MODERATED POSTERS

Basic Heart Rejection

MP001 DIFFERENTIAL EXPRESSION OF LONG NONCODING RNAS DURING CARDIAC REJECTION

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Background: Organ transplantation is the most effective treatment for end-stage diseases. Although various mechanisms have been suggested that induce immune tolerance during transplantation, the roles of long noncoding RNAs (LncRNAs), which modulate gene expression and regulate innate and adaptive immune responses, are not clearly understood in transplantation. Here we described the role of two essential LncRNAs, lncRNA-A930015D03Rik and mouselincRNA1055, in regulating Th1 response in graft rejection.
Methods: To understand the gene expression and lncRNA profile during transplantation, we performed microarray to profile lncRNA and mRNA of the heart graft and graft-infiltrating lymphocytes (GILs) in allogeneic and syngeneic mouse heart transplantation model. We screened the differentially expressed lncRNAs and mRNAs, and generated the network of lncRNA-mRNA co-expression and computationally predicted their association in transplantation. We further validated the selected T cell related lncRNAs by qPCR, which we identified in GSEA analysis. The functional validation of these lncRNAs in the regulation of Th1 response in transplantation was performed by shRNA-mediated inhibition.
Results: We established a profile of lncRNA and mRNA, which are differentially expressed during transplant rejection in mouse model of heart transplant. Consistent with the microarray results, we have confirmed and validated the expression of seven lncRNA by qPCR. The lncRNA-A930015D03Rik and mouselincRNA1055 were highly expressed in allogeneic heart graft and graft-infiltrating lymphocytes. We further identified that expression of IL-12Rb1 is strongly correlated with lncRNA-A930015D03Rik and mouselincRNA1055 in graft-infiltrating lymphocytes. Further analysis revealed the asso.

Basic Kidney Immunology

MP002 T-CELL AGEING PARAMETERS IN THE LYMPH NODE AND PERIPHERAL BLOOD OF END-STAGE RENAL DISEASE PATIENTS ARE STRONGLY CORRELATED BUT LATE DIFFERENTIATED T CELLS ARE MAINLY LOCATED WITHIN THE CIRCULATION

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Background: Ageing is associated with changes in the peripheral T-cell immune system, which can be significantly influenced by latent cytomegalovirus (CMV)-infection. Therefore, we investigated how changes in circulatory T cells correlate with T-cell composition of the lymph node (LN), which is crucial for a comprehensive understanding of the T-cell system.
Materials and Methods: T cells from peripheral blood (PB) and LN of 38 end-stage renal disease patients were analysed for frequency of recent thymic emigrants (i.e. $CD31^+$ naive T cells and T-cell receptor excision circle (TREC) content), relative telomere length and expression of differentiation markers.
Results: Compared with PB, LN contained relatively more $CD4^+$ than $CD8^+$ T cells ($p < 0.001$). The TREC-content in T cells was significantly higher in the LN ($p = 0.002$). The percentage of naive and central memory (CM) $CD4^+$ and $CD8^+$ T cells and thymic output parameters, showed a strong linear correlation between PB and LN ($p = 0.026$ for CM $CD4^+$ T cells and $p < 0.001$ for the other parameters). Highly differentiated $CD28^{null}$ T cells, being $CD27^-$, $CD57^+$ or $PD\text{-}1^+$ were almost exclusively found in the circulation but not in LN. An age-related decline in naive $CD4^+$ and $CD8^+$ T-cell frequency was observed ($p = 0.035$ and $p = 0.002$, respectively) within LN, concomitant with an increase in central memory $CD8^+$ T cells ($p = 0.033$). Latent CMV-infection dramatically increased the frequency of circulating terminally differentiated T cells, leading to increased frequencies of $CD4^+CD28^{null}$ and $CD8^+CD28^{null}$ T cells showing decreased expression of CD27 and increased expression of PD-1 and CD57. However, this effect of CMV was not observed in the LN-derived T-cell population.
Conclusions: Overall T-cell composition and measures of thymic function in PB and LN are strongly correlated. However, highly differentiated $CD28^{null}$ T cells, which may comprise a large part of circulating T cells in CMV-seropositive individuals, are almost absent from the lymph nodes.

MP003 END-STAGE RENAL DISEASE INDUCES A CONTRACTION OF THE TCR Vβ-REPERTOIRE

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Background: A diverse T cell receptor (TCR) repertoire is crucial for an effective immune response. Patients with end-stage renal disease (ESRD) have a defective T-cell mediated immunity. Recently, we showed by multiplexed DNA-based spectratyping that ESRD may significantly skew the TCR Vβ-repertoire. Here we assessed the impact of ESRD on the TCR Vβ-repertoire within different T-cell subsets using a flow cytometry-based approach with 24 TCR Vβ antibodies.
Methods: Frequencies of 24 TCR Vβ-families were tested by flow cytometry in circulating naive and memory T cell subsets of 10 ESRD patients and 10 age- and CMV-serostatus-matched healthy individuals (HI). In addition, the Gini-index, a measure used in the field of economics to describe the distribution of income, was calculated to determine the level of skewing at the subset level taking into account frequencies of all 24 TCR Vβ-families.
Results: Overall, a contraction of the TCR Vβ-repertoire was observed in most T-cell subsets from ESRD patients compared to HI, but ESRD did not affect one TCR Vβ family in particular. Interestingly, a significant ($p < 0.05$) overall decline in TCR Vβ-clones was observed within the total $CD4^+$, as well as naive and central memory $CD4^+$ T-cell subsets, the latter two being T-cell subsets that are severely depleted in ESRD patients. In ESRD patients, but not HI, the Gini-index of $CD8^+$ T cells was significantly higher than that of $CD4^+$ T cells, indicative of a more profound skewing pattern within $CD8^+$ T cells. Furthermore, increased skewing, as indicated by higher Gini-indices, was observed within the more differentiated T-cell subsets in both ESRD patients and HI.
Conclusion: ESRD is associated with a skewed TCR Vβ-repertoire as a result of an overall decline in TCR Vβ-family usage. This novel finding may have clinical implications for both the risk for acute rejection and infection after kidney transplantation.

MP004 TYPE 1 INNATE LYMPHOID CELLS (ILC1) DECREASE IN KIDNEY TRANSPLANT RECIPIENTS RECEIVING THYMOGLOBULIN FOR INDUCTION THERAPY

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Background: Innate lymphoid cells (ILC) are lymphoid cells which lack antigen-specific receptors and can be classified in three groups (ILC1, ILC2 and ILC3) based on cytokine pattern production. In this study we compare the ILC representation and its subsets in peripheral blood of kidney transplanted recipients at 14th post-transplantation day (KTR14) versus control subjects (CS) in order to identify possible frequency variations in the context of immune alloresponse and immunosuppressive therapy (IT).
Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from 77 KTR14 (13 received only triple therapy, 44 induction therapy with thymoglobulin (TMG) and 20 with Basiliximab) and 32 CS. PBMCs were stained to characterize ILC and its subsets by flow cytometry. Total ILC were identified as CD45+ Lin- (CD3−, CD19−, CD14−) and its subsets were defined as: ILC1 (CD117− CRTH2−), ILC2 (CD117− CRTH2+) and ILC3 (CD117+ CRTH2−). ILC1 and 3 were subdivided according to CD127 and NKp44 expression respectively.
Results:

- Total ILC frequency is similar in KRT14 when compared with CS ($p = 0.4$).
- ILC2 and ILC3 are higher in KRT14 versus CS ($p = 0.002$ and $p = 0.0001$, respectively) whereas ILC1 are lower ($p < 0.0001$) (Figure 1). These differences in ILC subsets frequencies are due to decreasing of ILC1 absolute number whereas ILC2 and ILC3 number are not statistically different between KRT14 and CS.
- ILC1 CD127+ frequency is higher ($p = 0.0004$) whereas NK/ILC1 CD127- frequency is lower ($p = 0.0009$) in KRT14 compared to CS. As regards as ILC3 subtypes, we observed a higher ILC3 NCR- frequency ($p < 0.0001$) and lower ILC3 NCR+ frequency ($p < 0.0001$) in KTR14 than CS.

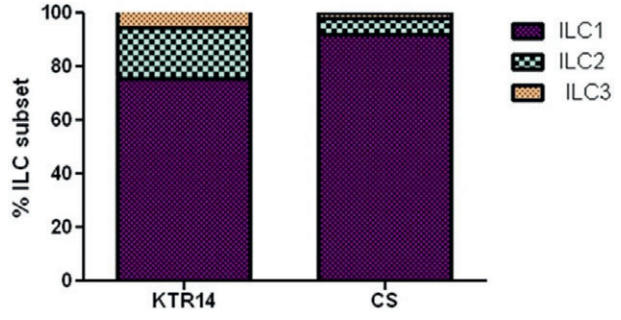

Figure 1. ILC1, ILC2 and ILC3 representation in KRT14 vs CS.

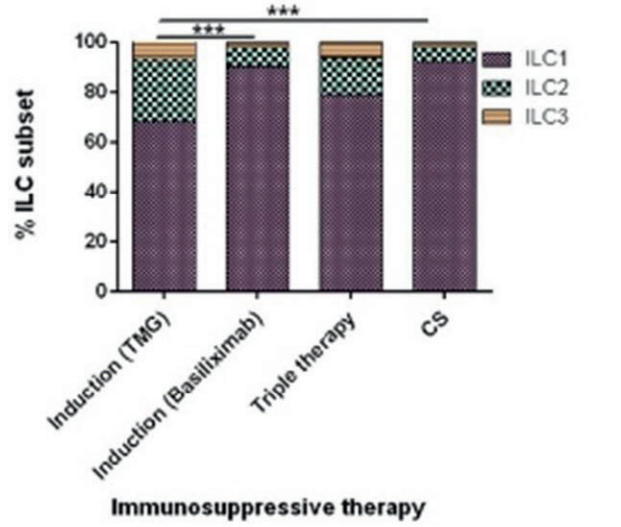

Figure 2. ILC1, ILC2 and ILC3 representation based on KRT14 immunosuppressive therapy vs CS.

• Decreasing of ILC1 in KRT14 was mostly due to patients who received TMG at pretransplant as induction therapy (Figure 2).

Conclusion: Different frequencies in ILC subsets and reduction in ILC1 number are observed in KRT14 versus CS. ILC1 (especially NK/ILC1 CD127−) decrease in KTR14 that receive TMG as induction therapy.

Basic Xenograft Other

MP005 INITIAL EXPERIENCE OF A1,3-GALACTOSYLTRANSFERASE GENE-KNOCKOUT (GTKO) PIG HEART TRANSPLANTATION IN BABOONS

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Background: Due to the shortage of human organ donors, xenotransplantation using the pig represents an alternative solution to the lack of donor human hearts. In the last two decades, significant progression in immunologic and genetic techniques achieved a prolongation of xenograft survival to several months. We performed heterotopic GT-KO pig heart transplantations in monkey (cynomolgus) to study the anastomotic techniques, immunosuppressive therapies.

Methods: We have performed 17 GT-KO pig-to-monkey heterotopic heart transplantations in the abdominal cavity. Baseline immunosuppression consisted of Rituximab, anti-thymocyte globulin (ATG), Anti-CD154, Cobra venom factor (CVF), tacrolimus, mycophenolate mofetil and methylprednisolone. Graft survival was estimated by detecting palpation and portable echocardiography. When recipient was dead or there was evidence suggesting graft failure, we performed an autopsy.

Results: The graft survival ranged from 1 to 43 days, with an average survival of 12.4 days. All grafts resumed a sinus rhythm initially with strong contractions after reperfusion. In initial 3 of the 17 cases, our technical complications occurred, e.g. massive air embolism, bleeding, torsion of graft. Two monkeys died from a peritonitis with a beating donor heart. All grafts developed thrombotic microangiopathy and consumptive coagulopathy. Large amount of hematoma was filled in four chambers.

Conclusion: Although abdominal graft implantation is technically easy and tolerable, other operative techniques, e.g. orthotopic and intrathoracic heterotopic transplantation need to be studied further. It seems to be studied immunologic mechanism about complement and/or coagulation cascades using GT-KO pigs that express human complement regulatory protein (hCRP) genes, such as human membrane cofactor protein (hCD46) or decay-accelerating factor (hCD55).

Basic Kidney Immunology

MP006 CHI3L1 PROTECTS TAA-INDUCED LIVER INJURY VIA REGULATING STAT3-MEDIATED T CELL DIFFERENTIATION

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Background and Aims: Chitinase 3–like 1 (CHI3L1) is a prototypic chitinase-like protein that has been retained over species and evolutionary time. The role of CHI3L1 in liver injury and liver failure has not been defined. This study was designed to dissect the functional role and potential mechanism of CHI3L1 in TAA-induced liver injury.

Methods: Murine liver injury model was established by injection of TAA. CHI3L1 as well as CHI3L1-ko mice were used to determine the role of CHI3L1 in liver injury. To investigate the mechanism of CHI3L1 in protecting liver injury, the level of STAT3 and IFN-r were measured.

Results: The CHI3L1 levels were decreased in patients after liver injury. High levels of CHI3L1 are associated with the improvement of liver function followed by medical treatment. To determine the role and mechanism of CHI3L1 in liver failure, mouse TAA-induced injury model was used. Injection of a recombinant CHI3L1 (rCHI3L1) reduced the liver damage and improved liver function. However, knockout of CHI3LI (CHI3LI KO) resulted in exacerbating liver injury, as evidenced by increasing serum ALT and liver damage histologically. Unlike in controls, rCHI3L1 treatment decreased the expression of T-bet, IFN-r, and Th17 but increased Foxp3 regulatory T cells (Foxp3 + Tregs). CHI3L1 treatment decreased the phosphorylation of STAT3, while CHI3L1 knockout increased STAT3 phosphorylation. Moreover, administration of recombinant mIFN-r or STAT3 inhibitor increased TAA-induced liver injury, which accompanied by increased Th17 and reduced Foxp3 expression in CHI3LI-deficient mice.

Conclusion: This study demonstrates that CHI3LI protects livers against TAA-induced liver injury. CHI3LI-mediated hepato-protection by inhibiting Th17 and promoting Foxp3 + Tregs in a Stat3-dependent manner. Our findings established the regulatory role of CHI3L1 in liver injury, and provides the novel therapeutic targets in liver injury and liver failure.

Basic Kidney Immunology

MP007 IMPACT OF IL-17F GENE POLYMORPHISMS IN OUTCOME OF KIDNEY TRANSPLANTATION IN TUNISIAN RECIPIENTS

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Background: Genetic polymorphisms of IL-17F, associated with functional and/or quantitative change in this glycoprotein have been described as predisposing to various autoimmune diseases. The proinflammatory interleukin-17 (IL-17) has some roles in renal transplantation, in this context, the relationship between the most common IL-17F polymorphisms with acute renal allograft rejection susceptibility in Tunisian renal recipients, has been investigated.

Methods: We examined 93 renal transplant recipients were enrolled and classified in: GI: 48 transplant who developed at least one episode of acute rejection and GII: 45 controls, kidney recipients also followed for at least one year with stable renal function. SNPs of IL-17F gene, including −1507 C/T (rs18889570), 7384 A/G (rs2397084), 7469 C/T (rs11465553), and 7489 A/G (rs763780) were evaluated by direct sequencing.

Results: No statistically significant association of the IL-17 F SNPs studied with the onset of acute rejection was observed. However, AA genotype on 7489A/G SNP showed anti-HLA antibodies less to other genotypes ($p = 0.020$) and a higher graft survival time ($p = 0.017$).
Conclusion: The AA genotype on 7489A/G SNP of IL-17F and the A allele would be associated with a lower risk of acute rejection with better graft survival.

Basic Cell Immunology

MP008 ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS HAVE A HETEROGENIC CYTOKINE SECRETION PROFILE

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Background: The tissue regenerative capacities and the immunomodulatory properties of adipose mesenchymal stem cells (ASC) make them interesting candidates for cell therapy after transplantation. These ASC mediate their functions via cell contact dependent and soluble interactions in which IL-6 and IFN-γ are important. However, ASC cultures are heterogeneous in phenotype and function, which impairs their therapeutic efficacy and offers the opportunity to isolate super-potent, pure ASC populations.
Methods: To determine the ASC heterogeneity, we analyzed the production of IL-6 and IFN-γ by ELISA, the frequency of IL-6 and IFN-γ producing ASC by ELISPOT, the expression of IL-6 receptor (CD126 and CD130 subunits) and IFN-g receptor (CD119) on ASC by flow cytometry, and studied whether this potential heterogeneity was affected by cytokine stimulation.
Results: ASC produced high levels of IL-6 and low levels of IFN-γ. The stimulation of IFN-g or TGF-β had no effect on IL-6 secretion, while TGF-β significantly increased IFN-g secretion. By ELISPOT analysis, it was demonstrated that 100% of ASC were capable of secreting IL-6. IFN-g or TGF-β did not increase the frequency of IL-6 producing ASC. 1.2, 1.4 and 1.7% of IFN-g producing ASC was detected among 4000, 2000 and 1000 seeded ASC, respectively. No CD126 was detected on ASC. A small population (10%) of the ASC expressed CD130, and IFN-γ stimulation resulted in an increased expression of CD130 to 18% of ASC. CD119 was expressed on 18% of ASC. IFN-γ or TGF-β stimulation resulted in a higher percentage of CD119 positive ASC, 26% and 31%, respectively.
Conclusion: These results demonstrate that ASC cultures are heterogeneous in their cytokine secretion and receptor expression profiles. This knowledge can be employed for selection of potent, cytokine-producing and responsive ASC, which is crucial to achieve better outcomes of cell therapy in organ transplantation.

Basic Cell Other

MP009 AN APPROACH IN REGENERATIVE MEDICINE USING TISSUE-ENGINEERED SCAFFOLDS AND MESENCHYMAL STEM CELLS

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Background: Regenerative Medicine approaches use bio-compatible and bio-degradable tissue engineered-scaffolds in combination with appropriate cells. Chitin is a naturally derived scaffolds has good mechanical stability, promotes wound healing, and is a candidate for replacement of tissue Damaged.
Aim: We have previously designed collagen-based hydrogels using human bone marrow derived mesenchymal stem cells (MSCs). The aim of this study was to use chitin-based tissue-engineered Wharton's jelly (WJ) of human umbilical cord MSCs, which can be obtained by a less invasive method, without posing harm to the mother or infant. The viability and function of the stem cells on the hydrogel construction were then investigated.
Methods: The WJ-MSCs were obtained from healthy infants-umbilical cords. The cells were isolated and characterized by cell surface marker, and differentiation potential. Chitin-porous scaffolds were prepared by dissolving the chitin solution in acetic acid. The morphology and biological effects of MSCs on chitin-based scaffold were investigated by scanning electron microscopy and cell proliferation assay.
Results: The cell-analysis showed viability between 98% and 100%. The cells were characterized by immunophenotyping marker CD34, CD80, CD86, and HLA DR, and differentiation potential into osteocyte and adipocyte. The result of scanning electron microscopy showed that MSCs could tightly adhere to chitin scaffold just shortly after seeding. The tissue-engineered construction induced cell-proliferation throughout the incubation period.
Conclusion: In this study, we have developed an in-vitro construction of transplantable chitin-based tissue-engineered disks, using WJ-MSCs for replacement of tissue damaged, has the potential to improve tissue defects in future.

Basic Kidney Immunology

MP010 B CELL POPULATION CHANGES AFTER THYMOGLOBULIN INDUCTION IN KIDNEY TRANSPLANT RECIPIENTS

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REDINREN RD16/0009/0027 from ISCIII-FEDER
Background: Humoral response has been recognized as the main responsible of kidney allograft losses. B cell production of donor specific antibody may be an important cause of allograft loss, but, in contrast, some B cell subpopulations relate to operational transplant tolerance. Rabbit antithymocyte globulin (rATG) is widely used for immunosuppressive induction in kidney transplant patients. Although in vitro studies have shown that rATG induces apoptosis of naïve, activated B cells and bone marrow plasma cells, the influence of rATG on B cell population in vivo is not completely defined.
Methods/Materials: Thirty-five patients undergoing kidney transplantation in our center were prospectively included from 2015. Patients who received Rituximab were excluded. Whole blood samples were taken before kidney transplantation and at 6 and 12 month post-transplantation. T and B populations were acquired by flow cytometry.
Results: Some 20 (57.1%) patients received rATG as induction therapy. Total lymphocytes (1530 vs. 928/μl, $p = 0.002$), CD3 (1235 vs. 683/μl, $p = 0.002$) and CD4 (718 vs. 171/μl, $p < 0.001$) significantly decrease at 6 month only in the group of patients who receive rATG. By contrast, CD19 population did not change at 6 month (121 vs. 97/μl, $p = 0.191$) and 12 month ($p = 0.546$) after rATG induction. One-year plasmatic cells remained significantly lower than pre-transplantation only in patients who received rATG (rATG 40 vs. 10/μl, $p = 0.007$, non-rATG 39 vs. 33/μl, $p = 0.463$). Other B cell subpopulations (naïve, unswitched/switched memory B cells, mature/immature) did not change significantly after rATG induction therapy.
Conclusion: rATG mainly exerts its strong immunosuppressive effect through T cell depletion. Peripheral plasmatic cells remain significantly low at 1-year after rATG induction, a finding previously reported in tolerant patients. By contrast, we did not find a significant effect of rATG induction on other B c.

MP011 URINE EXTRACELLULAR VESICLES FROM LIVING AND DECEASED KIDNEY DONORS REVEAL SIGNIFICANT DIFFERENCES AT THE PROTEOMIC AND MIRNA LEVELS

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Background: Chronic kidney diseases include diverse pathological processes leading to irreversible kidney failure, for which kidney transplantation (KTx) is the best therapeutic approach. Considering the origin of the graft, several studies have reported differences between living (LD) and deceased donors (DD) in graft and patient survival. Here, we have analysed the urine extracellular vesicles (uEVs) from LD and DD, with the aim to provide data on the EV-based status of the organ before nephrectomy.
Methods: Protein and miRNA content of size-exclusion chromatography isolated Urine EVs were analysed by liquid chromatography followed by mass spectrometry and next generation sequencing, respectively.
Results: EVs from DD contained less miRNAs than LDs-EVs, although most of the DD- expressed miRNAs were shared by LDs (96%). Noticeably, all samples contained miRNA-151b, a previously unreported miRNA in uEVs. Proteomics revealed that LD and DD only shared a 60% of the identified proteins. Major differences between groups were mainly due to overexpressed inflammatory molecules in DD.

Conclusions: The proteomic and miRNA profiles of uEVs may be indicative of the status of the organs, pointing to uEV analyses as a useful tool to evaluate the status of the organ before nephrectomy.

Basic Composite Tissue Immunology

MP012 VASCULOPATHY AND ENDOTHELIAL CELL RESPONSE IN VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

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Background: Vascular repair mechanisms are of pivotal importance for allograft survival and function. However, endothelial cell (EC) response has not been systematically investigated in vascularized composite allotransplantation (VCA), a unique type of graft composed of several tissues. Therefore, in this study we characterized the EC response in VCA and analyzed the role of recipient and donor bone marrow derived endothelial progenitor cells (EPC).
Methods: Orthotopic hind limb transplantations were performed and different clinical settings analyzed: (i) absence of immunoreaction (isograft); (ii) acute rejection (untreated allografts) and (iii) immunosuppressive therapy (1 mg/kg Tacrolimus, daily). Blood was collected weekly to measure the levels of plasmatic inflammatory and angiogenic cytokines and the numbers of EPC ($CD34^+KDR^+CD45^{low}$ cells) or circulating endothelial cells (CEC, $CD31^+CD45^-$ cells). At rejection or 30 days after transplantation, graft skin and muscle were analyzed to characterize tissue vasculopathy and EC response.
Results: Isografts were characterized by a mild release of cytokines without EPC and CEC response or tissue vasculopathy. Conversely, untreated allograft resulted in a massive release of cytokines with a significant increase of the numbers of CEC (mainly of recipient origin) and EPC (of donor and recipient origin). EC activation was parallel by a massive tissue vasculopathy and immunoglobulin deposition. Immunosuppressive therapy could significantly reduce EC activation, cytokines release and tissue vasculopathy. Interestingly, the therapy could significantly increase circulating EPC of donor and recipient origin.
Conclusion: Our data show that in VCA, similarly to SOT, EC damage is a main characteristic of acute rejection. This is paralleled by an increase of both inflammatory and angiogenic cytokines as well as by the release of CEC and EPC. Immunosuppressive therapy is able to mitigate EC damage promoting EC repair through the release of EPC.

Translational Liver Biomarkers and molecular changes

MP013 INTRAHEPATIC MICRORNA PROFILE OF LIVER TRANSPLANT RECIPIENTS WITH HEPATITIS C VIRUS CIRRHOSIS CO-INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS

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In patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) co-infection, HIV can modulate HCV replication and immune response as well as accelerate liver fibrosis. Recent studies have revealed that certain microRNAs (miRNAs) are responsible for the modulation of viral infection, replication, and host immune response. However, the role of miRNA in HIV/HCV co-infection is not fully elucidated. The aim of this study was to examine the differential expression of miRNAs in the liver, using samples from 20 patients who had a liver transplant (10 HCV-infected, 10 HIV/HCV-co-infected patients). Microarray revealed 22 miRNAs that were differentially expressed in the HIV/HCV-co-infected group compared with the HIV-infected group ($p < 0.05$). To confirm the microarray results, real-time polymerase chain reaction (PCR) was performed on miR101b, selected based on microarray results and biological significance in liver fibrosis. The expression of miR-101b was significantly decreased in the HIV/HCV-co-infected group than in the HCV-infected group (0.103 ± 0.09 vs. 0.0157 ± 0.0093, $p = 0.007$). In conclusion, HIV/HCV co-infection may promote liver fibrosis by modulating miRNA expression.

Clinical Kidney Biomarkers and molecular changes

MP014 PREDICTIVE ROLES OF PROINFLAMMATORY CYTOKINES IN ACUTE RENAL ALLOGRAFT REJECTION

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Background: Recent studies have suggested that increased levels of IL-18 in serum and renal parenchyma may predict acute rejection in patients with renal transplantation. This prospective observational study aimed to assess the relevance of serial interleukin-18 (IL-18) and Interferon gamma (IFN-γ) measurements for predicting renal allograft rejection in the first three years transplantation.
Patients and Methods: This study included 71 renal transplant recipients from living related donors. Blood and urine samples were collected immediately before and after transplantation at day 7 and month 1. Serum IFN-γ, IL-18, creatinine, cystatin C and urinary IL-18, cystatin C and creatinine levels were measured. Patients were assigned to 2 groups depending on their history and clinically diagnosed acute rejection [AR(+)] and without acute rejection [AR(−)].
Results: Among 71 recipients, 12 had AR(+) and 59 had AR(−). Serum IL-18 levels on day 7 and month 1 were higher in the AR (+) group, but the difference was not significant (249.2 ± 114.4 vs. 184.6 ± 87.8; 230.4 ± 91.9 vs. 197.8 ± 88.9, $p > 0.05$). Serum IFN-γ and urinary IL-18 levels were not significantly different. Serum creatinine and cystatin C levels on day 7 and month 1, urine cystatin C level on day 7, urine IL-18/ creatinine ratio on month 1 were significantly higher AR (+) group compared to AR (−) ($p < 0.001$, $p = 0{,}017$ for creatinine, $p < 0.001$ for cystatin C, $p = 0.006$ for urine cystatin C, $p = 0.018$ for urine IL-18/creatinine).
Conclusion: Proinflammatory cytokines measured in the serum at the early post-transplant period did not play a significant role in predicting the acute rejection observed within the first three years. Our findings suggest that well-known parameters such as creatinine and cystatin C still have a high predictive role in rejection diagnosis. We have shown that urine IL-18/creatinine ratio may be use an early, noninvasive biomarker of predicting the acute injury.

Translational Kidney Biomarkers and molecular changes

MP015 MICRORNAS DIFFERENTIATE BETWEEN ANTIBODY AND T-CELL MEDIATED RENAL ALLOGRAFT REJECTION

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Background: MicroRNAs are important regulators in encoding most genes and proteins. MicroRNAs has been associated with allograft rejection in kidney transplantation. We aim to investigate microRNA expression in different histopathological diagnosed kidney allograft rejection tissue. Analyse the differential expression levels and the cell source of the microRNA.
Methods: Microarray experiments and semiquantitative real-time reverse transcription polymerase chain reaction (QPCR) were performed with the total RNA isolated from 46 fresh rejecting kidney tissue. Initial microarray analysis and literature showed 5 microRNAs which were validated with QPCR. Cell location was determined using specific microRNA in situ hybridization on formalin fixated paraffin imbedded corresponding kidney biopsies from the same patients.
Results: Expression levels of miR-155 and miR-21 was significantly down-regulated in antibody mediated rejection group compared to acute cellular rejection whereas miR-195 was upregulated. miR-21 was significantly down-regulated in transplant glomerulopathy group compared to antibody mediated rejection group.
Conclusions: It has been reported that miR-155 is associated with the monocyte-macrophage lineage cells, as well as miR-21 has been described to be associated with renal fibrosis. Our results show that miR-155 and miR-21 are associated with rejection.

Translational Kidney Biomarkers and molecular changes

MP016 INFLUENCE OF KIDNEY DONOR REMOTE ISCHEMIC PRECONDITIONING ON DELAYED GRAFT FUNCTION BIOMARKERS

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Remote ischemic preconditioning (RIPC) is postulated to have protective impact on kidney allografts. The proposed mechanism involves systemic immune activation counterbalancing ischemia-reperfusion injury. Donor-performed RIPC-induced changes in delayed graft function (DGF) biomarkers were studied.
Material/Methods: 46 deceased donors (age 46.9 ± 15.9 years, 21F/26M) were included in the study. RIPC was performed by provoking lower limb ischemia through temporal closure of iliac artery in 17 donors (age 49.2 ± 18 years, median 58 years, 7F/10M). Control group included 29 donors (age 45.3 ± 15.0 years, median 46 years,14F/15M). Kidneys were received by 88 recipients (age 50.5 ± 13.9 years, 30F/58M). NGAL, IL-18, and clusterin were assessed by ELISA in serum and urinary samples obtained from donors before and after RIPC procedure preceding organ retrieval.
Results: The background serum/urinary levels (median, IQR) of IL-18 (476, 322–627 pg/ml/431, 99–683 pg/mg SCr) and NGAL (361, 200–500 ng/ml/279, 64–376 ng/mg SCr) were not affected by RIPC. Clusterin was increased in post-RIPC urine samples (2163, 1775–2616 vs. 946, 569–1160 ng/mg SCr; $p = 0.043$), while decreased in serum (199, 147–231 vs. 223, 185–279 pg/ml; $p = 0.050$).

Pre-retrieval serum IL-18 positively correlated to graft function up to 6 months post transplantation (eGFR ml/min, 3-month $r = 0.45$, $p = 0.003$; 6-month $r = 0.37$, $p = 0.023$), while urinary IL-18 negatively correlated with short-term function (eGFR ml/min, 7-day $r = -0.29$, $p = 0.029$; 1-month $r = -0.35$, $p = 0.017$). Urinary NGAL influenced both short- and long-term graft function (eGFR ml/min, 7-day $r = -0.31$, $p = 0.021$; 1-month $r = -0.45$, $p = 0.002$, 2-year $r = -0.56$, $r = 0.003$). Clusterin levels were not associated with graft function. DGF incidence was not related to any biomarker level.
Conclusion: Pre-retrieval donor IL-18 and NGAL levels are predictive of kidney allograft function. RIPC performed in kidney donors did not induce major changes in DGF biomarkers' levels.

Translational Liver Biomarkers and molecular changes

MP017 EARLY ENRICHMENT AND RESTITUTION OF THE PERIPHERAL BLOOD TREG POOL IS ASSOCIATED WITH REJECTION-FREE STABLE IMMUNOSUPPRESSION AFTER LIVER TRANSPLANTATION

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Background: Many attempts have been made to predict solid organ transplant outcomes by making point-measurements of immunological parameters in peripheral blood. A surprising and common feature of these studies has been the limited predictive value of mechanistically important biomarkers (e.g. Tregs) taken in isolation. This is partly explained by biologically relevant changes in such biomarkers being dwarfed by natural variation between individuals or time-points. Also, changes in donor antigen-reactive lymphocyte subset frequencies may be very small.
Methods: Blood samples were collected from 28 orthotopic liver transplant recipients on conventional triple immunosuppression. 9/28 had experienced one or more acute rejection episodes within one year of transplantation. Serial estimates of peripheral blood $CD4^+$ $CD25^+$ $CD127^{low}$ $FoxP3^+$ Treg frequencies were made by flow cytometry.
Results: Patients who did not experience a rejection episode during the first year after transplantation exhibited an early, significant enrichment of Treg within their total $CD4^+$ T cell compartment, which decayed to pre-transplant levels within 8 weeks. By contrast, no relative increase in Treg frequency was observed in patients registering one or more rejection episodes during the first year post-transplant.
Conclusion: Peaking at 2–3 weeks, the kinetics of Treg enrichment in non-rejectors is suggestive of a primary T cell response. Hence, we propose the early Treg enrichment phase represents a polyclonal activation and expansion of Treg, whereas the subsequent decay might reflect selection of a subset of donor antigen-reactive clones. In future, "high granularity" immune monitoring studies that capture kinetic information about evolving immune responses in the early post-transplant period may be particularly useful in predicting long-term transplant outcome.

Translational Kidney Biomarkers and molecular changes

MP018 KIDNEY AUTO-TRANSPLANTATION IN A DCD SWINE MODEL: EVALUATION OF FUNCTIONAL RECOVERY BY METABOLIC AND OXIDATIVE STRESS PROFILE DETERMINATIONS BY NUCLEAR MAGNETIC AND ELECTRONIC PARAMAGNETIC RESONANCE

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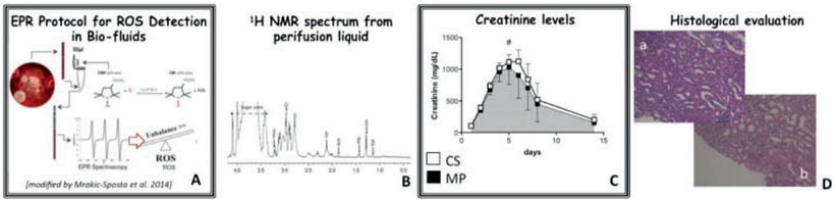
Introduction: Nowadays kidneys from DCD are considered worldwide to be a major resource to satisfy the need of enlarging the number of kidneys suitable for transplantation.
Methods: In order to mimic the condition of a DCD donor in a swine model (10 pigs), the left renal artery was isolated and clamped for 75 min. The kidney was then harvested, flushed with IGL-1 and stored for 8 hours in either Cold Storage (CS) or Machine Perfusion (MP) accordingly. Oxidative stress levels - oxygen radical species (ROS) quantitative measurement- by EPR (Electronic Paramagnetic Resonance) technique and the metabolic profile assessment by NMR (Nuclear Magnetic Resonance) technique were carried out every hour on perfusion solution samples. After 8 h the kidney was autotransplanted and a right nephrectomy was performed. All transplanted animals were observed for 14 days; creatininemia, and oxidative stress in plasma (ROS, protein carbonils (PC) and Thiobarbituric Acid Reactive Substances (T-bars) were assessed daily, metabolomics was performed on kidney biopsies collected before, after the preservation period and at the end of the observation.
Results: Function recovery in MP group (versus CS) was faster, ROS production, PC and T-bars levels were significantly lower and metabolites levels measured in the perfusate during the storage were significantly higher ($P < 0,05$).
Conclusions: All together these preliminary data confirm the importance of using the hypothermic pulsatile machine perfusion in renal conditioning in an animal model and suggests the potential significance of transferring such an experimental protocol to the human model. Furthermore the NMR-based profiling of metabolic content of perfusates proved predictive.



MP019

PROTEOMIC PROFILES OF DECEASED DONOR KIDNEY BIOPSIES OBTAINED PRIOR TO TRANSPLANTATION CORRELATE WITH ALLOGRAFT FUNCTION AT ONE YEAR

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Introduction: Cerebral injury during Donation after Brain Death (DBD) will induce a systemic inflammatory response affecting immediate kidney function and survival posttransplantation. Assessment of donor organ quality prior to transplant that can predict transplantation outcomes will improve donor organ utilisation and provide monitoring tools for novel targeted interventions.
Methods: DBD kidney biopsies obtained at retrieval (n = 38) were provided by the UK QUOD biobank. Biopsy samples were selected from donors from whom both kidneys were transplanted and had the same posttransplantation outcomes; either suboptimal (SO) or good (GO). Kidneys with SO (n = 19) had all developed delayed graft function (DGF) and had a mean 1 year eGFR = 35 ± 6 ml/min. Kidneys with GO (n = 19) had all functioned immediately and had a mean 1 year eGFR = 73 ± 18 ml/min. Demographical confounders for outcome include age, cold ischaemia, AKIN and Remuzzi scoring were matched. Samples were analysed by clinical proteomics (n = 10) and candidate markers then validated by immunoblotting on a separate cohort of donor kidney biopsies (n = 28).
Results: Remuzzi scoring and AKIN classification showed no evidence of acute or chronic kidney injury in the donor kidneys. However, proteomic analysis could differentiate between the donor kidneys that had SO from those with GO posttransplantation (Figure 1). Validation of candidate markers supported this discrimination and showed that degradation of glomerular basement membrane proteins, pro-fibrotic and apoptotic markers correlated with SO allograft function whilst increased antioxidant protein levels were associated with GO allografts.
Discussion: This study suggests that proteomic profiling of pretransplant donor kidneys may correlate with posttransplant outcomes at 1 year. Specific proteins can be identified and validated as potentially clinically relevant biomarkers.

Clinical Kidney Biomarkers and molecular changes

MP020

URINARY LIVER FATTY ACID BINDING PROTEIN REFLECTS PATHOLOGICAL TUBULO-INTERSTITIAL STATUS IN KIDNEY TRANSPLANT RECIPIENTS WITH STABLE GRAFT FUNCTION

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Background: For the early detection of rejection, toxicity of calcineurin inhibitor (CNI) or recurrent nephritis, we regularly undertake protocol renal allograft biopsy at 1, 3, 5 and 10 years after kidney transplantation. Because renal allograft biopsy is an invasive examination, it is ideal to be able to predict renal allograft status from some blood or urinary biomarkers. Urinary liver fatty acid binding protein (L-FABP) has been evaluated as a promising early biomarker of renal ischemia in human kidney. In some reports, urinary L-FABP predicts early renal allograft function. In this study we examined whether urinary L-FABP level predicts some pathological findings of renal allograft.
Methods: We prospectively enrolled 32 recipients with stable graft function who underwent protocol renal allograft biopsies at 1, 3, 5 or 10 years after kidney transplant (KT) between March 2016 and February 2017 in our center. We assessed the association L-FABP level in urine sampled at the same time of protocol biopsy with pathological findings of renal allograft biopsies. The concentration of L-FABP in urine samples were measured by enzyme immunoassay.
Serum creatinine
Results: In 8 (25%) of 32 patients, abnormal high value of urinary L-FABP were observed (33.5 (13.5-112)). Five (15.6%), 5 (15.6%), 2 (6.3%), 4(12.5%) and 3 (9.4%) patients were pathologically diagnosed with moderate interstitial fibrosis and tubular atrophy (IFTA), T cell mediate rejection (TMR), medullary ray injury induced CNI toxicity (MRI-CNI), recurrent IgA nephropathy (IgAN) and transplant glomerulopathy (TG). The urinary L-FABP level is significantly higher in patients with pathological tubulo-interstitial lesions (moderate IFTA, TMR and MRI-CNI) than in patients without pathological tubulo-interstitial lesion (26.7 vs. 4.2). But the difference in patients with and without pathological glomerular lesions (TG and IgAN) was not significant.
Conclusions: XXXX

Translational Liver Biomarkers and molecular changes

MP021

DEVELOPING A NON-INVASIVE SPECTROSCOPIC TECHNIQUE FOR DETECTING LIVER DAMAGE

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Background: With an increased utilization of extended criteria donors, there is a need for a detailed assessment of the liver grafts prior to transplantation. Furthermore, a real-time quantitative means of determining liver quality is lacking. Here, we present preliminary developments of a non-invasive, label-free [OG1] method for detecting liver damage.
Methods: The baseline ischemic injury was determined using a two-hour warm ischemia ($n = 1$). A porcine model of donation after circulatory death (DCD) with 45 minutes of warm ischemia was developed. *In situ* spectral data from eight pigs was obtained prior to cardiac arrest (CA) and at the end of warm ischemia using a 785 nm handheld Raman spectrometer. A minimum of two readings were obtained from the right lobe and quadrate lobe in all pigs. Liver and bile duct biopsies before CA and after warm ischemia were collected for analysis using nuclear magnetic resonance spectroscopy (NMR), immunostaining and Raman microspectroscopy.
Results: NMR spectroscopy was used to detect small metabolites in the tissue biopsies and the relative concentrations of a number of metabolites appears to correlate with the degree of ischemia (Figure 1). NMR combined with Raman detects morphological and biomolecular variations and Raman microspectroscopy identified cholangiocytes and lipid droplets in a label-free manner. Spectral readings taken in real time using handheld Raman showed a significant change after 45 minutes of warm ischemia, as determined by one-way ANOVA ($F(1,38) = 9.78$, $p < 0.01$).
Conclusion: Raman spectroscopy is a promising non-invasive technology that may provide real time information about the metabolic function of the liver grafts prior to transplantation.

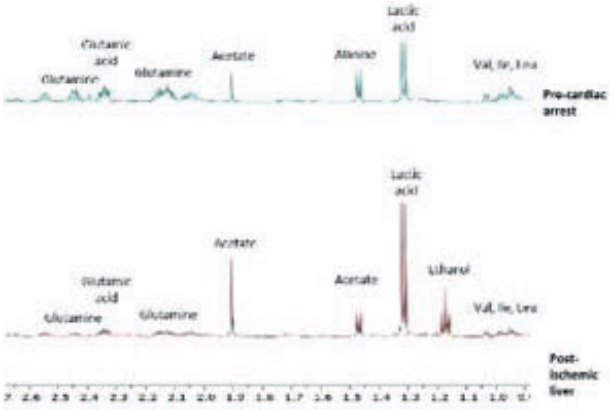

Figure 1. Spectroscopic metabolic signatures from liver biopsies pre-cardiac arrest and post ischemia.

Clinical Kidney Biomarkers and molecular changes

MP022

CARDIAC BIOMARKERS IN STABLE KIDNEY TRANSPLANT RECIPIENTS

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Background: Brain natriuretic peptide (BNP) and high sensitivity cardiac troponin I (hs-cTnI) are known cardiac biomarkers, but circulating galectin-3 (Gal-3) is a novel biomarker that reflects cardiac remodeling and fibrosis. It is

published that levels of all these biomarkers are increased in patients with renal dysfunction. Therefore it is challenging to interpret results of these tests among kidney transplant recipients (KTRs). The aim of study was to assess BNP, cTnI and Gal -3 levels in stable renal transplant patients along with transplant data and left ventricular ejection fraction (LVEF) as a marker for heart failure.
Methods/materials: We measured BNP, cTnI and Gal-3 levels in EDTA anticoagulated plasma samples at the same time point as serum creatinine during regular outpatient visits. An eGFR was calculated by equation of the Modification of Diet in Renal Disease Study. Four groups of patients were defined as following: Group 1 (eGFR >60 ml/min), Group 2 (eGFR 45–59 ml/min), Group 3 (eGFR 30–44 ml/min) and Group 4 (eGFR <30 ml/min). ANOVA was used to compare means among groups.
Results: This cross-sectional study included 71 white KTRs (mean age of 49.7 ± 1.5 years, mean body mass index 25.79 ± 0.56 kg/m^2). 48% were men, 7% had living donor, 16% had history of diabetes mellitus and 17% had history of cardiovascular disease. Mean serum creatinine was 139 ± 7 mmol/l, mean eGFR 48 ± 2.41 ml/min per 1.73 m^2 with mean kidney graft vintage 66 ± 8 (2–294) months and renal replacement therapy vintage 108 ± 9 (6–298) months. LVEF for Group 1, 2, 3, and 4 was 58%, 62%, 60% and 62%, respectively (p = NS). Mean levels of cardiac biomarkers are shown in table 1.
Conclusions: Kidney transplant dysfunction does not cause significant difference in levels of traditional cardiac biomarkers (BNP and hs-cTnI) and any increase in their levels should be interpreted the same significant as for patients without kidney disease. Galectin-3 as a new biomarker needs further evaluation.

Biomarker	Group 1	Group 2	Group 3	Group 4	p value
BNP (ng/ml)	250 ± 519	236 ± 650	74 ± 58	76 ± 140	NS
Hs-cTnI (ng/ml)	17 ± 36	177 ± 767	7 ± 6	6 ± 7	NS
Gal-3 (ng/ml)	25 ± 11	22 ± 10	17 ± 41	14 ± 3	.004

MP023 RELATIONSHIP BETWEEN GENE VARIABILITY OF ANTIOXIDATIVE ENZYMES AND RENAL FUNCTION DECLINE IN RENAL TRANSPLANT RECIPIENTS: TWO YEAR POST-TRANSPLANT FOLLOW-UP

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Background: Variability in genes encoding antioxidant enzymes may cause an interindividual difference in patients' susceptibility to oxidative damage. Whether this variability or not have an influence on graft function after renal transplantation (RT), it is not elucidated in its entirety. The aim of the study was to investigate the influence of the manganese superoxide dismutase (MnSOD) Ala16Val and glutathione peroxidase 1 (GPX1) Pro200Leu gene polymorphisms on estimated glomerular filtration rate (eGFR) in two year follow-up RT. Also, we analyzed whether investigated gene polymorphisms or not, could make graft more prone to potential tacrolimus (TAC) nephrotoxicity.
Methods/Materials: The study included 72 stable renal transplant recipients, who were on TAC based immunosuppression. The genotyping of MnSOD and GPX1 gene polymorphisms was performed by PCR–RFLP method and eGFR was calculated by the MDRD formula. Univariate linear regression analysis was performed with eGFR as dependent and TAC daily dose (TDD) as independent variable in order to estimate potential influence of these polymorphisms on TAC mediated renal function decline.
Results: The results of the study showed that only carriers of both variant alleles, Val of MnSOD (Ala/Val; Val/Val genotype) and Leu of GPX1(Pro/Leu; Leu/Leu genotype) gene polymorphism, had more pronounced decline of eGFR between the first and second year post–transplant compared to other patients($p < 0.05$). Additionally, the univariate regression demonstrated that TDD negatively affected eGFR in patients who carried both variant alleles, within two years after RT(R squared 6.7%; $p < 0.05$).
Conclusion: Our findings suggest that tested polymorphisms may represent significant predictors of renal function decline. Furthermore, reduction in graft function may be partly explained by potential TAC nephrotoxicity. Implementation of MnSOD and GPX1 genotyping in clinical practice may provide an identification of high risk patients for graft loss.

MP024 COULD URINARY BIOMARKERS SUCH AS NAG, NGAL AND KIM-1 HELP FOR THE EVERYDAY CLINICAL KIDNEY TRANSPLANTATION?

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Kidney transplantation (KT) is the best choice for patients that suffer End Stage Renal Disease (ESRD), because of the better survival rate compared to patients under dialysis. Our objective should be to improve allograft survival by acting in a preemptive way.
Methods: We analyzed the urinary biomarkers NAG (N-acetyl glucosaminidase), NGAL (Neutrophil Gelatinase associated Lipocaine) and KIM-1 (Kidney Injury Molecule-1) in urine samples from 70 kidney or kidney and pancreas transplant patients. The samples were taken at post-transplant days +1, +3, +5, +7, and the day of renal stabilisation in the first 3 months. The biomarker levels were related to the different complications after KT, such as acute rejection, delayed graft function (DGF), cold ischemia time, toxic drugs for kidney and the renal function on the day of stabilisation. The statistical test used was Pearson x2. The 70th percentile was taken and a significant value was established if $p < 0.05$. The statistical program used was IBM SPSS.
Results: During the first week after KT, in theory renal function improves as measured by creatinine levels. However, increasing levels of the urinary biomarkers NAG, NGAL and KIM-1 indicate possible tubular damage or other pathology of the kidney.

NGAL has shown to be a more sensitive marker than NAG and KIM-1 because it increases more than the other markers from the first day after KT.

Urinary values of NGAL at post-transplant day 7 predict statistically significant probability of suffering DGF.

Low NAG values at post-transplant day 3 have been related to a cold ischemia time of between 120 and 900 minutes, while high values in the first week post-transplant predict more renal function stabilisation time; the same occurs with NGAL.

In this study KIM-1 did not show any relationship with the events described above.
Conclusions: The use of the urinary biomarkers NAG and NGAL could help us to act in a preemptive way to avoid the KT complications and improve KT global results.

Basic Composite Tissue Other

MP025 ENGINEERING AN ENDOCRINE NEO-PANCREAS BY REPOPULATION OF A DECELLULARIZED RAT PANCREAS WITH ISLETS OF LANGERHANS

Benjamin Struecker[1], Hendrik Napierala[2], Nils Haep[2], Peter Tang[2], Madeleine Tintemann[2], Joseph Gassner[2], Max Noesser[2], Hannah Everwien[2], Dietrich Polenz[2], Steffen Lippert[2], Dominik Geisel[2], Anja Reutzel-Selke[2], Nathanael Raschzok[2], Johann Pratschke[2], Igor Sauer[2]
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Generation of functional endocrine Neo-Pancreata appears possible by decellularization of pancreata and repopulation of the extracellular matrices with islets and endothelial cells. We decellularized rat pancreata by perfusion with detergents and repopulated the decellularized matrices with intact islets. Decellularization was effective and comparable via three different perfusion routes (arterial, portal venous and ductular). Interestingly, repopulation of the matrices with whole islets was significantly better, when islets were infused via the pancreatic duct. Despite their magnitude, islets leaked from the ductular system into the peri-ductular decellularized space. Neo Pancreata were then perfused extracorporeally to evaluate viability and functionality of the grafts by Glucose stimulated insulin secretion and TUNEL staining. To the best of our knowledge, this is the first study to compare three different methods for perfusion decellularization of rat pancreata. Furthermore, we show proof-of-concept for the repopulation of decellularized pancreata with functional islets of Langerhans. The presented techniques is a significant step toward the ex vivo generation of functional endocrine pancreata and can serve as a bioengineering platform for further experiments.

Clinical Composite Tissue Other

MP026 RESULTS OF THE USAGE OF BIOLOGICAL MESH IN THE REPAIR OF COMPLEX ABDOMINAL WALL DEFECTS IN PATIENTS POST TRANSPLANTATION AND GENERAL SURGERY. THE FEASIBILITY OF USING MRI IN FOLLOW UP

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Infant Jesus Teaching Hospital, Poland

Immunosuppressive therapy, inflammation, and surgical site or general infection make the use of traditional methods for abdominal wall closure ineffective and preclude the application of synthetic mesh. In such difficult cases, the utilisation of the biological material, such as PermacolTM, a porcine acellular dermal collagen implant, may be a solution. The purpose of this study was to analyse the use of biological mesh in patients in whom the closure of abdominal wall defect with other methods was not possible and to compare outcomes in patients after transplantation and general surgery. The study group consisted of 14 patients, including 6 patients after transplantation. The evaluation of wound healing was based on a clinical examination and in selected patients on magnetic resonance imaging (MRI) that is an excellent examination however it should not be considered as an examination of choice in such cases. Only 3 uneventful outcomes were observed. Biological meshes may be used in patients in whom other ways of treatment had failed; still a prolonged time of wound healing should be expected. It seems, that it is safe to use PermacolTM in post transplantational patients. Implanting PermacolTM and negative pressure wound therapy can be combined.

MP027 PARATHYROID ALLOTRANSPLANTATION FROM CADAVERIC DONOR: CASE STUDY

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Background: Permanent hypoparathyroidism (PH) is a clinical condition accompanied by low parathormone (PTH) levels. Lifelong medication for this condition with either oral or intravenous calcium, calcitriol and active vitamin D is mandatory. Although this may not be beneficial or remain sufficient for many patients. Parathyroid allotransplantation (PTx) is relatively new and alternative approach for the treatment of PH. Moreover, PTx hasn't been effectively successful. The most significant problem in PTx is the development of immune response against the graft tissue. It may be a good approach to use the cadaveric donor to overcome this problem. Here we present a case of PH treated with PTx with short term immunosuppressant.
Methods/Materials: 41-year-old female was admitted to clinic with severe PH. Clinical findings were weakness, muscle spasms and dyspeptic symptoms. She was taking daily calcitriol, Ca-carbonate, vitamin D3 to relieve symptoms. She had underwent PTx from cadaveric 13 year old male. Cadaveric donor being matched to our patient in terms of ABO typing. Four glands were fragmented into the operating room and washed with 37°C saline. mixed with 2 ml platelet rich plasma solution obtained from the recipient. Afterward fragments were injected into the deltoid muscle onsite with a 14 gauge needle. Recipient has received 250 mg methylprednisolone one hour before PTx and hospitalized for two days. Methylprednisolone was only administered post transplantation on first two days (125 mg and 60 mg, respectively).Clinical features, Ca, PTH levels were followed.
Results: Daily medications were stopped after transplantation day 1. Recipient's clinical findings were observed during 24 months and no complications were seen. Increase of serum PTH and calcium levels were observed (Table 1).

Table 1. Ca and PTH levels

	Before PTx	PostPTx Day 1	PostPTx Month 1	PostPTx Month 3	PostPTx Month 6	PostPTx Month 12	PostPTx Month 24
Ca (mg/dl)	8.6	8.8	8.9	8.3	8.2	8.1	8.1
PTH (pg/ml)	5.9	13.7	34.8	41.8	37.1	82	49.0

Conclusion: Transplantation of healthy parathyroid glands from cadaver is a easy and fast application with short term immunosuppression. This method appears to be promising technique for the future.

Clinical Composite Tissue Other

MP028 REPORT OF THE SECOND INTERNATIONAL CHAUVET WORKSHOP

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The psychosocial assessment and management of VCA (vascularized composite tissue allotransplantation) patients is unique due to the fact that this surgery is considered as enhancing rather than life-saving procedure when compared with solid organ transplantation. The importance of the psychiatric assessment in this field of transplantation was determined at the First International Chauvet Workshop, which was held in Paris on March 21–22, 2014. The complexity of the psychosocial assessment and management in upper extremity transplantation was underscored at this meeting. During the first Chauvet workshop, attendees highlighted the critical domains in the psychosocial assessment and management of this unique population and the need for an international consensus on this.

During the second Chauvet workshop, the results of a survey, sent to all active transplant centers, were presented and discussed and a set of recommendations and guidelines for the assessment and management of upper extremity transplant recipients was created. The Second International Chauvet Workshop was held in Paris from 17 to 19, September 2016 and the attendees represented the majority of teams involved in upper extremity (14 teams), face (6 teams) and uterus (2 teams) transplantation.

An update on the psychosocial issues in face transplantation was followed by an in depth discussion organized around some salient issues such as suicide and face transplantation, body image, remodeling follow-up and support of the face transplant recipients and ethical issues in face transplantation. These discussions aimed at refining the patient selection process and determination of candidate eligibility, establishing the risk/benefit ratio, determining the patients' ability to understand risk and describing the optimal follow-up of face transplant recipients.

Clinical Others Surgical technique

MP029 THE STATE OF THE INTEROSSICULAR ARTICULATIONS OF TRANSPLANTED ALLOGRAFTS

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The aim of the presented research is the experimental study of the state of the anvil-stirrup joint during different terms after allotransplanting, as well as the analysis of the far-going results of the reconstructive operations on the middle ear in clinics with the usage of the ossicular allotransplants.

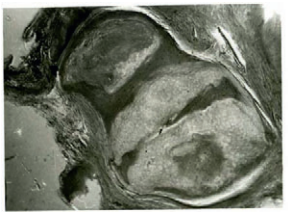
For studying the process of the anvil-stirrup reconstruction, we have conducted an experimental research on animals. 18 narcotized cats underwent allotransplanting of the anvil-stirrup complex.

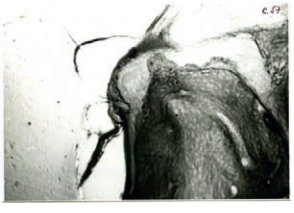
In cases when the transplanted auditory ossicles fulfilled their physiologic function, e.g. they were under the voice loading, in less than 9 months after transplanting the anvil-stirrup joint preserved its initial shape.

18 months later, instead of the joint capsular there formed a conjunctive tissue muff, which fixed the stirrup head to the long outgrowth of the anvil. Interjoint cavity was also filled with the conjunctive tissue. During this time complete reconstruction of the auditory ossicles took place.

This way, instead of the apparent joint – diarthrosis, in the process of reconstruction a movable joint from conjunctive tissue – syndesmosis – forms.

The anvil together with the small arc was implanted in 33 operations to the patients with the preserved stirrup, on the head of which the allotransplant was





fixed. Under this condition the "fork" of the transplanted complex as if clasps the head of the patient's stirrup. For inosculating the auditory ossicles in the place of their contact with the stirrup head, the hyaline cartilage had been ablated in advance. For additional fixation of the ossicles, the glue was used.

To sum up, when analyzing the data of the experimental research as well as the far-going results of the operations, it is possible to conclude that transplantation of the auditory ossicles' complexes which contain the anvil-stirrup joints, the latter preserve their anatomic structure and fulfill the function of sound-conductivity.

MP030 PENETRATING KERATOPLASTY (CORNEAL TRANSPLANTATION): THE LEARNING PERIOD

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Background: We aimed to present the results and complications of penetrating keratoplasty during the learning period.
Methods/Materials: The first 30 penetrating keratoplasty operations (30 eyes, 28 patients) performed by the same surgeon after completing one-year training in a registered Eye Bank were included. The diagnosis was keratoconus in 12 eyes, corneal opacities in 6 eyes, pseudophakic bullous keratopathy in 5 eyes, corneal dystrophy in 5, and secondary corneal perforation in 2 eyes. Mean follow-up time was 32 ± 12 months (12–49 months).
Results: Preoperative best corrected visual acuities (BCVA) were between hand movements and 20/200 (mean log MAR = 1.67 ± 0.38), and final postoperative BCVA ranged between 20/200 and 20/20 (mean log MAR = 0.39 ± 0.28) ($p < 0.01$). Mean final corneal astigmatism was 4.0 ± 1.5 dioptres (between +2.0 and −6.0 D). No complication was noted in 10 eyes (33%). In one eye during trephination an unintended iris incision occurred, and iris was sutured. Malign glaucoma appeared in one eye at the first postoperative day and was treated with laser iridotomy and medications. Early epithelial healing defects were present in 6 eyes (20%). Most resolved with eye lubricants and bandage contact lenses. The persistent defect in one eye was managed with an amnion membrane graft. Graft rejection occurred in 6 eyes (20%) (between 4 and 21 months, average = 15 months). All were treated with systemic and topical steroids and all grafts survived except for one. Graft insufficiency occurred in an eye with graft-iris synechia and the graft was replaced at month 16. Cataract developed in 8 of 19 phakic eyes (42%) (between 5 and 22 months, average = 12 months) and cataract surgery was performed in 7 eyes. Glaucoma developed in one eye and was treated with medications.
Conclusion: Penetrating keratoplasty significantly improves vision in most cases, and after a qualified period of training, complication rates of a beginner surgeon are comparable to those of experienced surgeons.

Translational Composite Tissue Biomarkers and molecular changes

MP031 META-ANALYSIS OF CLINICAL SIGNIFICANCE OF COMPLEMENT ACTIVATING ANTI-HLA DSA IN SOLID ORGAN TRANSPLANTATION

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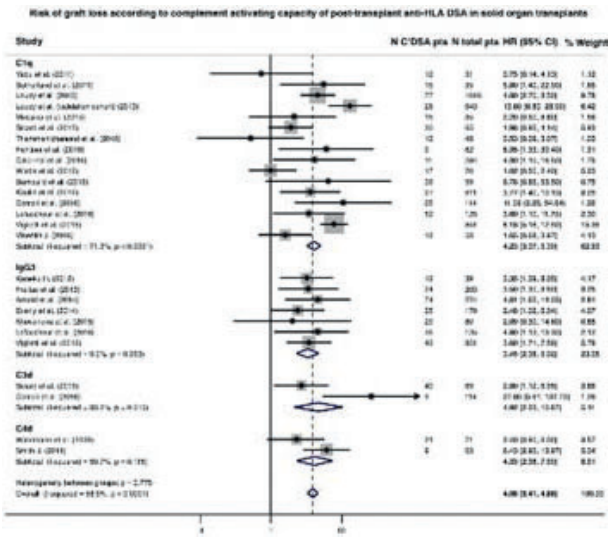
[1]Department of Nephrology-Dialysis and Transplantation Chu of Liège, Belgium; [2]Paris Translational Research Center for Organ Transplantation, France

Donor-specific anti-HLA antibodies (DSA) are currently recognized as the major limitation to access transplantation and the first cause of late transplant failures. The effect of complement activating anti-HLA DSA on allograft rejection and graft loss has been diversely reported with varying amplitudes between studies. We report the results of a meta-analysis of complement activating DSA and their association with graft outcomes in solid organ transplant recipients.

A comprehensive search has been conducted through several databases using the following search terms "solid organ transplantation, complement-activating DSA, IgG subclass, C1q, C4d, C3d, graft survival and antibody-mediated rejection". A total of 4.953 records published between 1971 and 2017 were identified by the search procedure. We eliminated non-related studies. We included studies using single–antigen flow bead techniques. Studies focusing on non-DSA were excluded. The primary outcome was graft survival and the secondary outcome was antibody-mediated rejection rate.

The search identified 44 cohort studies comprising a total of 9.221 solid organ transplant patients. Studies with available data on primary and secondary outcomes were finally used in the complete meta-analysis, summing up 27 studies and 6.283 patients.



Together, these studies demonstrate that patients with post transplant circulating complement activating anti-HLA DSA defined by C1q, C4d, C3d binding capacity or IgG3 subclass show an increased risk of antibody-mediated rejection with a pooled HR of 6.20 (95%CI: 4.57–8.42) and an increase risk of graft loss with a pooled HR of 4.08 (95%CI: 3.41–4.88) and i^2 of 58.9%.

Complement activating DSA are strongly associated with increased risk of antibody-mediated graft rejection and impaired long term allograft survival. Future studies are needed to define the place of complement activating DSA in the clinical-decision making for solid organ transplant recipients.

Clinical Composite Tissue Rejection

MP032 PARTIAL GRAFT LOSS OF A FACIAL TRANSPLANT AFTER DONOR SPECIFIC ANTIBODIES DEVELOPMENT

Emmanuel Morelon[1], Palmina Petruzzo[1], Jean Kanitakis[2], Stéphanie Dakpé[3], Olivier Thaunat[1], Valerie Dubois[4], Gabriel Choukroun[5], Sylvie Testelin[3], Jean-Michel Dubernard[1], Lionel Badet[1], Bernard Devauchelle[3]
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The role of antibody-mediated rejection is unclear in vascularized composite allotransplantation. Herein a case of late facial graft loss due to antibody-mediated rejection is reported.

The recipient was a 38 year-old woman severely disfigured by a dog bite. She received a face allotransplant on November 27, 2005. The induction immunosuppression included antithymocyte globulin, tacrolimus, mycophenolate mofetil and prednisone. From the eleventh month the immunosuppressive regimen consisted of prednisone, mycophenolate mofetil and sirolimus.

Ninety months after transplantation the patient was found on routine monitoring to have developed de novo class II-donor specific antibodies, without clinical signs of acute rejection. However, some months later she developed three biopsy-proven skin rejection episodes that were classified as histological Banff grade 3 acute rejection with lichenoid changes. These episodes were treated with glucocorticoid pulses followed by increased glucocorticoid maintenance. Despite rapid clinical improvement, eight months after the first acute rejection episode the sentinel graft underwent necrosis. Microscopic examination showed intimal thickening and thrombosis of the pedicle vessel. A facial skin biopsy showed C4d deposits on the endothelium of two dermal vessels, and magnetic resonance flow imaging of the facial graft showed a decrease in flow in the distal right facial artery. Three steroid pulses of 500 mg each, followed by Intravenous Immunoglobulins (2 g/kg), 5 sessions of plasmapheresis and Bortezomib 1.3 mg/m^2, were administered. Despite rescue therapy with Eculizumab, necrosis of the lips and the perioral area occurred, which led to surgical removal of the lower lip, labial commissures and part of the right cheek in May 2015.

This case of graft loss due to humoral rejection suggests that prevention of DSA occurrence is of utmost importance in VCA as well as in solid organ transplantation.

Clinical Others Surgical technique

MP033 SIX CASES OF UTERUS TRANSPLANTATION: FIRST YEAR REPORT OF THE 2-ARM CZECH UTERUS TRANSPLANT TRIAL

Jiri Fronek[1], Libor Janousek[1], Marta Novackova[2], Jan Matecha[2], Jaroslav Chlupac[1], Michael Olausson[3], Roman Chmel[2]

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Introduction: Uterus transplantation (UTx) may become another organ transplantation. There is very limited worldwide experience with some 24 cases performed so far. The Czech UTx trial has been setup and permitted by Ministry of health in 2015. This is the first trial worldwide comparing deceased and live donor UTx. In January 2016 the trial started with first uterus deceased donor training retrieval. The first UTx case we performed on 30th April 2016, in total 6/20 UTx we performed to the date.
Method: The plan is for 20 UTx procedures in total, of those 10 from life and 10 from deceased donors. The trial includes deceased donor retrieval training cases; we performed 5 of those, assessed the UTx graft for preservation injury and also trained the uterus graft quality testing including hysteroscopy and quality of the perfusion. To the date we performed 3/10 deceased donor UTx and 3/10 live donor UTx.

The deceased donor retrieval and UTx: Technically demanding procedure, multiorgan retrieval, pelvic cannulation, CIT 6:34, 2:26, 9:09 h, UTx took retrieval time 4:05, 4:10 and 4:54 h. The live donor UTx: The live donor hysterectomy is a difficult procedure, it took 5:23, 6:09 and 7:11 h, donors had BMI of 29.6, 35.6 and 35.9. The UTx took 3:29, 3:49 and 4:56. Immunosuppression is based on induction, early steroid withdrawal, MMF withdrawal at 6 months and long-term Tacrolimus monotherapy maintenance. We observed few mild rejections and some borderline changes in the biopsies.
Results: One of the deceased donor grafts thrombosed suddenly on 7 POD and had to be removed, other two are fine with follow-up of 5 and 2 months. All the live donor UTx cases are doing well, with follow up of 10, 4 and 1 month.
Conclusions: We cannot measure any function after the UTx. Also, visualizing the perfusion is not easy. The first embryo transfer is planned at least 6 months after UTx. We hope that UTx has got potential to serve women with Absolute Uterine Factor Infertility.

MP034 LIVING AND DECEASED DONOR UTERUS RETRIEVAL: THE TECHNICAL PITFALLS, EXPERIENCE WITH 8 DECEASED AND 3 LIVE DONOR CASES

Jiri Fronek[1], Libor Janousek[1], Michael Olausson[2], Roman Chmel[3]

[1]Transplant Surgery Department, Institute for Clinical and Experimental Medicine (Ikem) Prague, Czech Republic; [2]Department of Surgery, Sahlgrenska University, Göteborg, Sweden; [3]Department of Gynecology and Obstetrics, 2nd Faculty of Medicine, University Hospital Motol, Prague, Czech Republic

Introduction: Uterus transplantation (UTx) is the youngest organ transplantation. The Czech uterus transplant trial has been setup and permitted in 2015. In January 2016 the trial started with first uterus deceased donor retrieval. The first UTx we performed on 30th April 2016. The trial includes 20 uterus transplant procedures in total, of those 10 from life (LD) and 10 from deceased (DBD) brain dead donors.
Method: Deceased donor retrieval: Our trial started with four "training" decease donor uterus retrievals. All the deceased donors were between 18 and 60 years of age, brain dead, with plan for multiorgan retrieval. The first step of the retrieval is the uterus quality assessment, followed with detailed dissection including pelvic vessels, uterine arteries, uterine veins and ovarian veins. The cannulation prior to the perfusion must be performed using external iliac arteries bilaterally. The perfusion is slow, with need for additional ex-vivo perfusion.

Live donor retrieval: Via lower midline incision we approached the uterus, both ureters must be fully visualized, uterus with all related vasculature dissected. The perfusion is very slow, may take over one hour. In all three cases done so far we did not experience any complications.
Results: In one of the DBD transplant cases (our third one), we found cervical dysplasia, the graft was not transplanted. In total 8 DBD retrievals gave us a chance to explore the procedure in very detail. The first three LD retrievals and transplants went also well, with no complications. There were no major issues related to the LD and DBD uterus retrieval, both procedures are technically demanding.
Conclusions: Both deceased and live donor uterus retrieval are technically demanding procedures. There are tricks within both procedures. DBD retrieval requires lower pelvic cannulation and ex vivo additional graft perfusion as well as detailed graft quality assessment. LD hysterectomy is possibly relatively safe to the donor with fast recovery.

Clinical Composite Tissue Immunosuppressive agents

MP035 IMMUNOSUPPRESSION IN INDIA'S FIRST BILATERAL COMPOSITE TISSUE TRANSPLANTS (HAND)

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[1]Amrita Institute of Medical Sciences, India; [2]Amrita, India

Introduction: India's first three bilateral hand transplantation surgeries were performed at Amrita Institute of Medical Sciences. The aims of this article are to report the (1) immunosuppression protocol followed (2) clinical and histological assessment for rejection, (3) complications, and (4) graft survival.
Methods: We present 3 patients 26, 23 and 10 months after bilateral allogeneic hand transplantation.
Results: Lymphocyte cross match was negative. Induction regimen: with 50 mg of ATG for 6 days and 500 mg of methylprednisolone on the day of surgery. Maintenance immunosuppression: Tacrolimus 0.2 mg/kg and mycophenolate Mofetil 2 g/day (same in all cases). Patients underwent serial punch biopsies to look for skin rejections, skin being the most immunogenic tissue in composite tissue transplant.
Rejection Profile: Case 1–4 episodes of rejection on post op days 14, 118, 238 and 737 (3 ACR and one AMR). AMR was treated with 10 g of intravenous immunoglobulin and 1 dose of Rituximab. ACRs were treated with methylprednisolone (500 mg $\times$ 5 days, 250 mg $\times$ 3 days and 250 mg $\times$ 3 days respectively). He had acute appendicitis 26 months after transplantation and underwent an appendectomy. Case 2: 1 episode ACR on post op day 38, which was treated with methylprednisolone (500 mg $\times$ 3 days). Blood pressure stable (1 antihypertensive). He had acute diarrhoeal disease 18 months post op, and was diagnosed with giardiasis after extensive evaluation Case 3: 3 episodes of ACR on post op days 56, 106 and 171: were treated with methylprednisolone (250 mg $\times$ 3 days). Renal functions and blood pressure are both stable in all cases our patients have allograft survival with good functional recovery. There have been no major infections so far. CNI levels are being maintained at 10-15 ng/ml. We are tapering the immunosuppression as per renal transplant protocol.
Conclusion: Intermediate results of hand transplants have demonstrated good functional recovery with modified renal transplant immunosuppression protocol.

Translational Composite Tissue Other

MP036 CONFIRMATION OF SAFETY OF HUMAN CORD BLOOD DERIVED EX-VIVO CREATED DI-CHIMERIC CELLS BEFORE AND AFTER CRYOPRESERVATION IN THE IMMUNODEFICIENT MOUSE MODEL: A PRELIMINARY STUDY

Joanna Cwykiel, Natalia Filipek, Erzsebet Szilagyi, Uygur Safak, Maria Siemionow

University of Illinois at Chicago, USA

Background: We developed a new supportive therapy of *ex vivo* fused human cord blood-derived di-chimeric cells (DCC) to prolong Vascularized Composite Allograft (VCA) survival. The aim of this study was to assess the safety of DCC before and after cryopreservation in the immunodeficient mouse model.
Methods: Twenty-four fusions of human umbilical cord blood (UCB) were performed. UCB from 2 unrelated donors were stained with PKH26 and PKH67 dyes. Fused with polyethylene glycol, double (PKH26/PKH67) stained DCC were sorted and cryopreserved for 6 months in CryoStor®CS10. The safety of DCC before and after cryopreservation was tested in vitro (COMET assay; CA) and in the immunodeficient mouse model. Forty-eight animals divided into 8 groups received intramuscular (IM, Groups 1–4) or intraosseous (IO, Groups 5–8) injection of 0.5–3 $\times$ 10^6 of DCC or UCB (Groups 2,6 and 1,5, respectively) or cryopreserved DCC or UCB (Groups 4,8 and 3,7, respectively). Mice were observed for 90 days for changes in weight, activity, posture and hair loss. Three times per week mice were evaluated by palpation and at 90 days by magnetic resonance imaging (MRI). The presence of DCC in the peripheral blood was determined by flow cytometry (HLA class I) at 90 days after cell delivery. Lymphoid organs, lungs, and liver were harvested at 90 days and assessed by H&E staining for the presence of tumor-like growth.
Results: CA showed no damage in the DNA of DCC after fusion. No DCC or UCB derived tumor-like growth was detected up to 90 days post-injection. IO delivered DCCs (<1%) were observed up to day 90 in the blood of the recipients.
Conclusions: The safety of DCC before and after long-term storage was confirmed, thus allowing for the future DCC application as the "off-the-shelf" product. The unique concept of DCC, which presents phenotype characteristics of transplant donor and recipient, is a new promising approach for tolerance induction and prolonging solid organ and VCA survival.

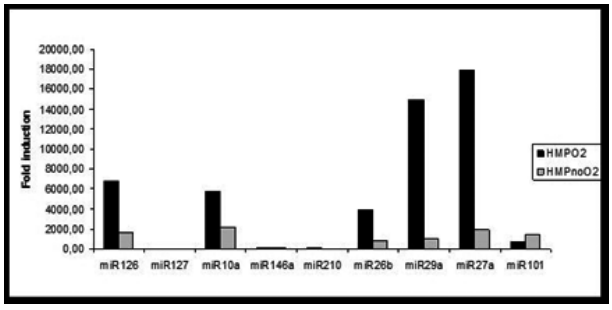
Basic Kidney Ischemia-reperfusion and preservation

MP037 MIRNAS EXPRESSION AS USEFUL MARKERS FOR ADVANTAGEOUS PERFUSION IN A PRECLINICAL DCD MODEL OF OXYGENATED HYPOTHERMIC MACHINE PERFUSION

Maria Victoria Gomez Dos Santos[1], Vital Hevia[2], Edurne Muñoz[3], Maria Laura García Bermejo[3], Esperanza Macarena Rodríguez-Serrano[3], Adolfo Martinez[4], Ana Saiz[5], Jose Manuel Del Rey[6], Victor Diez-Nicolás[2], Sara Alvarez[2], Jennifer Brasero[7], Francisco Donis[7], Francisco Javier Burgos[1]
[1]Urology Department, Irycis, Hospital Ramón Y Cajal, Alcala University, Spain; [2]Urology Department, Irycis, Hospital Ramón Y Cajal, Spain; [3]Biomarkers and Therapeutic Targets Group, Irycis, Hospital Ramón Y Cajal, Spain; [4]Transplant Coordination, Hospital Ramón Y Cajal, Spain; [5]Pathology Department, Irycis, Hospital Ramón Y Cajal, Spain; [6]Biochemistry Department, Irycis, Hospital Ramón Y Cajal, Spain; [7]Urology Department, Hospital Ramón Y Cajal, Spain

Introduction: Extended criteria donors (ECD) and donation after cardiac death (DCD) donors provide organs which tend to be more sensitive to the stress of preservation, with increased rates of delayed graft function (DGF) and primary non-function (PNF). There is a lack of evidence about the potential role of oxygen in preservation techniques. However, it seems that it might be particularly beneficial in kidneys which have suffered a period of warm or cold ischemia, like ECD and DCD grafts. Biomarkers and Therapeutic Targets Group have characterized several miRNAs as key mediators of the proximal tubule response to ischemia-reperfusion (I/R) injury.
Material and Methods: An orthotopic auto-transplantation model mimicking Maastricht type III DCD under hypothermic machine perfusion (Life-port® kidney transporter) was developed. Real time PCR detection was performed using SYBR Green and specific commercially available probes for each miRNA of interest in preservation solution and kidney biopsies from non-oxygenated and oxygenated grafts.
Results: miRNAs miR29a, miR101, miR-126 and miR-10a, modified their expression most significantly. These miRNAs that are related to cell adhesion, intracellular trafficking and kidney fibrosis, showed a differentially expression between oxygenated and non-oxygenated kidney grafts (Fig. 1).

Although, biopsies from renal tissue taken pre and post-perfusion were optically normal and didn't show any structural difference, cell cultures and the corresponding MitoTracker Orange staining showed a clear decrease on cell proliferation and vitality post-perfusion regardless of the addition of O2.



Conclusions: miRNAs expression levels attending to oxygenation in preservation solution could be predictive of allograft homeostasis maintenance that result in cell adhesion and intracellular trafficking modifications. There is a need for further investigation into the inner mechanism of HMP and oxygen use and their influence in I/R injury.

Clinical Liver Ischemia-reperfusion and preservation

MP038 EXTRACTION TIME DURING LIVER TRANSPLANTATION IMPAIRS OUTCOME

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[1]University Hospitals Leuven, Belgium; [2]Ku Leuven, Belgium; [3]Eurotransplant, The Netherlands

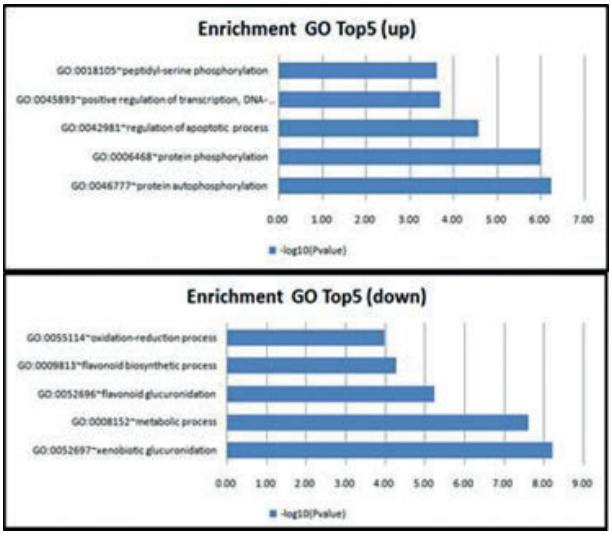
Background: During donor hepatectomy, despite topic cooling to 10–20°C, the liver still sustains warm ischemia that might be harmful.
Methods: We investigated the relationship between extraction time (ET) and transplant survival in 12974 recipients of deceased-donor livers transplanted in Eurotransplant (2004–2013). Cox regression analyses were corrected for donor, preservation, recipient variables. Transplant center was included as random effect (stronger impact on outcome than donor center). ET was the time from start of cold flush and end of hepatectomy. Transplant survival was defined as all-cause graft failure.
Results: Median follow-up was 4.04 years (IQR 2.4–6.2). Median ET was 41 min (32–52). ET was longer in livers donated after circulatory death (DCD) compared to brain-dead donors [50 min (35–68) vs. 40 min (32-51), $p < 0.001$]. ET independently associated with transplant loss (adjusted hazard ratio (aHR) 1.03 for every 10 min increase, 95%CI 1.02–1.05; $p < 0.0001$), as well as graft and patient survival. Other independent risk factor for transplant loss were donor/recipient age, donor last sodium/peak AST, DCD, split liver, cold ischemia time, lab MELD, acute liver failure, retransplant, cholestatic disease, viral hepatitis, recipient BMI. The magnitude of ET-effect was comparable to the effect of each hour of additional cold ischemia time (aHR 1.04, 95%CI 1.02–1.05; $p < 0.0001$). DCD livers (aHR 1.14, 95%CI 1.06–1.22 for DCD, 1.03, 95%CI 1.00–1.05 for brain dead donor livers, $p = 0.008$ for interaction) and livers with a higher donor risk index (aHR 1.10, 95%CI 1.01–1.19, $p = 0.024$ for interaction) were more susceptible to the effect of ET on graft survival. The probability for transplant loss is visualized in Fig.
Conclusions: ET impairs liver transplant outcome – especially in higher risk livers. This is likely due to insufficient cooling and/or rewarming of the graft in the donor. Reducing ET or keeping the liver cold during extraction might improve outcomes.

Basic Kidney Ischemia-reperfusion and preservation

MP039 A STUDY ON GENE EXPRESSION OF THE PROTECTIVE EFFECT OF NITRIC OXIDE RELEASING NANOFIBER IN RAT RENAL ISCHEMIA-REPERFUSION INJURY

Hyung Joon Ahn, Kyung Hwan Jung, Sun Hyung Joo
Kyung Hee University, Korea

Background: Renal ischemia-reperfusion injury (IRI) is very important issue in kidney transplantation. Nitric oxide (NO) was well known to be a protector of IRI. The aim of this study was to investigate the gene expression of the protective effect of the new delivery system of the NO releasing nanofiber on renal IRI in a rat models.
Methods: Male Sprague-Dawley rats were divided into three groups: (1) sham group (SG, $n = 5$); (2) control group, renal IRI without any treatment (CG, $n = 4$); (3) NO group, the renal IRI with wrapping the liver using NO rapid releasing-polymer nanofiber matrix (NG, $n = 6$). After Rt nephrectomy, NO releasing sheet was applied by wrapping Lt kidney one hour before clamp of renal artery. Renal ischemia was sustained during 55 min, followed by reperfusion. NO sheet was removed 24 h. And 48 h after surgery, the rats were sacrificed. We investigated the changes of gene expression between the groups with RNA Quant-Seq analysis.
Results: Mean of creatinine in SG, CG and NG at 48 h after operation were 0.48(±0.08), 4.67(±0.33) and 2.60(±1.0) respectively. There were significant differences between groups ($p = 0.002$). The top five terms of upregulated gene ontology (GO) among this study that the gene content of CG is more than two times than that of SG, and the gene content of NG is less than two times than that of CG were GO:0046777~protein autophosphorylation,



GO:0006468~protein phosphorylation, GO:0042981~regulation of apoptotic process, GO:0045893~positive regulation of transcription, and GO:0018105~peptidyl-serine phosphorylation. The top five terms of downregulated GO were GO:0052697~xenobiotic glucuronidation, GO:0008152~metabolic process, GO:0052696~flavonoid glucuronidation, GO:0009813~flavonoid biosynthetic process, and GO:0055114~oxidation-reduction process.
Conclusion: NO nanofiber has the protective effect agai.

MP040 HOW TO PROMOTE REGENERATION AFTER ISCHEMIC REPERFUSION INJURY: DUAL ROLE OF CD 137 AGONISTIC ANTIBODY

Jong Soo Lee[1], Kyung Sun Park[1], Hyun Chul Chung[1], Ho Jong Park[1], Byungsuk Kwon[2], Hye Jeong Kim[2], Sang Joon Park[1], Jongha Park[1], Hong Rae Cho[1]

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Acute sterile inflammation can be induced by tissue damage caused by ischemia-reperfusion. In transplantation ischemia reperfusion injury (IRI) is unavoidable procedure. IRI causes delayed graft function and increases rejection rates, finally shortens long-term graft survival. Previously we reported an inflammatory loop between tubular epithelial cells (TECs) and inflammatory cells during kidney IRI. We demonstrated that blocking this pathway attenuated the severity of AKI in previous reports. Although sterile inflammation plays a dominant role in damaging TECs in the early injury phase of AKI, blocking this inflammatory pathway may turn out the signal of the regeneration. Here, we focused on CD 137 – CD137 ligand and reverse pathway which plays a dual role in both early inflammatory and late regenerative phase. In the early phase of IRI, CD 137 ligand signal on tubular epithelial cell amplifies inflammation and augments the intensity of tubular damage. During the healing phase, CD 137 signal promotes regeneration of TEC by decreasing G2-M arrest of TEC, switching M2 macrophage from proinflammatory M1 macrophage, and expansion of regulatory T cell. Early administration of CD 137 agonistic antibody decreases the intensity of TEC injury via blocking CD 137 ligand signal of TEC. In addition, delayed treatment with CD 137 agonistic antibody promotes regeneration via enhancing CD 137 regenerative signal of immune cell. In this work we provide evidence that treatment with CD 137 agonistic antibody can protect from IRI and its' progression to chronic graft dysfunction by attenuating AKI severity and promoting regeneration of damaged tissue.

MP041 PROTECTING RENAL ENDOTHELIAL CELLS FROM COLD AND WARM ISCHEMIA BY PHARMACOLOGICAL AGENTS

Meryl Thomas, Gwenaelle Antetomaso, Guillaume Demarne, Patrick Berna

Balmes Transplantation, France

Ischemia-reperfusion (IR) is a complex pathophysiological process encompassing activation of inflammatory responses and cell death programs, the severity of which critically affects the transplant outcome in the short and long runs. Given major organ shortages, preserving the function of kidney grafts is of utmost concern, and new therapeutic strategies targeting IR injuries (IRI) – a main hurdle to broaden quality organ procurement – are required. While technologies under clinical development focus on reducing cell damage after IRI had developed by treating the transplant receiver, we think approaches directed at limiting injuries straight at the organ level will be highly valuable, and result in lesser organ degradation and optimal function recovery.

Using primary renal glomerular endothelial cells – the most prominent target of IRI – we developed a high-throughput phenotypic screening assay that models the transplant process. To slash discovery time and thus delivery to the patients' bedside, we screened about 2,000 compounds among well-established pharmaceutical agents. The assay was performed under two distinct temperatures (37 and 4°C) given that IR damages upon kidney transplant result from exposure to both warm and cold ischemia. We identified several cytoprotective compounds which we further tested as mixtures to assess potential synergistic effects. This led to discover BT0214, a potent combination therapy against the deleterious effects associated with IRI at 4 and 37°C.

The fixed-dose combination BT0214 could become a new paradigm in the treatment of renal IRI and would easily be added to existing preservation solutions. Mechanism of action studies of identified IR pharmacological modulators and ex vivo assay on isolated perfused kidney are underway, that will help extend the knowledge about molecular and immunologic consequences of BT0214 treatment on renal IRI.

Basic Cell Ischemia-reperfusion and preservation

MP042 THE IMPACT OF RED LED IRRADIATION ON HEPATOCYTES PRESERVATION

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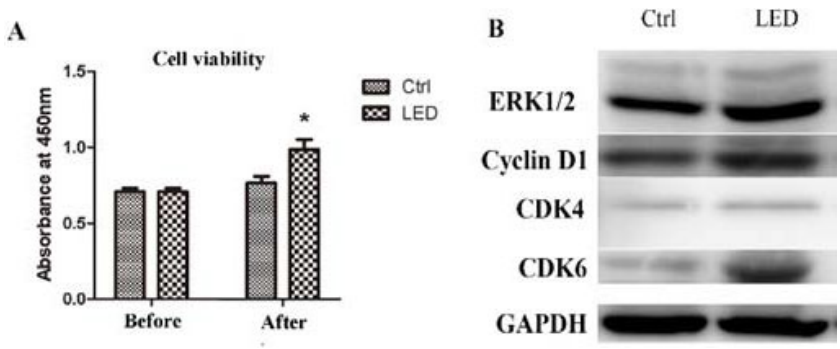
Background: Cell-based transplantation is an alternate method of liver transplantation to delay end-stage liver diseases. However, autogeneic hepatocytes as the ideal cell source for therapy are quite fragility and rare go proliferation to obtain enough cell number for treatment. Photobiomodulation therapy has been used to accelerate wound healing. However, the effect of light emitting diodes (LED) for hepatocytes is unclear. Thus, the aim of this study is to investigate the effect of LED irradiation on hepatocytes.
Methods: Mice hepatocytes were isolated form collagenase I digested liver and cultured in collagen type I coated 35 mm dishes (5×10^4/dish) with DMEM containing 10% fetal bovine serum. Then the 635 nm wavelength LED irradiation was performed 3 times in 48 h (5 mins, 15 mW/cm^2). The cell viability, reactive oxygen species (ROS) levels, extracellular signal-regulated kinase1/2 (ERK1/2) and cell cycle related cytokines (cyclin D1, CDK4 and CDK6) were observed. ROS and ERK1/2 inhibition were performed to determine the effect of ROS and ERK1/2 during LED irradiation.
Results: LED irradiation significantly increased hepatocytes proliferation ($p < 0.05$, figure A). The intracellular ROS staining showed higher intensity of fluorescence in LED group. The levels of ERK1/2 and cell cycle related cytokines levels significantly increased after LED irradiation (figure B). Along with LED irradiation, the ROS inhibitor impeded hepatocytes proliferation. Also, the ERK1/2 and cell cycle related genes expression were not as elevated as only received LED irradiation. ERK1/2, as the downstream of ROS, were inhibited by its inhibitor also restrained the hepatocytes proliferation and cell cycle related cytokines expression during LED irradiation.
Conclusion: 635 nm LED could accelerate the proliferation of hepatocytes through ROS/ERK pathway and be a 'pre-conditioning' method before cell transplantation.



Clinical Kidney Ischemia-reperfusion and preservation

MP043 PRELIMINARY RESULTS OF MEDICAL CORRECTION OF ISCHEMIA-REPERFUSION INJURY IN DIFFERENT TYPES OF DONORS

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Background: Damaging factors in clinical Tx are: ischemia-reperfusion injury (IRI), activation of the innate or adaptive immune systems, the complement system, acute rejection and its chronic dysfunction, apoptosis phenomena. Transplant Center team offered an idea of transplants medical protection by pretransplant elimination of the following factors out of donor's bloodstream: activated neutrophils, chemokines and cytokines, adhesion molecules at kidneys and liver vascular endothelium.
Materials and Methods: In 2015 the concept of medicamental correction of IRI was practically applied to the brain-dead donor (BDD) for the first time. A high dose of «Thymoglobulin» (Sanofi Genzyme, France) (quadruple to the therapeutic dose) has been injected to the donor's organism during brain death diagnosis procedure (6 hours). Complete blood count was done before the administration and right before the cold perfusion initiation. Each of the two comparison groups consists of 10 BDDs, first was treated with Anti-thymocyte globulin (ATG), second was a control. Our Transplant biobank team is forming a prospective collection of samples from different types of donors, including those with medical correction of IRI applied protocol and recipients for the ongoing research: enzyme-linked immunosorbent assay, PCR, immunoblotting.
Results: Complete blood count of comparison groups demonstrates leucopenia and thrombocytopenia in ATG group. Results of KTx has improved dramatically: in 75% of cases after ATG injection immediate function was observed, while in control group – only 50% of cases. Suggested protocol offers a tool that can improve the quality of grafts, their transplant-applicability, increase transplant function time by preventing early transplant nephropathy, expand donor pool through using expanded criteria donors.
Conclusion: Research offers a positive chance to transplant kidneys from expanded criteria donors, thus expanded criteria donors, thus expanding donor pool.

MP044 NEW PORTABLE PERFUSION SYSTEM (PPS) FOR EMERGENCY CONTROLLED BLOOD REPERFUSION ORGANS IN DONORS WITH IRREVERSIBLE CARDIAC ARREST

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Background: The crucial problem in program of donation from donors after cardiac death (DCD) is the warm ischemic time (WIT), especially in donors with sudden irreversible cardiac arrest. The real perspectives of use this kind of donors are restricted by technical obstacles for routinely and fast initiating ECMO in ICUs. In order to reduce the time for the start of perfusion "in situ" for organ resuscitation we use the new portable axial pump perfusion device (PPS).
Material and Methods: In our clinical practice, the PPS has been used in 2 cases (DCD). Donors were 2 women: №1 – 27 years, brain damage, №2 – 48 years, cerebral-vascular diseases. The distances to donor's hospitals were 9 and 12 kilometers (20 min and 17 min – arrival time team OPO). The system's assembly time was 10–15 min (including priming perfusion contour time). Primary WIT were 55 and 63 min, respectively. For ECMO of abdominal donor's organs "in situ" we were using the PPS with leukocytes depletion and modified donor's blood. The times of perfusion were 140 and 142 minutes, respectively. The PPS provided 5–6 l/min. flow perfusion rate that excluded to need using a double-balloon catheter. The flow oxygen was set constant 350 ml/min. The levels of hemoglobin and hematocrit were 34.1 g/l and 37.2–0.30 g/l – 0.32, respectively. The KTx were performed by 4 recipients on hemodialysis (3 women, 1 man). The average age of the patients was 46.75 (0.75) years.
Results: IGF was observed in all of 4 cases. 1-year outcomes of KTx from DCD with using the PPS are completely satisfactory: graft survival rate was 100%. The average level of serum creatinine was 0.084 (0.013) mmol/l.
Conclusions: By the use new the PPS, we can reduce up to 10 min the time for initiating abdominal reperfusion (ECMO). Routinely use of the PPS in clinical practice in ICUs could relevantly expand the donors' pool.

MP045 PRESERVATION OF GLYCOCALYX IN EXPERIMENTAL KIDNEY TRANSPLANTATION

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Background: The endothelial glycocalyx is a dynamic network of glycoproteins and proteoglycans that plays a central role in vascular permeability, adhesion of leukocyte and coagulation. The glycocalyx can be disrupted by various conditions such as ischemia-reperfusion injury and fluid overload. In particular, reperfusion of the kidney graft has been associated with endothelial damage within the microcirculation. The present study evaluates the effect of variations in perioperative fluid load on early kidney function and the preservation of the glycocalyx, during kidney transplantation in pigs using kidneys from brain dead donors.
Methods: Recipient pigs were randomized to either high volume fluid therapy (HVF) or individualized goal directed fluid therapy (IGDT) in addition to either continuous low dose nor-epinephrine (NE)-infusion or no NE. A model simulating the normal clinical conditions of a brain death donation was used. Tissue samples including cortex and medulla were taken from the recipient kidney 10 h post reperfusion. Kidney graft function was evaluated using measured GFR (mGFR). The preservation of glycocalyx in kidney tissue was evaluated 10 h after reperfusion by semiquantitation of the immunofluorescent staining for alpha-linked N-acetylgalactosamine.
Results: Results showed no significant differences in mGFR between the four groups. Mean fluorescence labeling for glycocalyx in the glomeruli 10 hours after reperfusion revealed a significantly greater mean level of fluorescence in the animals receiving IGDT without NE compared to HVF with NE. A great between animal variation was also observed.
Conclusion: In conclusion, the study showed no difference in early kidney graft GFR as a result of the different principles of fluid administration and pressor therapy. A structural difference on the glycocalyx was observed between the IGDT without NE and HVF with NE. This may suggest that a reduced fluid volume may result in better preservation of the glycocalyx.

Basic Heart Ischemia-reperfusion and preservation

MP046 ASSESSING MITOCHONDRIAL FUNCTION DURING HUMAN ORGAN TRANSPLANTATION

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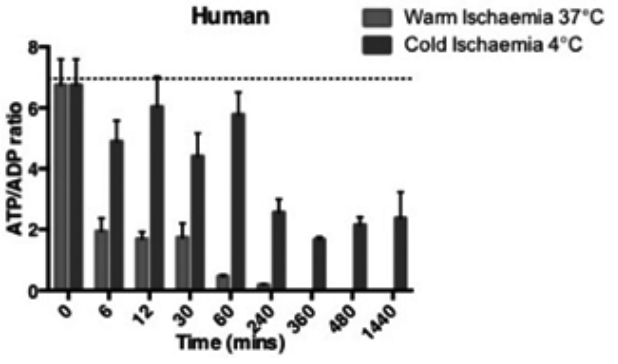
Background: Severe ischaemia reperfusion injury (IRI) during transplantation causes irreversible damage to donated organs. Thereby, the lack of oxygen results in a block of the mitochondrial respiratory. Upon reperfusion, a burst of reactive oxygen species causes oxidative damage to mitochondria, which can cause opening of the permeability transition pore and release of mitochondrial damage associated molecular patterns (mtDAMPs) like mitochondrial DNA (mtDNA). These activate the innate immune response when released to the circulation.
Method: To investigate the effect of IRI during transplantation, hearts from anaesthetised mice, pigs and rejected DBD donor hearts were exposed to warm (37°C) or cold (4°C) ischaemia (n = 5–8) prior to transplantation.
Results: We show that ischaemia leads to decreased ATP/ADP ratios and a reduction of the mitochondrial membrane potential. ATP/ADP ratios are 1.5-fold lower within 6 mins warm ischaemia than after 240 mins cold ischaemia.



This is conserved in different species and can also be shown in murine kidney tissue. Prolonged ischaemia causes oxidative damage on isoprostanes and mtDNA after reperfusion. Less than 20% of mtDNA can be amplified after warm ischaemic damage prior to heart transplantation in mice. Additionally we show that reperfusion of the organ in a recipient results in the release of mtDAMPS into the circulation. Blood plasma analysis of mice 24 h after heart transplantation including 12 mins warm ischaemia, show 2-fold more mtDNA in the circulation than without additional ischaemia.
Conclusion: These results indicate that the severity of ischaemia affects the outcome of transplantation. Compared to cold ischaemia, warm ischaemia results in a greater decrease in ATP/ADP ratios and more oxidative damage. This correlates with improved transplantation outcome after cold ischaemia. Altogether, this opens new ways to ameliorate IRI to improve the outcome of organ transplantation.

Basic Kidney Ischemia-reperfusion and preservation

MP047 NOX4 AND OXIDATIVE STRESS MEDIATED TGF-β INDUCED HUMAN KIDNEY PROXIMAL TUBULAR EPITHELIAL CELL APOPTOSIS IN ISCHEMIC REPERFUSION INJURY

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Aims: Ischemia/reperfusion injury, resulting from hypoxic damage within a graft, is the leading cause of cell death and graft rejection. In this study, we investigated whether Nox4 have a great role in ischemic injury in a cellular model in which experimental hypoxia was induced using CoCl2.
Main Methods: The ischemic injury induced in HK-2 cells by CoCl2 was validated by reduced cell viability at different times and doses. Reverse transcription polymerase chain reaction for Nox4 was performed. Western blotting for Nox4 and Smad pathway were done. ROS production was detected using a DHE stain and Amplex red assay. HK-2 cells were transfected with siNox4 and pretreated with GKT137831(most specific Nox1/4 inhibitor). ELISA has been used to measure TGF-β1 levels. The effect of treatment with TGF-β1 type 1 tyrosine kinase inhibitor SB431542 on Nox4 expression was observed.
Results: Expression of Nox4 in HK-2 cells significantly increased by hypoxic stimulation. TGF-β1 was secreted endogenously by hypoxic HK-2 cells. SB4315432 significantly inhibited Nox4 expression in HK-2 cells via Smad2/3 dependent cell signaling pathway. Silencing of Nox4 recued production of reactive oxygen species (ROS), downregulation of proinflammatory markers and reduced caspase 3/7 activity in hypoxic HK-2 cells. Pretreatment of GKT137831 replicated theses results.
Conclusions: Hypoxia induces HK-2 cell apoptosis through the signaling pathway involving Nox4 dependent ROS generation and TGF-β1 via Smad pathway. Therapies targeting Nox4 may be effective against ischemia induced kidney injury.

Basic Liver Ischemia-reperfusion and preservation

MP048 INSTITUT GEORGES LOPEZ SOLUTION PREVENTS FROM PROTEASOME ACTIVATION IN FATTY LIVER PRESERVATION

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Background: During the cold storage the fatty liver graft is deprived of oxygen, leading to ATP depletion and therefore harmful repercussions; including the activation of UPS. The aim of this study was to investigate the effect of two different preservation solutions: Institut George Lopez (IGL-1) and Histidine Tryptophan-Ketoglutarate (HTK) on the liver ubiquitin proteasome system (UPS) activation when liver grafts were subjected to cold storage.
Methods: Steatotic livers from Zucker Obese rats (10 weeks old) were preserved for 24 h (at 4 °C) in IGL-1 or in HTK solutions. Liver injury (AST/ALT) and ATP content were measured. Proteasome Chymotrypsin like ATP-dependent (26S) and ATP-independent (20S) activity were assessed. Also, the expression of the proteasome subunits 19S and core 20S, and levels of poly-ubiquitinated proteins were determined by western blot analyses.
Results: Data showed that IGL-1 prevents liver injury (AST/ALT). Higher levels of ATP were found in livers preserved in IGL-1 significantly when compared to HTK solution. The Chymotrypsin-like activity of 26S and 20S proteasome subunits increased in the HTK preserved livers by a 13% and 38%, respectively when compared to livers preserved in IGL-1 solution. Accordingly, the western blot analyses of ubiquitin showed a dramatic increase in the levels of polyubiquitinated proteins in HTK solution compared to the livers preserved in IGL-1. The expression of the subunit 19S of the proteasome was unchanged, while the core 20S subunit was significantly increased in livers preserved in HTK.
Conclusion: IGL-1 preservation solution provides a more efficient prevention of proteasome activation in cold ischemic steatotic grafts than livers preserved with HTK.

Basic Kidney Histology

MP049 TGF-Β1 INDUCES TRANSPLANT KIDNEY INTERSTITIAL FIBROSIS THROUGH ENDOTHELIAL-TO-MESENCHYMAL TRANSITION VIA TGF-Β/SMAD AND AKT/MTOR/P70S6K PATHWAYS

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Background: Chronic allograft dysfunction (CAD) induced by kidney interstitial fibrosis is the main cause of allograft failure in kidney transplantation. Studies suggested that endothelial-to-mesenchymal transition (EndMT) may play an important role in kidney fibrosis. We undertook this study to characterize the functions and potential mechanism of EndMT in transplant kidney interstitial fibrosis.
Methods: The EndMT was assessed by proteins and mRNAs extracted from human umbilical vein endothelial cells (HUVECs) treated with transforming growth factor-beta1 (TGF-β1) at different doses or at different intervals using western blotting, qRT-PCR and ELISA assays. Cell motility and migration were evaluated with motility and migration assays. The mechanism of EndMT induced by TGF-β1 was measured by western blotting. In addition, human kidney tissues from control and CAD group were also assessed by HE, Masson's trichrome, immunohistochemical, indirect immunofluorescence double stainings and western blotting assays.
Results: TGF-β1 significantly promoted the development of the EndMT in a time-dependent and dose-dependent manner and promoted the motility and migration abilities of HUVECs. The TGF-β/Smad and Akt/mTOR/p70S6K signaling pathways were revealed to be associated with the pathogenesis of EndMT induced by TGF-β1, which was also proven *in vivo* by analysis of the specimens of the control and CAD groups.
Conclusions: The progression of EndMT, characterized as the change in cell morphology and promotion of endothelial cells' motility, migration and extracellular matrix (ECM) secretion, could promote transplant kidney interstitial fibrosis by targeting TGF-β/Smad and Akt/mTOR/p70S6K signaling pathways, hence resulting in development of CAD in kidney transplant recipients.

Basic Kidney Histology

MP050 THE PROGNOSTIC VALUE OF INDIVIDUAL HISTOLOGIC LESIONS AND COMPOSITE SCORES IN 0-BIOPSY FOR LONG-TERM KIDNEY ALLOGRAFT FUNCTION

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Background: We aimed to study the relation of individual and composite histologic lesions in 0-biopsy with long-term kidney graft function.
Methods/Materials: We included in the study recipients of cadaver ($N = 101$) and living ($N = 29$) kidney transplanted in 2005–2010, with adequate 0-biopsy and follow-up data. We studied the association of individual lesions, Banff scores for indication (ind-Banff) and 0-biopsies (0-Banff), Remuzzi score, donor damage score (DDS), chronic damage score (CDS), and chronic allograft damage index (CADI) with graft function. GFR was estimated at 6 months and annually over 5 years. The endpoints were the eGFR at 6 months, the drop in eGFR of ≥25%, and the slope of eGFR.
Results: DDS, CDS, chronic and total ind-Banff and 0-Banff scores predicted lower eGFR at 6 months ($p < 0.05$); chronic 0-Banff score had the strongest impact ($\beta = -0.235$). Global glomerulosclerosis (GS), and ATN were associated with eGFR at 6 months in univariate analysis ($p < 0.05$). Chronic 0-Banff score ($\beta = -0.172$) and global GS ($\beta = -0.192$) remained significant after adjustment for clinical variables. 33.8% of patients lost ≥25% of GFR, which

was predicted by acute, chronic and total 0-Banff score, chronic and total ind-Banff score, and DDS ($p < 0.05$). Glomerular thrombi (GT), mesangial matrix (MM) increase, arteriolohyalinosis (AH), percentage of injured glomeruli (global GS+segmental GS+chronic ischemia) each predicted 25%-loss of GFR ($p < 0.05$). Acute 0-Banff score (OR = 2.04), GT (OR = 2.54) and AH (OR = 2.54) were significant after adjustment for clinical variables. 15% of patients had eGFR slope >−5 ml/min/year. Fibrinoid necrosis (FN) in glomeruli, adhesions, MM increase, and eGFR at 6 months predicted rapid loss of GFR ($p < 0.05$), whereas composite scores had no impact. FN (OR = 6.38) was significant in multivariate analysis.
Conclusions: Individual and composite histologic lesions in 0-biopsies differently predict worse 6 months kidney graft function, and further GFR loss.

MP051 INFLUENCE OF HEPATITIS C VIRUS INFECTION ON THE DEVELOPMENT OF INTERSTITIAL FIBROSIS AND TRANSPLANT GLOMERULOPATHY IN PEDIATRIC RENAL TRANSPLANT PATIENTS

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Baskent University, Turkey

Background: The long-term impact of HCV infection in renal allograft remains controversial. Some reports have indicated that HCV-infected kidney recipients had poorer graft survival. However, the impact of HCV on the development of interstitial fibrosis (IF) and transplant glomerulopathy (TG) is not clear enough. Therefore, we designed this study to identify the influence of HCV on the development of IF and TG in pediatric recipients.
Methods: Total 40 patients with a mean age of 16.7 ± 2.2 years (12–20) included in the study. Development of both IF and TG in follow-up biopsies compared between HCV-positive ($n = 16$) and negative ($n = 24$) recipients. Also, the number of acute rejection (AR) episodes and graft survival compared between HCV-positive and negative patients.
Results: The frequency of the development of IF and TG, 12 and 24 months after Transplant (Tx) was higher in HCV-positive patients compared to HCV-negative recipients ($p < 0.01$). The time between Tx and the development of IF and TG was 14.9 ± 4.4 and 25.9 ± 12.3 months respectively for HCV-positive patients. It was 29.4 ± 11.6 and 42.6 ± 5.4 months respectively for HCV-negative patients. A significant difference found between these two groups in regards to the development of IF and TG ($p = 0.001$). HCV-positive patients (1.44 ± 1) had greater numbers of AR episodes than did HCV-negative recipients (0.67 ± 0.9) ($p < 0.05$). Overall the 3-, 5- and 10-year graft survival was 69%, 50%, and 0% respectively for HCV-positive patients. It was 96%, 50%, and 11% respectively for HCV-negative patients ($p < 0.05$).
Conclusion: HCV-positive patients had higher incidences of the early development of IF and TG. They also had higher AR episodes and shorter graft survival than did HCV-negative recipients. The negative role of HCV in the long-term renal allograft survival can be explained by the possible triggering effect of HCV on the development of early IF and TG through augmenting AR episodes.

Clinical Kidney Immunology

MP053 COMPARISON OF PANEL REACTIVE OF ANTIBODIES: VIRTUAL CALCULATORS VS BEADS. UNMASKING POTENTIAL FALSE NEGATIVE RESULTS IN ANTI-HLA ANTIBODY SCREENING

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Background: The anti-HLA antibody screening in patients on waiting list and after solid organ transplantation is a routine procedure in presumed no-sensitized patients. However, sometimes the results of anti-HLA screening differs from more specific Single Antigen (SA) assay. This study ought to compare the antigen frequency of class-I antigens in screening test with virtual calculators from Spanish program(PATHI) and Eurotransplant (EURT) and the possible bias in the anti-HLA assay results.
Methods: The antigen frequency of broad HLA class-I antigens of EURT were taken from website whereas the antigen frequency of split and broad HLA class-I antigens from PATHI and LABScreen Mixed (MIX) Lot 020(One Lambda) were calculated. The global frequency of class-I and A-, B- and C-antigens were compared. Ten patients with mismatched results (negative in screening but positive after SA class-I test) were assessed.
Results: A positive correlation of broad HLA class-I antigen frequency between MIX, PATHI and EURT was observed ($p < 0.05$). However, comparing split HLA class-I antigens between PATHI vs MIX, the A locus maintained significant positive correlation ($r = 0.63$; $p = 0.002$) but B ($r = 0.09$; pNS) and C loci ($r = 0.32$; pNS) no significant correlation was achieved. Despite the correlation in A locus, 3 patients with negative screening for A32 (70.06 mfi ± 21.52) but positive after SA test (4736 mfi ± 3693) were detected.
Conclusions: Although differences in the frequency of HLA antigens used in anti-HLA screening could be a source of discrepant results with under-represented or misrepresented HLA antigens. Without differences in HLA-A antigens between MIX and Spanish donors, a lack of accurate to detect anti-A32 reaction in this study is demonstrated suggesting a SA test in the post-transplant monitoring with A32 donor. Large studies should be addressed to detect potential HLA antigens with false negative reactions in anti-HLA screening test.
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MP054 THE IMPACT OF PRE-TRANSPLANT DONOR SPECIFIC ANTI-HLA ANTIBODIES ON OUTCOME OF KIDNEY TRANSPLANTATION – SINGLE CENTER EXPERIENCE

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Background: Pre-formed antibodies directed against human leukocyte antigens (HLA) have a major impact on allograft survival and form a significant barrier in renal transplantation. The clinical significance of pre-transplant donor specific antibodies (DSA) despite negative cytotoxic cross match (CDC-XM) would be useful for clinical decision making. We aimed to determine the impact of pre-transplant DSA despite a negative CDC-XM on the outcome of kidney transplantation.
Methods: One hundred and eleven patients were prospectively randomized for renal transplantation at Hamed Al-Essa organ transplant center in Kuwait between January 2011 and December 2013. Patients were divided into 2 groups. Group 1 included 50 recipients with positive DSA at the time of transplant and were subjected to desensitization protocol. Three local protocols were utilized; first protocol: Plasma exchange, high dose intravenous immunoglobulin (H-IVIG), and Rituximab (RTX); second protocol: Immunoadsorption plus RTX, and third Protocol: H-IVIG and RTX. Group 2 included 61 recipients with negative DSA at the time of transplant. All recipients had negative CDC-XM and flow-cytometry cross-match (FCXM) at the time of transplant. Mean florescence intensity was considered positive from ≥1500. Serum creatinine, proteinuria, PRA and DSA levels were carried out basal and at 3, 6, 12, 24 months for all patients.
Results: There was a statistical significant difference between the two studied groups when comparing the mean PRA positivity for class I and II anti-HLA antibodies at baseline and during follow ups despite similar mean number of HLA mismatches. There was no statistical significant difference regarding early post-transplant graft function, patient survival, and graft survivals between the two groups.
Conclusion: Renal transplantation after successful desensitization was comparable to low immunologic risk transplantation for both graft and patient survival.

MP055 A PROSPECTIVE OBSERVATIONAL TRIAL TO EVALUATE A CMV-SPECIFIC ELISPOT ASSAY IN SOLID ORGAN TRANSPLANT (SOT) RECIPIENTS: THE PROTECT STUDY

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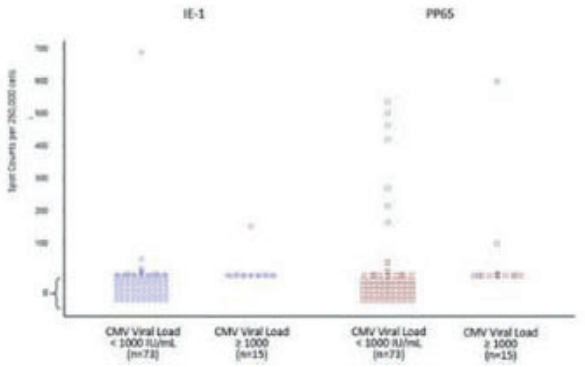
[1]Toronto General Hospital, Canada; [2]University of California San Francisco, USA; [3]Erie Country Medical Center, USA; [4]Mass General Hospital, USA; [5]University of Washington Medical Center, USA; [6]Houston Methodist, USA; [7]Queen Elizabeth, UK; [8]Emory University, USA; [9]Oxford Immunotec, USA

Background: CMV replication in transplant recipients is primarily controlled by the T-cell response. We evaluated the role of a novel CMV-specific ELISPOT assay to predict protection against CMV infection in SOT recipients.
Methods: This is an ongoing, multi-center (43 sites), prospective, observational study of 250 high-risk (D+/R−) kidney transplant recipients. Subjects were enrolled either pre-transplant or during initial anti-viral prophylaxis. Cell-mediated immunity (CMI) was determined using an ELISPOT assay that evaluates responses to CMV-specific antigens IE-1 and pp65, from the capture of IFN-γ enumerated as spot counts (T-SPOT.*CMV*, Oxford Immunotec). CMV CMI and quantitative PCR (Roche Cobas) were assessed at 1, 2, 3, 4 and 6 months following completion of anti-viral prophylaxis. CMV infection was defined as a central viral load of ≥1000 IU/ml. T-SPOT values taken at the end of prophylaxis were analyzed to predict a viral load threshold ≥1000 IU/ml within 3 months of completion of prophylaxis.

Results: Of the first 100 subjects to reach completion of prophylaxis, 12 subjects had missing T-SPOT and/or CMV PCR values and were excluded from the analysis. The majority of patients were white (80%), male (73%), and median age was 55. Most patients received 6 months of antiviral prophylaxis (75%) vs 3 months of prophylaxis (25%). Of the 88 eligible subjects, 9 had a pp65 response of ≥100 spots at the completion of prophylaxis. In this group, 2/9 developed CMV infection during the first 3 months following completion of prophylaxis, resulting in a positive predictive value (PPV) for protection against CMV infection of 77.8% with a specificity of 86.7% ($p = 0.26$) (see Figure). Using the same cut-off for IE-1, the PPV for protection against CMV infection was 50% and specificity of 93.3% ($p = 0.5$).
Conclusion: These preliminary data from an interim analysis demonstrate a trend toward predictive utility of T-SPOT.*CMV* for CMV viremia in D+R- kidney recipients.

Figure – Scatterplot of counts at completion of prophylaxis vs CMV infection (3 months forward)



MP056 OUTCOMES OF PATIENTS WITH SUCCESSFUL DESENSITIZED RENAL TRANSPLANTATION: A SINGLE-CENTER STUDY

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Introduction: Desensitization can improve transplantation rates in sensitized kidney transplant recipients. However, long-term outcomes are lacking. Here we analyze outcomes in desensitized living donor kidney transplant recipients.
Materials: We treated 41 highly sensitized patients between June 2007 and September 2016. All patients underwent desensitization using pretransplant plasmapheresis (PP) and low-dose intravenous immunoglobulin (IVIG; 100 mg/kg), rituximab and Bortezomib (one patient). Demographics, immunologic characteristics of patients, allograft function, acute rejection (AR) episodes, survival, and adverse events were evaluated.
Results: There were 41 desensitized (42 ± 14 years-old, 31 female) and 41 low-risk patients, matched as age, gender and transplantation time, included. Protocol biopsy could be performed in 23 desensitized patients at 1, 3, 6 months and 1 year. Average follow-up was 22 (0–55 months).The rate of rejection was significantly higher in the desensitized group ($n = 10$; 24%) than the control group ($n = 1$; 2.5%) ($p = 0.007$). Subclinical antibody mediated rejection was revealed in 13 patients performed protocol biopsy. Graft loss was founded 5 (12%) patients in the desensitized group, and no in the control group. Graft loss due to drug incompliance was found in only one out of 23 patients who underwent protocol biopsy. The mean serum creatinine concentration at last follow-up was similar in desensitized and control group. (1.1 ± 0.3 mg/dl vs 1.0 ± 0.4 mg/dl, respectively; $p = 0.57$). The rate of BK viruria was similar in both group. BK virus nephropathy ($n = 1$) and cytomegalovirus infection ($n = 1$) were found only one desensitized patient. Four years graft survival was 91% and 100% protocol biopsy and conventional follow-up groups respectively.
Conclusion: When protocol biopsy is performed, desensitization has good long-term results with graft outcomes similar to non-HLA-sensitized patients despite higher immunologic risk.

MP057 BK INFECTION OCCURRENCE AND IMMUNOLOGIC MONITORING IN KIDNEY TRANSPLANTATION

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Background: Kidney transplant patients are more prone to infections due to immunosuppressive therapy, with BK virus being one of the most important causes of infection. Although Epstein-Barr virus and cytomegalovirus can directly cause activation of serum complement according to *in vitro* experiments with fibroblasts, the same hasn't been confirmed for BK polyomavirus.
Methods/Materials: In a prospective study of a cohort of 185 patients of our center in the Community of Madrid who underwent kidney transplantation in the period from 2012 to 2016 we identified infections caused by various bacteria, fungi and viruses in the first 6 months post-transplant using hospital's registry of isolated pathogens. Levels of IgG, IgM, IgA and complement levels of C3 and C4 were measured at the time of transplantation, at day 7, 30, 90 and 180 after transplantation.
Results: From 6 months, incidence of BK infection was 24.9%. In this single center study, we measured higher C4 levels at early study points after transplantation in patients who developed BK virus infection: at day 7, 24 ± 5 vs 21 ± 6 mg/dl, $p = 0.049$; and at day 30 24 ± 5 vs 21 ± 6 mg/dl, $p = 0.008$. Later measurements were similar: at day 90 22 ± 5 vs 21 ± 6 mg/dl, $p = 0.59$ and day 180 22 ± 6 vs 21 ± 6 mg/dl, $p = 0.59$. No correlations between immunoglobulin G or C3 levels and BK virus infection was observed.
Conclusion: The results obtained in our study imply that early C4 levels might be correlated to BK virus infection in kidney transplanted patients. Previous studies have suggested that in a subset of patients with BK nephropathy the presence of C4d deposition correlates with marked viral cytopathic effect. The potential role of higher serum C4 levels in correlation with BK infection warrants further investigation in future studies. IgG concentration was not correlated with development of BK polyomavirus infection in kidney recipients.

Clinical Kidney Rejection

MP058 RENAL TRANSPLANTATION IN AVERAGE SENSITIZED RECIPIENTS

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This prospective study sought to compare the efficacy of two desensitization protocols: double filtration plasmapheresis with low-dose of intravenous immunoglobulin (IVIG) (100 mg/kg) and high-dose IVIG (2 g/kg) only among patients with low level of preexisting antibodies (PRA ≤30%).

Study group includes 19 patients with average PRA was 25.1 ± 6.1%. They received low IVIG dose. Comparison group includes 23 patients with PRA 18.9 ± 4.4). They received high IVIG dose. Crossmatch was negative in both groups.

6 episodes of acute rejection and 1 episode of infection were registered in the study group, 13 episodes and 3 episode were in the comparison group respectively. Acute rejection caused total loss of graft function in 4 patients of study group and in 8 patients of comparison group.

In the study group overall renal graft survival was 79%. It was 65% in the comparison group. Annual graft survival consists 94% and 62%, respectively.

Graft function was significantly better in the study group. 3 months after transplantation patients of the main group had a significantly lower level of daily proteinuria ($p < 0.001$); 6 months – higher GFR ($p = 0.001$) and lower daily proteinuria ($p = 0.01$). 1 year after transplantation patients of the main group had lower creatinine plasma level ($p = 0.001$), higher GFR ($p = 0.001$), lower daily proteinuria ($p = 0.001$) versus patients of control group.

Our study showed that increasing of acute rejection episodes and graft survival rate can be observed also in low sensitized recipients. Graft survival is higher in study group. Thus, double filtration plasmapheresis with low-dose intravenous immunoglobulin is more effective treatment compared to high IVIG dose regimen. It also has a beneficial effect on anti-HLA donor-specific antibodies production de novo.

Further investigations are needed to evaluate the long-term clinical efficacy of this approach.

MP059 ANTIBODY-MEDIATED REJECTION OF KIDNEY GRAFTS: COMPARISON OF STANDARD THERAPY AND THERAPY WITH ADDITION OF BORTEZOMIB AND/OR RITUXIMAB

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Background: Plasmapheresis (PP) or immunoadsorption (IA) with intravenous immunoglobulin infusion is the essential therapy in antibody-mediated rejection (ABMR) of kidney grafts. The aim was to compare an outcome of standard therapy (PP/IA and Cytomegalovirus hyperimmune intravenous IgG) (ST group) and additional therapy with bortezomib (B) and/or rituximab (R) (B/R group) in ABMR.
Methods: Kidney transplant recipients with ABMR, treated at our institution in years 2005–2017, were included into retrospective analysis.
Results: We analyzed 78 patients with ABMR, 41 men and 37 women with average age 49.5 ± 13.8 years. ST group consisted of 48 patients (62%) and B/R of 30 (38%). Median time from Tx was 84 months (IQR 39–240) in ST and 98 months (IQR 24–276) in B/R group. At time of kidney graft biopsy, serum creatinine was 267 ± 164 μmol/l in ST and 208 ± 112 μmol/l in B/R group, with 27% and 13% dialysis-dependency, respectively. There were 24 (50%) acute ABMR in ST and 10 (33%) in B/R group, where majority of rejections were late-presenting. All patients were treated with standard therapy, i.v. methylprednisolone and immunosuppression modification. Thirty patients were additionally treated either with B (9/30%), B and R (16/53%) or R (5/17%). Concomitant T-cell mediated graft rejection (TCMR) was present in 30 (62%) and 13 (43%) patients in ST and B/R group respectively. Patient survival at 2 years was 89% in ST and 100% in B/R group ($p = 0.125$). Cumulative proportion of kidney graft survival at 1 and 2 years was 67% and 53% in ST group and did not significantly differ from B/R group (73% and 48%, respectively; $p = 0.641$). Chronic ABMR ($p = 0.004$) was significant, while dialysis dependency ($p = 0.072$), serum creatinine ($p = 0.082$) and presence of DQ-DSA ($p = 0.062$) at biopsy were borderline significant predictors of worse graft outcome.
Conclusions: Short-term kidney graft survival at two years after ABMR therapy was not significantly influenced by B/R added to standard protocol.

MP060 THE EFFICACY OF ANTI-LYMPHOCYTES GLOBULINS COMPARED WITH OTHER APPROACHES IN REVERSING ACUTE GRAFT REJECTION POST LIVE-DONOR RENAL TRANSPLANTATION: A 10 YEARS FOLLOW UP

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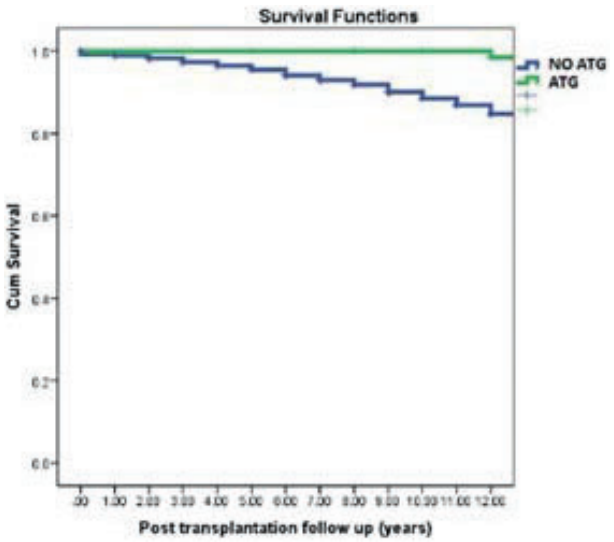
Background: Transplantation proved to be superior to hemodialysis on long term patient survival & quality of life. However, the advances in immunosuppression, acute rejection still occupies the main risk factor of poor graft outcome. Acute graft rejection is treated according to the histopathologic score (Banff classification). In steroids resistant acute graft rejection and in high grade acute graft rejection; polyclonal immunoglobulins are usually administrated. Our aim was to compare the efficacy of anti-lymphocytes globulins compared with other approaches in reversing first acute graft rejection post living-donor renal transplantation.
Patients & Methods: A retrospective analysis was performed on 2500 patient data records to evaluate the efficacy of anti-thymocyte globulins versus other approaches in reversing acute graft rejection.
Results: Among our kidney transplanted patient series 23.3% suffered acute cellular rejection ($N = 646$), 4.17% patients received ATG ($N = 27$) and 95.8% received pulse methyl prednisolone ($N = 619$). Long term graft survival (10 years) was significantly improved in the ATG group (98.5%) versus non-ATG group (86.5%) when ATG was given in the first rejection episode ($p = 0.018$). Late ATG administration in the second or third acute cellular rejection episodes was associated with similar outcome of graft survival compared to non-ATG group ($p = 0.465$). Incidence of bacterial & viral infections showed insignificant differences between ATG & non-ATG group on long term follow up.

In conclusion, early administration of polyclonal anti-lymphocytes globulins provokes better graft survival compared to delayed administration & to other approaches.



Clinical Kidney Surgical technique

MP061 THE SINGLE INCISION, SINGLE SURGEON NEPHRECTOMY: AN ADVANCED, OPEN DONOR NEPHRECTOMY TECHNIQUE

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Kidney transplantation remains the treatment of choice for end-stage renal disease. We present an advanced living donor nephrectomy technique that is less invasive than the conventional open flank incision. This technique involves only one incision much smaller that the one used in the laparoscopic technique. The introduction of a specially designed self-retaining retractor and the use of the harmonic scalpel allows the surgeon to perform the nephrectomy without the need of an assistant. Surgeons in attendance are taught how to perform the surgery and are not used as assistants. The procedure is applicable in all potential donors regardless of the body mass index of the donor or the size of the surgeon's hands. It provides excellent graft functions and allows to expand living donor programs. It allows the donor a quick recovery and an excellent cosmetic outcome. This technique is not associated with complications such as pancreatitis, bowel obstruction, pneumonia, adult respiratory distress syndrome, splenic lacerations, pneumothorax, liver lacerations, and diaphragmatic injuries unlike in the laparoscopic nephrectomies.

MP062 OUTCOMES OF KIDNEY TRANSPLANTATION USING GRAFTS WITH MULTIPLE RENAL ARTERIES

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Background: Transplantation of the kidney grafts with multiple renal arteries may be associated with increased incident of early complication and may influence the results of transplantation. The aim of this study was to detect if the multiple kidney graft arteries have an impact on transplantation outcome.
Methods/Materials: We retrospectively analyzed 205 consecutive cases of deceased donor renal transplantations performed from 01.01.2008 to 31.12.2011. Patients were divided into two groups: group A – patients who have kidney graft with multiple arteries (41 patients) and group B – patients who have kidney graft with one artery (164 patients). All patients were observed for 5 years. Groups were compared for the early vascular complication (bleeding and thrombosis), graft function and 5-year graft survival. All grafts passed "back-table" vascular reconstruction prior to transplantation.
Results: The incidence of early vascular complications in group A and group B was 12.2% and 7.3% for bleeding ($p = 0.235$) and 2.5% and 1.8 % for arterial thrombosis, respectively ($p = 0.490$). Group A and B patients had similar 5-year graft survival (65.5% and 68%, $p = 0.357$) as well as similar serum creatinine levels after 5 years for functioning grafts (0.168 ± 0.06 mmol/l and 0.170 ± 0.10 mmol/l, $p = 0.554$).
Conclusion: Our study results show that kidney transplantation using grafts with multiple renal arteries is safe and has similar outcomes as using grafts with single renal artery.

MP063 LAPAROSCOPIC NEPHRECTOMY IN HEMODIALYSIS PATIENTS WITH POLYCYSTIC KIDNEY DISEASE AS A STANDARD FOR INCLUDING IN THE KIDNEY WAITING LIST

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Background: The surgical tactics for patients with polycystic kidney disease (PKD) are disputable in many aspects. There are our preliminary findings for defining best algorithm for including those patients in the waiting list.
Materials and Methods: There was two groups of patients ($n = 32$) who underwent nephrectomy of a polycystic-changed kidneys. The first group (15 patients) performed open surgery using midline laparotomy and lumbotomy (16 operations), the second group (17 patients) – laparoscopic nephrectomy (24 operations). Surgical intervention in both groups was performed for emergency and planned patients receiving renal replacement therapy with dialysis.
Results: The average duration of laparoscopic and open surgeries was 146 ± 14 and 134 ± 15 min ($p > 0.05$). The average size of the removed polycystic-changed kidneys was 22.5 ± 4.27 cm in the first group, and in the second – 21.5 ± 3.9 cm ($p > 0.05$). The frequency of postoperative complications in the first and second groups consisted of 43.75% and 12.5%, respectively. Was observed in 1 (6.25%) case of lethal outcome in the first group. Average postoperative hospital stay in the first group 13-14 (to 13.7 ± 1.3), the second – 7-8 (7.6 ± 0.4, $p < 0.05$). Patients after laparoscopic procedures activated in 2-3 days (2.5 ± 0.13), after open operations on 4-5 (4.13 ± 0.39, $p < 0.05$).
Conclusions: The use of laparoscopic nephrectomy in patients with PKD allows to reduce the frequency of postoperative complications and mortality.

Clinical Liver Surgical technique

MP064 SUCCESSFUL LIVER TRANSPLANTATION USING A JUMP GRAFT FOR DIFFUSE PORTAL VEIN THROMBOSIS DUE TO PORTO-BILIARY FISTULA CAUSED BY RADIOFREQUENCY ABLATION

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Portal or hepatic vein thrombosis after radiofrequency ablation (RFA) is caused mainly by RFA heat damage to the endothelial cells of the portal or hepatic vein near the ablation zone. The vascular injury involves the intimal layer of the portal or hepatic vein, which in turn leads to platelet aggregation and the subsequent formation of thrombosis. During liver transplantation, a jump venous graft from the superior mesenteric vein (SMV) is indicated when the portal flow can be restored in cases of extensive portal vein thrombosis or when there is no suitable engorged collateral coronary vein available. We report a case where successful liver transplantation using a jump graft for diffuse portal vein thrombosis due to porto-biliary fistula cause by RFA.

A seventy three-year-old patient diagnosed with hepatocellular carcinoma and liver cirrhosis due to hepatitis B was admitted to our hospital. He had previously underwent liver segmentectomy and later received chemoembolization and RFA due to tumor recurrence. Due to refractory ascites and hepatic encephalopathy, he underwent deceased donor liver transplantation. The MELD score was 28 and CTP score was 11. Preoperative CT scan revealed extensive portal vein thrombosis in both liver lobes extending to main portal vein and proximal SMV. During the operation, venotomy of the anterior wall of the main portal vein revealed bile in addition to diffuse thrombosis. During anastomosis, jump venous graft from the superior mesenteric vein (SMV) was used to restore portal flow. After normalization of liver function tests and rehabilitation, he was discharged on the 83rd postoperative day.

In conclusion, due to the possibility of porto-biliary fistula and the development of diffuse portal vein thrombosis, RFA should be judiciously performed for potential transplant candidates.

Clinical Kidney Surgical technique

MP065 GRAFT VOLUME INCOMPATIBLE KT: SUCCESSFUL 2 CASES

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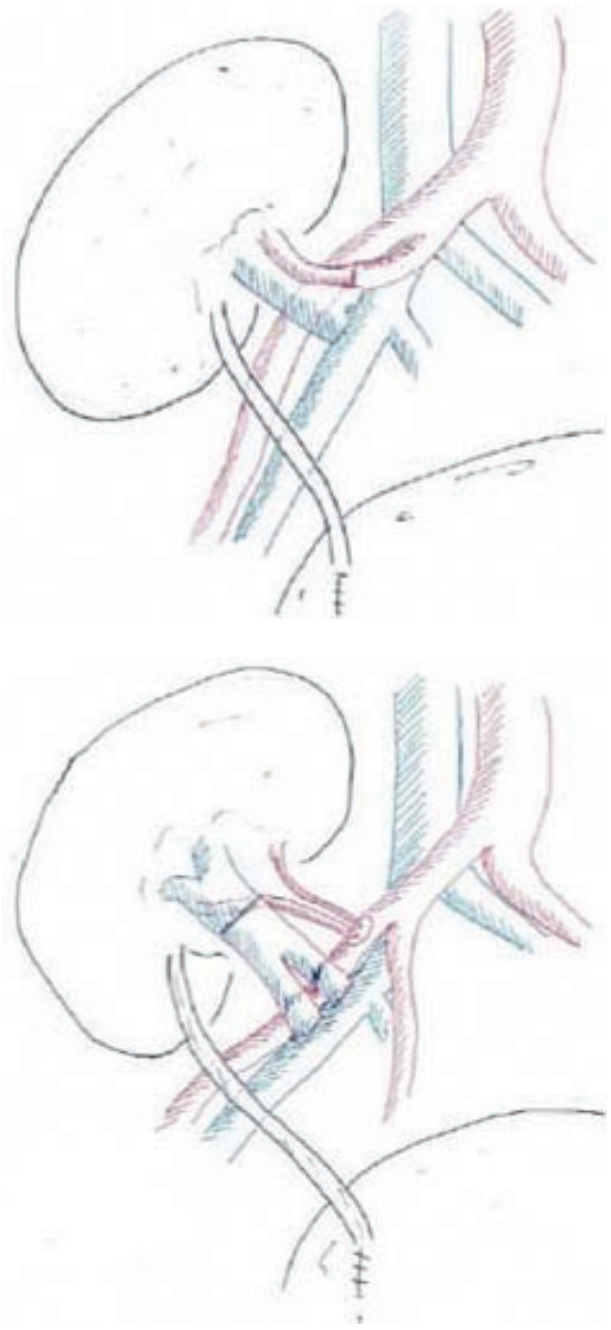
Introduction: Kidney transplantation (KT) is an effective treatment that reduces mortality in end-stage renal disease patients. However, unlike living donor KT, deceased donor KT (DDKT) is difficult to fit appropriate cases for many factors such as age, gender, weight, and histocompatibility between donor and recipient. Especially, pediatric kidney donors have small kidneys and all tissues are thin, immature, and fragile. We announced that there are two cases of successful transplant from children to adult.

Case 1. 6-year-old donor's weight is 23 kg and cause of brain death is hypoxic damage. 56-year-old female HD patient underwent DDKT (Her weight



is 58 kg). Graft weight is 75 g and has single artery and vein. An end - to - end anastomosis of the renal artery to the donor internal iliac artery and an end to side anastomosis of the renal vein to the external iliac vein were performed. The ureteroneocystostomy was made by using the U-stitch method that cutting a part of the bladder and fixing and bundling the ureter. There was no initial surgical complication, and normal renal function was maintained for 3 months (Fig. 1).

Case 2. 5-year-old donor weight is 22 kg and traumatic SDH is cause of brain death. 58-year-old male HD patient performed DDKT (His weight is 65 kg). Graft weight is 80 g and has double artery and vein. Anastomosis of the common iliac artery of the recipient was performed on a portion of the aorta that the two renal arteries showed together. The common iliac artery portion of the donor was resected, and the upper portion was attached to the renal vein in an inverted V shape, and the lower portion was anastomosed to the common iliac vein of the recipient end to side. The ureteroneocystostomy was performed by using the U-stitch method. There was no initial surgical complication, and maintained normal graft function for 3 month (Fig. 2).

Conclusion: Kidney transplantation donated by children is not easy, but it can be done successfully.

MP066 PARTIAL ALLOGRAFT NEPHRECTOMY FOR A DE-NOVO RENAL CELL CANCER DEVELOPING IN A RENAL TRANSPLANT RECIPIENT

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Introduction: Although renal transplant recipients are at an increased risk of malignancies, these usually arise from native tissue. Renal cell carcinoma arising de-novo in the renal allograft is rare, occurring in 0.14 to 0.2 per cent of renal transplant recipients. We present one such case managed surgically at our centre and share our experience.

Methods: A review of case notes and investigations.

Results: We present the case of a 70-year-old gentleman who received a cadaveric transplant six years ago. His immunosuppression involved prednisolone, mycophenolate and tacrolimus for a year following which he was switched to sirolimus. He had undergone an ultrasound of his transplant kidney which raised the suspicion of a solid lesion and this was confirmed on an MRI to be a 4.4 cm tumour involving the upper pole of the allograft. A biopsy revealed a grade 1 renal cell carcinoma and a full staging CT scan was performed.

Following detailed multidisciplinary and patient discussions it was decided to proceed to a partial nephrectomy with a curative intent.

As a part of the procedure planning his immunosuppression was changed to tacrolimus and he underwent a successful partial transplant nephrectomy. Postoperatively he had a slow recovery and requiring prolonged hospitalisation due to recurrent chest and cardiac complications. His graft function initially deteriorated and then stabilised without the need of any other form of renal replacement therapy and he remains dialysis free 6 months postoperatively.

Discussion: Partial allograft nephrectomy remains a viable option for de-novo malignancies although extreme caution and careful planning is required.

MP067 THE USE OF HAND ASSISTED RETROPERITONEOSCOPIC NEPHRECTOMY TECHNIQUE FOR SINGLE INCISION SIMULTANEOUS NATIVE NEPHRECTOMY AND LIVING DONOR KIDNEY TRANSPLANTATION: REPORT OF TWO CASES

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Native nephrectomy during the kidney transplant (KT) process has been shown to be a feasible option especially when performed by laparoscopy. Recently, hand-assisted retroperitoneoscopic nephrectomy (HARP) technique has been introduced which combines control and speed of hand-guided surgery with benefits of retroperitoneal access. One of the lesser-known benefits of the HARP technique is the potential use of the hand port incision for simultaneous ipsilateral KT.

Herein, we present two KT recipients requiring unilateral native nephrectomy, who underwent simultaneous left nephrectomy using HARP technique and living donor KT on the left side by using the same hand port incision. Patient 1 was a 40 year-old female with end-stage renal disease (ESRD) secondary to membranoproliferative glomerulonephritis. Her pre-transplant evaluation revealed a left renal mass suspicious of malignancy. Before proceeding to KT, left nephrectomy and frozen section was planned to rule out malignancy. Patient 2 was a 52 year-old male with a history of small bowel resection due to mesenteric embolism, which has led to nephrolithiasis induced ESRD. His flank pain was predominant on the left side and computer tomography revealed prominent stone formation in the left kidney. Because of the history of multiple abdominal operations, he was a good candidate for left nephrectomy using the retroperitoneoscopic approach. The patients had two 12 mm. trocar incision (subxiphoid, anterior subcostal) and lower paramedian incision (6–7 cm) for hand port insertion that was used for transplantation of kidney after extending 3–4 cm. more. Both patients underwent simultaneous HARP left nephrectomy and KT with an uneventful postoperative course.

The transplant centers who are well experienced in HARP donor nephrectomy, can use their skills at the recipient surgery to perform combined surgery of HARP native nephrectomy and KT from a single incision. With the combination of the two surgical procedures, the recov.

Clinical Liver Surgical technique

MP068 EFFECTS OF THROMBECTOMY IN EARLY HEPATIC ARTERY THROMBOSIS AFTER LIVER TRANSPLANTATION IN LONG-TERM OBSERVATION

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Medical University of Silesia in Katowice, Poland

Hepatic artery thrombosis (HAT) is the most severe vascular complication and one of the major causes of early graft loss and recipient mortality after orthotropic liver transplantation (OLTx). The number of retransplantations and donor deaths can be decreased with an urgent thrombectomy of the hepatic artery.

We aimed to analyze short- and long-term outcomes of the surgical revascularization of thrombosed hepatic artery in patients after OLTx.

Material and Methods: 287 OLTx in 268 patients performed between 2005 and 2016 were analyzed. Doppler assessment of the graft vessels patency was performed daily within first 5 days after transplantation in all recipients. If Doppler diagnosed HAT was confirmed in angio-CT, recipients were referred to surgical revascularization, depending on circulatory stability.

Results: Early HAT was diagnosed in 11 cases (3.8%), occurring most frequently between 1st and 3rd day after transplantation. One patient died of multiorgan failure, and another underwent retransplantation due to fulminant graft failure. Nine patients were treated with surgical thrombectomy. In long-term observation 3 recipients died (two of them with satisfactory graft function), and 3 other underwent retransplantation.

The late biliary complications were diagnosed in 5 recipients: four common bile duct stenoses and one bile leakage. All recipients were treated endoscopically with good long-term effect in 2 patients.

Only one recipient had no complications after revascularization.

Conclusion: Urgent surgical revascularization of early HAT after OLTx allows to avoid urgent retransplantation. However, this approach is connected with high risk of biliary complications, what indicates the need for intense follow up.

Clinical Kidney Surgical technique

MP069 A NEW SURGICAL TECHNIQUE OF EN BLOC KIDNEY TRANSPLANTATION FROM DECEASED INFANT DONORS INTO ADULT RECIPIENTS

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Tongji Hospital, China

Background: In view of high discarding rate of renal grafts from infant donors due to frequent vascular complications such as thrombosis and embolism, we attempted to use a new simplified technique of en bloc dual kidney transplantation (EBKT).

Materials and Methods: The death causes of the donors (both age 1 month) were intra- cranial hemorrhage and cerebral hypoxia. The dual kidneys were recovered en bloc with the abdominal aorta and inferior vena cava (IVC) after in-situ perfusion. The functional warm ischemia times were 16 minutes and 18 minutes. After splitting the rear wall of the donor aorta and IVC, suitable size of patches were made by cutting along the vascular openings of renal arteries and renal venous. All the nonrenal tributaries of the donor aorta and IVC patch were ligated. Two adult recipients (age 46 years and 61 years) received dual kidney grafts. All kidneys had the length less than 6 cm. We anastomosed the donor aorta patch to the external iliac artery in an end-to-side fashion. The donor IVC patch was also anastomosed to the recipient's external iliac vein in an end-to-side fashion. A single 3F double J stent was placed in each ureter and the distal end of ureter graft was implanted into the bladder separately. The total time including back table preparation and anastomosis were less than 2 hours.

Results: Two recipients underwent excellent renal function recovery post-operation without any vascular complications. The creatinine levels were 60 and 65 μmol/l at 12 months of follow up respectively.

Conclusions: Instead of traditional EBKT in which proximal side of aorta was sewn, this new method can be attempted for implanting en bloc dual kidney

grafts from infant donors into adult recipients with lower risk of vascular complications. Owing to simplified technique, dual kidneys can be transplanted like a single kidney with a shorter overall operative time.

MP070 «SHORT» RIGHT RENAL VEIN IN LIVING DONOR KIDNEY TRANSPLANTATION

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Background: Depending on the type of kidney transplantation (cadaveric or living) and anatomic peculiarities, the problem of «short» right renal vein (RRV) can be resolved in different ways. Surgical techniques include the use of segments of cadaveric vena cava inferior or iliac veins, recipient iliac vein transposition, renal vein elongation with saphenous or gonadal vein or polytetrafluoroethylene graft.
Methods/Materials: We represent 4 cases of «short» RRV in living donor kidney transplantation with various ways of elongation. In 2 patients the superficial femoral vein (SFV) was identified in the upper third of the thigh with subsequent harvesting of 5 cm segment up to the deep femoral vein orifice. Autovein was anastomosed in reversed position on back-table end-to-end of kidney transplant vein with next end-to-side anastomosis with recipient external iliac vein. In another 2 patients the problem of «short» RRV of living donor kidney transplant was resolved by recipient internal iliac vein (IIV) interposition.
Results: Good immediate results were obtained after all described «short» right kidney transplant vein elongation. During follow-up from 48 to 96 months no kidney transplant venous outflow violations were stated at duplex ultrasonography scanning.
Conclusions: The SFV and IIV in the most cases geometrically coincide with renal vein, thus providing the necessary venous outflow parameters of kidney transplant. Technically, SFV harvesting or IIV mobilization cannot create difficulties for AN experienced surgeon. Thus, the autologous SFV and IIV can be used successfully in occasional cases, which require «short» right renal vein elongation in living donor kidney transplantation.

Clinical Kidney Surgical technique

MP071 EXTRA-RENAL MYCOTIC PSEUDOANEURYSM AFTER KIDNEY TRANSPLANTATION, A SERIOUS LIVE THREATENING CONDITION OR A CONTROLLABLE COMPLICATION. CASE PRESENTATION AND LITERATURE REVIEW

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[1]Hamed Al Essa Organ Transplant Center, Canada; [2]Hamed Al Essa Organ Transplantation Center, Kuwait

Vascular complications after kidney transplantation affect 3–15% of patients. They carry the worse prognosis as they commonly lead to graft loss. Arterial mycotic pseudoaneurysm is rare serious complication occurs in less than 1% of patients. Literature records are few, sporadic case reports and small series, mainly concerned on patient survival and limb salvage rather than graft survival.

Etiologically it is multifactorial. Its infectious etiology is diagnosed when positive tissue culture of the pseudoaneurysmal or the arterial wall was found. We report on a case of 35 years old lady who presented 63 days after deceased donor kidney transplantation, with mild deterioration of her renal function. She had a history of fever and positive urine and blood cultures growing klebsiella pneumonia one week post transplantation, treated with 14 days of meropenem. Duplex ultrasound scan, reported pelvicalyceal (PC) dilatation and perigraft fluid collection of $7 \times 10 \times 11$ cm, and failed to demonstrate the flow inside the aneurysm.

Renal scintigraphy diagnosed delayed clearance of the isotope in the (PC) system due to partial obstruction.

Retrograde ureteric stent was introduced with no improvement; non-contrast Computed tomography scan displayed a globular mass between the graft, urinary bladder and pelvic wall. During surgical exploration anastomotic aneurysmal sac was opened and reperfusion of the allograft with patchplasty failed to improve the kidney vascularity, graft nephrectomy done with repair of the external iliac artery with PTFE patchplasty. Renal artery wall Tissue culture grew Serratia marcescens sensitive to ciprofloxacin, given for four weeks.

Prompt on time intervention and appropriate individualized plane for treatment modality, increases chances for graft salvage, and avoid potential fatal complications. Once pseudoaneurysm is diagnosed intervention is indicated. Experienced and competent diagnostic tools are essential for early detection of such serious complic.

Clinical Kidney Surgical technique

MP072 LATE DEHISCENCE OF ARTERIAL ANASTOMOSIS IN KIDNEY TRANSPLANTATION: CASE REPORT

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Background: Immunosuppressive therapy cause a delay in tissue remodelling and in scar tissue formation. Patients undergoing kidney transplantation are exposed to a great risk, considering that at least three anastomosis are crafted during this intervention. Anastomotic dehiscence usually appears in the early post-operative period; we shows a case of an anastomotic leak occurred many days after the intervention.
Methods: A 67 year end stage kidney disease patient, who underwent living donor left kidney transplantation. In post operative day (POD) 22 an emergency intervention was carried out for arterial anastomosis dehiscence; the arterial patch on the common iliac artery was removed and crafted new renal-iliac arterial anastomosis with autologous greater saphenous vein graft (Fig. 1).
Results: Cultural and histological examination of excised arterial patch, showed mucoid degeneration of the vascular wall and a positivity for *E. Coli.* The MRI examination noted a voluminous collection in the right hip with greater axis of about 20 cm. The treatment was initially conservative and then by ultrasound-guided drainage. Discharged in POD 48 and 27 respectively after transplantation and reintervention, in good clinical condition, with good renal function and perirenal collection reduction.
Conclusion: An infectious insult in the immediate pre-transplant, expose the patient to a major risk in the post-operative period. In particular facilitating a dangerous anastomotic dehiscence in patients whose immunosuppressive therapy already slows down tissutal repair. The only option in this unfortunate event is the surgical one and a temporary suspension of immunosuppressive therapy. In fact, an anastomotic leakage after many days from the intervention cannot be only the result of a delayed consolidation due to immunosuppressants, but must be the consequence of concomitant events such as infectious diseases.