRESSURE CONTROL IS A KEY TO IMPROVE THE OUTCOME OF ADULT LIVING DONOR LIVER TRANSPLANTATION (A-LDLT) WITH SMALLER GRAFTS
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Introduction: Because of small-for-size (SFS) graft idea, larger size grafts, i.e. right lobe grafts, became the standard graft type in A-LDLT. However, graft selection has recently changed in our institute; i.e. smaller grafts can be utilized in a certain conditions.

Methods: A total of 621 A-LDLT were performed since 1998. Large series of portal vein pressure (PVP) control was introduced in 2006, and 138 cases were analyzed. PVP was controlled mainly by splenectomy, and by creating port-systemic shunt additionally if indicated. The optimal PVP is set ≤20mmHg after reflow of graft. Graft size, graft type and patient survival were analyzed with or without PVP control.

Results: Prior to PVP control, only 10.1% (47 out of 464 A-LDLTs) were graft/recipients weight ratio (GRWR) <0.8, with 74.4% and 70.0% survival rate at 1- and 5-years after A-LDLT. In contrast, after 2006 (introduction of PVP control), the selection of SFS grafts increased up to 24.6% (34 out of 138 A-LDLTs), with 84.5% survival rate at 1-year. Although only 4.7% of A-LDLT used left lobe grafts before 2005, 31.2% of A-LDLT utilized left lobe grafts after 2006 with better outcomes. Not only the survival improvement in SFS grafts, but also those in GRWR <0.8% were observed 87.2% vs. 74.8% at 1-year after A-LDLT (after 2006 vs. before 2005).

Retrospectively, we analyzed the patient survival at 15mmHg of final PVP, and PVP <15mmHg demonstrated significantly better 1-year survival than ≥15mmHg (94.4% vs. 71.1%).

Conclusions: PVP control can improve patient survival in SFS grafts as well as in appropriate size grafts in A-LDLT. As PVP control can overcome size mismatching, it may be applied not only in A-LDLT, but also in DDLT or split-liver transplant when the graft size is considered smaller for recipient.

O-228 DUAL GRAFT LIVING DONOR LIVER TRANSPLANTATION WITH RIGHT AND LEFT LIVER GRAFT COMBINATION
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Background: In this study, we reviewed the outcome of DLDLT with combined right and left liver (RL/LL) grafts in the context of donor safety and recipient outcome.

Patients and methods: From 2000 March to 2007 June, 213 cases DLDLT were performed from in our institution. Of them, 37 cases (17.4%) were performed by using combined RL/LL grafts. We analyzed short-term and long-term outcome of RL/LL DLDLT. We compared outcomes including donor morbidity, graft and patient survival and recipient morbidity rate with those of single right lobe LDLTs and DLDLTs with another graft combination.

Results: In 37 cases, modified right lobe graft was used in 33 patients and right lobe (RL) graft in 4 patients. Mean GRWR of RL/LL DLDLT was 1.10% which was slightly higher than those of single RL LDLTs (0.94%) and DLDLT with another graft combination (1.02%), but there was no statistical significance. There was no donor mortality. And morbidity rate of RL donor was 3.5% and one of LL donor was 1.2%. Overall 1- and 3-year survival rate was 97.3% and 96.8% which were comparable to that of single RL LDLT. Of 37 cases RL/LL DLDLT, 3-year graft survival rate (92.3%) of RL graft was slightly lower than that of LL graft (97.8%). Morbidity rate of each graft was similar. And overall morbidity rate of RL/LL DLDLT was not different from single RL LDLT and DDLT with another graft type.

Conclusion: RL/LL DLDLT is feasible option for overcoming small-for-size graft in adult LDLT. In the context of donor safety, it did not increase donor morbidity rate in right liver donor.

O-229 REAPPRAISAL OF EFFICACY AND SAFETY OF SELECTIVE HEMI-PORTOCAVAVAL SHUNT IN ADULT LIVING DONOR LIVER TRANSPLANTATION
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Aim: The purpose of this study is to evaluate the efficacy and safety of hemi-porto-caval shunt (HPCS) as an inflow modulation in small-for-size graft liver transplantation.

Background: We have developed selective HPCS based on portal vein pressure and actual graft volume to overcome small-for-size syndrome. The number of the patients with HPCS reached to double what we reported before and 3-years mean follow-up was achieved.

Patients and methods: From July 2003 to January 2009, twenty patients (mean age 46.0 years old, mean body weight 73.2kg) underwent living donor liver transplantation (LDLT) with HPCS. All patients except one who had fulminant hepatic failure underwent LDLT due to liver cirrhosis (the mean MELD
The use of left livers for transplantation in adult recipients remains controversial and challenging. We present a single center experience of left liver transplantation over a 12 year period.

Patients and methods: From March 1996 to November 2008, 27 adult patients, 13 males and 14 females with a mean age of 49 years (range: 18-67), a mean body weight of 59 kg (range: 40-84) received a left liver lobe transplant from 17 split liver and 10 living donors. Mean graft-to-recipient Ratio (GWR) and mean MELD score were 0.88% (range: 0.57%-1.28%) and 18 (range: 6-32) respectively. Main indications for liver transplantation (LT) were alcoholic (n=10) and viral-related (n=6) cirrhosis. 4 patients died prior to transplantation, 2 patients were re-transplanted and 1 patient had late retransplantation. In 12 patients, a venous splanchic decompression (2 porta-caval shunts and 10 meso-caval shunts with porto-mesenteric disconnection) was performed at the end of the transplant procedure in order to decrease portal pressure to the graft. The 12 patients, a venous splanchic decompression (2 porta-caval shunts and 10 meso-caval shunts with porto-mesenteric disconnection) was performed at the end of the transplant procedure in order to decrease portal pressure to the graft. The 12 patients, a venous splanchic decompression (2 porta-caval shunts and 10 meso-caval shunts with porto-mesenteric disconnection) was performed at the end of the transplant procedure in order to decrease portal pressure to the graft. The 12 patients, a venous splanchic decompression (2 porta-caval shunts and 10 meso-caval shunts with porto-mesenteric disconnection) was performed at the end of the transplant procedure in order to decrease portal pressure to the graft. The 12 patients, a venous splanchic decompression (2 porta-caval shunts and 10 meso-caval shunts with porto-mesenteric disconnection) was performed at the end of the transplant procedure in order to decrease portal pressure to the graft.

Results: After a mean follow-up period of 43 months, 18 (66.6%) out of the 27 patients are alive; 3 patients had early retransplantation for technical complications, small-for-size syndrome and venous portal steal syndrome. Six deaths (22.2%) occurred in the peri-operative period from graft ischemia due to excessive portal compression in the 2 patients with porta-caval shunt, sepsis after re-LT in 2 cases, small-for-size syndrome in 1 case and cardiac failure in 1 case. In univariate analysis, risk factors for early patient death were GWR below 0.8% and portal decompression. When no portal decompression was performed, all 15 recipients survived without need for retransplantation.

Conclusions: When appropriate graft/recipient matching is performed, excellent outcome can be expected in adult patients following left liver lobe transplantation. Portal decompression should be used in very selected cases.

O-232 RECIPIENT OUTCOMES IN DOMINO LIVER TRANSPLANTATION WITH DONOR VENA CAVA PRESERVATION

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Introduction: Domino liver transplantation (LT) is an accepted resource to increase donor pool using whole livers from live donors with Familial amyloid polyneuropathy (FAP). Hepatectomy with venous cava preservation (VCP) is considered a safer procedure and none of the donors developed long-term sequelae. The aim of this paper is to report on our experience in 30 adult recipients with VCP this requires vascular reconstruction of the graft's outflow. The aim of the study is to retrospectively evaluate complications and outcomes of FAP liver recipients with donor VCP.

Materials and methods: From Jan-01 to Dec-08, 30 patients received a LT from a FAP donor with VCP. One patient was excluded due to primary non-function of the graft. Venous outflow reconstruction of the FAP-graft was performed using a venous patch from the deceased donor, by 2 techniques: using suprarepatic VC (n=27) or the iliac bifurcation(n=3), both associated with venoplasty of the three hepatic veins. Progression of ascites postoperatively was defined by production of >500 ml/day for more than 10 days. Patient demographics, ischemia time, vascular complications and outcomes were analyzed.

Results: No clinical or radiological signs of venous outflow obstruction were found in our series with a median follow up of 39.7 months (range 2.6-98.1). The main causes of liver failure were HCV (43.3%) and ETOH (36.7%); HCC was present in 39.3% of the patients. Recipient’s mean age was 62.5 years old with a mean MELD score of 18.4. The mean ischemia time was 8 hours (570 minutes). Of the 6 patients presenting with ascites pre-LT, 2 persisted short term and were treated conservatively. The 1 year patient survival was 96.7% and the one year graft survival was 100%.

Conclusions: Domino LT is associated with good results. Donor VCP doesn’t compromise recipient outcomes despite the increased difficulty related to the vascular reconstruction. In our series, there wasn’t recurrence of FAP in the recipient.

O-233 AUXILIARY LIVER TRANSPLANTATION FOR ACUTE LIVER FAILURE: A REAPPRAISAL

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Background: Auxiliary liver transplantation (AxLT) is an attractive option in patients with acute liver failure since it allows delayed regeneration of the native liver and discontinuation of immunosuppression. However, use of AxLT remains controversial due to technical complexity, increased morbidity and insufficient regeneration of the native liver in some cases. The aim of our study was to assess the role of AxLT based on our experience in 27 patients.

Patients and methods: From 1992 to 2008, AxLT was performed in 27/154 (17%) patients who had emergency liver transplantation. There were 15 females, 12 males; mean age 32 years (range 16-62). Indications for AxLT were paracetamol overdose (3), HBV infection (8), drug-induced (9), mushroom poisoning (2), hepatitis of unknown origin (4) and others (1). Criteria for AxLT were: absence of multi-organ failure, a potential for regeneration and availability of an optimal allograft. 16 patients received a right graft, 3 received a whole graft and 8 received a left graft. We considered 2 periods: before (first) and after (second) 2000.

Results: Mean duration of surgery was 10.9 hours; mean blood units transfused was 5.3. Mean follow up was 110 months. Early post-transplant mortality rate (within 3 months) was 44% in the first period and 22% in the second. Survivors who had complete regeneration and were free of immunosuppression were 4/10 (40%) in the first period and 4/6 (66%) in the second. Among these, 8 auxiliary grafts were transplanted on conventional LT and most patients could be weaned off immunosuppression. Although applicable to a minority of patients, AxLT should be considered in those with a potential for regeneration.
O-234 LIVER TRANSPLANTATION (LT) FROM DONATION AFTER CARDIAC DEATH (DCD) DONORS: MULTICENTRE BELGIAN EXPERIENCE 2003-2007

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Introduction: We retrospectively reviewed the DCD-LT Belgian experience in terms of patient and graft survivals, and of biliary complications.

Patients and methods: From 2003 to 2007, 58 DCD-LT were performed in Belgium, 56 of the 60 donors had donors of age 44 years. Mean donor BMI was 24.5. All DCD procedures were performed in the OR. Mean delay between respiratory withdrawal and cardiac arrest was 14.7 min.

Results: Mean cold ischemia was 451 min. Peak of transaminases was 2,241 U/mL. Global patient survival was 91.3%, 81.2% and 68.1% at 1 month, 1 year and 2 years, respectively. Graft survival was 84.4%, 70.3% and 49.7% at 1 month, 1 year and 2 years, respectively. Causes of early mortality were operative death (n=2), PNF, MOP and ARDS. Late deaths were due to accident (n=2), malignant tumour (n=5) and biliary sepsis. Two patients needed early reLT for PNF and HAT. Six patients needed later reLT for diffuse bile duct lesions. Eleven patients developed biliary stenoses requiring endoscopy and/or surgery. In univariate analysis, significant donor factors for death were delay between respiratory withdrawal and cardiac arrest of more than 15 min, and cold ischemia of more than 6 hours. In the recipient factors, HU status of the recipient was the only significant risk factor for early death. Censoring the graft losses within the first 3 months, the overall rate of symptomatic ischemic bile duct lesions was 38% (19/50).

Conclusion: DCD donors may be a source of viable liver grafts. However actual results are inferior to the results of DBD LT, and prognostic criteria should be evaluated to improve these results.

O-235 NEW NATURAL BANDING METHOD OF PORTO-CAVAL SHUNT BY USING A PARAUMBILICAL VEIN AS SHAPE MEMORY GRAFT IN SMALL-FOR-SIZE GRAFT LIVING DONOR LIVER TRANSPLANTATION

Yoshinobu Sato, Satoshi Yamamoto, Hiroshi Oya, Takashi Kobayashi, Katsuyoshi Hatakeyama. First Dept. of Surgery, Niigata University School of Medicine, Niigata, Japan

In order to obviate a small-for-size graft syndrome (SFSGS), a portacaval (PC) shunt had been considered in a case of adult-to-adult living donor liver trans-plantation (AA-LDLT). However, the permanent PC shunt sometimes revealed the graft atrophy in the late period of LDLT, thereby resulting in liver dysfunction. Therefore, we have already reported the effect of a time-lag ligation of PC shunt after LDLT. But this procedure has a problem of management of long duct lesions. A thick cover of hilar plate around the graft duct preserves the peri-ductal arterial plexus, and prevents their retraction. Bilomas or strictures that required surgical or radiologic intervention and developed within 6 months after transplantation were defined as post-transplant biliary complications. There were 52 males and 16 females with a mean age of 50.6±6.7 years. Hepatocellular carcinoma with B-viral hepatitis was the most common underlying liver disease (31/68; 45.6%). The incidence of complications are shown in Table 1.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Group 1 (n=38)</th>
<th>Group 2 (n=16)</th>
<th>Group 3 (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile leak (Biloma)</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Bile duct stricture</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Both</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15 (39.5%)</td>
<td>3 (18.8%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

There was significant difference in complication rates between group 1 and 3 (p=0.011), however, there was no significant difference between group 2 and 3 (p=0.310). This new technique of ‘Hilar plate looping with glissonian overlapping anastomosis’ (Group 3) technique. ‘Hilar plate looping’ loops the complete hilar plate and glissonian sheath around the hepatic duct after full dissection of the right hepatic artery and portal vein. This thick cover of hilar plate around the graft duct conserves the peri-ductal arterial plexus, and prevents their retraction. Bilomas or strictures that required surgical or radiologic intervention and developed during donor surgery significantly reduces recipient biliary complications in LDLT.

O-236 THE EFFECT OF DIFFERENT TECHNIQUES FORBILE DUCT ANASTOMOSIS ON POST-TRANSPLANT BILIARY COMPLICATION IN LIVING DONOR LIVER TRANSPLANTATION

Jinsub Choi1,2, Soo Jin Kim1,2, Ji Hong Cho1,2, Kyung Ock Jeon1,2, Jong Hoon Lee1,3, Kiil Park1,2, Soon Il Kim1,2, Yu Seun Kim1,2, 1Surgery, Yonsei University College of Medicine, Seoul, Korea; 2The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea; 3Surgery, Kwandong University Myongji Hospital, Goyang, Korea

Biliary complications remain a major cause of morbidity after liver transplantation. And, suboptimal blood supply to the bile duct by technical cause is one of the important pathological of biliary complication. The objective of this study is to verify the effect of different techniques for bile duct anastomosis on post-transplant biliary complication. Among the 121 liver transplants done at our center from August, 2005 to August, 2008, 68 right lobe LDLT recipients were enrolled in this study. Different techniques for biliary anastomosis were done during different periods. The first 38 recipients used ‘Classic dissection with size-matched anastomosis’ (Group 1), the next 16 recipients used ‘Hilar plate looping with glissonian overlapping anastomosis’ (Group 2) technique. ‘Hilar plate looping’ loops the complete hilar plate and glissonian sheath around the hepatic duct after full dissection of the right hepatic artery and portal vein. This thick cover of hilar plate around the graft duct conserves the peri-ductal arterial plexus, and prevents their retraction. Bilomas or strictures that required surgical or radiologic intervention and developed within 6 months after transplantation were defined as post-transplant biliary complications. There were 52 males and 16 females with a mean age of 50.6±6.7 years. Hepatocellular carcinoma with B-viral hepatitis was the most common underlying liver disease (31/68; 45.6%). The incidence of complications are shown in Table 1.

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There was significant difference in complication rates between group 1 and 3 (p=0.011), however, there was no significant difference between group 2 and 3 (p=0.310). This new technique of ‘Hilar plate looping with glissonian overlapping’ during donor surgery significantly reduces recipient biliary complications in LDLT.

O-237 POLYCYSTIC LIVER AND KIDNEY DISEASE: LIVER TRANSPLANTATION ALONE OR COMBINED LIVER KIDNEY TRANSPLANTATION?

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Polycystic liver disease (PLD) is frequently associated with autosomal dominant polycystic kidney disease (ADPKD). Clear indications for combined liver and kidney transplantation (CLKT) are dialysis-dependent status and overt terminal renal failure. If renal insufficiency is less pronounced, the indication for associated kidney transplantation (KT) is controversial. In this study we review our experience with isolated LT and CLKT in patients with PLD.

Methods: Between 1995 and 2008, 37 patients underwent LT for PLD. 3 patients with isolated PLD received LT alone. 34 patients had courtined PLD and ADPKD: 19 underwent isolated LT and 15 CLKT. Among the 15 CLKT patients, 10 were dialysis-dependent at time of transplantation whereas KT was performed preemptively in 5 (creatinine clearance (CrCl) 34.2, 32.1, 38.3, 27.5 and 15.4 mL/min, respectively).

Results: The 1 and 5 year patient and liver graft survival are 95% and 90%, respectively. Of the 19 patients who underwent isolated LT for combined PLD and ADPKD: 3 received a KT 9.9 and 8 years post-LT because of evolving ADPKD and calcineurin inhibitor (CNI) toxicity (pre-LT CrCl 106, 58.6 and 103.9 mL/min, respectively); 2 developed terminal kidney failure and 9.5 years post-LT (pre-LT CrCl 68.4 and 52.4 mL/min, respectively); 1 developed acute renal failure immediately post-LT, requiring permanent dialysis (pre-LT CrCl 47.6 mL/min).

Conclusions: This series (the largest reported so far) demonstrates that short and long term survival after LT and CLKT for PLD is excellent. Terminal kidney
Session 28. New organ donation challenge: from living donor to non heart beating donor

**O-238 SUCCESSFUL EXPANSION OF THE LIVING DONOR POOL BY ALTERNATIVE LIVING DONOR PROGRAMS**

Joke I. Roodnat, Judith A. Kai-van Gestel, Wilij Zuidema, Marijn A.A. van Noord, Jacqueline van de Watering, Jan N.M. IJzermans, Willem Weimar, J. Internal Medicine, ErasmusMC Rotterdam, Rotterdam, Netherlands; 2Surgery, ErasmusMC Rotterdam, Rotterdam, Netherlands

**Introduction:** The development of alternative living donor programs considerably increased the number of renal transplantations in our centre.

**Method:** This retrospective study includes all 786 patients and 1059 potential donors that attended our pre-transplant unit between January 1st 2000 and December 31st 2007, with the request for a living-donor renal transplantation.

**Results:** More than 50% of the potential donors were first degree family members. The recipients brought one potential donor in 77.2% of cases, 2 donors in 15.9%, 3 or more donors in 6.8% of cases. In the regular living donor program a compatible donor was found for 467 recipients. Without considering alternative donation 579 donors would have been refused, 13 donations pending. Alternative living donor kidney transplant programs led to an increase in the number of compatible combinations. The kidney-exchange program (35), ABO-incompatible donation (25), altruistic donation (37) and domino-paired altruistic donation (17) increased the number of compatible combinations with 114 (24.4%). Contrary to the direct donation program, donors in the alternative programs were primarily partners and other non family members (p < 0.001). Eventually for 54.9% (581/1059) of our donors a compatible combination was found. 458 donors were definitely refused, 20 donations pending.

In 26.4% of cases the donor was refused because another donor was more compatible. Donor-recipient incompatibility comprised 19.4% in the final refused population. Without considering alternative donation 38.4% of the refused donors would have been declined on incompatibility. This means that 20% of the whole potential donor population was incompatible with their first choice recipient.

**Conclusion:** The implementation of alternative living donation programs led to a significant increase in the number of transplantations, while transplantations via the direct donation program steadily increased. This success compensates for the fact that almost two donor screenings had to be done for every transplantation. A major increase in the proportion of alternative living donations can be expected.

**O-239 DONOR ETHNICITY AND PARTICIPATION TO (ALTERNATIVE) LIVING KIDNEY DONATION PROGRAMS**

Joke I. Roodnat, Marijn A.A. van Noord, Judith A. Kai-van Gestel, Wilij Zuidema, Jacqueline van de Watering, Jan N.M. IJzermans, Willem Weimar, J. Internal Medicine, ErasmusMC Rotterdam, Rotterdam, Netherlands; 2Surgery, ErasmusMC Rotterdam, Rotterdam, Netherlands

**Introduction:** In Rotterdam 30% of inhabitants are immigrants Immigrants represent 33% of the patients on the waiting-list for transplantation.

**Method:** This retrospective study includes all 1059 potential donors that attended our pre-transplant unit between January 1st 2000 and December 31st 2007, with the request for a living-donor renal transplantation procedure. Ethnicity was classified as: Caucasian, Asian or Turkish. Living donor programs in our center are: Direct, Kidney-exchange, Domino-paired, ABO-incompatible, and Altruistic donation.

**Results:** Predominantly Caucasian donors attended our out-patient clinic (79%). From all 1059 potential donors 581 eventually were coupled to a compatible recipient. In the population of actual donors, the preponderance of Caucasian donors was even more striking (85%). Only 39.4% of non-Caucasian potential donors actually donated, compared to 58.9% of the Caucasian potential donors (p < 0.001). Among non-Caucasian ethnicities, Arabian potential donors were least likely to donate (23.5%) and Asian potential donors were most likely to donate (50.0%). Non-Caucasian donors significantly less often participated in the alternative living donor programs (3.6% respectively 12.6%, p < 0.001). In the donor population Caucasians were 50.6 ± 12.8 years old and non-Caucasians were 41.6 ± 11.3 years old (p < 0.001). In the non-Caucasian donor population first degree relatives predominated, whereas first degree relatives and “no family” were equally represented in the Caucasian donor population (p < 0.001). In comparison to the Caucasian population, partners are under-represented in the non-Caucasian donor population (p < 0.001).

Reasons for donor decline were not different.

**Conclusion:** Non-Caucasian recipients less often attend the pretransplant outpatient clinic with a living potential donor, and these potential donors are less likely to donate. Non-Caucasian couples less often participate in alternative living donor programs. Non-Caucasian donors are primarily represented by first-degree relatives, whereas only half of the Caucasian donors are first degree relatives. Partners are under-represented in the non-Caucasian donor population.

**O-240 APPROACHES FOR LAPAROSCOPIC LIVE DONOR NEPHRECTOMY SHOULD BE SELECTED ACCORDING TO SURGEON’S SKILL AND DONOR’S PHYSICAL STATUS**

Masayoshi Miura, Hiroshi Harada, Norikata Takada, Nobuyuki Fukuzawa, Toshimori Seki, Nashiko Shimoda, Tatsu Tanabe, Kouichi Kanagawa, Katsuya Nonomura, Department of Urology, Hokkaido University Hospital, Sapporo, Japan; 2Department of Urology and Renal Transplantation, Sapporo City General Hospital, Sapporo, Japan; 3Department of Urology, Asahikawa City Hospital, Asahikawa, Japan

**Objectives:** Donor’s safety, quality of life and the graft quality are the primary basis of live donor nephrectomy. Therefore, we have investigated the outcomes of different surgical approaches to find the best method for laparoscopic live donor nephrectomy.

**Methods:** A total of 259 donors who underwent laparoscopic live donor nephrectomy were included in this retrospective study. Three approach have been performed: transperitoneal hand-assisted (HALDN, n=131), retroperitoneal hand-assisted (HARPDN, n=24). Selection of the approach was based on the history of abdominal surgery, patient’s choice or surgeon’s preference. The followings were compared: age, gender, body mass index (BMI), laterality, operation time (OT), blood loss (BL), warm ischemic time (WIT), the number of analgesics use (NA), early graft function measured with radiocisotope renal scan on day 1 (the peak uptake time, the 20 min to peak uptake ratio, effective renal plasma flow, and donor complication. Comparison between OT and the thickness of perirenal fat (TPNF) measured with CT scan was studied. The difference in the above outcomes among 5 surgeons was also compared.

**Results:** There was no difference in age, gender, BMI, laterality or BL. OT and WIT were significantly longer in RPLDN than HARPDN in HALDN. NA was significantly larger in HALDN than in the other approaches. Early graft function was equivalent in all the approaches. The frequency of minor or major
complications and open conversion were significantly larger in HARPDN than in the others. OT, WIT, and the complication rates were significantly different depending on the surgeon. OT significantly correlated with TPNF in RPLDN but not in HALDN.

Conclusions: RPLDN is the first choice of approach based on less pain. However, HALDN should be chosen depending on surgeon's skill and TPNF.

O-241 COMPLICATION RATES IN 1019 CONSECUTIVE LIVING DONOR NEPHRECTOMIES
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1 Department of Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway; 2 Department of Surgery, Oslo University Hospital Rikshospitalet, Oslo, Norway

Purpose: Living kidney donor nephrectomy (LDN) has been performed at our hospital since 1960. We wanted to assess early postoperative complication rates during the last decade.

Materials/Methods: We retrieved the files from live kidney donors in the period 1997-2008. Complications related to the donation were identified, registered as a total of 169 new combinations with the negative grading system for surgical complications (grade ≥3 = major complication).

Results: During the time period there were a total of 1023 LDN performed at our hospital. We were able to collect data on 701 of the donors (593 females). Mean age was 47.7 years (SD 11.8) and mean BMI was 25.4 (SD 3.7). There was no peri- or postoperative mortality. A total of 30 major (2.9%) and 181 (18.1%) minor complications were registered. In 329 (32.3%) donors the right kidney was removed. Kidney vessel anomalies were present in 236 (23.4%) donors. Laparoscopic nephrectomy was introduced in 1998 and 244 (23.9%) nephrectomies were done laparoscopically. Three of these needed urgent conversion. There was a higher frequency of major complications in the laparoscopic group (4.1% vs. 2.6%), but the difference was not statistically significant. There have been fewer major complications during the last years of laparoscopic nephrectomy indicating a “learning curve” for the procedure. In the postoperative period 17 donors underwent re-operation. Wound infection developed in 38 (3.7%) donors. Significant peri- and postoperative bleeding occurred in 16 (1.6%) patients, eight (0.8%) of these received blood transfusions. There were seven cases of renal artery lesion.

Conclusion: The risk of major complications after LDN is low (2.9%), but might represent a potential hazard to the donor. A vigilant surveillance of peri- and postoperative care is mandatory in living donor nephrectomies.

O-242 5-YEARS EVALUATION OF THE NATIONAL LIVING DONOR KIDNEY EXCHANGE PROGRAM IN THE NETHERLANDS
Marry de Klerk 1, 2, Marian D. Witvliet 2, Bernadette J.M. Haase-Kromwijk 2, Frans H.J. Claas 1, Willem Weimar 1, 2, 3
1 Dept. of Internal Medicine–Transplantation, Erasmus MC, Rotterdam, Netherlands; 2 Dept. of Surgery, Erasmus MC, Rotterdam, Netherlands; 3 Dept. of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands

Background: Living donor kidney exchange has become an efficient solution for recipients with a blood type or cross-match incompatible donor. However, no information is available on the practical probables inherent to these programs. Here we describe our 5 years experiences with 312 couples enrolled from the seven transplant centers.

Methods: Our protocol consists of five steps: registration, computerized matching, cross matching, donor acceptation, and transplantation. We prospectively collected data of each step of the procedure.

Results: Out of the 312 registered pairs we created 194 computer-matched combinations. However, 72/194 recipients proved to have a positive cross match with their new donor, which was not predicted by the screening results of the recipient centers. Alternative solutions were found for 47 couples, resulting in a total of 169 new combinations with the negative cross matches. Thereafter, due to 24 individual clinical problems, the exchange procedure had to be discontinued for 59 couples while only 21 of them alternative solutions were found. Since 10 of the day 131 patients (42%) had received exchange kidneys, 75 (24%) were transplanted outside the program, 67 (21%) are still on the crossover waitlist and 39 (13%) had left the program for medical or psychological reasons.

Conclusion: A living donor kidney exchange program is a dynamic process. Many clinical hurdles and barriers are encountered that for a large part were not foreseen but should be taken into account when programs are initiated based on computer simulations. Success is dependent on a flexible organization able to create alternative solutions when problems arise. Centralized allocation- and cross match procedures are instrumental in this respect.
**O-245** VERY LONG-TERM DATA ON LIVING KIDNEY DONORS: A SINGLE CENTER EXPERIENCE SINCE 1959

Catherine Fournier 1, Henri Kreis 1, Sylvie Pucheu 2, Eric Thervet 1, Frank Martinez 1, Marie-France Marmer 1, Renaud Sanouj 1, Lynda Berehi 1, Arnaud Mepam 1, Christophe Legrand 1, 1Service de Transplantation Adulée, Université Paris Descartes, Hôpital Necker, Paris, France; 2Service de Psychiatrie, Université Paris Descartes, Hôpital Georges Pompidou, Paris, France; 3Service d’Urologie, Université Paris Descartes, Hôpital Necker, Paris, France.

**Background:** Very long-term data on living kidney donors are important with regard to safety reasons. The aim of this study was to survey our entire experience with living kidney donors since its inception in 1959.

**Methods:** We retrospectively looked at all kidney donors from June 1959 until December 2007. Whenever these donors were living and located, we called them to ask if they were willing to fill a questionnaire and to perform serum creatinine and albuminuria dosage.

**Results:** Out of 397 living kidney donors, we were able to get informations in 297 cases (75%); 42 were dead of whom one went on hemodialysis for 2 years and 255 were still alive. A questionnaire was sent: 5 refused to fill it and so far 177 answered. Mean age was 56 years (21-86). Mean current serum creatinine was 98 mol/l (53-153) and mean eGFR was 69 ml/min/1.73m² (22-125). Mean proteinuria was 0.06g/l (0-1). Two patients were on hemodialysis. Nearly all living kidney donors never regret their donation.

We focused on 68 individuals who gave a kidney more than 30 years (mean 39 years). Mean current age was 72 years (57-77). Diabetes mellitus was absent in 86% of cases, present in 7% and unknown in 7%. Dyslipidemia was absent in 62% of cases, present in 27% and unknown in 11%. Hypertension was absent in 65% and present in 35%. Mean serum creatinine was 94 mol/l, mean eGFR was 63ml/min and mean proteinuria was 0.07g/l.

**Conclusion:** These data coming from one of the longest experience in the world bring important data regarding long term safety of living kidney donation. The prevalence of hypertension was not different from an age-matched population in our country nor was the incidence of end stage renal failure.

**O-246** HOW FRANCE LAUNCHED ITS NHBD PROGRAM

Corinne Antoine 1, Lionel Badet 1, Laurent Jacob 1, Benoit Barrou 1, Frederic Brun 1, Emilie Savoye 1, Gaelle Cheissen 1, Frederic Andres 1, Bernard Loty 1, National Steering Committee Non Heart Beating Donors 1, 1Direction Médicale et Scientifique, Agence de la Biomédecine, Saint Denis, France; 2Service d’Urologie, Hôpital Edouard Herriot, Lyon, France; 3Service d’Anesthésie Réanimation, Hôpital Saint Louis, Paris, France; 4Service d’Urologie, Hôpital La Pitié Salpêtrière, Paris, France; 5Service d’Anesthésie Réanimation, Hôpital Kremil Binchet, Kremil Binchet, France; 6SAU, Hôpital Avicenne, Bobigny, France.

Non-heart-beating donor (NHBD) renal transplants have been introduced into clinical practice in France since 2006. After change of the French law, NHBD program was founded in an original and multicentric way with a national medical protocol. Only uncontrolled donors with normal anamnestic period <30 months and total normal ischemia time <150 minutes were considered. In situ kidney perfusion must be realized by a double-balloon catheter and in situ cooling was used, present in 7% and unknown in 7%. Delayed normothermic circulation; kidneys must be retrieved in less than 180 min. All kidneys must be machine-perfused using the continuous-hypothermic pulsatile preservation system before transplantation. Methods used to assess the organ viability included perfusion parameters and morphologic assessment.

After 2 years of activity for 9 first pilot sites, first results show no in-hospital donor recruitment. Majority of donors were men (90%) with a mean age of 41 years and 70% belonged to the Maastricht class I. Organ’s retrieval was done in 86 out of the 200 listed donors, procuring 98 kidneys which were grafted to 96 recipients and 73 harvested kidneys have been discarded because of morphological aspect, viability tests or positive serology. We observed a frequent protocol’s failure (nearly 50%), because of too short delay, difficult situation at initial medical evaluation, and procedure simple relative’s opposition. Concerning recipients, they were mainly long waiting patients with a cold ischemia mean time around 14 hours. The overall graft survival was 80% with 90% of delayed graft function. NHBD kidneys are a valuable additional source of organs for transplantation. An improvement of organ quality perfusion after the warm ischemia period and a decrease of the DGF rate could be obtained with a preferential use of regional normothermic circulation as perfusion modality for this type of donor.

**O-247** PREDICTION OF DEATH IN POTENTIAL CONTROLLED NON-HEARTBEATING DONORS: PRELIMINARY RESULTS OF A PROSPECTIVE STUDY

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**Introduction:** In controlled non-heartbeating donors (NHBD), liver and lung donation is possible if the donor dies within 60 minutes after withdrawal of treatment. Kidney donation is not possible after 120 minutes. Donor efforts in this group are associated with unnecessary cost and are disappointing for the relatives and involved health care personnel. The objective of this study is to identify patients who do not die within 60 and 120 minutes.

**Method:** This is a prospective cohort study of potential controlled NHBD in the Netherlands from April 2007 until October 2008. Patient and treatment characteristics were analysed as potential risk factors for time of death. Also the prediction of the intensivist was registered. Univariate and multivariate logistic regression techniques were used.

**Results:** 142 potential donors were studied of whom 74% died within 60 minutes, 7% between 60 and 120 minutes and 19% thereafter. In the univariate analysis, controlled ventilation, use of norepinephrine, absence of reflexes, cardiac co-morbidity and a neurological diagnosis other than post-anoxic encephalopathy were associated with death within 60 minutes (P<0.05). Exclusion and use of sedation were not associated with early death. In the multivariate analysis, controlled ventilation (OR 4.4, 95% CI: 2.0-10, P<0.001) and a neurological diagnosis other than post-anoxic encephalopathy (OR 2.9, 95% CI:1:4-6.0, P=0.007) remained independent risk factors for early death. The prediction of the intensivist was not accurate.

**Conclusion:** The vast majority of potential controlled NHBD die within one hour after withdrawal of treatment. Controlled ventilation and a neurological diagnosis other than post-anoxic encephalopathy were independent risk factors for early death. However, it was not possible to identify potential donors with a very low likelihood of early death in whom donation efforts are futile.

**O-248** TRENDS IN ORGAN DONATION AND TRANSPLANTATION IN RUSSIA. ANALYSIS OF 2006-2008 NATIONAL REGISTRY DATA

Sergey V. Gaule 1, Yan G. Moysyuk 1, Marina G. Minina 1, Oleg N. Reznik 2, 1Clinical Organ Transplantation, Shumakov State Scientific Center of Transplantology and Artificial Organs, Moscow, Russian Federation; 2Moscow Coordinating Center of Organ Donation, Moscow, Russian Federation; 3Transplantation Department, Danyelize State Research Institute for Emergency, S.Petersburg, Russian Federation.

**Background:** Prior to 2006, organ donation and transplantation activity in the country (142.0 million inhabitants) has remained at the critically low level (kidney transplantation rate did not exceed 3.0 pmp). Due to the reasons of organizational, legal, economic, educational character positive trends have been observed from 2006. For the first time there’s the data of the national registry provided by 34 kidney transplant (tx) centers, 6 liver tx centers, 4 heart tx centers, 2 pancreas tx centers.

**Results:** Results are presented in the table.

<table>
<thead>
<tr>
<th>Organ transplants (tx)</th>
<th>2006</th>
<th>2007</th>
<th>Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney tx (pmp)</td>
<td>556</td>
<td>664</td>
<td>40.7</td>
</tr>
<tr>
<td>Living kidney tx %</td>
<td>25.0</td>
<td>20.9</td>
<td>18.5</td>
</tr>
<tr>
<td>Living liver tx %</td>
<td>88</td>
<td>117</td>
<td>42.0</td>
</tr>
<tr>
<td>Heart tx %</td>
<td>51.1</td>
<td>41.0</td>
<td>37.6</td>
</tr>
<tr>
<td>Living pancreas tx %</td>
<td>38.8</td>
<td>38.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Total tx</td>
<td>669</td>
<td>942</td>
<td>40.8</td>
</tr>
</tbody>
</table>

Total number of kidney transplants has increased almost 1.5 times, primarily through deceased donor organs, as a result of the increasing activity of local donation programs in some large regions (Moscow, St. Petersburg, Ekaterinburg, Novosibirsk). Practical application of transplant coordination and acceptance of brain death criteria is reflected in the increasing value of extrarenal transplantations performed in a few transplant centers. Up to now the problem of donor organ shortage is solved by using of living donor kidneys (18 centers) and liver (2 centers). During this period 19 kidney tx centers (55%) have increased their activity. 15 kidney tx centers perform more than 20 operation per year and only 6 kidney tx centers – more than 50 ones. Shumakov Institute is the largest center: in 2008 there were 164 solid organ transplantsations (106 kidneys; 43 livers incl.8 split tx; 15 hearts).

**Conclusion:** Up to now despite the increasing number of transplant operations population provision is still very unsatisfactory. Unrealized potential of-
ceased donor organ creates good preconditions for the significant growth of solid organ transplants based on development of regional and national transplant coordination system. The main national challenge is to extend the donor hospital number and turn its staff attitude to organ donation.

Session 29. Pharmacology & immunological monitoring

O-249 IMPACT OF STEROID WITHDRAWAL ON THE IMMUNE RESPONSE OF RENAL TRANSPLANT PATIENTS

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Superior kidney graft outcome has been described after steroid withdrawal in a large prospective study within the Collaborative Transplant Study. To analyze effects of steroid withdrawal on clinically relevant immune parameters, we assessed CD4 helper activity, SCID30, immunoglobulin-secreting cell formation, neopterin and intracellular cytokine production in a prospective randomized study of 84 renal transplant recipients (CsA/Aza, CsA/MMF, Tacr/Aza; steroid tapering >6 months posttransplant) at 2 years posttransplant.

Two-year graft function was better in patients off steroids (creatinine clearance: 62±7 versus 47±4 ml/min, p=0.03). Lower steroid dosage was significantly related to lower serum lipid levels, systolic and diastolic blood pressure (p=0.002).

Multivariate logistic regression showed that steroid-free therapy was independently associated with enhanced T cell proliferative capacity (p=0.004) and CD4 cell IL-4 responses (p=0.07, p=0.03, univariate), which was previously shown to predict a low risk of acute rejection. Enhanced CD4 cell IL-2 production on steroid-free treatment (p=0.02, univariate) could not be confirmed in the multivariate analysis. Logistic regression, however, confirmed a downregulated IL-2R (CD25: p=0.02, univariate; p=0.01; logistic regression) and CD40 expression on B cells (p=0.03, univariate and logistic regression) in steroid-free patients. Interestingly, patients on steroid treatment exhibited even higher CD25 expression on CD4 cells than healthy controls (p=0.02). MMF compared to Aza showed only a minor effect on B cell CD25 downregulation (p=0.05; logistic regression). Steroid-free treatment had no impact on monocyte activation, CD4 helper activity, SCID30 and immunoglobulin-secreting cell formation.

Our data show that steroid-free maintenance immunosuppression enhances T cell proliferation but provides graft protective immunologic effects via enhanced CD4 cell IL-2 production and suppression of CD25 and CD40 expression. Upregulation of IL-2R and the B cell costimulatory pathway by steroids might result in B cell responses against the graft during periods of infection-induced IL-2 release.

O-250 THE ASSOCIATION OF EARLY SUBTHERAPEUTIC MPA EXPOSURE (<30 mg*h/L) AND ACUTE REJECTION: A COHORT ANALYSIS OF THE CLEAR STUDY

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Adequate early mycophenolic acid (MPA) exposure may be associated with a decreased rate of acute rejection (AR). A greater proportion of patients may achieve higher MPA levels with the use of a post-transplant mycophenolate model (MMF, CellCept®) loading dose.

Purpose: This cohort analysis of a randomized trial examines the efficacy and safety of a 5-day 3-g MMF loading dose to increase the proportion of renal transplant patients exceeding the MPA therapeutic level of 30 mg*h/L by Day 5 versus standard post-transplant 2-g daily dosing.

Methods: The loading-dose arm (n=68) received MMF 1.5 g BID days 1–5, then 1.0 g BID. The standard-dose arm (n=67) received MMF 1.0 g BID.

In patients with MPA AUC <30 mg*h/L at Day 5, 50.0% (8/16) had suspected and treated AR vs. 15.5% (13/84) in patients with MPA AUC ≥30 mg*h/L at Day 5 (p=0.0047). Higher MPA AUC at Day 5 was significantly associated with an increased incidence of AR in the first 6 months post renal transplant.

Session 29. Pharmacology & immunological monitoring

O-251 PHARMACOMETRICS OF VOCLOSPORIN IN A PHASE 2B RENAL TRANSPLANT TRIAL

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Purpose: Voclosporin (VCS) is a next generation calcineurin inhibitor (CNI) being developed for solid organ transplantation. Established therapeutic drug monitoring of CNI-based immunosuppression is based on trough concentration ranges that may not adequately determine clinical outcome. However, VCS has been developed using a pharmacometric approach which balances VCS concentration (PK), calcineurin inhibition (PD) and defined clinical outcomes—graft rejection (BPAR) and new onset diabetess (NODAT)—to determine an ideal therapeutic window.

Results: PROMISE was a 12 month, randomized, concentration-controlled study in de novo renal transplant patients comparing three oral voclosporin dosing groups (low, mid, and high dose) to tacrolimus. A total of 334 patients were enrolled in the Phase 2B study of which 248 were randomized to the VCS arms. VCS trough concentrations and calcineurin activity (CNA) were determined using an LC/MS based assay.

Results: PK/PD modelling of BPAR and NODAT versus trough concentration (C0) of VCS predicted an optimal C0 range of between 32-60 ng/mL.

In addition, Cox regression analyses of calcineurin activity (CNA) and BPAR suggested patients are 1.7 times more likely to reject if CNA was above 1.3 pmol/min/mg.

Figure 1. VCS C0 vs incidence of rejection & diabetes.
CYP3A5 and ABCB1 POLYMORPHISMS INFLUENCE TACROLIMUS CONCENTRATIONS IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN RENAL TRANSPLANT PATIENTS

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Purpose: Peripheral blood mononuclear cells population (PBMCs) is expected to be a more specific biological matrix than whole blood in order to reflect the pharmacological efficacy of tacrolimus (Tac). The lymphocyte expression of P-glycoprotein (P-gp), an efflux transporter encoded by the ABCB1 gene, might influence Tac intracellular concentration and therefore its immunosuppressive activity. This study investigated the effect of genetic polymorphisms in CYP3A5 and ABCB1 genes on Tac blood and intracellular concentrations seven days after renal transplantation.

Methods: 96 renal recipients were genotyped for three different ABCB1 gene polymorphisms (1199G→A, 3435C→T, and 2677T/A) and the CYP3A5*3 gene polymorphism. Trough blood and PBMCs Tac concentrations were monitored by immunoassay and LC-MS/MS respectively, and compared according to recipient genotypes.

Results: Dose-adjusted Tac PBMCs concentrations correlated significantly with dose-adjusted Tac blood concentrations (r²=0.6138, P=0.0057). The ABCB1 1199A carriers presented a 1.4 fold increased Tac PBMCs levels (P=0.0014). The ABCB1 3435T and 2677T/A carriers were both associated to a 1.3 fold increased Tac PBMCs levels (P=0.0089 and P=0.0122 for 3435T and 2677T/A carriers, respectively). Dose-adjusted Tac PBMCs levels were significantly lower in patients expressing CYP3A5*3 compared with patients who did not (CYP3A5*3/3, P=0.0021). Tac dose requirement, based on blood TDM, and dose-adjusted Tac blood levels were both lower in CYP3A5*1 carriers (P=0.0005). The impact of ABCB1 genetic polymorphisms on Tac blood concentrations was negligible.

Conclusions: Our results confirm the impact of CYP3A5 polymorphism on Tac blood pharmacokinetics parameters. This study reports for the first time the influence of and ABCB1 polymorphisms on Tac intracellular concentrations. As Tac PBMCs concentrations could be a better marker of Tac efficacy than whole blood, it might be interesting to genotype recipients for ABCB1 in order to better individualize the Tac immunosuppressive therapy in renal transplantation.

O-254 AN INTENSIFIED DOSING OF ENTERIC-COATED MYCOPHENOLATE SODIUM IN RENAL TRANSPLANT PATIENTS RESULTS IN IMPROVED EFFICACY WITHOUT COMPROMISING SAFETY

O-254

Conclusion: Prospective adaptation of Tac daily dose according to CYP3A5 polymorphisms is associated with a higher proportion of patients reaching the targeted C0 and a numerical better renal function at M1. Longer follow-up is being analyzed.
Methods: MPA plasma concentrations at baseline (C0h), 30 minutes (C0.5h), 1 (C1h) and 2 hours (C2h) were obtained by high-performance liquid chromatography (HPLC) in 22 patients treated with pantoprazole 40mg and MPA 200mg. Measurable flow cytometry, we observed a dose-dependent increase in the level of phosphorylated(P)-STAT5 in the CD4+CD25hiFoxP3+ and CD4+CD25intFoxP3-Tcells. At 2000 U/mL, median P-STAT5 levels were more increased in CD4+CD25hiFoxP3+ T-cells (3 to 70%) than in CD4+CD25intFoxP3-Tcells (1 to 43%), p=0.02. In the presence of a clinically relevant CP-690,550 dose of 200 ng/mL, the IL-2-induced P-STAT5 was partially inhibited in CD4+CD25hiFoxP3+ T-cells, while almost completely blocked in the CD25int-T cells (decreased by 63% vs. 90%, median, p=0.02). Analysis showed a higher IC50 CP-690,550 level for the CD4+CD25hiFoxP3+ T-cells (136 ng/mL) compared to the CD4+CD25intFoxP3-Tcells (58 ng/mL), p=0.05. In the presence of CP-690,550, co-culture of CD25int-Tcells with CD4+CD25hiFoxP3+Tcells at a 10:1 ratio inhibited the proliferative response by 48% (median, 39-51%), which was comparable to 54% (44-64%) in the absence of CP-690,550. Thus, CP-690,550 inhibits effector T-cell function but spares the suppressive activity of CD4+CD25hiFoxP3-Tcells. JAK/STAT inhibition provides a novel mechanism for modulation of anti-donor responses in transplant-patients.

O-258 IMPACT OF BASILIXIMAB THERAPY ON REGULATORY T-CELLS EARLY AFTER KIDNEY TRANSPLANTATION: CD25 DOWN-REGULATION BY RECEPTOR MODULATION

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Purpose: Basiliximab is a monoclonal anti-CD25 antibody successfully used to prevent acute graft rejection after organ transplantation (Tx). Its application might not only affect activated/effector T-cells, but also regulatory T-cells (Tregs) of the CD4+CD25CD127lowFoxP3+ phenotype. We investigated the influence of Basiliximab on the frequency of peripheral Treg cells in patients early after kidney Tx.

Methods/Materials: Blood from Basiliximab-treated patients (n = 13; injection on days 0 and 4) and from a non-Basiliximab group (n = 6) was collected preoperatively and at defined points within 3 months after Tx. All patients received initial triple immunosuppression consisting of a calcineurin inhibitor, mycophenolate mofetil and steroids. The frequency of Tregs was determined by multi-color flow cytometry using monoclonal antibodies (mAb) to CD4, CD25, CD127, FoxP3 and P-STAT5.

Results: Transplantation of patients with Basiliximab resulted in a decrease in the percentage of CD4+CD25hiFoxP3+ Tregs, which lasted for about three months. This decrease was accompanied by a rise in CD4+CD25hiFoxP3+ Tcells expressing the CD127dim phenotype. The frequency of CD4+FoxP3+ T cells remained stable suggesting that the drop of CD4+CD25hiFoxP3+ Treg may result from blocking of anti-CD25 mAb by Basiliximab or down-regulation of CD25 molecules rather than from elimination of the cells. In vitro, pre-incubation of CD4+CD25hiFoxP3+ cells with Basiliximab did not inhibit staining by anti-CD25 mAb. However, when CD4+CD25hiFoxP3+ Tcells were cultured at 37°C in the presence of Basiliximab, down-regulation of CD25 occurred within 48h, thus indicating receptor modulation.

Conclusion: Basiliximab therapy has a direct effect on CD4+CD25hiFoxP3+ Treg. Although the functional consequences of CD25 internalization and/or shedding are not known, these observations raise questions about the use of Basiliximab in tolerance promoting protocols.

O-259 PREFERENTIAL INCREASE IN MEMORY AND REGULATORY T-CELLS DURING CD4+ T-CELL IMMUNE RECONSTITUTION AFTER THYMoglobulin induction Therapy in Renal Transplant Patients Receiving sirolimus vs Cyclosporine

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Thymoglobulin induction with sirolimus maintenance therapy is effective but no study has compared immune reconstitution with sirolimus vs calcineurin inhibitors.

In RTx treated with sotrastaurin (200mg bid) combined with standard- or reduced-exposure of tacrolimus, T-cell activation and proliferation were inhibited post-transplantation by 95% (p<0.001) and 73% (p<0.001), respectively, compared to pre-transplantation. Inhibition was similar in combination of sotrastaurin with tacrolimus standard- or reduced-exposure.

Conclusion: We developed a T-cell function assay which quantifies the effects of sotrastaurin in combination with tacrolimus. Future studies in larger cohorts are needed to show if this assay might help to tailor the sotrastaurin dose for individual transplant recipients to optimize its efficacy and safety.
Session 30. New approaches to diagnostic prediction

**Methods:** In a 12-month, randomized, open-label, single-center pilot study, peripheral lymphocyte reconstitution was compared in de novo kidney transplant patients receiving sirolimus (n=9) or CsA (n=10). All patients received Thymoglobulin (2.5mg/kg/day for 1 day, 1.25mg/kg/day for 3 days), MMF and corticosteroids. Lymphocyte count was recorded at day 0, during days 1-14, and at months 1, 2, 3, 6 and 12. Cell counts were compared between treatment groups using a Fishers test on a compacted data set, allowing a single comparison across all post-baseline timepoints.

**Results:** Total lymphocytes were profoundly depleted in both groups. Reconstitution was greater in the CsA arm vs sirolimus (p=0.004). CD4+ T-cell count recovery in the CsA cohort was also higher (p=0.025). At baseline, naive T-lymphocytes (CD4+ CCR7+ CD45RA-1) were more numerous in the sirolimus cohort vs the CsA arm (p=0.028) but became less numerous vs CsA after Thymoglobulin therapy (p=0.019). In contrast, memory cells (CD4+ CD45RO+) were less frequent in the sirolimus group vs the CsA arm at baseline (p=0.006) but were more frequent after Thymoglobulin (p=0.05). The number of regulatory T cells (CD4+ CD25 High) similar at baseline in the two groups, was significantly increased after Thymoglobulin in the sirolimus cohort vs the CsA arm.

**Conclusion:** The pattern of homeostatic reconstitution after Thymoglobulin induction differs between sirolimus and CsA, with a disproportionately high recovery of memory and regulatory T-cell subsets on sirolimus. These data suggest that the beneficial effect of sirolimus that favours T-regulatory cells during immune reconstitution could be counterbalanced by a parallel increase of memory subsets, more resistant to immune regulation.

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**O-261 MICA ANTIBODIES ASSOCIATE WITH BIOPSY-PROVEN CELLULAR REJECTION IN RENAL TRANSPLANT RECIPIENTS**

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**Introduction:** The human MHC class I chain-related genes, MICA and MICB, encode stress-related molecules recognised by NK cells. MIC molecules are also expressed in renal transplants and induce antibody (Ab) responses. We aimed to predict for donors and methods: We performed retrospective analysis of various renal transplant groups. In the post-transplant sera of 299 renal transplant patients, using high resolution Luminox-based screening and single antigen commercial kits (LABScreen). We performed high resolution-based MICA typing was also performed on 223 donor and recipient pairs.

**Results:** MICA Abs were detected in 62/299 (20.7%) patients. Post-transplant biopsies were performed on all patients and the incidence of cellular rejection was significantly increased in MICA Ab+ve patients (18/62, 29.0%) vs MICA Ab -ve patients (40/237, 16.9%) (Chi Square = 4.64, P = 0.03, OR = 2.0). Of those patients with MICA Abs and biopsy proven cellular rejection 12/18 (66.6%) did not have detectable anti-HLA class I or II Abs. The production of detectable MICA Abs could also be attributed to certain mismatched amino-acid residues in the 2nd and 3rd extra-cellular MICA protein domains.

**Conclusions:** The production of MICA Abs in renal transplant recipients acts as a correlate of cellular rejection and donor-recipient mismatching for specific MICA epitopes may affect graft outcome.

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**O-262 HLA-DP ANTIBODY FORMATION BEFORE AND AFTER RENAL TRANSPLANTATION**

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HLA-DP antibodies are expressed on renal endothelial cells and supposed to present a target for humoral immune response in kidney transplantation, because they are usually described after failure of a transplant. Until the Luminex Single Antigen assay became available, anti-HLA-DP antibodies have been difficult to detect. This study analyses frequency, specificity and time of occurrence of DP ab by LSA.

410 transplant patients were tested during transplant screening for presence of HLA-DP ab. The pattern of ab specificities was correlated to particular motifs of the hyper variable regions (HVR A-F) in exon 2 of the HLA-DP1 gene, which are shared between groups of DPB1 alleles. HLA-DP ab were demonstrated in 48 (12%) patients: 30 were recipients of a first, 7 of a second graft. 11 showed a very low positive reaction in the pre-typing protein conformation of HLA class-I alleles using the Modeller program. The electrostatic charge on the molecular surface of each molecule was calculated using the DelPhi algorithm. HLA-DP specificities evaluated, with a close correlation between increasing number of aa polymorphisms and the number of positive reactions in the antibody screening.

We correlated the frequency and specificity of probable donors of HLA-DP Ab with the HLA-DPB1-specific antibody levels by Luminex Single Antigen assay.

**Conclusions:** The determination of MICA Abs in renal transplant recipients acts as a correlate of cellular rejection and donor-recipient mismatching for specific MICA epitopes may affect graft outcome.

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**O-263 EFFICACY OF AN ACCEPTABLE MISMATCH PROGRAM (AM) FOR THE ACCESS TO RENAL TRANSPLANTATION OF HIGHLY SENSITIZED PATIENTS: THE FRENCH EXPERIENCE**

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The AM program started in France on 04/2005, adapted from the AM program of EuroTransplant, to facilitate hyperimmunized patients to the access of a renal transplant and to achieve a good graft survival. Any eligible hyperimmunised patient (class I IgG >80% PRA), after determination of permissible Ags by Single Antigen Assay, has a national priority to receive any graft without...
any HLA class I mismatch (MM) between the donor and the combination of the recipient own Ag and its acceptable Ags. A maximum of 1 DR MM is accepted. Between 01/04/2005 and 31/12/2008, 235 renal transplantations has been performed using this program. In the positive cross match (XM) patients as compared to the transplanted patients, patients are more likely to be waiting for a second graft (84% vs 75%) and are more highly immunized (62 versus 50% of all HLA class II PRA). Transplanted patients had on average 6 MM with their donor, only considering their own HLA antigens, and 36% had no DR MM. After 44 months, we observed an improved 2 years access to transplantation for highly immunized incident patients, increasing from 42 to 51%, whereas this rate decreased for the non immunized or more slightly immunized patients. Among the 424 XM performed, 185 XM were positive. The vast majority of patients (40%) were B or 0-rematched for HLA class II, (n=12, age=41.6±7.1), 16 patients derived from untreated living donors (n=8, age=44.6±6.9) were also included in the study. Donor treatment consisted of 250 mg methylprednisolone i.v. at time of consent for organ donation and thereafter 100 mg/h i.v. Intraoperative biopsies were taken 30 min. after reperfusion and were immediately snap-frozen until analysis by real-time RT-PCR. In comparison to the normal collective, elderly deceased organ donors revealed a significant de novo gene expression of several immune cells and resident cells but also that several miRNAs were specifically up-regulated and down-regulated in PBMCs and, pro-inflammatory cytokines induced a down regulation of miR-30a-3p in HRECs. Finally, several of the differentially expressed miRNAs could be patented in urine samples from kidney transplant recipients as promising markers for the diagnosis of miRNAs as novel non-invasive biomarkers of allograft status.

O-265 ALTERATIONS IN INTRAGRAFT microRNA EXPRESSION DURING ACUTE REJECTION OF THE RENAL ALLOGRAFT: IMPLICATION FOR DIAGNOSIS AND MECHANISM OF THE ALLO-IMMUNE INJURY

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Acute rejection (AR) is exemplified by alterations in the expression of protein encoding genes. Because the noncoding microRNAs (miRNAs) regulate the expression of a vast array of genes, we investigated whether AR is associated with alterations in intragraft miRNA expression. miRNA expression patterns of human renal allografts were ascertained using microfluidic cards (N=7 biopsies). A subset of 17 miRNAs were differentially expressed (P value < 0.01; 10 miRNAs were expressed at a lower level and 7 miRNAs were expressed at a higher level in AR samples, and the presence or absence of AR could be predicted using miRNA expression profiles. Differentially expressed miRNAs were validated in an independent set of 26 biopsies. Levels of over-expressed miRNAs correlated with the intragraft levels of miRNA for CD3 and CD20, and levels of under-expressed miRNAs correlated with the miRNA for renal tubule proteins (N=12). As well as with kidney allograft function. Further in vitro experiments in activated PBMCs and in human renal epithelial cells (HRECs) subjected to pro-inflammatory cytokines provided new insights in the regulation of miRNA in human cells and suggested that the over- or under-expression of miRNAs in AR samples not only reflected the variation in the proportion of immune cells and resident cells but also that several miRNAs were specifically regulated in response to the activated status of the cells. Upon PHA activation, miR-155 was upregulated whereas miR-223 and let-7c were down-regulated in PBMCs, and pro-inflammatory cytokines induced a down regulation of miR-30a-3p in HRECs. Finally, several of the differentially expressed miRNAs could be patented in urine samples from kidney transplant recipients as promising markers for the diagnosis of miRNAs as novel non-invasive biomarkers of allograft status.

O-266 POST TRANSPLANT HLA AND MICA IMMUNISATION AND CHRONIC REJECTION IN HEART/LUNG TRANSPLANTATION: ONE CENTER STUDY

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During the 15th IHWS, we participated to the component studying the affects of post Transplant HLA and MICA antibodies (Ab) on long term Heart/Lung graft survival. This component was chaired by Prof. P.Terasaki and Dr M. Ozawa. We report here on data from our own center. We have included 161 patients (45 Heart, 21 Heart+Lung, 107 isolated Lungs) in this study between 1997 and 2007 with a follow up of at least 6 months with a good organ function. Clinical information was obtained and blood collection for HLA and MICA immunization study was collected during summer 2007. One year later, clinical outcome was requested. HLA and MICA Ab screening was performed with Luminex technology (LABScreen Mixed One Lambda) and the specificity analysis (DSA versus NDSA) was performed with LABScreen Single Antigen Assay. HLA Ab screening was performed in 25 patients. Seven out of these 25 patients (28%) displayed complications. When DSA class II and MICA Ab were both present, clinical complications were observed (3/3). HLA Ab screening was negative in 161 patients (63% of the cohort). Nine patients (5%) displayed complications. HLA Ab were not associated with complications. In summary, immunological complications occurred preferentially in Heart transplantation and are associated with HLA DSA class II. Moreover, HLA NDSA were preferentially anti class I and not associated with complications. The incidence of MICA immunization is low (9%) but when MICA Ab are associated with HLA DSA class II, chronic complications were observed.

O-267 INTRAGRAFT ECTOPIC LYMPHOID TISSUE DURING CHRONIC REJECTION: HJACKING OF AN EMBRYONIC DEVELOPMENTAL PROGRAM PROMOTES THE DEVELOPMENT OF A LOCAL AGGRESSIVE ALLOIMMUNE RESPONSE

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Introduction: During chronic rejection, inflammatory infiltrates organize themselves into a functional ectopic lymphoid tissue within rejected organs. In the present study we postulated that this process could rely on the recapitulation of the developmental program triggered in the embryo during the ontogeny of secondary lymphoid organs, i.e. lymphoid organogenesis. Methods/Results: We prospectively collected 20 human renal grafts explanted for terminal chronic rejection and 12 controls (6 native kidneys and 6 renal grafts removed for non-immune failure). Patchy nodular CD20+ B cell infiltrates were evidenced in all chronically rejected grafts but in none of the controls. The level of expression of the genes involved in lymphoid organogenesis (LO) was measured by Q-PCR. LO genes were not expressed in the 12 control tissues. On the contrary, the 20 chronically rejected grafts were distributed into 3 clusters corresponding gene respectively to a stepwise increase in the number and the level of expression of LO genes. The samples in which the complete set of LO genes were expressed displayed a highly functional intragraft lymphoid tissue: 17 miRNAs were the local maturation of B cells from naive to memory or plasmacells; and ii) the local generation of alloantibodies. In contrast, in samples in which the LO recapitulation was incomplete B cell maturation was blocked. Accordingly, we observed that the time of transplantation (from trans-
DONOR NATURAL KILLER CELLS DETERMINE LONG-TERM HUMAN KIDNEY TRANSPLANT OUTCOMES THROUGH HLA-C SUB-GROUP DEPENDENT RECIPIENT DENDRITIC-CELL MATURATION

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Introduction: Natural killer (NK) cells have a critical role in the maturation of which is the tumbor response. It also controls that NK cells are a major determinant of long-term kidney transplant outcome through interactions between NK-cell killer immunoglobulin receptors (KIR) and their ligand HLA-C. HLA-C has two subgroups C1 and C2: based on HLA-C specificity, HLA-C2 is a more potent inhibitor of NK cell activation than HLA-C1.

Methods & results: (i) In 760 kidney transplant recipients, those with HLA-C2 genotype had better 10-year graft survival than those with HLA-C1 genotype (66% & 44% respectively, p=0.002, HR=1.51, 95%CI=1.16-1.97). A multivariable analysis confirms this association. Donor HLA-C genotype did not influence long-term graft survival. (ii) Isolated NK cells (by CD56 staining) were present in a peri-tubular distribution in kidneys (n=5) donated for transplantation (pre-perfusion). (iii) In an allogeneic (indirect) NK-Dendritic Cell (DC) in-vitro co-culture system, the possession of HLA-C2 by DC was associated with anti-inflammatory cytokine production (IL-12a, IL-6), diminished DC maturation (CD86, HLA-DR), and absent CCR7 expression. In contrast, possession of HLA-C1 by DC was associated with pro-inflammatory cytokine synthesis (TNF-α IL-12p40/70), enhanced DC maturation and CCR7 expression. These responses were IL-15 dependent.

Conclusion: These data indicate that donor derived NK cells differentially interact in situ with recipient DC through KIR/HLA-C interactions in the presence of IL-15 (which is present in the kidney early after transplantation). HLA-C2 recipients sustain less priming for indirect allogrecohnition than HLA-C1 recipients and have better long-term outcomes. As the PK (PKR)/DC/HLA-C synapse is not inhibited by current immunosuppressive protocols, it represents a potent new therapeutic target in human kidney transplantation.

THE IMMUNOREGULATORY MOLECULE HLA-G INHIBITS THE mTOR PATHWAY AND CELL CYCLE OF ACTIVATED T CELLS THROUGH SHP2

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Aim: HLA-G is involved in regulating T cell responses and is associated with lower rejection in human transplantation. We have shown that HLA-G regulates T cell regulation by inhibiting their cell cycle progression. We have analysed the pathway involved in the cell cycle inhibition.

Results: Soluble HLA-G (HLA-G5) inhibited both CD4 and CD8 T cell proliferation. This effect is due to the interaction of HLA-G and the inhibitory receptor ILT2 since siRNA to ILT2 allow activated T cells incubated with HLA-G to proliferate. ILT2 is a transmembrane receptor with an intracellular domain with ITIM motifs which can recruit phospatases. Immunoprecipitation of ILT2 in presence of HLA-G co-precipitates the phosphatase SHP2. In addition, incubation of T cells with HLA-G is associated with the occurrence of the P-SHIP2 which is functional form. It also correlates with the dephosphorylation of mTOR but not with CD3ζeta or ERK. Moreover, the inhibition of SHP2 with NF448777 in this condition inhibits the dephosphorylation of mTOR as well as the transfection of siRNA to SHP2. In addition, siRNA to SHP2 inhibited the p27 expression observed in presence of HLA-G. Altogether, these data indicates that SHP2 is implicated in the regulatory effect of HLA-G in T cells.

Conclusion: The immunoregulatory molecule HLA-G regulates the cell cycle of T cells by modulating the mTOR pathway through the activation of the phosphatase SHP2.
at transplant (history of AMI, CHF, coronary revascularization, CVA, peripheral arterial disease surgery), donor type, obesity, history of cancer, and time on ESRD therapy, the adjusted relative risk of MACE was 0.84 (95% CI: 0.72-0.98; p=0.03), 0.85 (0.65-1.01; p=0.06), and 0.92 (0.76-1.10; p=0.32) for stroke (median; IQR: 10.12 vs 5; 5; p=0.05) were twice as high in OSA versus non OSA patients.

**Conclusions:** The prevalence of OSA is similarly high in transplanted and non-transplanted patients. OSA may contribute to increased cardio-cerebro-vascular risk in transplanted patients.
O-275 HOW TO PREDICT THE CARDIOVASCULAR (CV) RISK AFTER RENAL TRANSPLANTATION

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Aims of this study is in 359 renal transplant recipients, with a graft functioning for at least 1 year (KTX), were: 1) to evaluate the incidence of post-tx CV events; 2) to identify current main CV risk factors; 3) to assess the predictive role of existing CV risk scores.

Methods: Major Acute Clinical Events (MACE: angina, AMI, ictus cerebri, cardiac death) and routine biochemistry were prospectively yearly analyzed in 359 KTX who received a renal transplant in a single center between January 1997 and December 2007, median follow up time was 70 months. All transplant candidates with positive cardiac history or age over 50 years were pre-tx evaluated with pharmacological ecocardiostress; positive pts underwent then coronary angiography followed by PTCA or CABG, as indicated.

Results: The incidence of MACE increased over post-tx time: MACE affected 0.27%, 2.41% and 8.94% of KTX within the first 5 months, 6 years and 10 years post-tx, respectively. At univariate analysis, risk factors associated with MACE were male gender (P=0.0051), age > 55 y (P=0.033), BMI > 27 (P=0.046), pre-tx positive CAD history (P=0.001), pre-tx total cholesterol >204 mg/dl (P=0.003), pre-tx systolic blood pressure >142 mmHg (P=0.002), presence of left ventricular hypertrophy before tx (P=0.003), post-tx diabetes on therapy (P=0.0002), post-tx serum creatinine >1.7 mg/dl (P=0.05). Evaluating the Follow-up and the INDANA CV risk score indexes, only INDANA could significantly (P=0.05) predict the MACE observed in our population, as this CV score index is also including renal function.

Conclusions: MACE after renal tx relates to traditional pre and post-tx CV risk factors. The individual increase of MACE 5 years after tx indicate the need for an aggressive cardiac re-evaluation: INDANA index may help to select the population at high CV risk.

O-276 METABOLIC SYNDROME AFTER RENAL TRANSPLANTATION: CHANGES IN MARKERS OF INFLAMMATION AND ADHESION MOLECULES

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Purpose: In kidney transplant recipients (KTR) the high incidence of metabolic syndrome (MS) is related to a clustering of cardiovascular risk factors or metabolic abnormalities and has been reported to have adverse effects on patient and graft survival. This study was focused on the incidence and character-istics of MS in a population of KTR, evaluating the levels of circulating markers of cardiovascular risk with or without a diagnosis of post-transplant MS.

Methods: The study recruited 565 KTR transplanted between 1996 and 2004, without pre-transplant diabetes, stable renal function at 1 year post-transplant and at least 4 years follow-up. MS was diagnosed in the presence of ≥3 of the following risk factors: obesity, dyslipidemia (raised triglycerides level, reduced HDL cholesterol), hypertension, impaired glucose tolerance. The serum levels of the following biomarkers of cardiovascular risk were compared across patients with or without MS: CRP, Lp(a), IL-6, IL-10, TGF-beta, TNF-alpha, IFN-gamma, MCP-1, P-selectin, sCD40L, HPA, VCAM-1.

Results: Ninety-seven patients (17.2%) had MS at 1 year post-transplant, 70 of them with ≥3 and ≥27 with 4 risk factors. Three patients with MS died of cardiovascular disease.

In univariate analysis, MS patients showed significantly higher levels of CRP, Lp(a), IL-6, VCAM and P-selectin and lower levels of IL-10 and TGF-beta (Table 1). Using multivariate regression analysis to determine the independent associ-ation of the aforementioned parameters with the risk of MS, CRP, sVCAM and P-selectin remained independent predictors of MS, while IL-10 resulted as protective factor for the development of MS.

Conclusions: Our study suggests an association in KTR of MS with elevated levels of adhesion molecules and altered balance between pro-inflammatory and anti-inflammatory molecules. The predictive value of these biomarkers in relation to post-transplant MS needs to be better assessed in further longitudinal studies.

Session 32. Composite tissues & xenotransplantation

O-277 FIRST FACE ALLOGRAFT: A THREE YEARS FOLLOW UP

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The first human face allotransplantation was performed in Amiens (France) on November 2005. We report outcomes up to 3 years after transplantation. The recipient, a 38 years old woman, was mutilated by a dog bite, received a facial allograft (nose-lip-chin) from a brain dead woman. A vascularized sen-teral donor graft was also performed. Donor bone marrow was infused on days 4 and 11 post transplantation. Initial immunosuppression protocol included Thymoglobulins, tacrolimus, prednisone and mycophenolate mofetil (MMF). At 3 years the patient is doing well with prednisone (5 mg/d), MMF (1500 mg/24h) and sirolimus (through level 8-10 mg/ml).

Results: Functional and aesthetic results: the patient has a complete recovery of sensibility and a motor recovery which allowed a complete mouth closure, with a possibility to drink and to eat and a normal phonation. Aesthetic results allowed the patient to live a normal social life. Immunological follow-up: i) two episodes of acute rejection, which occurred at day 18 and 212, regressed successfully after 3 boluses of steroid. ii) Macroscopic aspect and histology of the biopsies from mucosa and sentinel flap skin graft didn’t show any sign rejection. iii) anti-HLA antibodies have remained neg-ative. iv) Study of peripheral blood T lymphocyte subsets showed an increase in CD8+DR+ and CD4+CD25+CD127- T reg during the rejection episodes. Then, T reg decreased while CD8+DR+ remained at high level despite the absence of chronic rejection.

Side effects of immunosuppression: i) the patient started a degradation of re-nal function at 6 months, which improved after the switch from tacrolimus to sirolimus. At 3 years GFR (MDRD) is 79 ml/min/1.73m². ii) The patient devel-oped mild hypertension, and alteration of lipid status related to sirolimus, easily controlled by statins.

Conclusion: Three years after transplantation the balance between the results and the complications is satisfactory.

O-278 CHRONIC REJECTION IN COMPOSITE TISSUE ALLOGRAFT

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Composite tissue allograft (CTA) showed little evidence of chronic rejection and it was reported only in non compliant recipients. The course of chronic rejection on bones, muscles, nerves, tendons and vessels may have yet undescribed implications. For this reason we have studied all these structures in four bilateral hand grafted patients (9, 6, 2 years and 6 months of follow-up respectively) without macroscopic and histological signs of chronic rejection in the skin. Bone quantitative parameters and architecture were studied by quantitative computed tomography and dual-energy-X-Ray absorptiometry. Magnetic resonance imaging (MRI) allowed for visualization of bones, muscles, tendons and nerves, and magnetic resonance angiography for vessels, which were also assessed by Doppler ultrasounds. Nerves were also investigated by ultrasonogra-phy and electromyography. Microcirculation was studied by nailfold capillary microscopy. Bone quantitative parameters at radius and tibia distal level did not show any
significant reduction in graft bone density, and bone architecture was preserved in all patients. Nerves and tendons did not show any structural modification. Angio MRI and Doppler ultrasounds showed patency of all examined blood vessels, and no signs of microvascular damage were reported but only minor alterations such as venular stasis. In the present study we evidenced only fatty degeneration of some intrinsisc muscles in the majority of patients which seems to be clearly correlated to the period of muscular denervation. Although the different times of follow-up the results were similar for all the recipients. These data confirm that when there are no signs of chronic rejection in the skin there are neither in the other components of CTA.

O-279 VASCULARIZED BONE MARROW TRANSPLANTATION: AN ALTERNATIVE TO CONVENTIONAL CELLULAR BONE MARROW TRANSPLANTATION

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Current protocols for bone marrow transplantation (BMT) involve removing the bone marrow component directly from its donor microenvironment and then injecting such components into the circulatory system of the recipient. This procedure is usually preceded by conditioning protocols (body irradiation, immunosuppression, or both). Vascularized bone marrow transplantation (VBMT), in comparison with conventional marrow transplants, has the advantage of providing a microenvironment and immediate engraftment of both mature and progenitor hematopoietic cells at the time of transplantation in the absence of immunomodulation or irradiation. The aim of the study was to follow the development of microchimerism after allogeneic VBMT vs conventional BMT. One group of a VBMT model consisted of a donor Brown Norway (BN) rat hind limb heterotopic transplanted on recipient Lewis rats was used. An intravenous infusion of donor bone marrow cells in suspension equivalent to that grafted in the vascularized femur limb was administered i.v. on recipient rats in the second group. Cellular microchimerism was investigated in recipients of VBMT vs BMT. Donor-derived cells could be detected in VBMT recipients at 30 and 60 days but not in recipients of i.v. suspension BMC grafting. VBMT provides a theoretical alternative to conventional cellular bone marrow transplantation by addressing crucial clinical problems such as failure of engraftment or graft versus host disease. It may be possible to develop a new approach for bone marrow transplantation based primarily on a microsurgical procedure (transplantation of vascularized bone marrow flaps).

O-280 TRANSLATION OF hCD46 TRANSGENIC PORCINE ISLETS INTO DIABETIC NONHUMAN PRIMATES RESULTS IN LONG-TERM NORMOGLYCEMIA UNDER LIMITED IMMUNOSUPPRESSION

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Purpose: Intraportal xenotransplantation of porcine islets is characterized by an inflammatory graft loss and the need for intensive immunosuppression to avoid acute rejection. We investigated if the transgenic expression of a human complement regulatory protein (hCD46) on porcine islets would improve islet xenotransplantation.

Methods/Materials: In 9 cynomolgous monkeys, diabetes was induced by i.v. streptozocin (1500mg/kg). Four (Group A) were transplanted with nontransgenic porcine islets, and five (Group B) with hCD46 islets. Both groups received equal numbers of islets (85,000-100,000IEQ/kg) and limited immunosuppression (ATG, anti-CD154 monoclonal antibody, MMF). Follow up was for 5 months, except for 1 Group B animal that was followed >1yr to verify the durability of the normoglycemic status.

Results: Islet-dependent normoglycemia was achieved in 3 of 4 Group A monkeys for 5, 17, and 36 days, respectively, compared to 4 of 5 Group B monkeys for 87, 91, 92, and 396 days (P=0.004) (figure1). Fasting blood glucose values were well-controlled (<120mg/dL). In the fifth Group B monkey, exogenous insulin needs were reduced >50% for 3 months with detectable porcine C-peptide. Post-transplant fasting porcine C-peptide levels were 1.10±0.41ng/mL (Group A), vs. 0.90±0.51ng/mL (Group B) (P<0.046). After an i.v. glucose challenge these levels failed to increase in Group A (1.02±0.32ng/mL), while in Group B they markedly increased to 4.07±1.46ng/mL (P=0.02). There was no response by monkey beta cells. Animals stayed healthy and gained weight. Post-mortem liver histology showed many viable islets free from complement deposition in Group B, in contrast to marked C4d staining in Group A.

Figure 1. 1 year follow-up after islet xeno-Tx.

Conclusion: hCD46 expression on porcine islets significantly prolonged normoglycemia in monkey recipients (up to >1yr) and allowed for limited immunosuppression with minimal adverse events, thus advancing islet xenotransplantation toward clinical application.

O-281 IMPROVEMENT IN CARDIAC FUNCTION AFTER PRECLINICAL ORTHOTOPIC CARDIAC XENOTRANSPLANTATION

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Purpose: Preclinical 90-day median survival of pig-to-primate orthotopic cardiac xenotransplants is a likely standard for clinical application. In this report we examine the recovery of cardiac function after successful pig-to-primate cardiac xenotransplantation.

Methods: Five successful (CD46,n=3; GalK0/CD55,n=2) orthotopic pig-to-baboon heart transplants were performed. Immunosuppression consisted of ATG induction, tacrolimus, sirolimus, tapering steroids and iGal therapy in the CD46 transplants. Heart function was monitored biochemically, echocardiographically, and by intramyocardial electrodacardiography.

Results: The five recipients survived 57, 40, 34, 22 and 14 days in a healthy condition. Mortality resulted from bowel infarction, pneumonitis, respiratory failure of a surgical bleed and sudden death due to unknown cause, respectively. Autopsy revealed minimal or mild rejection in 4 recipients and mild to moderate in the 5th. All recipients exhibited a transient spike in serum troponin C after transplant. An improvement in LV ejection fraction was noted in 4 of 5 recipients within 7 to 14 days of transplant. Ejection fractions were normal at the time of death in 3 recipients and 45% and 45% in the remaining two.

Conclusions: These orthotopic recipients represent the longest survivors to date. In successful transplants early perioperative ischemia/reperfusion injury was shown to be completely recoverable indicating that normal cardiac reparative processes function across the xenotransplant barrier. Cardiac xenograft rejection was controlled in survivors who remained healthy on clinically used immunosuppressants. The model is challenging but these results support the potential viability of orthotopic cardiac xenotransplantation with its attendant advantages of complete implantability, intrinsic power supply and no anticoagulation. These early results along with the high impact that successful cardiac xenotransplantation would have justified continued preclinical studies.

74 Tuesday, 1 September 2009 Session 32. Composite tissues & xenotransplantation
**Session 33. How can we make the lungs breathing better?**

**O-282**

**LIVER XENOTRANSPLANTATION USING α1,3-GALACTOSYLTRANSFERASE GENE KNOCK-OUT (GTKO) PIGS**

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**Purpose:** We have explored orthotopic transplantation (Tx) in baboons using liver. Further genetic-engineered pigs to determine whether these livers could ‘bridge’ a patient to alloTx.

**Methods:**

**Group 1:** AllOtx in wild-type (WT) pigs (n=2) or baboons (n=1).

**Group 2:** XenoTx in baboons using livers from GTKO (n=1) or GTKO transgenic for CD46 (GTKO/CD46, n=3) pigs. Immunosuppression consisted of thymoglobulin induction and tacrolimus, mycophenolate mofetil, and steroids maintenance.

**Results:**

In Group 1, the two non-immunosuppressed WT pigs were effectively euthanized at 3 days; liver function tests (LFTs) were normal and liver histology showed minimal acute cellular rejection. The immunosuppressed baboon was also euthanized at 30 days; LFTs and histology were normal. In Group 2, the baboons survived for 4, 6, and 7 days. When 24h, albumin fell to the normal pig level (2.2), but was maintained at the normal baboon level (3.7-0.7/g/d) by a human albumin. LFTs remained in the normal range. Western blot demonstrated that pig proteins (albumin, plasminogen, fibrinogen, haptoglobin) were produced by the liver. Complement activity (CH50 test), PT, FII, and INR were normal. Production of numerous pig coagulation factors was confirmed. However, severe thymoglobulinemia (platelets <20,000/mm³) developed within 5h, with subsequent spontaneous internal hemorrhage, necessitating euthanasia. At necropsy, liver histology showed patches of hemorrhagic necrosis, platelet-fibrin thrombi, monocyte/macrophage margination, and vascular endothelial cell hypertrophy. In vivo studies demonstrated activation of baboon platelets leading to platelet-monocyte aggregates.

**Conclusions:** GTKO/CD46 pig livers function adequately in baboons for up to 7 days, but severe thymoglobulinemia, results in internal bleeding, Activation of pig vascular endothelial cells and/or baboon platelets, with increased tissue factor activity, may result in platelet/WBC-aggregation and sequestration in the liver. Further genetic-engineered modifications of the pig, e.g., adding the TFPI gene, may help overcome the current limitations.

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**Session 33. How can we make the lungs breathing better?**

**O-283**

**INCIDENCE AND OUTCOME OF ABDOMINAL SURGICAL INTERVENTIONS FOLLOWING THORACIC TRANSPLANTATION**

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**Purpose:** Abdominal complications after heart (HTx) or lung (LuTx) transplantation are associated with high risk of mortality. Aim of the present study was to analyze the frequency and outcome of abdominal interventions following HTx or LuTx.

**Methods:** Retrospective analysis was performed on 281 patients after HTx and 754 patients after LuTx (total n = 1035) undergoing abdominal surgery at the tertiary medical school, Hannover, Germany, between January 2000 and December 2008.

**Results:** In the course of transplantation 71 patients (6.9%) were in need of surgical interventions due to abdominal complications. The incidence was comparable in both groups of patients (5.7% after HTx vs. 7.3% after LuTx). Following HTx 3 individuals received emergency surgery due to bowel perforation, appendicitis and ileus. No patient died in relation to the disease. Effective operations (n=17) without incidence of mortality were performed based on varying diagnoses. Following LuTx 35 individuals were operated on 43 cases of emergency indication. Leading diagnosis was bowel perforation (n=10) with surgery performed 10.4 months after LuTx, although 7 of 10 patients were operated within the first four weeks post transplantation (time between LuTx and operations in general: 15.2 months). In recipients of LuTx emergency intervention were associated with a mortality of 25.6%, thereof 45.5% after bowel perforation. Elective surgical treatments (n=31) after LuTx were diverse and had no influence to mortality.

**Conclusions:** Early abdominal complications after LuTx correlate with a high mortality. Peroration of the bowel was the leading diagnosis with severe impact on the patient’s outcome. In findings of an acute abdomen after HTx and LuTx we propose a broad indication for further diagnostics and a low barrier to force an early explorative laparotomy.

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**O-284**

**INCIDENCE OF DE NOO MALIGNANCIES IN LUNG TRANSPLANT RECIPIENTS IN ITALY: A SINGLE-INSTITUTION EXPERIENCE, 1991-2008**

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**Background:** Patients who underwent transplantation present an increased cancer risk. The present study aims on two different drug protocols, and to quantify the incidence rate (IR) and the excess risk of de-novo malignancies (excluding non-melanoma skin cancers) in patients who received lung transplantation (LTx).

**Methods:** We collected data on baseline demographics, transplantation, last follow-up and eventual cancer) on 261 patients (68.2% males) who underwent LTx (28 combined heart-lung, 114 single- and 119 double-LTx procedures) at Policlinico “San Matteo” of Pavia (Northern Italy) (1991-2008). Period at risk of developing cancer (person-years, PY) was computed from 30 days post-LTx to date of cancer diagnosis, death, or last follow-up. Observed and expected cancer were compared through sex- and age-standardized incidence ratios (SIRs) and 95% confidence intervals (CI) using Italian Cancer Registries data as baseline IR

**Results:** Overall, 1,079 PYs were accumulated (median follow-up, 3.4 years). 26 patients (24 males) developed at least one confirmed de-novo-malignancy (29 single diagnoses). Among those 7 Non Hodgkin-NHL, 7 Kaposi’s Sarcoma-KS, lung and colorectal cancers (4 diagnoses each) and 1 Hodgkin lymphoma-HL. Interestingly 10/16 malignancies occurred in early post-transplanted period (8.6 years) and in early post-transplanted period (8.6 <2 years vs. 2.2 <2 years post-LTx).

**Conclusions:** LTx patients are at higher risk for cancer (mainly viral-related malignancies). Further investigation is needed to highlight the relationship between immunosuppression and cancer risk in LTx.

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**O-285**

**PROTOCOL-DRIVEN RECIPROCAL OUTCOMES OF MALIGNANCY AND CHRONIC REJECTION AFTER LUNG TRANSPLANTATION**

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**Background:** The incidence of malignancy after solid organ transplantation is high, generally regarded as a consequence of immunosuppressive drugs given. We evaluated the cancer incidence and possible risk factors in lung transplantation patients, with emphasis on two different drug protocols.

**Methods:** The histopathological results of all lung transplant patients (1990-2007) were screened for malignancies using a national pathology registration. Risk factors for malignancy were evaluated in univariate and multivariate analyses. Immunosuppression from 1990 – 2001 was ATG, followed by ciclosporine, azathioprine and prednisolone (protocol 1). In case BOS in protocol 1, ciclosporin was switched to tacrolimus. In 2001 the protocol was changed into anti-CD25 (induction), tacrolimus, azathioprine and prednisolone. In addition patients received CMV prophylaxis and EBV/gyedated tapering of ciclosporine.

**Results:** Of the recipients, 25.5% developed a malignancy. These were mainly MMRC (12.4%) and PTLD (8.5%). Differences in risk factors, including donor age and gender (6.6%). The standardized incidence ratio was 30.01. Both protocol 2 and older recipient age were significant independent risk factors for the development of a non-PTLD malignancy. Protocol 2 resulted in a significantly improved outcome
with regard to overall 1- and 5-years graft survival, freedom from BOS and development of PTLD. Remarkably, the percentage of malignancies in solid organs was higher in patients receiving tacrolimus, as standard and rescue therapy (8.0%) compared to those not receiving tacrolimus (3.6%). This difference was not statistically significant.

Conclusion: The risk of developing a malignancy in our lung transplantation program was comparable to that of the normal population. A protocol change in 2003 resulted in a favourable outcome with respect to survival, BOS and PTLD. This was counterbalanced by a dramatic increase of non-PTLD malignancies. The switch to tacrolimus alone could not be identified as an independent risk factor.

**O-286**

**STABILIZATION OF KIDNEY FUNCTION IN A COMBINATION TACROLIMUS-EVANS BASED IMMUNOSUPPRESSION AFTER LUNG TRANSPLANTATION**

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Objective: Nephrotoxicity is a limitation in Calcineurininhibitor (CNI) based immunosuppression. Protective effects of Everolimus on kidney function were achieved in heart and renal recipients, but no data concerning renal function in heart immunosuppressed patients after lung transplantation (LUTX) are published.

Aim of this study was to evaluate the impact of a CNI reduced, everolimus (EV), mycophenolate mofetil (MMF) and steroid based immunosuppression in LUTX with chronic renal failure.

Methods: In 42 LUTX (23 m/19 f: age: 51.9±12.0 yrs) with deterioration in renal function CNI (CsA: 15 pts/Tacrolimus: 27 pts) was stepwise halved and EV (median 1.9 yrs after LUTX). Routine laboratory values, GFR, CNI-EV trough levels were monitored monthly before CNI-EV, at time of conversion and 12-months after CNI-EV switch. We evaluated safety, side effects and biopsy proven rejections retrospectively.

Results: A stabilizing effect was achieved in all 42 LUTX (GFR pre switch: 37.9±12.5 ml/min; GFR 12 months post switch: 37.9±15.0 ml/min). The greatest benefit was seen in LUTX patients with < 40 ml/min renal function deteriorated significantly within 12 months after switch to CNI-EV (p<0.001). 3 LUTX (7.3%) died significantly within 12 months after switch to CNI-EV.

Conclusion: CNI-EV immunosuppressive regimen is a safe feasible therapy to stabilize renal function. The best protective renal effect can be achieved in patients with GFR greater than 40 ml/min.

**O-287**

**SUBLINGUAL TACROLIMUS AS AN ALTERNATIVE TO INTRAVENOUS ROUTE IN THORACIC ORGAN TRANSPLANTATION**

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Objective: Tacrolimus (TRL), currently used in transplantation is characterized by narrow therapeutic index, low bioavailability (10-25%) and pharmacokinetic variability. Intravenous (IV) TRL is needed whenever oral route is strictly unavailable. The low amount (0.01-0.03 mg/kg) of the infusion formulation (5mg/mL), resulting in high dilution and careful infusion technical management, increased variability and overdose risk. Sublingual (SL) TRL administration was introduced as IV alternative. This study addressed the feasibility to provide SL TRL in transplanted patients.

Methods: Retrospective study conducted during 2005-2008 in 16 transplanted patients as 13 lung (10 cystic fibrosis), 3 heart, receiving SL TRL controlled by regular therapeutic drug monitoring as trough blood levels (C0) analyzed by MEIA. Four full AUC were determined. Patients received SL TRL on a dose-to-dose basis from the oral formulation powder content and asked not to swallow for at least 15 minutes after intake.

Results: Mean age was 35±15±6.8 yrs in 14M/3F. The 146 C0 samples collected during SL period (duration 15.8 days [2-46]) showed 90.4% concomity form 5-15 ng/mL, 5.5% supratherapeutic and 4.5% subtherapeutic levels. Mean dose, C0 and AUC were respectively 0.116±0.096 mg/kg/d, 12.5±3.5 ng/mL and 230±74 ng/mL, with 1h average peak time concentration. These results were consistent with oral references and SL literature. Neither acute rejection nor renal toxicity or drug interaction management difficulties were notified, except some unpleasant taste reports. Whatever the contribution of TRL passive swallowed oral absorption, SL route was effective to replace IV delivery, even in one cystic fibrosis patient with digestive interruption due to endotracheotomy.

Conclusion: This study supported the convenience of TRL SL administration, even in unconscious patients. Limited short-term IV infusion resulted in potential clinical improvement and cost savings. Further investigations are needed to confirm SL dose ranging (0.1mg/kg/day) and critical intensive intestinal drug interactions evaluation during oral-SL switch.

**Session 34. Pancreas transplantation: clinical aspects**

**O-288**

**TRANSPLANTATION OF TISSUE-ENGINEERED TRACHEAE -DECELLULARIZATION BY ULTRA-HIGH PRESSURE IMMUNOSUPPRESSION AFTER LUNG TRANSPLANTATION**

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Background: Tissue-engineered tracheal grafts are expected to be applied for the treatment of extensive tracheal defects. Here, we tried to elucidate the feasibility of a novel airway replacement using tissue bioscaffolds processed by ultrahigh pressure method (UHP) and mesenchymal stem cells.

Methods: Rat study-1: B-N rat tracheae were decellularized by i) cryopreservation (CR), ii) Triton X-100 (TX) or iii) UHP (880 MPa for 10 min. at 4 °C). Fresh (FR) or treated each trachea was transplanted in the subcutaneous space of the Lewis rat. CR and UHP tracheae were also orthotopically engrafted to Lewis rats. Rat study-2: Syngeneic mesenchymal stem cells (1.0-10^5) suspended in thrombin solution were sprayed with fibrogenin solution on UHP tracheal grafts at the time of allagogenic orthotopic transplantation. Pig study: Decellularized porcine tracheae by CR, TX or UHP were served for the pathological study, compression test or PCR assay for porcine endogenous retrovirus (PERV) DNA.

Results: In both rat and pig studies, cellular contents in TX and UHP tracheae were clearly excluded except in deep capillar. Rat CR, TX and UHP tracheae showed minimum allo-rejection 4 weeks after subcutaneous transplantation, although severe wall thickness with marked cell infiltration was observed in rat FR tracheae. Structural strength of porcine TX tracheae declined by about 60% compared with those of porcine CR or UHP tracheae. Orthotopic CR and UHP grafts were reepithelialized by 4 weeks after transplantation. Furthermore, UHP grafts seeded mesenchymal stem cells showed recellularization of the cartilaginous region within 4 post-transplant weeks. PERV-DNA was undetected only in porcine UHP tracheae.

Conclusions: UHP could be an option for the preparation of allagogenic or xenograftic tracheal bioscaffolds. Seeding of syngeneic mesenchymal stem cells seems to accelerate regeneration of tracheal cartilage of decellularized grafts.

**O-289**

**A RANDOMIZED, PROSPECTIVE TRIAL OF ALEMUTUZUMAB VERSUS RABBIT ANTI-THYMCYCTE GLOBULIN INDUCTION IN KIDNEY-PANCREAS TRANSPLANTATION: A SINGLE CENTER EXPERIENCE**

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Objective: To review our single center experience with alemtuzumab (SHP-104 versus iATG induction in simultaneous kidney-pancreas transplantation (SKPT).

Methods: From 2/05 thru 10/08, 46 SKPTs (45 with portal-enteric drainage) underwent a randomized, double-blind, double-dummy trial of single dose Alem vs multiple dose iATG antibody induction therapy in combination with tacrolimus, MMF and early steroid elimination.

Results: 28 patients (pts, 61%) received Alem and 18 (38%) received iATG induction. There were no significant differences between the 2 groups in 1 year (92% Alem vs 100% iATG) or overall (92% Alem vs 92% iATG) pt survival; 1 year (91% Alem vs 92% iATG) or overall (87% Alem vs 85% iATG) kidney graft survival; and 1 year (87% Alem vs 87% iATG) vs overall (83% Alem vs 90% iATG) pancreas graft survival rates (all p=NS). The 1st year and overall acute rejection (AR) rates (both 17% Alem vs 39% iATG, p=0.10) and infection rates (30% Alem vs 67% iATG, p=0.09) were slightly lower in the Alem
COMPARING RISK FACTORS AND INCIDENCE OF CANCER IN KIDNEY-PANCREAS AND KIDNEY TRANSPLANT RECIPIENTS REPORTED BY UNITED NETWORK FOR ORGAN SHARING (UNOS) BETWEEN 1986-2006

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Kidney-Pancreas (KP) transplantation is the treatment of choice in type 1 diabetic patients with end-stage renal failure. However, recipients are at increased risk of infection and cancer. Aim of the study was to identify risk factors and incidence of cancer in KP transplant recipients and compare it with renal transplant (RT) recipients.

The data were analyzed using a combination of descriptive and comparative statistics. The groups were compared using the chi-square test.

Results: Similar quadruple immunosuppressive regimen was used in all recipients. There were no differences in baseline donor and recipient characteristics. Simultaneous pancreas-kidney (SPK) transplantations were performed in 180 recipients, 81 younger than 50 and 99 aged 50 years or more. The one year mean serum creatinine (1.1 vs. 1.2 mg/dl), calculated MDRD GFR (5716 vs 5514 ml/min) and glycocyhe- moalbumin in >50 years (5.5% vs. 5.1%) levels were similar in the Alem and RTG groups respectively.

Conclusion: Excellent results can be achieved with either Alem or iATG induction in DKTP, although Alem may be associated with fewer AR episodes and infections, and more bleeding complications.

IMPACT OF DONOR AGE IN PANCREAS TRANSPLANTATION: A UK SINGLE CENTRE EXPERIENCE

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Background: The shortage of cadaveric donors for pancreas transplantation has prompted to use organs from donors previously regarded as suboptimal. The aim of this study was to compare the outcome and complications in pancreas transplant recipients transplanted with organs from different donor age groups.

Objectives: The study was performed in a selected group of recipients aged 50 years or more seeking for PTx that traditionally has been regarded as poor candidates for SPKtx.

Material and method: 166 pancreas transplants were performed in our unit between 2001 to December 2008. 128 simultaneous pancreas kidney (SPK), 30 pancreas after kidney (PAK) and 8 pancreas transplantation alone (PTA). Clinical data was collected prospectively into an electronic database (Microsoft Excel). Patients were grouped according the donor age and analysed. Group I (n=25): donor age ≤ 18, Group II (n=116): donor age 18-45 and Group III: (n=25) donor age >45 years old. Clinical outcomes including early and long term surgical morbidity (e.g. bleed, thrombosis, infections, etc), graft, patient survival and hospital stay were compared between all groups.

Results: The one year patient survival rate in Group I was 100%, 89% in Group II and 88% in Group III. The one year pancreas graft survival rate was 84%, 76% and 68% respectively.

The median HDU/ITU stay was shorter in group I (3.5days) and II (2.5days) compared to group III (5.5 days), the median hospital stay was similar (18, 15.5 and 17.5 days respectively).

Summary: Both patient and graft survival rate was higher in the group receiving transplant from pediatric donors. Similarly the rate of major surgical complication tended to be lower. Recipients with organs received from younger donor have shorter HDU/ITU stays.

LONG FUNCTIONING PANCREAS AND/OR KIDNEY GRAFT PREVENTS CARDIOVASCULAR DEATH IN SPKTX RECIPIENTS

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This study evaluates long term survival and cardiovascular death incidence among spktx recipients in relation to function of their grafts.

Between 1988 and 2008 101 spktx were performed. Recipients who had follow – up longer than 18 month were included (n=62). There were three groups: group I (n=33) with good function of both grafts, group II (n=19) who had lost transplanted pancreas while having good functioning kidney graft and group III (n=10) who lost both transplanted organs. Survival rates and incidence of cardiovascular death between groups were compared. The cumulative survival rates for group I, II and III after 5, 10, 15 years were: 100%, 87%, 68% vs 100%, 83%, 62%, 34%, 51% respectively. The survival rate was significantly higher in group I and II than in group III (log-rank test; p<0.01).

There were no significant difference in survival rates between group I and II. In group I deaths due to cardiovascular event and leukemia were noted. In group II death was due to complications of graft.
Session 35. Clinical immunosuppression: renal function

**O-294** THE IMPACT OF PANCREAS ONLY TRANSPLANTATION ON EXISTING RENAL FUNCTION: DOES IT DETERIORATE?

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Introduction: The long-term effects of pancreas only transplantation on renal function among transplanted patients is still debatable.

Aim: To examine trends in renal function and factors which may contribute during early post-transplant years.

Method: From 2001 to 2008 we performed 36 pancreas only transplants in 31 patients (28 PAK & 8 PTA). Serum creatinine at given time points (pre-transplant, one week, 1, 3, 6, 12 and 24 months) were evaluated and estimated glomerular filtration rate (eGFR) was calculated. Several potential risk factors effecting renal function were analysed. Induction immunosuppression was the same in all patients.

Results: The median eGFR remained unchanged throughout (48, 56, 47, 47, 47 and 48 at pre, 7, 30, 90,120,360 and 720 days of operation respectively). The pattern was similar for median serum creatinine (138, 118, 136, 139, 138 and 144) at above time points. Analysing PAK and PTA separately showed that: In PAK subgroup the median eGFR remained unchanged (44 and 48 at pre-operative and at 24 months respectively). This was mirrored in the functioning PAK group where the eGFR increased from 47 to 50. In the PTA group however there was a marked drop at one year (84 to 66) of overall PTAs compare to functioning PTAs which showed no changes in eGFR at one year (81 to 76). A large reduction of eGFR (>20%) was seen in 2 patients who had received PAK (baseline eGFR of 26 and 42) and one PTA (baseline eGFR of 62).

Summary: In our series the renal function does not deteriorate after solitary pancreas transplantation. In the PTA subgroup only the loss of graft seems to be detrimental to native renal function. Reduction in eGFR following pancreas transplantation was pronounced in patients with low baseline eGFR.

**O-295** IMPROVEMENT IN RENAL FUNCTION FOLLOWING CONVERSION FROM LOW-DOSE MYCOPHENOLATE ACID TO HIGHER-DOSE ENTERIC-COATED MYCOPHENOLATE SODIUM (EC-MPS) WITH CONCOMITANT TACROLIMUS REDUCTION IN MAINTENANCE KIDNEY TRANSPLANT RECIPIENTS

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Due to pharmacokinetic interactions, mycophenolic acid (MPA) dose is frequently reduced in tacrolimus-treated recipients. An alternative could be increasing EC-MPS dose and reducing tacrolimus, but the impact on renal function is unknown.

Methods: A multicenter, randomized, open-label study was undertaken in maintenance (>12 months) kidney transplant recipients with stage 3 eGFR (MDRD, 30-50mL/min/1.73m²) receiving MPA 5mg/day or EC-MPS 720mg/day with tacrolimus (C0 5ng/mL ± 18%) corticosteroids. Patients were randomized to unchanged treatment (converting from MPA to equimolar EC-MPS if required) or standard or switch to high-dose EC-MPS (1440mg/day) with reduced tacrolimus (2mg/m²-C0 4.5mg/mL).

Results: 94 patients were randomized (high EC-MPS 46, standard 48), with similar time post-transplant in both groups. Mean EC-MPS dose was 1406-1440mg/day and 711-720mg/day in the high-EC-MPS and standard groups, respectively. Mean tacrolimus C0 in the high-EC-MPS group was 4.9±1.6ng/mL, 4.5±1.7ng/mL and 4.1±1.7ng/mL at months 1, 3 and 6 post-transplantation (p<0.001 vs standard group). Mean tacrolimus C0 in the standard group throughout (range 6.9±8.1ng/mL). Mean eGFR was 46.4±11.2 at baseline and 49.1±11.1mL/min/1.73m² at month 6 with high-EC-MPS vs 45.3±9.5 and 44.7±11.5mL/min/1.73m² in the standard arm. The primary endpoint, adjusted change in eGFR from baseline to month 6 was -0.48±0.93mL/min/1.73m² in the standard arm vs 2.48±0.95mL/min/1.73m² in the high-EC-MPS patients (-2.96mL/min/1.73m², 95% CI -3.60 to -0.32, p=0.028, ANCOVA). There were no deaths, graft losses or biopsy-proven acute rejections. A similar incidence of adverse events was suspected to be related to the study drugs occurred with high EC-MPS (17.8%) vs standard treatment (17.0%); rates of infections were 20.0% vs 29.8% respectively.

Conclusions: Conversion to high-dose EC-MPS with concomitant reduction in tacrolimus exposure can improve renal function without compromising efficacy.

**O-296** RENAL FUNCTION, EFFICACY AND SAFETY OF SIROLIMUS AND MYCOPHENOLAT MOFETIL THERAPY AFTER EARLY CALCINEURIN-INHIBITOR WITHDRAWAL IN NOVEL RENAL TRANSPLANT PATIENTS: ONE-YEAR ANALYSIS OF A RANDOMIZED MULTICENTER TRIAL

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This prospective, randomized, multicenter study was designed to examine, whether an early conversion approach from a CNI-based immunosuppression with cyclosporine, mycophenolate mofetil (MMF) and corticosteroids (ST) to a CNI-free immunosuppression regime with sirolimus, MMF and ST avoids the long-term detrimental effects of cyclosporine on renal function, while simultaneously providing adequate efficacy and safety.

141 patients were randomized to receive either sirolimus or low-dose cyclosporine in conjunction with MMF and ST after induction with ATG-F and a short course of standard-dose cyclosporine/MMF. The primary end point was eGFR at 12 months. Secondary end-points included patient/allo graft survival, acute rejection and safety parameters.

The mean calculated GFR (Nankivell) was higher in patients receiving sirolimus (64.5±25.2mL/min) than in patients receiving cyclosporine (53.4±18.0mL/min). There was no difference in patient and death-censored graft survival (86.6% for both groups). The rate of biopsy-proven acute rejection after conversion was similar in both groups (17% vs. 16%). A higher rate of discontinuations was noted for the sirolimus group 35.7% vs. 19.7%. However, infectious complications after conversion (43.5% vs. 54.9%) and CMV viraemia (5.8% vs. 26.8%) were found to be higher in the cyclosporine group. A regimen of delayed sirolimus, MMF and ST is beneficial for maintenance of renal function and reduction in infectious complications, as compared to a cyclosporine-based immunosuppression.

**O-297** RENAL FUNCTION IN EVEROLIMUS/ENTERIC-COATED MYCOPHENOLATE SODIUM TREATED DE NOVO RENAL TRANSPLANT RECIPIENTS AFTER CALCINEURIN INHIBITOR WITHDRAWAL: THE ZEUS STUDY

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Aim of study: Assessment of an Everolimus/Enteric-coated mycophenolate sodium (EC-MPS) regimen on renal function after Calcineurin Inhibitor Withdrawal in renal allograft recipients at month 12 post transplantation.

Methods: In this study 300 renal allograft recipients were randomized to an immunosuppressive regimen consisting of either Everolimus(EC-MPS) or CsA/EC-MPS. After induction therapy with Basiliximab all patients (pts) received CsA, EC-MPS and corticosteroids for the first 4.5 months post transplantation when pts were randomized 1:1 to either a) continue CsA/EC-MPS
therapy (n=145) or convert to Everolimus/EC-MPS (n=155). Dosing for EC-MPS was 720mg BiD. Everolimus and CsA trough levels were 6-10ng/ml and 100-150ng/ml, respectively. As primary endpoint renal function was assessed by calculated Glomerular Filtration Rate (gFR; Nankivell-method). In addition, renal function was determined by cGFR according to Cockcroft-Gault and MDRD method, serum creatinine and slope of creatinine.

Results: At randomization renal function was comparable in both groups. At month 12 dGFR (Nankivell formula) was 72±8 for the Everolimus/EC-MPS and 62±7ml/min/1.73m² for the CyA/EC-MPS treatment group, respectively. The observed GFR slope from month 4.5 to month 12 was 0.8±[95%CI; -0.4, -1.1] for the Everolimus/EC-MPS pts and -1.8±[95%CI; -4.9, 1.3] ml/min/1.73m² for CsA/EC-MPS pts. ANCOVA model

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<th>Nankivell formula</th>
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<td>Mean ± SD</td>
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At baseline no significant differences in proteinuria were observed between the Everolimus/EC-MPS (35±±259 mg/d) and the CyA/EC-MPS (366±774 mg/d). Mean age in the IGF group was younger than the SGF group in Everolimus/EC-MPS (456±510 mg/d) and slightly higher in the CsA/EC-MPS treatment group (284±472 mg/d), respectively. Proteinuria was reported by the investigator in 16% of Everolimus treated pts and 17% in CsA treated pts.

Conclusion: Our results confirm the expected improved of renal function after CNI withdrawal after introduction of the non- nephrotoxic Everolimus/EC-MPS regimen in de novo renal transplant patients.

**O-298** SLOW RECOVERY OF GRAFT FUNCTION IS ASSOCIATED WITH POOR GRAFT AND PATIENTS SURVIVAL IN LIVING DONOR TRANSPLANTATION

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**Background:** The definition and clinical outcome of slow recovery of graft function (SGF) in living donor kidney transplantation are unclear. This study was performed to evaluate the clinical characteristics and pathologic findings in patients with SGF.

**Methods:** 310 recipients were included. According to estimated GFR at day after transplantation, recipients were categorized into immediate graft function (IGF group, eGFR >60 ml/min) and SGF (eGFR <60 ml/min). Clinical characteristics, pathologic findings, and clinical course were compared between groups.

**Results:** Mean age in the IGF group was younger than the SGF group in recipient age and donor age (p<0.05). BMI ratio of donor to recipient was higher in SGF (p<0.05). BMI ratio of donor to recipient was higher in SGF (p<0.02). Graft rejection in first year (p<0.001). Ten-year patient survival rate was significantly different between groups, but occurrence of acute rejection within one year significantly decreased the long-term graft survival rate in the SGF group compared with the IGF group (74% vs. 97%, p<0.001).

**Conclusion:** SGF observed in early posttransplant period is responsible for decreased long-term grafts and patients survival.

**O-299** COMPARISON OF HISTOLOGICAL LESIONS ON TEN YEAR PROTOCOL BIOPSIES IN KIDNEY TRANSPLANT RECIPIENTS WITH OR WITHOUT CYCLOSPORINE

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**Purpose:** Very few studies have compared long-term lesions on protocol biopsies from kidney transplant recipients who either received cyclosporine or not.

**Methods:** Two pathologists, unaware of treatment group, retrospectively analyzed histological lesions present on protocol biopsies at 10 years in patients who received cyclosporine (n=53) or not (n=93).

**Results:** Mean 10-year serum creatinine was significantly higher in the cyclosporine (CyA) group: 173 versus 113 µmol/L. The glomerulosclerosis percentage was higher in the CyA group (30 versus 17%, p=0.026), as was the percentage of patients with interstitial fibrosis versus 7% in the CyA group (p<0.002). The mean fibrointimal thickening score (CV) was similar in the two groups: 1.7 (CyA group) versus 1.5 (CsA group). The arterial hyaline score was higher in the CyA group (2.0 versus 1.1, p=0.001). In the CyA group 91% of patients displayed AH versus 64% in the other group (p<0.001). In these patients, deposits were strongly under-endothelial in 55% of cases, and subendothelial and muscular in 45% of cases, whereas in cyclosporine-treated patients, deposits were subendothelial and muscular in 71% of cases (p=0.007). If only muscular deposits are considered, the proportion of patients displaying CyA arteriopathy was significantly 28% in the control group and 64% in the CyA group (p<0.001). This pattern of arteriopathy was more frequent in patients treated for hypertension (49% vs. 31%, p=0.04).

**Conclusion:** This unique long-term comparative study shows that 1) lesions suggestive of CyA nephrotoxicity are not universally encountered ten years after transplantation, 2) the specificity of arteriopathy must be questioned since a significant proportion of patients who never received any cyclosporine display muscular arteriolar hyaline deposits.

**O-300** INTERSTITIAL FIBROSIS AND FIBROSUS INTIMAL THICKENING IN DE NOVO RENAL ALLOGRAFTS UNDER SIROLIMUS OR CYCLOSPORINE: RESULTS OF A RANDOMISED, CONTROLLED TRIAL (FIBRASIC)

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Calcineurin inhibitors are a major cause of chronic allograft nephropathy and long-term graft failure. In a prospective, randomised trial of sirolimus (SRL)-versus cyclosporine (CsA)-based immunosuppression in de novo renal allograft recipients, we morphometrically determined the fractional interstitial volume (VvInt) and the arterial intima/media ratio (IM) in implantation and protocol biopsies at 6 months. The concomitant immunosuppression, including dailizumab, steroids and mycophenolate mofetil, was similar in 24 SRL and 21 CsA treated patients. Graft function (eGFR) was evaluated at 6 and 12 months with the MDRD formula (Jellife). At implantation VvInt (SRL: 27±6.4% vs. CsA: 27.2±6.1%) and IM (SRL: 38.8±16.4% vs. CsA: 50.3±40.7%) were comparable in SRL and CsA treated grafts. In contrast, at 6 months VvInt (SRL: 20.9±7.1% vs. CsA: 27.5±5.9%; p = 0.055) and IM (SRL: 27.5±11.3% vs. CsA: 53.3±30%; p = 0.02) were lower in the SRL treated grafts. Graft function at 6 months VvInt (SRL: 50.2±17 ml/min vs. CsA: 50.2±20 ml/min) and at 12 months SRL: 49±13 ml/min vs. CsA: 53±21 ml/min. Thus, Sirolimus appears to protect the renal allograft against the development of interstitial fibrosis and arterial vessel intimal hyperplasia in the early phase after transplantation. However, this beneficial effect was not associated with a superior graft function at 6 months and 1 year. Longer follow-up may be needed to translate the histological improve into better graft function.

**O-301** IMMEDIATE VERSUS DELAYED EVEROLIMUS- BASED IMMUNOSUPPRESSION: COMPARABLE RENAL FUNCTION AND WOUND HEALING COMPLICATIONS IN KIDNEY TRANSPLANT RECIPIENTS AT RISK OF DELAYED GRAFT FUNCTION

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Increased incidences of delayed graft function (DGF) and wound healing complications (WHC) were associated with the proliferation signal inhibitor (PSI) Sirolimus. Everolimus is also a PSI but with a different pharmacokinetic profile. The CALLISTO study reports a 12 month data for WH, DGF and renal function in de novo deceased-donor renal transplant recipients (RTRx) at risk of DGF with either immediate initiation of everolimus 1.5mg/day (IE) post-RTx or delayed everolimus (DE) after 4 weeks of treatment with mycophenolic acid.

**Results:**
Methods: 139 RTxR were randomized to IE (n=65) or DE (n=74) with cyclosporine+steroids+anti-IL-2R antibody. Everolimus target C0 levels were 5-9ng/mL.

Results: Both groups showed similar incidences of DGF (IE 24.6%; DE 33.9%). MedianCrCl (Cockcroft-Gault) was comparable at Month 12 (IE 39.9±11.4mL/min [7.8–98.4]; DE 43.1±11.4mL/min [6.9–92.7]). Maximum creatinine clearance was stable by Week 2 through to Month 12. Median nadir serum creatinine of 138µmol/L (57-637) and 133µmol/L (51-695) was reached within 90 days (IE and DE) respectively. Proteinuria/creatinuria ratio (g/mmol) was similar between the two arms at Month 12 for IE 0.2 (0.1-1.3) and DE 0.3 (0.4-4.5) with 24-proteinuria of 0.2±0.04g/L for both. Sixteen IE and 24 DE patients underwent at least 1 dialysis session (excluding D1). Mean dialysis sessions/patient (5±1-26) IE vs 3±1-12 DE) and median duration of dialysis (11.5 days [1-28] IE vs 5.5 days [1-29] DE) were comparable. WHC were balanced at Month 3 and Month 12 for both arms and almost unchanged from Month 3 onward (Table).

Results:

<table>
<thead>
<tr>
<th>Month 3</th>
<th>Month 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>IE (%)</td>
<td>DE (%)</td>
</tr>
<tr>
<td>DGF</td>
<td>24 (36.9)</td>
</tr>
<tr>
<td>Fluid cl</td>
<td>24 (36.9)</td>
</tr>
<tr>
<td>Wound dehiscence</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Urine leak</td>
<td>2 (3.1)</td>
</tr>
</tbody>
</table>

Conclusion: In renal transplant recipients at risk of delayed graft function the 12-month analysis of the immediate everolimus regimen showed comparable incidences of delayed graft function, wound healing complications and renal function.

EC-MPS is associated with superior efficacy outcomes compared to MMF in de novo kidney transplant patients: a pooled analysis

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Dose adjustment of mycophenolic acid (MMP) is associated with increased risk of kidney allograft rejection and graft loss. Enicat-coated mycophenolate sodium (EC-MPS) delays release of MPA vs MMF and may permit higher dosing in de novo patients, with consequent efficacy benefits.

Methods: A pooled data analysis was undertaken based on 1891 de novo kidney transplant patients receiving EC-MPS (n=1268) or MMF (n=602) with CsA and steroids in multicenter studies ERL B301 (n=423), ERL B2405 (n=1107), RAD B201 (n=196) and RAD B251 (n=196). Entry criteria were consistent between trials. Starting dose was bioequivalent for MMF (2000mg/day) and EC-MPS (1440mg/day). Induction was permitted in ERL B301 as per center practice. Multivariate logistic regression analysis including treatment type was performed to identify other potential explanatory variables (recipient age, gender and race, induction therapy, diabetes at baseline and all variables by treatment interaction).

Results: Using MFM equivalents, mean MPA dose during months 0-12 was similar with EC-MPS (1.6±0.37mg/kg/day) or MMF (1.66±0.29mg/kg/day). On univariate analysis, graft loss, biopsy-proven acute rejection (BPAR) and a composite of death, graft loss or BPAR were each significantly less frequent with EC-MPS at month 12 post-transplant (Table). Multivariate analysis demonstrated a significantly lower rate of all efficacy endpoints with EC-MPS vs MMF (Table). Similar results were observed at month 6 post-transplant. Age by treatment interaction was significant for death, BPAR and the composite endpoint. The incidence of serious adverse events was similar with EC-MPS (56%) versus MMF (53%).

At 12-month follow-up, 30.9% of patients were on 'dual-therapy' Adavgral regimen (5.2% corticosteroids; 25.7% MMF). Kaplan–Meier graft and patient survival and freedom from BPAR were high (98.9%, 100%, and 99.5%, respectively), and corticosteroid-related infections were lower (11.5%). Hypertension (8.4%), increased creatinine (7.3%), and bacterial urinary tract infections, non-insulin dependent diabetes mellitus and hyperlipidaemia (all 5.2%). Creatinine clearance (Cockcroft–Gault) and serum creatinine remained stable throughout (table 2).

Conclusion: These data continue to support the efficacy and safety of once-daily prolonged-release Adavgra in kidney recipients, with excellent graft and patient survival and renal function.

Basiliximab versus Daclizumab combined with triple immunosuppression in deceased donor renal transplantation: a prospective randomized study

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Introduction: In this prospective, randomized, single-center study, we compared the efficacy and safety of two anti-interleukin-2 receptor monoclonal antibodies combined with triple immunosuppression in adult recipients of at least 1 HLA-mismatched deceased donor renal transplant.

Methods: Patients taking cyclosporine microemulsion (CsA-Neoral), mycophenolate mofetil and methylprednisolone were randomly assigned to induction with either basiliximab or daclizumab, given in standard doses. An intention-to-treat analysis of 1-year data assessed incidence of acute rejections, graft function, safety of this therapy, and patient and graft survival.

Results: Two hundred and twelve patients were studied. At 12 months, eleven (10.3%) patients in the basiliximab group and ten (9.5%) patients in the daclizumab group experienced biopsy-confirmed acute rejection. Mean serum creatinine was 104±32 µmol/L in the basiliximab and 107±37 µmol/L in the daclizumab group. The basiliximab and the daclizumab groups had similar incidences of graft loss (5.6% and 9.5%), respectively) and patient death (2.8% and 2.9%). Incidences of infections that required hospital treatment were sim-
EFFECT OF DONOR BONE MARROW CELLS INFUSION ON ALLOIMMUNITIZATION IN KIDNEY ALLOGRAFT PATIENTS

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Objectives: The aim of this study was to investigate the role of donor bone marrow cells infusion in post-transplantation anti-HLA antibody induction and outcome of kidney allograft patients.

Methods: Between June 2006 and May 2007, a total of 40 living donor kidney transplants; 20 recipients with Donor Bone Marrow Cells (DBMC) infusion (2.1 × 10^7–1.3 × 10^8 MNCs/body including 3.5 × 10^7±1.6 × 10^7CD34+ progenitor cells) and 20 without infusion as control, were entered into study and followed prospectively for one year. Both groups received the same baseline immunosuppressant consisting triple drug regimen. WBC cross match, Panel Reactive Antibody (PRA) and HLA-DNA typing were performed for all patients. Pre and post transplantation sera samples were screened for the presence of anti-HLA antibodies, and subsequently antibody identification was determined for positive patients.

Results: Incidence of acute rejection (AR) was 30% in controls versus 15% in DBMI patients. All patients with AR had a pre-transplant anti-HLA antibody in both groups. 35% in DBMI and 30% in controls had pre-transplant antibodies but without acute rejection. In controls, 2 patients with AR and 2 without AR were positive for both Donor Specific Antibody (DSA) and non DSA. All 3 patients with AR in DBMI showed non DSA post operatively, but with a lower strength to HLA antigens. Mean percentages of post-transplant PRA was 16.5% vs. 38.5% in controls. The lower titer of antibodies and lower average serum creatinine were found for patients with AR in DBMI compared to controls.

Conclusion: Infusion of DBM mononuclear cells was perfectly tolerated, but the descending rate of creatinine level was slower than control group. The absence of GVHD and lower percentages of PRA in DBMI group are possible manifestations of functional immune modulation achieved by the DBMC infusion protocol.

O-305

Factors Associated with Graft Loss in Chronic Kidney Transplant Recipients Converted to MMF (CellCept®)

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Introduction: In renal transplantation, there is evidence for the benefit of introducing MMF into the immunosuppressive regimen even some years post-transplantation, in particular in association with CNI reduction and in patients requiring intervention due to progressive renal function decline. Here we explore factors associated with a failure of MMF introduction to save the graft, defined as graft loss or death up to 4 years after intervention.

Methods: TranCept is a prospective, multicenter observational study of patients switched to MMF more than 6 months after transplantation with the objective to document outcomes up to 4 years after switch. In an analysis of 1710 evaluable patients we studied factors leading to graft loss by multivariate Cox regression. Backwards variable selection based on Akaike’s Information Criterion was used to optimize the regression model.

Results: The yearly graft loss rate was 4% in Kaplan-Meier estimates. In the Cox regression (table 1), proteinaemia at the time of switch has a strong and significant association with graft loss, whereas chronic allograft nephropathy did not reach significance (p=0.069). A deteriorating renal function before MMF introduction and a low mean eGFR value were also independently graft loss predictors, as well as time from transplantation to switch (which ranged from 0.5 to about 20 years).

Table 1

<table>
<thead>
<tr>
<th>Hazard ratio</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>CNI: Tacrolimus (vs. CsA)</td>
<td>0.5434327</td>
</tr>
<tr>
<td>Time from Tx to switch [years]</td>
<td>1.0605374</td>
</tr>
<tr>
<td>Pre-switch eGFR slope [ml/min/year]</td>
<td>0.9478793</td>
</tr>
<tr>
<td>Mean pre-switch eGFR [ml/min]</td>
<td>0.9758587</td>
</tr>
<tr>
<td>Donor type: Living (vs. deceased)</td>
<td>0.8203826</td>
</tr>
<tr>
<td>Age at switch [years]</td>
<td>1.0008686</td>
</tr>
<tr>
<td>Donor age [years]</td>
<td>1.0112781</td>
</tr>
<tr>
<td>Gender: male (vs. female)</td>
<td>1.4132710</td>
</tr>
<tr>
<td>Renal function decline as switch reason (vs. others)</td>
<td>1.0516974</td>
</tr>
<tr>
<td>HB at switch [g/dl]</td>
<td>0.8161374</td>
</tr>
<tr>
<td>Proteinaemia at switch (vs. no proteinaemia or unknown)</td>
<td>2.367330</td>
</tr>
<tr>
<td>Pre-switch BPAR (vs. none)</td>
<td>0.6886865</td>
</tr>
<tr>
<td>Biopsy-proven chronic allograft nephropathy (vs. none)</td>
<td>1.6195566</td>
</tr>
<tr>
<td>Interaction CNI: time to switch</td>
<td>1.0553180</td>
</tr>
<tr>
<td>Interaction pre-switch eGFR slope: mean value</td>
<td>0.9996777</td>
</tr>
</tbody>
</table>

Low hemoglobin has also a significant association with graft loss. The CNI type at switch (cyclosporine or tacrolimus), pre-switch biopsy proven acute rejection (BPAR) were not significant predictors (BPAR was significant in an analysis of death-censored graft loss). The explanatory variables that were significant in this analysis remained significant after the variable selection procedure.

Conclusion: In this observational study, proteinaemia and unfavorable renal function evolution prior to switch to MMF-based regimens appear to be the most relevant risk factors for graft loss.