TRANSPLANT
INTERNATIONAL

023 KIDNEY

O01 FLUOROQUINOLONE PROPHYLAXIS IN PREVENTING BK POLYOMA VIREMIA AFTER RENAL TRANSPLANT: A SYSTEMATIC REVIEW AND META-ANALYSIS

Turun Song[1], Zhongli Huang[1], Dongyang Zeng[1], Zhengsheng Rao[1], Yang Qiu[1], Lei Fu[1], Libo Xie[1], Xianding Wang[1], Tao Lin[2]
[1]West China Hospital, Organ Transplantation Center; [2]Organ Transplantation Center West China Hospital

Objectives: The available studies concerning preventing BK viremia after renal transplantation with fluoroquinolone yielded conflicting results. The purpose of this systematic review was to examine the evidence regarding the effect of fluoroquinolone prophylaxis in BK viremia prevention after renal transplantation.
Methods: We searched PubMed, Embase and the Cochrane central register of controlled trials for research articles published prior to January 2015 using the key words such as fluoroquinolone, BK viremia and renal transplantation. We selected all type of studies published in English language. The primary outcome was BK viremia at one year, and the secondary outcomes were BKVN, graft failure, fluoroquinolone resistant infection and renal function.
Results: Eight trials included a total of 1477 participants with a mean duration fluoroquinolone prophylaxis of more than one month. At one year, fluoroquinolone prophylaxis was not associated with lower incidence of BK viremia (HR = 0.84, 95%CI 0.58–1.20). No difference was detected in BKVN between fluoroquinolone prophylaxis and control group (HR = 0.88, 95%CI 0.37–2.11). There was no difference in the risk of graft failure due to BKVN (HR = 0.49, 95%CI 0.05–4.46) and fluoroquinolone resistant infection (HR = 1.16, 95%CI 0.60–2.22). Both group had similar estimated glomerular filtration rate (MD = −10.03 ml/min/1.73 m^2, 95%CI −21.89–1.83).
Conclusion: This study suggests that fluoroquinolone prophylaxis is ineffective in BK viremia prevention after renal transplantation.

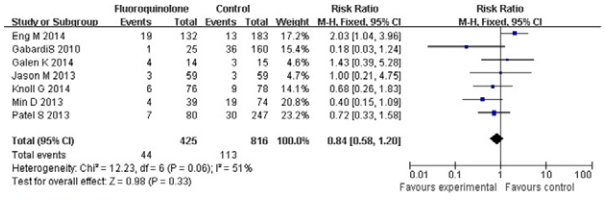


Figure1. BK viremia at one year.

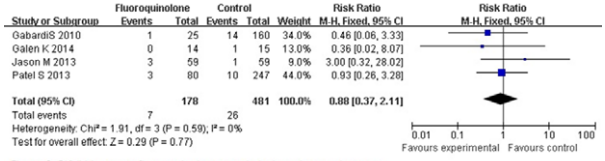


Figure 2. BKVN between fluoroquinolone prophylaxis and control group

O02 SUCCESSFUL COST EFFECTIVE PREVENTION OF CYTOMEGALOVIRUS DISEASE IN KIDNEY TRANSPLANT

Osama Gheith, Torki Alotaibi, Medhat A Halim, Hany Adel, Ahmed Mosaad, Hasanein Atteya, Zakareia Zakareia, Tarek Said, Prasad Nair, Naryanan Nampoory
OTC

Introduction: Prophylaxis for cytomegalovirus infection is highly recommended for kidney transplant recipients. Using valgancyclovir in low dose is still under investigation.
Aim of Work: To assess the cost effectiveness of 450 mg valgancyclovir prophylaxis compared with 900 mg for kidney transplants.
Patients and methods: In this prospective trial, 201 kidney transplants were randomized (1:1) to receive 450 mg valgancyclovir prophylaxis (group1, $n = 100$) or 900 mg daily (group2, $n = 101$) for the first 6 months post-transplant. Patients were studied for incidence of CMV disease, leucopenia attacks, rejection episodes and graft outcome and associated costs in one year duration. Direct costs associated with acquisition of immunosuppressive medications, diagnosing rejection, and hospitalizations were included. For each type of rejection (steroid-responsive or resistant), there sources used were categories composed of hospitalization, diagnostic tests, and prescribed drugs used to treat the rejection episode. The cost data from our hospital records and the costs were measured in US dollars.
Results: Demographic features of the studied groups were comparable. More patients have received tacrolimus in group1, while in group 2 more patients were maintained on cyclosporine (p0.001). We found that the cost of CVM prophylaxis in patients of group 1 was significantly lower (by 50% at 6 months, $p < 0.001$) with lower leucopenia attacks (p 0.04) and lower doses of granulocyte colony stimulating factor (by 30% at 6 months, p 0.03) compared to group 2. Higher doses of mycophenolate mofitel (p 0.04) among group 1 patients were protective therefore they experienced less rejection episodes (p0.01). In group2; there were more cytomegalovirus infections requiring full treatment (p0.052) and more BK virus nephropthy (p0.03). Graft and patient outcomes were satisfactory in both groups. Mean estimated glomerular filtration rates were above 60 ml/min at baseline, at 6 months and at 12 months post-transpla

O03 CONCERTED T-CELL ACTIVITY AGAINST EARLY AND LATE BKV-ANTIGENS IS NECESSARY FOR VIRAL CLEARANCE

Benjamin Weist[1], Michael Schmueck[2], Mikalai Nienen[2], Avidan Neumann[2], Timm Westhoff[3], Petra Reinke[4], Nina Babel[5]
[1]Berlin Brandenburg Centrum fuer Regenerative Therapien Charite Universitaetsmedizin Berlin; [2]Berlin Brandenburg Centrum fuer Regenerative Therapien, Charité Universitaetsmedizin Berlin; [3]Medical Clinic, Marien Hospital Herne, Ruhr University Bochum; [4]Berlin Brandenburg Centrum für Regenerative Therapien Charité Berlin; [5]Berlin Brandenburg Centrum fuer Regenerative Therapien Charite Berlin

Background: Polyomavirus-BK (BKV) associated nephropathy is a known cause of graft failure. No specific therapy is established so far and viral load monitoring accompanied by adjustment of immunosuppression is the only known effective therapeutic option. We previously demonstrated BKV-specific T-cells as a factor predicting BKV-clearance and disease recovery. However, due to technical limitations, data on the role and specificity of different T-cell subsets including cytolytic/helper CD4 and CD8 T-cells is scarce. Moreover, the impact and contribution of T-cells specific to early (LT, st) and late (VP1-3) viral antigens is also not defined.
Methods: We implemented a multi-parameter flow cytometry protocol and investigated the sensitivity and robustness of variable effector molecules (IFNg, TNFa, IL2, IL4, IL17, GranzymeB) and receptors (4-1BB, CD40L, TIM3, PD1) as BKV-specific activation markers under immunosuppression. By detecting BKV-specific T-cells according to the expression of the receptors CD137 and CD154 in combination with the effector molecule GranzymeB, we were able to detect specific T-cells more sensitively (compared to IFNg-based approaches) and categorized them into cytolytic/helper T-cells. Subsequently, antiviral immunity of 37 kidney transplant patients in clinical follow up and of 15 healthy volunteers was dissected into cytolytic and helper T-cell responses. Next, we dissected cytolytic and helper T-cell responses according to early (st, LT) and late (VP1-3) BKV-antigen specificity and investigated the Transcription factors that drive cytolytic CD4+ and CD8+ T-cell responses against BKV.
Results: Our approach increased the sensitivity of detecting of BKV-specific T-cells by 4.9-fold (median) in comparison to previously used INFg-based detection by flow cytometry. Of importance, we showed that BKV clearance was observed only when both, cytolytic and helper, T-cells were simultaneously detected. Interestingly, CD4 T-cells significantly contribute

O04 THE VALUE OF PERFUSION FLUID CULTURE ANALYSIS IN DECEASED DONOR RENAL TRANSPLANTS. SIX YEARS SINGLE CENTRE EXPERIENCE

Ammar Almidani[1], Sophie Collier[2], Neal Banga[2]
[1]Royal Free Hospital; [2]Royal Free NHS Foundation

Backgound: Microbiological analysis of kidney perfusion/transport solution is not routinely performed in all UK transplant centres. The aim of this study is to assess the impact of routine analysis on patient management and outcome.
Methods: Data were collected retrospectively on all deceased donor transplants performed between 2008 and 2013. Organisms detected were classified

as either pathological, uncertain pathogenicity, or contaminants. Treatment was guided by the microbiology team. Outcomes including type and duration of treatment, morbidity and mortality, length of hospital stay (LOS) and biopsy-proven acute rejection (BPAR) were compared between recipients receiving grafts with culture-positive (PF+) and culture-negative (PF-) perfusate. PF+ were treated if there was pathological cultures.
Results: In total 397 deceased donor transplants were included, of which 330/379 (83.2%) had perfusate samples analyzed. Organisms were cultured in 57/330 (17.3%) of these samples of which 15/23 (67%) were pathological the most common of which were Escherichia coli and 3/57 (5%) cases involved candida. LOS in patients with PF+ was 11 days ($p = 0.08$). There was one graft loss but no difference in morbidity, BPAR rates between the 2 groups. Directly attributable major complications of pathogenic PF+ was graft loss secondary to Candida. A statistically significant proportion of PF+ samples came from donors after circulatory death (DCD) perfusate 37/57 (65%) compared with brain-dead (DBD) perfusate 20/57 35% ($p = 0.0004$ χ^2 test).
Conclusion: Identification of organisms in perfusate is common (17.3%). The similarity in outcomes in this study between PF+ and PF- graft recipients may be secondary to the pro-active treatment. It is important to recognise candida and and have a treatment protocol. PF+ was more frequent in DCD grafts compared to DBD grafts possibly as a result of bacterial translocation during warm ischaemia. Further studies are needed to assess the impact of pro-active treatment

O05 ONE-YEAR RESULTS OF A PROSPECTIVE RANDOMIZED OPEN TRIAL DESIGNED TO REDUCE THE INCIDENCE OF CYTOMEGALOVIRUS (CMV) INFECTION IN DE NOVO KIDNEY TRANSPLANT RECIPIENTS

Ronaldo Esmeraldo, Maria Luiza Mb Oliveira, Petrucia Ma Pinheiro, Celi M Girao
Hospital Geral de Fortaleza

Background: CMV infection is a frequent complication after organ transplantation. Here we report the results from a 1-year, prospective, single centre, open study designed to compare the effects of 2 immunosuppressive regimens on the incidence of CMV infection in renal transplant recipients (RTxR).
Methods: 120 low immunological risk *de novo* RTxR were randomized (1:1) within 24 h post-transplantation to either one of the two reduced tacrolimus (Tac) exposure regimens: (G1, $n = 60$)) everolimus (EVR, 1.5 mg bid, C0 3–8 ng/mL) with very low dose Tac or (G2, $n = 60$) sodium mycophenolate (MPA) with low dose Tac. All patients received Thymoglobulin (6 mg/kg/day) ± steroids. For the first 3 months (M) all patients received Tac (0.1 mg/kg/day, C0 4–7 ng/mL). After that, G1 Tac was targeted to C0 2–4 ng/mL whereas G2 continued the initial regimen unchanged. The primary outcome in this study was the incidence of CMV infection or disease during the first year of transplantation. None of the patients received CMV prophylaxis. CMV infection was monitored fortnightly using a quantitative CMV PCR assay (DNAemia expressed in IU /mL) for the first 3 M, then monthly until M6.
Results: Intention to treat population of 120 RTxR (G1, $n = 60$; G2, $n = 60$) showed comparable baseline characteristics, with a mean age of 43.5 ± 14 years with 77% male and 97% recipients from deceased donor (DD). CMV serum status D+/ R- was observed in 7 patients in G1 and 3 patients in G2. CMV infection occurred in 9 (15%) patients in G1, 8 asymptomatic DNAemia and 1 syndrome, versus 31 (52%) in G2, 27 asymptomatic DNAemia and 4 syndrome ($p < 0.001$). There was no case of invasive CMV disease. Overall graft survival was 98%. Biopsy proven acute rejection was seen in 4 (7%) in G1 and 2 (3%) in G2.
Conclusion: This 1-Year analysis indicates that patients receiving everolimus are at lower risk to develop CMV infection [RR = 0.29 (95% CI: 0.152 a 0.556); $p < 0.001$].

O06 LIVING DONOR KIDNEY TRANSPLANTATION FROM HBSAG(+) DONOR TO HBSAG(-) RECIPIENT WITH OR WITHOUT ANTI-HBS

Jin Kong[1], Joon Jeong[1], Byung Kim[2], Sung Son[1], Soo Yoon[1], Eun Whang[1]
[1]Hanseo Hospital; [2]Maryknoll Hospital

HBsAg positivity is currently regarded as a contraindication of kidney donation to HBsAg negative patients. We developed a protocol that enables transplantation from a HBsAg (+) living donor to a HBsAg (-) recipient. Transplant candidates without protective titer (≥10 mIU/ml) of anti-HBs antibody were given hepatitis B vaccination to develop protective level of antibody. Viral load of donors was reduced by entecavir to be undetectable by real time PCR before transplantation. Recipients were also given entecavir before and during 3 months after transplantation for prophylaxis. Hepatitis B immune globulin was injected intravenously to recipients in the morning of transplant day. Seven living donor kidney transplantations from HBsAg(+) donor to HBsAg(-) recipient were performed. Anti-HBs was positive in 6 recipients and negative in 1 recipient at initial presentation. In the anti-HBs(-) recipient, hepatitis B vaccination was administrated, and anti-HBs became positive (15 mIU/ml) before transplantation. All the recipients had undetectable HBV DNA after transplantation and remained HBsAg(-)/anti-HBs(+) during the median follow up of 39 (1–63) months. Kidneys from HBsAg(+) living donors can be safely transplanted to HBsAg(-) recipients with or without anti-HBs.

O07 RITUXIMAB SPARING PROTOCOL FOR PATIENTS WITH LOW TITERS IN ABO INCOMPATIBLE KIDNEY TRANSPLANT

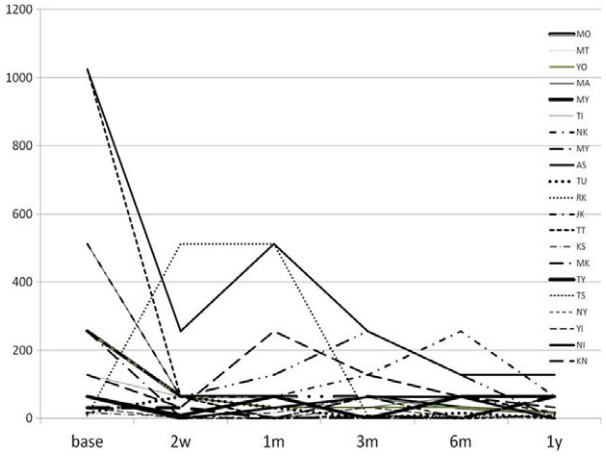
Shunji Narumi[1], Yoshihiko Watarai[1], Norihiko Goto[1], Takahisa Hiramitsu[1], Makoto Tsujita[1], Takayuki Yamamoto[1], Manabu Okada[1], Kenta Itoh[1], Kenta Futamura[1], Takaaki Kobayashi[2]
[1]Nagoya Daini Red Cross Hospital; [2]Applied Immunology, Nagoya University Graduate School of Medicine

Introduction: Acute antibody mediated rejection (AMR) has been a major problem in ABO-incompatible kidney transplantation (ABOi-KTx). We established rituximab sparing protocol for patients with low ABO titers.
Patients and Methods: One hundred sixty-nine adult patients, 58 females and 111 males underwent ABOi-KTx between January 2007 and December 2013. All patients received 2 weeks of mycophenolate mofetile and prednisolone before transplant, followed by calcineurin inhibitor with basiliximab induction. Patients with starting titer of 32× or more (SD group: $n = 124$) received low dose rituximab (100 or 200 mg/body x2) and 4 times of pre-transplant plasmapheresis (PP). SD group included 7 patients with pre-existing donor specific antibody (DSA). For the patients with starting titer below 16× (LR group: $n = 45$), rituximab was excluded from desensitization therapy and pre-transplant PP was reduced to 2 times.
Results: With a mean observation period of 41 (2–94) months, 3 and 5 years patient survival are 97.7% and 95.8% in SD group and 100% in LR group. Graft survival at 3 and 5 years are 96.1% and 90.7% in SD group and 95.8% and 95.8% in LR group. Clinical and subclinical AMR were observed in 3.2% (4/124) of SD group and successfully treated with additional PP/IVIG and steroid pulse therapy but none developed AMR in LR group. Chronic active AMR was observed in 2 patients of SD group. Cytomegalovirus viremia/disease was observed 29.8% (37/124) of SD group and 17.7% (8/45) of LR group ($p = 0.11$). Conclusions: This study demonstrated that rituximab sparing desensitization protocol of ABOi-KTx in a low risk patient population is safe and effective. Amount of rituximab can be reduced in standard and high risk patients without significant increase in prevalence of AMR. Furthermore, reduction of pretransplant IgG titer to <32× may reduce the prevalence of AMR.

O08 POSTOPERATIVE REBOUND OF ANTI-BLOOD TYPE ANTIBODIES AND ANTIBODY-MEDIATED REJECTION AFTER ABO-INCOMPATIBLE LIVING RELATED KIDNEY TRANSPLANTATION. -IS PROPHYLACTIC TREATMENT NECESSARY?

Ishida Hideki, Tomokazu Shimizu, Taiji Nozaki, Kazuya Omoto, Masayoshi Okumi, Kazunari Tanabe
Tokyo Women's Medical University Hospital

Background: The purpose of this study is to examine whether postoperative anti-blood type antibody rebound is attributed to kidney allograft rejection in ABO blood type-incompatible (ABO-I) living related kidney transplantation (KTx).
Materials and Methods: A total of 191 ABO-I recipients who received ABO-I living related KTx between 2001 and 2013 were divided into two groups: Group 1 consisted of low rebound (≦1:32), $N = 170$, and Group 2 of high rebound



(≧1:64), $N = 21$, according to the levels of the rebounded anti-blood type antibodies within one year after transplantation. No prophylactic treatment for rejection was administered for elevated anti-blood type antibodies, regardless of the levels of the rebounded antibodies. We investigated the relevance of the postoperative anti-ABO titer rebound and acute rejection in ABO-I KTx to conclude the necessity of B cell targeting therapies for the rebounded anti-ABO antibodies.
Results: Within one year after transplantation, T cell-mediated rejection was observed in 13 of 170 recipients (13/170, 8%) in Group 1, and in 2 of 21 recipients (2/21, 10%) in Group 2 (Groups 1 vs. 2, p = 0.432). Antibody-mediated rejection was observed in 15 of 170 recipients (15/170, 9%) and 2 of 21 recipients (2/21, 10%) in Groups 1 and 2, respectively (p = 0.898). No significant differences in graft function were observed at 3 months, 6 months, 1 year, 3 years, 5 years, or 10 years between the two groups. The graft survival of recipients with high rebound titers was slightly lower than those with low rebound titers, although not statistically significant.
Conclusion: In this study, we found no correlation between the postoperative anti-blood type antibody rebound and the incidence of acute rejection. We concluded that no treatment is necessary for rebounded anti-blood type antibodies.

O09 RITUXIMAB INDUCTION FOR KIDNEY TRANSPLANT RECIPIENTS AT HIGH IMMUNOLOGICAL RISK: A SINGLE CENTRE EXPERIENCE

Daniel Gingerich[1], Inge Derad[2], Philip Muck[2], Malte Ziemann[3], Martin Nitschke[2]
[1]University of Luebeck; [2]University of Luebeck Transplant Center; [3]University of Luebeck, Transfusion Medicine

For renal transplant recipients with a high immunological risk (e. g. high PRA or donor specific antibodies (DSA)) lymphocyte depleting induction schemes are recommended. These and blood group incompatible patients (AB0i) are assumed to be at high risk for rejection, organ failure or development of donor specific antibodies. On this background, we started a B-cell depleting induction protocol in 2006, combining Rituximab (500 mg single dose) and anti-CD25 (basiliximab) with a standardized tacrolimus based triple therapy. Here we present our data on graft function, patient survival and adverse events of our Rituximab treated patients. From January 2006 until December 2013 78 (19%) of the 408 kidney recipients at our centre met the criteria described above: 27 patients with an AB0-incompatible graft and 51 patients with an HLA incompatible graft. Patients with a positive T-cell crossmatch (CDC) were excluded. The mean follow up was 44.8 months (1 – 90). Hospitalisation due to infectious complications (e. g. pneumonia 9%) or cardiovascular complications (e. g. myocardial infarction 5%) were rare. Table 1 shows patients characteristics and primary outcome data.
Conclusion: In this high risk group of kidney graft recipients primary nonfunction was twice as high as our center standard. Despite the high early rejection rate kidney function at discharge and in the long term is reasonable good. Patient survival is excellent and albeit the more intensive immunosuppression infectious or cardiovascular complications are comparable to low risk patients of our centre. Humoral rejection or appearance of donor specific antibodies is rare. Our Rituximab induction protocol seems to be safe with satisfactory transplant survival.

	$n = 78$ (%)	STD
time on dialysis (month)	46.6	38.90
recipient age	47.54	12.04
donor age	48.29	14.37
living donation	37 (47)	
AB0 incompatible	27 (35)	
retransplant	42 (54)	
HLA immunized (>5% PRA)	53 (68)	
HLA immunized (>30% PRA)	45 (58)	
mismatch (HLA A, B, DR)	2.86	1.80
delayed graft function	30 (38)	
primary nonfunction	8 (10)	
creatinine1 (μmol/l)	141	60.46
creatinine2 (μmol/l)	140	69.38
early rejection (day 1–30)	20 (26)	
late rejection (after day 30)	12 (15)	
humoral rejection any time	10 (13)	
organ failure due to rejection	5 (6)	
graft survival*	57 (73)	
patient survival*	75 (96)	
DSA tested any time after Tx	68 (87)	
de novo DSA after Tx	13 (19)	

(1) at discharge, (2) at latest follow up

O10 POST-TRANSPLANTATION IMMUNOADSORPTION CAN BE WITHHELD IN ABO-INCOMPATIBLE KIDNEY TRANSPLANT RECIPIENTS

Annelies De Weerd, Madelon Van Agteren, Jan Ijzermans, Willem Weimar, Michiel Betjes
Erasmus Medical Center

Background: After ABO-incompatible kidney transplantation, postoperative plasma exchange (PE) or immunoadsorption (IA) is performed per protocol or depending on postoperative A/B-titers to prevent acute rejection. However, the need for postoperative PE or IA is not known.
Methods: Since 2006, 30 consecutive patients received three standard postoperative IAs. Starting from 2009, the last 46 patients received only preoperative IA. Preoperative desensitization consisted of rituximab, tacrolimus, mycophenolate mofetil, prednisone and intravenous immunoglobulins. Antigen-specific IA was performed pre-operatively with the Glycosorb® device. Biopsy-proven acute rejections either antibody-mediated (AMR) or mixed cellular and antibody-mediated (MAR) within three months were recorded.
Results: The postoperative titer in patients with postoperative IA did not exceed 1:16 (IgG 1:4 [<2–16] median and range). The postoperative IgG titer was not significantly different after abandoning postoperative IA, although three patients had titers of 1:32 and one patient even 1:128. Rejections tended to be more frequent in the group with postoperative IA: 6 AMR and 3 MAR were recorded in 30 patients, versus 4 AMR and 1 MAR in the 46 patients without postoperative IA (30 vs. 11%, p = 0.067). Baseline characteristics differed however: in the group with postoperative IA the vast majority had blood group O (87 vs. 52%, p = 0.003). Also the IgG titer on the day of transplantation was higher (1:4 [<2–16] vs. 1:2 [<2–32], p = 0.007). All 14 patients with AMR and MAR rejections had postoperative IgG titers ≤1:16.
Conclusion: Postoperative removal of A/B-antibodies can be safely removed from the ABOi transplantation protocol using strict preoperative criteria for antibody lowering.

O11 OUTCOMES OF ABOI RENAL TRANSPLANTS: A SINGLE CENTRE EXPERIENCE OVER A SEVEN YEAR PERIOD

Faryal Afridi, Rachael Coates, Alison Brown, Chera Arunachalam, Laura Baines, Neil Sheerin, David Talbot
Freeman Hospital

Introduction: ABOi transplantation has been popularised in recent years in view of widening organ shortage and increasing reports of safety of incompatible transplantation. This series looks at outcomes for consecutive ABOi renal transplants in our regional transplant centre.
Methods: Patients ($n = 35$) who received an ABOi live donor kidney transplant from 2006 to 2013 were included. Data relating to patient demographics, antibody titres (pre and post transplant), HLA incompatibility, antibody levels, immuno-suppression regimen including induction and antibody removal procedures was obtained from a prospectively maintained database and analysed using SPSS v21. Primary outcome measure was stable graft function at follow-up with number of treatments needed pre transplant and episodes of rejection measured as secondary outcomes. Data on non-local patients ($n = 9$), graft loss ($n = 2$) and death with functioning transplant ($n = 1$) was excluded.
Results
Of 35 patients follow-up data was available for 23. Graft function remains good in 51% (11% moderate and 3% poor). Mean creatinine in this cohort is 125 (Range 46–334). 46% patients required no pre-op antibody removal, the remaining 54% received 1–14 treatments with most receiving 2 or 3.
Conclusion: As a leading centre in ABOi transplantation in the UK we are utilising new techniques (including the recent approval of the use of Eculizumab) to build up on a promising seven year experience with dialysis independent outcomes for the majority of patients.

Graft Function	$n = 23$
Good	18 (78%)
Modertae	4 (17%)
Poor	1 (3.4%)
Pre_op antibody removal sessions	
0	16 (46%)
1	2 (5.7%)
2	6 (17%)
3	5 (14.3%)
5	2 (5.7%)
8	1 (2.9%)
14	1 (2.9%)
n/a	1 (2.9)
Outcomes	
Death	3%
Failed graft	5.7%
Current Creatinine	Median 125 (Range 46–334)

O12

FLEXIBLE DESENSITIZATION PROTOCOL IS THE SAFE AND EFFECTIVE WAY TO PERFORM ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION

Alexander Sushkov, *Alexey Sharshatkin, Yan Moysyuk*
V.I. Shumakov Center of Transplantology and Artificial Organs

Most desensitization protocols for ABO-incompatible kidney transplantation (iABO) provide uniform precondition pattern for all candidates. Since 2011 we use flexible desensitization strategy that individualized for initial anti-A/B titer.31 iABO were performed between 01/01/2011 and 31/12/2014. Patients received pre-transplant desensitization according to the original protocol (Table). Target anti-A/B titer was 1:8 or less. In the same timeframe 158 blood group compatible living-donor kidney transplants (cABO) were performed.

The median of desensitization time in iABO group was 14 days (5 - 30) and the median of rituximab dose was 260 mg/m^2 (92 - 400). There were no serious adverse events during desensitization. Acute rejection rates in iABO and cABO groups were 6.5% and 4.4%, respectively, Fisher exact p = 0.6432. The 1- and 3-year graft survival in iABO group – 93.1% and 83.7%, in cABO group – 96.6% and 93.9%, respectively, Log-rank p = 0.1877. The flexible approach allowed to acquire good clinical results in iABO group and in the same time to reduce precondition time and not to use high rituximab doses in patients with low anti-A/B titers.

Initial anti-A/B titer	Rituximab, mg	Minimal desensitization time, days	Anti-A/B removal procedure	For all iABO candidates:
≤1:8	200	7	no	IVIG 500 mg/kg Tacrolimus + MMF + Steroids (start at the same time with rituximab)
1:16 ? 1:64	500	10	Plasmapheresis	
≥1:128	375 mg/m2	14	Immunoadsorption	

025 LIVER

O13 ABO MISMATCH INCREASES BILE DUCT COMPLICATION RATE IN DECEASED DONOR LIVER TRANSPLANTATION

Andreas Brandl[1], Matthias Pemler[2], Robert Öllinger[3], Felix Aigner[3], Matthias Biebl[3], Sascha Weiß[2], Robert Sucher[3], Johann Pratschke[3], Stefan Schneeberger[2]

[1]Charité Berlin - Campus Mitte - Department of Visceral, Thoracic and Vascular Surgery; [2]University Hospital Innsbruck, Department of Visceral, Thoracic and Transplant Surgery; [3]Charité Berlin - Campus Virchow Klinikum - Department of General, Visceral and Transplant Surgery

Background: Acceptance of liver allografts with a blood group mismatch in high urgency allocation (HUA) is common practice and helps to receive an organ in time. While patient and graft survival in patients transplanted with an ABO incompatible graft have been previously described, we herein present novel insights into bile duct complications in this setting.
Methods: This retrospective analysis included 413 consecutive adult recipients of deceased donor liver grafts between 1/2005 and 12/2012 at our centre. Statistical analysis was performed with fisher´s exact and Mann-Whitney test, survival was estimated with Kaplan-Meier plot. Linear regression was performed using backwards model and Wald-test.
Results: A total of 13 (3.1%) patients received liver grafts with ABO mismatch. Patient characteristics differed in hospital discharge (35 vs. 23 days; p = 0.004), cirrhosis (69.2 vs. 93.5%;p = 0.01), CMV mismatch (76.9 vs. 48.0%;p = 0.04), Re-transplantation (46.2 vs. 9.0%;p = 0.001), HUA (30.8 vs. 4.3%;p = 0.003) and gender mismatch (61.5 vs. 33.0%;p = 0.04). Five-year patient and graft survival for ABO mismatch (76.9, 76.9%) was not significantly different (79.5, 75.9%;p = 0.26, p = 0.47). Bile duct complications (53.8 vs. 19.8%;p = 0.008), leakage (30.8 vs. 10.3%;p = 0.04), non anastomotic stenosis (23.1 vs. 4.8%;p = 0.03), anastomotic stenosis (28.6 vs. 10.3%; p = 0.04), re-operation rate (23.1 vs. 4.8%;p = 0.04) and mortality (23.1 vs. 2.0%;p = 0.003) were higher in ABO mismatch recipients. Linear regression model identified ABO mismatch (HR 4.518;p = 0.02), recipient BMI (HR 1.078; p = 0.03), hepaticojejunostomy (HR 3.975; p = 0.001), arterial complication (HR 3.975;p = 0.001) and venous thrombosis (HR 10.432;p = 0.002) as independent factors for bile duct complications.
Conclusion: Patients receiving liver grafts with ABO mismatch showed similar patient and graft survival compared to the control group. ABO mismatch was identified as independent factor for the development of bile duct complications.

O14 ABO INCOMPATIBLE LIVER TRANSPLANTATION – THE CZECH EXPERIENCE

Jiri Fronek, Libor Janousek, Martin Oliverius, Milos Kucera, Matej Kocik, Eva Kieslichova, Ondrej Viklicky, Pavel Trunecka
Institute for Clinical and Experimental Medicine (IKEM) Prague

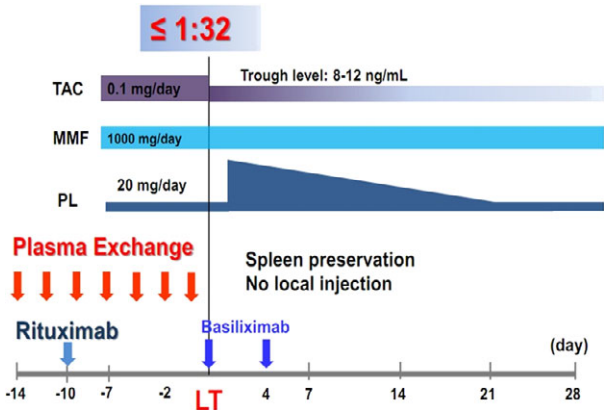
Introduction: The AB0-incompatible liver transplantation (LTx) is alternative method which can be used in some of the fulminant liver failure (FLF) cases, in some countries even for elective transplants. For overcoming the blood group barrier various techniques can be used.
Methods: Czech Republic is country with 10 million inhabitants and some 200 deceased donors per year. At our institution we performed some 119 LTx in 2014, our LTx program counts over 1000 LTx since 1995. To increase the chance for survival, in some of the fulminant liver failure cases we used the AB0i graft. In all except one case we used plasma-exchange, in one case non-specific immunoadsorption.
Results: In total 10 patients received AB0i liver graft, 3/10 received hemiliver, 1 patient as auxiliary graft, 2/10 died shortly after the LTx, none due to the incompatibility-related problems, 1/10 was re-transplanted 16. POD, 8/10 patients are alive with well functioning graft. Conclusions:In a small country with limited number of liver grafts per year, the AB0i liver transplantation is justified in FLF setting. Such technique gives reasonable chance for survival, the final outcome depends on severity of the FHF as well as primary diagnosis. Both apheretic as well as plasma-based techniques can be used with success to overcome the AB0i barrier. Both full size and hemiliver grafts can be used for transplant, one case was full split liver for two adults because two FHF patients occurred at the same time, both these two patients (husband and wife) are alive and well. One case was left hemiliver as auxiliary graft, the patient is well, already after graft removal.

O15 RESULTS OF ABO INCOMPATIBLE LIVER TRANSPLANTATION USING A SIMPLIFIED PROTOCOL AT A SINGLE INSTITUTION

Juhan Lee[1], Jae Geun Lee[1], Seung Hwan Song[1], Jung Jun Lee[2], Jin Sub Choi[1], Myoung Soo Kim[1], Dong Jin Joo[1], Man Ki Ju[1], Soon Il Kim[1], Gi Hong Choi[1]

[1]Yonsei University College of Medicine; [2]CHA Bundang Medical Center

Background: ABO incompatible (ABOi) living donor liver transplantation (LDLT) has become a feasible option for patients with end-stage liver disease due to development of various desensitization strategies. However, there has been no united desensitization protocol for ABOi LDLT. We have established a simplified protocol that does not incorporate splenectomy or local infusion therapy and analyzed the outcomes in a single-center clinical study.
Methods: We analyzed 19 ABOi LDLT cases that had been performed without concurrent splenectomy and local infusion between January 2012 and December 2013. We used a single dose of rituximab (375 mg/m^2) 10 days before transplantation and several series of plasma exchange to adjust the patients' isoagglutinin titer to a target titer of 1:32.
Results: The mean initial immunoglobulin (Ig) M and IgG anti-ABO titers were 76.63 ± 78.81 (range 8~256) and 162.53 ± 464.1 (0~2048) respectively. We performed preoperative plasma exchange on 16 patients and postoperative plasma exchange on nine patients. One case of mortality occurred due to pneumonia. There were four cases (21.1%) of acute rejection, all of which were treated successfully with steroid pulse or antithymocyte globulin. Antibody-mediated rejection or graft failure did not occur. A total of six postoperative complications occurred, including three infections (15.8%), two cases of anastomotic biliary stricture (10.5%), and one case of portal vein stenosis (5.3%).
Conclusion: ABOi LDLT following a simplified protocol can be safely performed without an increased risk of antibody-mediated rejection or other complications compared to other ABOi LDLT protocols.



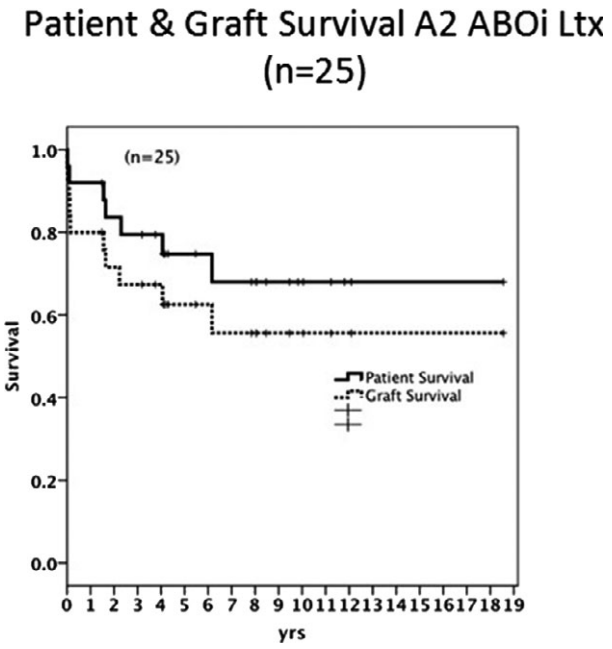
O16 IS IT JUSTIFIABLE TO USE A2 DONOR ABO-INCOMPATIBLE LIVER GRAFTS IN NON URGENT SITUATIONS?

Dahlgren Ulrika Skogsberg[1], Johan Mölne[2], Lennart Rydberg[3], Styrbjörn Friman[4], William Bennet[4]

[1]Transplant Institute Sahlgrenska University Hospital; [2]Department of Pathology, Sahlgrenska University Hospital; [3]Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital; [4]Transplant Institute, Sahlgrenska University Hospital

Introduction: The use of A2 donor ABO-incompatible (ABOi) liver grafts have previously been reported with good patient and graft survival. In order to decide whether this blood group combination can be considered safe to use also in non-urgent situations, we analysed all A2 donor ABOi liver transplantations (LT) performed at our center.
Patients and Methods: From 1996 to 2013 25 adult ABOi LT using A2 donor grafts were performed in Gothenburg, Sweden. Median MELD-score was 26 (range 6–40). Six patients were treated in the ICU at the time of transplantation. All patients received tacrolimus, 20 patients received prednisolone, 15 mycophenolate mofetil, 6 rituximab (anti-CD20 antibody, single dose day 0), 13 IL-2 receptor antagonists and 2 patients received a single dose of antithymocyte globulin day 0. Two patients were treated with plasmapheresis (PP) or immunoadsorption (IA) before transplantation and 10 patients with PP and/or IA after transplantation.

Results: The 1-, 5-, and 10 year patient (PS) and graft survival (GS) were 92/80%, 75/63% and 68/56%, respectively with a 63 months mean follow up (range 0 days–145 months). One patient had a humoral rejection. Eight patients (32%) had acute cellular rejections. Three patients (12%) developed hepatic artery thrombosis, 1 patient (4%) portal vein thrombosis and 5 patients (20%) biliary complications.
Conclusion: A2 donor ABOi liver grafts can be used in urgent situations with relative good patient-, and graft survival. However, since we cannot rule out an increased risk for humoral rejection, vascular and biliary complications we cannot encourage the use of A2 donor ABOi liver grafts in non-urgent patients. Future studies are needed to clarify if an antibody reducing protocol can enable the use of A2 donor ABOi liver grafts also in non-urgent situations.



O17 VARIATIONS OF DESENSITIZATION FOR ABO-INCOMPATIBLE LIVER TRANSPLANTATION IN CHILDREN

Sergey Gautier, Irina Tsirulnikova, Dmitry Akhaladze, Olga Tsirulnikova, Olga Shevchenko
Federal Research Center of Transplantology and Artificial Organs named after V.I. Shumakov

Background: ABO-incompatible (ABOi) living donor liver transplantation (LDLT) is a valuable option for children without ABO-compatible (ABOc) living related donors. The aim of the study was to elaborate effective methods of desensitization in children for ABOi LDLT.
Methods: Since 2010 44 patients (21 boys and 23 girls) have passed ABOi LDLT. Median age was 13.25 (4–52) months and median weight - 8.2 (5–15) kg.
Results: In 22 patients initial anti-ABO titres were 1:8 or less, no special preparation was performed. In 9 children with moderately elevated anti-ABO (1:16–1:32) the desensitization included only transfusion of AB(IV) fresh frozen plasma. In 13 patients with high anti-ABO titres plasmapheresis was carried out preoperatively; 5 of them also received rituximab and in one case splenectomy was performed during transplant procedure.

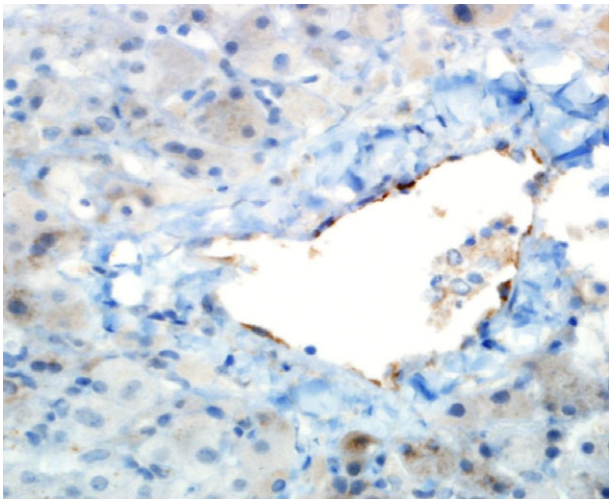
Basic immunosuppressive protocol included basiliximab, tacrolimus and steroids; in 11 patients MMF was added. In 38 of 44 children the ABO antibodies disappeared during 1st week postoperatively. The rest 6 patients needed plasmapheresis after LDLT. The requirement of plasmapheresis didn't depend on the way of desensitization.

6 patients died during first year after LDLT due to different non-immunological reasons. Other 38 patients are alive with good graft function. The mean follow-up period is 19.9 (1–56 months). Acute rejection occured in 4 patients, in 2 of them it was successfully treated by steroid pulse therapy, in two other – in combination with plasmapheresis (due to increased level of ABO antibodies). The patient and graft survival, so as the incidence of rejection and vascular, biliary and infectious complications, don't exceed those in ABOc LDLT in similar recipients.
Conclusion: The optimal variation of desensitization in children depends on the initial anti-ABO titres. ABOi LDLT using our desensitization protocols can be a safety and effective method of treatment for children with good short and long-term results.

O18 ABO-INCOMPATIBLE LIVER TRANSPLANTATION IN CHILDREN, ROLE OF HLA ? SINGLE CENTRE EXPERIENCE

Mohammad Shagrani[1], Martin Burdelski[1], Mohammad Al Sebayel[1], Eldeen Zahr, Firas[1], Yasser Elsheikh[1], Hamad Albahili[1], Hessah Alhussaini[2], Talal Algouff[2], Dieter Broring[2]
[1]King Faisal Specialist Hospital & Research Center, Organ Transplant Centre;
[2]King Faisal Specialist Hospital & Research Center, Pathology Department

Background: The no. Of children waiting for liver transplantation without having a suitable living donor is increasing. Spilt liver transplantation from deceased donors is very limited due to shortage of such high quality donor organs. A Second option would be to perform ABO-Incompatible (ABO-I) transplantation in children where only an ABO-I liver donor is available.



Patients	Date Of Liver TX.	Donor Type	ABO	Donor Blood Group	Age At Transplantation	Status	
1	07/12	Living -Mother	O Positive	A Positive	34 Months	Alive	
2	11/12	Living -Nephew	B Positive	A Positive	10 Months	Alive	
3	12/12	Living-Mother	B Positive	AB Positive	60 Months	Alive	
4	02/13	Living-Mother	B Positive	A Positive	37 Months	Alive	
5	04/13	Living-Cousin	B Positive	A Positive	7 Months	Alive	
6	06/13	Living-Uncle	A Positive	B Positive	12 Months	Alive	
7	09/13	close Living-Uncle	O Positive	B Positive	5 Months	Dead	
8	09/13	Living-Father close	B Positive	A Positive	9 Months	Alive	
9	10/13	Deceased Donor	O Positive	A Positive	8 Months	Alive -AMR	Antibody Mediated R.
10	11/13	Living-Mother close	O Positive	A Positive	20 Months	Alive	
11	10/14	Living-Mother	O Positive	A Positive	20 Months	Alive	
12	11/14	Deceased Donor	O Positive	A Positive	18 Months	Alive	
13	11/14	Living-Father	O Positive	B Positive	30 Months	Alive	
14	11/14	Living-Brother	O Positive	B Positive	19 Months	Alive	
15	01/15	Living-Uncle	O Positive	A Positive	22 Months	Alive	

Menthod: Between 2011 and 2014, 15 children underwent ABO-I liver transplantation (T.1). Blood type and cross-match with antibody screen were performed prior to transplantation. Isohemagglutinins titers were drawn pretransplantation. Isohemagglutinins titers against ABO antigen were monitored post transplantation. We defined indications for plasmapheresis as increased isohemagglutinin titers associated with allograft dysfunction and picture of hemolysis.
Post-Transplantation Immunosuppression: Two doses of basiliximab day 1 and day 4. I.V. Methylprednisolne was given during anhepatic phase, followed by prednisolone tapered slowly. Mycophenolate mofetil was started at 10–20 mg/kg/day on postoperative day1 and increased to reach 600 mg/m^2. Oral Tacrolimus began on postoperative day 7 aiming for a trough level 7–9 ng/ml at the first 3 months. In the absence of rejection, steroids were planned to discontinue within 12 months after transplantation. As A protocol blood bank supply our patients if needed packed red blood cells from O positive donors and blood products from AB donors to avoid any increment in the ABO Isotitre.
Result: Post transplantation surgical complications were nil either vascular or biliary complications. Pt.No.9 was one of tow Cadaveric ABO-I had AMR-Antibody Mediated Rejection-confirmed by C4D stain treated with Steroid, I.V. Rituximab and immunoglobulin. Pt. No.7 died at home ?Sepsis. All patients had low ABO Isotitre.
Conclusion: Crossing blood groups will play a significant role in avoiding pediatric waiting mortality in emergent patients. We do believe in role of HLA matching in our cases.

009 ETHICS/LAW/PSYCHOSOCIAL/PUBLIC POLICY

O19 NON-DISCLOSURE OF MENTAL HEALTH DISORDERS IN LIVING KIDNEY DONATION

A. Doshi[1], H. Maple[2], N. Mamode[3]
[1]King's College London; [2]Department of Transplantation, Guy's Hospital; [3]Department of Renal Transplantation, Guy's and St Thomas' NHS Foundation Trust

Aims: British living donation guidelines recommend a formal mental health assessment for live kidney donors (LKDs) with a psychiatric history to ensure donor safety. This is dependent on donor questioning and disclosure. The aim of this study was to determine the level of disclosure of mental health issues (MHI) to the transplant team.
Methods: LKDs donating at a London centre between August 2012–13 were included. Patient records were interrogated to determine whether LKDs had been asked about MHI by transplant clinicians and whether MHI had been disclosed. Data were correlated against results from a LKD psychology study which ran concurrently. The discrepancy between disclosure to the clinical and research teams was assessed.
Results: 104 LKDs were included (age: 44.66 year (SD: 13.026); 58 M:46 F). Donations were most frequently parental (40) or spousal (22). Only 50% of LKDs had a documented MHI discussion by the clinical team (vs 100% research team). 16 LKDs disclosed MHI to the clinical team, compared with 29 to the research team. There was no difference in age ($p = 0.138$) or donor-recipient relationship ($p = 0.072$). All 29 LKDs disclosing a MHI to the research team had sought professional help and had received treatment. Mood disorders were most common (75.9%). Of the 13 donors who did not disclose a MHI to the clinical team, 3 had denied and 10 had never been asked MHI. Non-disclosed MHI included depression requiring ongoing treatment and a history of severe depression requiring electroconvulsive therapy. 3 of these LKDs were referred to mental health services prior to donation.
Conclusion: This study has shown that a large number of LKDs are not routinely asked about MHI by transplant clinicians. A proportion of important MHI are therefore never disclosed. Compulsory questioning regarding MHI must be included as part of LKD workup to ensure that donors are encouraged to disclose MHI and so that adequate support measures can be implemented.

O20 DO DONORS REALLY BENEFIT FROM LIVING DONATION?

Hannah Maple[1], Nizam Mamode[2]
[1]Guy's Hospital London; [2]Guy's Hospital, London

Introduction: Living kidney donation is, in part, justified by the assumption that the donor experiences some psychological benefit after donation. However, there are no studies that emphatically demonstrate this to be the case. The aims of this study were to determine whether donors demonstrate a psychological benefit after donation and whether this was quantifiable. We hypothesized a decrease in scores for distress, depression, stress and anxiety and an increase in scores for wellbeing, life satisfaction, self-esteem and social comparison.
Methods: 100 living kidney donors completed 11 validated psychological questionnaires at 3 time points: pre-operatively and 3 and 12 months after donation.
Results: Participants included 45 women and 55 men; average age 45 year (s.d. 12.98; range 18–70 year). Of the above listed factors, social support was the only measure that demonstrated a statistically significant change across the 3 time points and this was found to decrease (72.0 vs. 71.0 vs. 67.5; X_2 (2, 70) = 10.29, $p = 0.006$). There was no significant difference in scores for wellbeing (29.5 vs. 29.5 vs. 29.5; $p = 0.81$), distress (10.2 vs. 9.4 vs. 10.7; $p = 0.09$), mood (0 vs. 0 vs. 0; $p = 0.15$), stress (4.5 vs. 4.5 vs. 5.2; $p = 0.074$), life satisfaction (27.5 vs. 27.0 vs. 26.0; $p = 0.92$), self-esteem (22.7 vs. 21.8 vs. 21.8; $p = 0.37$), anxiety (10.0 vs. 10.0 vs. 11.0; $p = 0.36$), optimism (21.2 vs. 20.7 vs. 20.2; $p = 0.72$) and social comparison (68.6 vs. 66.8 vs. 66.7; $p = 0.89$).
Discussion: This is the most comprehensive prospective study conducted with an aim of demonstrating a post-operative psychosocial benefit to donors. The results indicate that whilst there is no apparent psychological benefit from living donation, there is also no obvious harm. A failure to demonstrate benefit after living donation calls into question the moral and ethical justifiability of the practice.

O21 CROSS-BORDER QUEST: PATIENTS GOING ABROAD FOR PAID ORGAN TRANSPLANTS

Linde Van Balen[1], Frederike Ambagtsheer[1], Ninoslav Ivanovski[2], Susanne Lundin[3], Martin Gunnarson[3], Ingela Byström[3], Willem Weimar[1]
[1]Erasmus MC - University Medical Center Rotterdam; [2]University of St. Cyril and Methodius; [3]Lund University

Background: Patients travel worldwide for living kidney transplants. Although travelling abroad does not directly imply an illegal transplant, it is commonly seen as an illegal and immoral endeavour involving risks. Lack of data hampers drawing conclusions about its nature and potential illegality. We aimed to describe how, where and by whom transplants abroad were facilitated, and to describe the motivations, experiences and characteristics of patients travelling abroad.
Methods: From March-May 2014 interviews were performed with patients from Sweden, Macedonia and The Netherlands who travelled abroad for kidney transplantation.
Results: 22 patients (19 men; born between 1949 and 1985) travelled abroad from Sweden ($N = 5$), Macedonia ($N = 10$) and The Netherlands ($N = 7$) for transplantation between 2000 and 2011. The most frequently reported countries were Pakistan ($N = 13$), India ($N = 3$) and Iran ($N = 2$). 7 patients went to their motherland. For 6 patients a facilitator organized their transplant abroad, the others received help from family or friends. 17 patients directly paid the doctor, hospital or a broker; some paid for the whole transplant service. 14 patients met the supplier; 4 patients said to have paid their supplier. Reported total costs varied from €280-€45.000. Almost all patients mentioned a lack of hygiene and poor hospital conditions. 11 transplants were uncomplicated; 11 patients had severe complications (e.g. infections, kidney loss). Motivations to go abroad were the long wait time for deceased organs, lack of regular transplant activities, complications of dialysis and discrimination by the health care system.
Conclusion: Despite the worldwide prohibition of organ trade, patients still purchase organs. Knowledge about the facilitation of these transplants helps to disrupt and prevent illegal transplant networks. Warning patients against the medical, ethical and legal risks and increasing the organ supply are strategies to prevent patients from purchasing organs abroad.

O22 UK DONATION ETHICS COMMITTEE (UKDEC) PUBLISHED GUIDANCE ON PRE-MORTEM INTERVENTIONS TO OPTIMISE ORGAN QUALITY AND IMPROVE TRANSPLANT OUTCOMES IN DCD

Antonia Cronin[1], Dale Gardiner[2], Amanda Gibbon[3], Penney Lewis[4]
[1]MRC Centre for Transplantation King's College London; [2]Nottingham University Hospital; [3]UK Donation Ethics Committee; [4]King's College, London

Background: In 2009 in the UK Dept of Health (DH) issued guidance on legal issues relating to DCD, stating that if a person (P) wished to be a donor, actions which facilitate donation may be in their best interests (BI) if they do not cause or place them at a material risk of experiencing harm or distress. Since then the number of DCD transplants performed in the UK has more than tripled. Pre-mortem interventions to optimise organ quality may also improve DCD transplant outcomes. This has prompted further consideration of the BI test in this context.
Methods: A UKDEC legal working group undertook a review of the existing guidance. An appointed clinical working group conducted a review of relevant literature relating to pharmacological and mechanical pre-mortem interventions.
Results/Discussion: UKDEC has published new guidance to apply when the continuation of life-sustaining treatment is no longer in P's BI & organ donation would be in P's BI. It states that to decide if an intervention would be in P's BI, the potential benefits to P must be balanced against the potential (risk of) harm or distress. The potential benefits encompass both the prospective benefit of knowing their wishes will be facilitated, and the future benefit attaching to their legacy. Potential harm may include pain, shortening P's life & worsening P's medical condition. Potential distress may include feelings of suffocation, panic, & invasion of privacy. Factors affecting the balancing assessment include: the strength of P's desire to become a donor; the potential of an intervention to optimise organ quality & improve transplant outcomes; & the possibility of the alleviation of symptoms or avoidance of distress. It is anticipated that this published generic guidance may also be a useful guide for clinicians and clinical scientists when considering the ethical & legal implications of applying novel interventions and developing translational pathways for emerging and future biotechnologies.

O23 THE IMPORTANCE OF DONORS' AND RECIPIENTS' MEDICAL PROCESS FOR LIVING DONOR PSYCHOLOGICAL OUTCOMES

Lotte Timmerman, Mirjam Laging, Reinier Timman, Willij Zuidema, Denise Beck, Jan Ijzermans, Michiel Betjes, Jan Van Busschbach, Willem Weimar, Emma Massey
Erasmus Medical Center

Background: A minority of living kidney donors have poor psychological outcomes after donation. There is mixed evidence as to the influence of the medical process on these psychological outcomes. We examined whether objective indicators of the donors' and recipients' medical process and socio-demographic characteristics predicted change in donors' mental health (psychological symptoms and wellbeing) between pre-donation and one year post-donation.
Methods: One-hundred forty-five donors completed questionnaires on psychological symptoms (BSI, PANAS-NA) and wellbeing (MHC-SF, PANAS-PA) a median of 2.5 months pre-donation, and 3 and 12 months post-donation. To describe the medical process, we obtained the number of recipient re-hospitalizations and donor complications (divided into none; minor; and severe) from medical records at 3 and 12 months after the operation. Multilevel regression analyses were used to examine whether these indicators and socio-demographic characteristics predicted change in donors' mental health over time.
Results: Donor complications ($p = 0.003$) and recipient re-hospitalizations ($p = 0.001$) predicted an increase in donors' psychological symptoms over time. Recipient re-hospitalizations also predicted a decrease in wellbeing ($p = 0.005$) over time. In addition, donors without a partner showed a greater increase in negative affect over time ($p = 0.002$).
Conclusion: Donors experiencing complications themselves and/or recipient re-hospitalizations are at risk for a lower mental health than before donation. Furthermore, donors without a partner are at higher risk of poorer psychological outcomes than those with a partner. Professionals should monitor donors who experience unfavorable medical outcomes, and offer additional support when needed.

O24 ORGAN DONATION AFTER EUTHANASIA IN THE NETHERLANDS

Jan Bollen[1], Hanneke Hagenaars[2], Gert Van Dijk[2], Ernst Van Heurn[3], Jan Ijzermans[2], Wim De Jongh[3], Walther Van Mook[3]
[1]Maastricht University; [2]Erasmus Medical Center; [3]Maastricht University Medical Center

Neither many physicians nor patients know that it is legally and medically possible to donate organs after performing euthanasia. Even though this is not a frequent occurrence, often being limited by the patient's underlying pathologies, the combination of euthanasia and organ donation has nevertheless been practiced several times over the last decade in both Belgium and the Netherlands.

In anticipation of the situation in which a request for a combined euthanasia and organ donation is made, and contributing to awareness of the possibility of this combination among general practitioners and medical specialists, the Maastricht University Medical Center (MUMC) and Erasmus Medical Center, in close collaboration with all stakeholders, have developed a multidisciplinary guideline on organ donation after euthanasia.

This guideline consecutively lists the various criteria to fulfill, and the strict rules and regulations that the different participants involved, e.g. the patient, the performing physician, the transplant coordinator, the municipal coroner and the intensive care physician, need to comply with to meet all due diligence requirements.

The Dutch Acts on Euthanasia and Organ Donation provide sufficient opportunities for patients to donate their organs after a euthanasia procedure, in the absence of contraindications. Given the right of self-determination, such combined procedure is also ethically justifiable. A physician who is confronted with a patient who wishes to undergo euthanasia may consider raising the possibility of organ donation, if no contra-indications are identified. Patients who fulfill the due diligence requirements for euthanasia and who want to donate their organs should undergo several preparatory measures. Depending on their medical history, their age, and their own wish, they can be able to donate their lungs, liver, kidneys and pancreas.

021 ISLET/CELL TRANSPLANT

O25 COMBINED PANCREATIC ISLET–LUNG TRANSPLANTATION: A NOVEL APPROACH TO THE TREATMENT OF END-STAGE CYSTIC FIBROSIS

Philippe Baltzinger[1], Thibault Bahougne[1], Michel Greget[1], Tristan Degot[1], Jeremie Reeb[1], Pierre Bonnette[2], A. Gut[2], Dominique Grenet[2], F. Bouilloud[2], Thierry Berney[3], Marc Stern[2], Romain Kessler[1], Gilbert Massard[1], Laurence Kessler[1]

[1]CHU de STRASBOURG; [2]Hôpital FOCH, Paris; [3]CHU de GENEVE

Introduction: Cystic fibrosis related-diabetes (CFRD) is a major factor of morbi-mortality in lung transplantation. We report the follow-up of five patients with end-stage CF who were treated with combined pancreatic islet-lung transplantation.
Patients and Method: All CF patients have an end stage respiratory insufficiency and an uncontrolled diabetes with low C peptide levels (<0.5 ng/ml or absence of response after glucagon stimulation). Bipulmonary bloc and pancreas are procured from the same donor. During the lung transplantation, the pancreas is shipped to the laboratory for islet isolation and culture. One week after lung transplantation, the islets are injected by percutaneous transhepatic catheterization of the portal vein under local anesthesia. Immunosuppression associates steroids and basiliximab, tacrolimus and mycofenolate mofetil.
Results: From Oct. 2011 to Oct. 2014, five CF patients (2 F/3M, age: 31 $\pm$ 5 years, IMC: 18.8 $\pm$ 2 kg/m^2) with respiratory insufficiency (FEV1: 25.6 $\pm$ 4%) and brittle diabetes (HbA1c = 8.6 $\pm$ 1%, insulin requirement = 43 $\pm$ 14 IU/day) underwent combined pancreatic islet-lung transplantation with an amount of 2940 $\pm$ 850 IEQ/kg. The follow up is from 6 to 36 months and 4 patients reached 12 months follow up. Improvement in lung function was observed for all patients with a FEV1 reaching 62 $\pm$ 16% and 67 $\pm$ 15% respectively 3 and 12 months after lung transplantation. The five patients showed immediately islet graft function with an increase in C peptide plasma levels up to 2.34 $\pm$ 1 μg/l and 0.86 $\pm$ 0.1 μg/l respectively 3 and 12 months after transplantation. No complications related to the islet injection were observed. All patients presented an improvement in the metabolic control with a decrease in HbA1c to 6.4 $\pm$ 0.6% at 12 months in absence of hypoglycemic events and a 30 $\pm$ 14%decrease in the exogenous insulin needs.
Conclusion: In CF patients, combined transplantation restores both pulmonary and metabolic control without immediate increase in morbidity.

O26 ABSENCE OF AMYLOID DEPOSITION IN HUMAN ISLETS TRANSPLANTATION AFTER 13 YEARS INSULIN INDEPENDENCE

Yannick Muller, Philippe Morel, Thierry Berney
Geneva University Hospitals and University of Geneva

Long-term insulin independence after islets of Langerhans transplantation is rarely achieved. Amyloid deposition was described around transplanted islets that had lost their function. The aims of this study were to analyze the histological features and the amyloid deposition of transplanted islets in a type 1 diabetic patient who died of a cerebral hemorrhage after >13 years insulin-independence. Insulin-positive islets were found throughout the right and left liver. Two- and three-dimensional analysis showed that islets lost their initial rounded and compact morphology, had a mean diameter of 136 μm and were constituted of an unfolded epithelial band of 39.1 μm. Islets were also present in the pancreas, but were negative for insulin; exceptionally, isolated beta cells could be seen in the pancreatic parenchyma. Glucagon positive cells were present in both organs, and rare somatostatin cells were observed in islets implanted in the liver. Congo red staining revealed near-absent amyloid deposits around the islets in the liver. This data demonstrate that insulin-independence was mediated by the islet graft and not through the regeneration of the native islets favored by chronic immunosuppression. As expected from the literature data, amyloid deposition was only rarely observed in this patient.

O27 INFUSION OF THIRD-PARTY MESENCHYMAL STROMAL CELLS AFTER LIVER TRANSPLANTATION: A PHASE I CLINICAL STUDY

Olivier Detry[1], Morgan Vandermeulen[1], Marie-Hélène Delbouille[1], Arnaud Deroover[1], Joan Somja[2], Noella Bletard[2], Alexandra Briquet[3], Chantal Lechanteur[3], Pierre Honore[1], Yves Beguin[3]

[1]Dapartment of Surgery & Transplantation, University of Liege, CHU Liege; [2]Dapartment of Pathology, University of Liege, CHU Liege; [3]Dapartment of Hematology, University of Liege, CHU Liege

Background: Mesenchymal stromal cells (MSC) are multipotent bone marrow progenitors that have demonstrated significant immunosuppressive effects in various in vivo and in vitro studies. This study aimed to be the first evaluation of the safety and tolerability of MSC infusion after liver transplantation in a prospective, controlled phase-1 study.
Methods: 10 liver transplant recipients under standard immunosuppression (TAC-MMF-low dose steroids until day 30) received 1.5–3 $\times$ 10^6/kg third party MSC on post-operative day 3 $\pm$ 2. These patients were prospectively compared to a group of 10 control liver recipients. Primary endpoints were MSC infusion toxicity, and incidence of cancer and opportunistic infections at month 6. Secondary endpoints were patient and graft survivals and rejection at month 6, as well as the effects of MSC on recipients' immune function and on immunohistology of at month 6 graft biopsies.
Results: No MSC infusional toxicity was observed. Both groups were comparable in terms of donor and recipient characteristics. There was no difference in primary end-points between control and MSC groups. No patient developed de novo cancer. There was no statistical difference in patient and graft survivals or in rejection rates. There was no graft rejection in the MSC group. Month-6 graft biopsies were not different according to Banff and fibrosis scores.
Discussion: This phase 1 study showed excellent tolerability and safety of a single infusion of third-party MSC after liver transplantation. There were no graft safety issues and no excess of immunosuppression after MSC injection. Further analyses of consequences of MSC injection on the immune profile are needed. The possibility of avoiding calcineurin-inhibitors with repeated MSC injections as main immunosuppressive therapy and/of tolerance induction by MSC infusion should be investigated by further studies.

This study is in part supported by an ESOT Senior Clinical Research Grant and by the University of Liege.

O28 CHARACTERIZATION AND EFFECTS OF PORCINE ADIPOSE TISSUE MESENCHYMAL STEM CELLS ON KIDNEY GRAFT RECOVERY IN A PRECLINICAL PORCINE MODEL OF RENAL TRANSPLANTATION

Xavier Matillon[1], Edouard Baulier[2], Jonathan Rodriguez[3], Odile Damour[3], Jerome Roumy[4], Delphine Bon[5], Frederic Favreau[6], Thierry Hauet[7], Lionel Badet[1]

[1]INSERM U1082 IRTOMIT/Hopital E Herriot, Service Urologie et chirurgie de Transplantation, Lyon; [2]INSERM U1082 IRTOMIT/CHU de Poitiers/Faculté de medecine et Pharmacie, Poitiers; [3]Hôpital Edouard Herriot, Laboratoire de Cellules et de Substituts Cutanés, Lyon; [4]INSERM U1082 IRTOMIT/CHU de Tours, service de radiologie, Tours; [5]INSERM U1082 IRTOMIT/Faculté de medecine et Pharmacie, Poitiers; [6]INSERM U1082 IRTOMIT/CHU de Poitiers, service biochimie/Faculté de medecine-Pharmacie, Poitiers; [7]INSERM U1082/CHU Poitiers, Biochimie/Faculté de medecine/FHU SUPPORT/IBiSA INRA Surgeres

Background: Ischemia reperfusion (IR) is a pathological process involved in acute and chronic renal graft dysfunction. The aim of this study was to characterize mesenchymal stem cells from porcine adipose tissue (pASC) and their role in the graft function recovery in conditions mimicking deceased after cardiac arrest donors.
Methods: In vitro, morphology, proliferative capacities, phenotype by flow cytometry and the metabolic profile with Nuclear Magnetic Resonance (NMR) of pASC were determined. Their resistance to a sequence of hypoxia-reoxygenation (HR) was characterized on their viability and metabolic profile in NMR. In vivo, a porcine preclinical model was used with 1 h of renal warm ischemia followed by 24 h of graft storage at 4°C in UW solution and renal auto-transplantation with contralateral nephrectomy. The effects of autologous injection of 106 pASC/kg in the renal artery after cold preservation were determined on renal blood flow, renal graft function and histological outcomes.
Results: The cell extraction technique was reproducible and allowed a sufficient extraction rate of pASC characterized by mesenchymal stem cells phenotype. The metabolic profile in NMR of pASC was stable during the first passages. The cell viability after a sequence of HR exceeded 70% underlined the feasibility of a direct injection in the renal artery at reperfusion time. The injection of 106 pASC/kg at passage 2 was practicable 15 days after removal of adipose tissue. The function recovery was significantly improved and the histological lesions were reduced at day 7 in the group treated by pASC.

Conclusion: Injection of pASC in renal graft artery at reperfusion of the grafts in a porcine model mimicking deceased after cardiac arrest donor conditions improves graft function recovery and limits tubular damages. These therapeutic potentials will be confirmed by further studies at the end of the follow-up at 3 months.

O29* IMPACT OF TIMING ADMINISTRATION OF MESENCHYMAL STROMAL CELLS ON SERUM CREATININE FOLLOWING RENAL ISCHEMIA/ REPERFUSION IN RATS

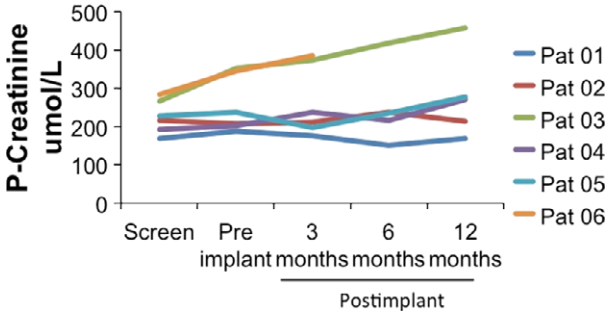
Pauline Erpicum, Pascal Rowart, Laurence Poma, Jean-Marie Krzesinski, Olivier Detry, Francois Jouret
University of Liege Hospital (ULg CHU)

Experimental models of renal ischemia/reperfusion (I/R) have suggested protective effects of mesenchymal stromal cells (MSC) therapy. Still, parameters of MSC injection, including volume, route and timing of cell administration, remain largely debated. Particularly, MSC infusion in mouse has been shown to be beneficial "a priori" but deleterious "a posteriori" of renal I/R injury. In order to further investigate the influence of the timing of MSC administration, we used 10-week-old Lewis rats categorized in 4 groups. Groups 1 (MSC D-7, $n = 10$) and 2 (MSC D + 1, $n = 7$) received caudal i.v. injection of MSC (1.5×10^6 in 1 ml of saline) 7 days before or 1 day after renal I/R, respectively. Control groups 3 (saline D-7, $n = 6$) and 4 (saline D + 1, $n = 6$) received equal volume of saline at similar time points. Left renal ischemia (by clamping of the renal pedicle) lasted 45 min. Right nephrectomy was simultaneously performed. Blood sample was collected from inferior vena cava at 48 h post reperfusion. MSC phenotype was confirmed by FACS analysis. In groups 1 and 3, serum creatinine (SCr) reached 1.4 ± 0.7 versus 2.4 ± 0.8 mg/dl, respectively ($p < 0.05$). In groups 2 and 4, SCr was 4.9 ± 0.7 versus 3.3 ± 0.9 mg/dl, respectively ($p < 0.001$). Furthermore, SCr levels were statistically worse when MSC were administered after renal I/R in comparison to a priori infusion ($p < 0.0001$). In conclusion, MSC administration 7 days prior to renal I/R attenuates kidney injury in comparison to (i) saline infusion or (ii) MSC infusion 1 day after renal I/R. Conversely, on the basis of SCr levels, MSC therapy performed after renal I/R worsens kidney injury in rats.

O30 A "FIRST-IN-HUMAN STUDY" OF IMPLANTATION OF NEO-KIDNEY AUGMENT, AN AUTOLOGOUS SELECTED RENAL CELL POPULATION, IN TYPE-2 DIABETIC CKD STAGE 3–4 PATIENTS

Torbjörn Lundgren[1], Pontus Blomberg[2], Torkel Brismar[2], Jonas Wadström[2], Peter Stenvinkel[3]
[1]Karolinska; [2]Karolinska University Hospital; [3]Karolinska University Hospitall

Background: Animal models of CKD show that a selected population of bioactive renal cells (SRC) can be delivered through parenchymal injection resulting in a decrease in disease progression. It has been shown to 1) reduce chronic infiltration by monocytes/macrophages and T-lymphocytes and attenuate the NFκB response 2) promote tubular cell expansion. We used a laparoscopic technique to perform a study with Neo-Kidney Augment (NKA).
Methods: Six type-2 diabetic (108 ± 11 kg) patients (64 ± 6 years) with CKD 3–4 were selected. After evaluation of iohexol clearance, MRI, renal scintigraphy and albumine-creatinine ratio (ACR) patients underwent a regular renal biopsy. Two cores were shipped to the Tengion (Winston Salem, NC, USA) for tissue separation, cell isolation and product preparation. NKA was shipped back to Karolinska (range 59–87 days after biopsy) for intracortical injection using a laparoscopic hand-assisted retroperitoneal technique (HARS).
Results: No complications occurred at biopsies. All resulted in material being used to obtain NKA. Implantation of 8 ml NKA into the left kidney was uneventful. No bleeding occurred at the site. A postoperative complication was observed in one patient (ileocecal volvolus, leading to a right-sided hemicolectomy). Infectious complications (hospitalizations) were observed in three patients in the first three months. Antihypertensive medication has been reduced 3/6 patients during the first 6 m following implant. S-creatinine has remained stable at 6 and 12 months after autologous renal cell implantation in four out of the five first patients. In patient 03 the rise in s-creatinine has been related to postrenal obstruction.
Conclusion: NKA was safely implanted in six T2DM patients. In this population complications after the implantations were related to the surgical procedure. Longer follow-up and more patients are needed to reveal if this technique can arrest porogression of CKD and delay the start of renal replacenent therapy.



017 INTESTINE

O31* INTESTINAL TRANSPLANTATION: 20 YEARS OF REGISTRY DATA IN THE UNITED KINGDOM

Sally Rushton[1], Elisa Allen[1], Rebecca Curtis[1], F. Mirza Darius[2], Andrew Butler[3], Hector Vilca Melendez[4], Peter Friend[5], Jonathan Hind[4], Stephen Middleton[3], Girish L. Gupte[6], Khalid Sharif[6], Simon Travis[5]
[1]NHS Blood and Transplant; [2]Queen Elizabeth Hospital Birmingham; [3]Cambridge University Hospitals; [4]King's College Hospital, London; [5]Oxford University Hospitals; [6]Birmingham Children's Hospital NHS Foundation Trust

Introduction: Intestinal transplant activity in the United Kingdom has increased since the designation of two additional transplant centres in 2008. Soon after, a dedicated Bowel Advisory Group to the National Health Service Blood and Transplant authority and comprehensive national data collection were established.
Methods: We report on patterns in activity over the last 20 years and outcomes across all four UK intestinal transplant centres, as recorded on the UK Transplant Registry.
Results: Between 1994 and 2014, a total of 181 intestinal transplants were performed in the UK; 98 in paediatric recipients (9 re-transplants) and 83 in adult recipients (5 super-urgent, 5 re-transplants). Of these, 100 (63 adults, 37 paediatric) were performed in 2009–2014, compared to 81 (20 adults, 61 paediatric) over the 15 years prior to that. Detailed patient data were available for the last decade. Adult and paediatric transplants were performed for short bowel (48%, 40%), motility disorders (12%, 13%), mucosal disorders (0%, 7%), liver disease (5%, 15%), malignancy (8%, 0%), with 66% and 63%, respectively, admitted from home for transplantation. 70% of adults and 78% of children had undergone previous abdominal surgery. Survival after adult primary intestinal transplantation was 87% at 90 days and 60% at 5 years. In children, 90 day survival was 92% and 5 year survival was 66%. Recipient age at transplant and the organs included in the transplant were found to be predictive of survival post-transplant.
Conclusions: UK intestinal transplant activity has increased over the last 20 years. The majority of the growth has occurred in adult transplantation with a corresponding decrease in children, which mirrors the worldwide trend[1]. Early post-transplant outcomes for adults and children are good, with longer-term outcomes less good.

[1]Intestine Transplant Registry 2013 Bi-Annual Report, Intestine Transplant Association

O32 15 YEARS OF INTESTINAL AND MULTIVISCERAL TRANSPLANTATION – A SINGLE CENTRE EXPERIENCE

Undine Gerlach, Ulrich-Frank Pape, Dinah Joerres, Christian Lojewski, Timm Denecke, Johann Pratschke, Andreas Pascher
Charité - Universitaetsmedizin Berlin

Intestinal transplantation is the only causal therapy for patients with a short bowel syndrome who fail parenteral nutrition. Treatment-refractory rejections, late graft enteropathy and the sequels of overimmunosuppression challenge long-term survival. We describe 15 years of a single centre experience of intestinal and multivisceral transplantation. 118 patients with anatomical or functional short bowel syndrome presented. Indication for transplantation was given for patients with complications under total parenteral nutrition (TPN). Post-transplantation, induction therapy, immunosuppression, graft biopsies, HLA and non-HLA antibody-screening, infection prophylaxis and enteral nutrition were initiated according to the centre's standards. We subdivided our experience into 2 Eras: I: 2000–2005 and II: 2006–2015. 37 patients (f: m = 13:24; mean age 38 $\pm$ 10 years) underwent isolated intestinal (ITX) or multivisceral transplantation (MVTX). Comparing era I vs. II, there were 21 ITX (13 vs. 8) and 16 MVTX (2 vs. 14). Recipients in era I had spent less time on TPN (32 $\pm$ 31 vs. 58 $\pm$ 73 months), less time on waitlist (219 $\pm$ 172 vs. 433 $\pm$ 226 days) but had received more organ offers (9 $\pm$ 14 vs. 6 $\pm$ 6). Waitlist mortality for ITX/MVTX was 0%/0% in era I but 0%/24% in era II. Cold ischaemia time was similar (301 $\pm$ 92 vs. 396 $\pm$ 108 min). Induction therapy in era I included Basiliximab or Alemtuzumab, and Thymoglobulin + Infliximab in era II. Immunosuppression comprised Tacrolimus and Mycophenolat Mofetil or Sirolimus in both eras. The actuary 1-, 5-, 10-, 15-year survival rate for era I was 60%; 60%; 53%; 53% and improved in era II: 86%; 73%; 73%. Era I showed more cellular rejections (57% vs. 26%), but less antibodymediated rejections (7% vs. 42%), which may have resulted from different inductions or more awareness towards donor-specific HLA-antibodies. Defining the indication for ITX in timely manner is important to prevent waitlist mortality, especially for MVTX. Due to expanded therapeutic opti

O33 QUALITY OF LIFE AFTER INTESTINAL TRANSPLANTATION FROM THE ADULT PATIENTS' PERSPECTIVE: SYSTEMATIC REVIEW

Laurens Ceulemans, CéLine Lomme, Jacques Pirenne, Sabina De Geest
University Hospitals Leuven

Background: Quality-of-Life (QoL) has become one of the primary endpoints after intestinal transplantation (ITx). In contrast to the pediatric population, the state of science in this field has not yet been summarized for the adult population. Therefore we performed, for the first time, a systematic literature review on the evidence on QoL from the adult ITx patients' perspective.
Methods: Pubmed, EMBASE, CCTR, CINAHL and PsycINFO were searched until October 2014. Structured data abstraction was performed and methodological quality was assessed using a standardized checklist.
Results: Nine eligible studies were identified. Study-aims were: (i) QoL comparison pre- and post-ITx (n = 5); (ii) QoL comparison between ITx and home parenteral nutrition (HPN) patients (n = 6), healthy subjects (n = 1), or general population (n = 1). Methodological quality was suboptimal. Prospective (n = 3), retrospective (n = 2) and cross-sectional (n = 7) designs were used. Non-probabilistic ITx sampling was applied, partially matched for healthy controls (n = 1) and general population (n = 1). The terminology, operational definitions and assessment methods of QoL were diverse. Findings suggest that *(i) post-ITx QoL improved versus pre-ITx* (e.g. anxiety, sleep, social support, leisure); *(ii) post-ITx QoL improved with longer follow-up* (e.g. anxiety, impulsiveness/control); and *(iii) QoL between ITx and HPN patients was similar for most domains* yet ITx patients excelled for energy, social functioning and travel ability.
Conclusion: Although results are encouraging, QoL research in adult ITx needs methodological improvement by implementation of multicenter and prospective studies, adequate QoL conceptualization and appropriate measurement.

O34 TEN YEARS AFTER A SUCCESSFUL INTESTINAL TRANSPLANTATION: WHAT IS THEIR LIFE?

Lorenzo Norsa[1], Girish Gupte[2], Buloda Esther Ramos[3], Francisca Joly[4], Olivier Corcos[4], Jacques Pirenne[5], Gustaf Herlenius[6], Florence Lacaille[1]
[1]Necker-Enfants Malades Hospital, Paris; [2]Birmingham Children's Hospital; [3]University Hospital La Paz, Madrid; [4]Beaujon Hospital, Clichy; [5]University Hospital, Leuven; [6]Sahlgrenska University Hospital, Goteborg

Introduction: Intestinal transplantation (ITx) is a difficult procedure, it can lead to physical or psychological sequelae. Aim is evaluate graft function, impact on life and psychosocial status in long term survivors. Patients: All European patients, who received ITx before 18 years of age and enjoy a good function after 10 years or more of follow-up.
Results: 38 patients with mean age of 18 years were enrolled from 5 centers. 1 needs parenteral nutrition while 7 are on complementary enteral nutrition for feeding disorders. 6 patients had 5 or more stools a day, and 3 are experiencing stool incontinence. 1 patient has a gastrostomy and 4 an ileostomy. The mean standard deviation for height is -1.2 and -1.1 for weight. In the last 2 years 50% had endoscopy and 29% stool balance study. In the last year 11 patients needed hospitalization for complications and 22 in the last 5 years, with a medium stay of 19 days in 5 years. Most frequent complications were acute diarrhea and infections. 53% of patients are taking 5 or more drugs daily. Special medical or psychological assistance is needed in 9 and 11 patients. Regarding personal life 31 patients are in training and 3 are working; 29 patients are living with parents. 2 have alcohol and drugs addiction, 1 have been in prison and one is morphine dependant.
Discussion: In patients with good long term graft function we observed a significant burden of health care. Only a few of them needed nutritional support, due to feeding disorders, few had still a stoma. However bowel movements can be a problem in few, number of drugs is still high in most. One third needed emergency hospitalization recently and more than half in the last five years. Majority of patients is still dependent from parents but they are still young. More than half of them need a special medical or psychological assistance and some developed frightening addictions. These long term results should help us to prevent and treat these problems in the future.

O35 SPLANCHNIC ARTERY EMBOLIZATION SAFELY FACILITATES EXENTERATION-PHASE DURING MULTIVISCERAL TRANSPLANTATION

Laurens Ceulemans, Ina Jochmans, Diethard Monbaliu, Marleen Verhaegen, Wim Laleman, Frederik Nevens, Sam Heye, Geert Maleux, Jacques Pirenne
University Hospitals Leuven

Background: Multivisceral transplantation (MvTx) for diffuse portomesenteric thrombosis is a surgically and anaesthesiologically challenging procedure, partly because of the risk of massive bleeding from the collateral circulation during visceral exenteration. We hypothesized that *pre-operative embolization*

of the celiac trunk (CT) and superior mesenteric artery (SMA) might reduce this risk.
Methods: In three consecutive patients who underwent MvTx, the preoperative embolization procedure was performed by an interventional radiologist in the same operating theatre as the transplantation. Embolization of the visceral arterial end-branches (first CT, then SMA) was performed with trisacryl gelatin microspheres, polyvinylalcohol microparticles and glue. Analyzed data were: demographics, serum D-lactate, pH, hemoglobin, blood pressure, transfused packed cell (PC) units, intervention time, ischemic time and outcome. Results are reported as median (range).
Results: All recipients were male (43-, 22-, 47-years). Portomesenteric thrombosis was due to antiphospholipid syndrome, neuro-endocrine tumor, and liver cirrhosis. A peri-transplant D-lactate peak of 6.1 mmol/l (5.1–7.6) and lowest pH of 7.24 (7.18–7.36) were observed. Values normalized within 3 h post-transplant. Embolization and exenteration times were 80 min (70–90) and 140 min (130–165), respectively during which blood pressure remained stable, lowest hemoglobin level was 6.1 g/dl (6.1–7.6) and 3PC (2–4) were administered. All procedures were uneventful. Cold and warm ischemic times were 5 h 19 min (5 h–6 h 15 min) and 28 min (24–30), respectively. Follow-up was 7 mo (4–9). The first patient died 4 mo post-Tx to an intracranial bleeding; the other patients are doing well and free from total parenteral nutrition.
Conclusion: Our experience suggests that embolization of the CT and SMA in the operating room immediately prior to MvTx is a valuable technique to safely facilitate exenteration of the native splanchnic viscera.

O36 RECENT PROGRESS OF 4 LIVING DONOR INTESTINAL TRANSPLANTATIONS IN OUR CENTER

Yohei Yamada[1], Ken Hoshino[1], Masahiro Shinoda[2], Taizo Hibi[2], Tomoaki Kato[3], Kiyotomo Abe[1], Nobuhiro Takahashi[1], Naoki Shimojima[1], Takumi Fujimura[1], Motohiro Kano[1], Takahiro Shimizu[1], Akihiro Fujino[1], Yuko Kitagawa[2], Tatsuo Kuroda[2]
[1]Department of Pediatric Surgery/KEIO University School of Medicine; [2]Department of Surgery/KEIO University School of Medicine; [3]Transplant Surgery/Columbia University

Background: Living donor transplantation is the primary type of transplantation in Japan. We have performed 4 living donor intestinal transplantations (ITx) between 2006 though 2014 and discuss recent progress and future perspective.
Patients characteristics: The indication of 4 ITxs was intestinal failure due to intestinal hypoganglionosis and the lack of central venous access. Graft types were jejunum without ileocecal valve, between 110 and 150 cm in length. All recipients underwent manometry and radiological gastrointestinal contrast study pretransplant aiming to preserve their native bowel (NB) as long as possible. Induction treatment for the first patient (Pt) 1–3 was basiliximab/simulect, then thymoglobulin for the Pt 4.
Results: All donors are fine without any comorbidities, except one episode of ileus requiring laparotomy. All 4 Pts are alive. Pt 1: 14 years old at the time of ITx. Now 8 years post ITx, the stoma was reversed and currently independent of TPN (total parental nutrition). NB: 26 cm of ileum and S-colon. Pt 2: 11 years old at the time of ITx, now 7 years post ITx. He developed anastomotic stricture probably due to ischemic ulceration, requiring endoscopic balloon dilatation every three months. Still on TPN. NB: 15 cm of ileum and S-Colon. Pt 3: 15 years old at the time of ITx. The graft was removed due to chronic rejection at 20 months post ITx. He is currently TPN dependent. NB: 26 cm of ileum and D-Colon. Pt 4: 10 years old at the time of ITx. He developed moderate ACR, which was successfully reversed. Now 4 months post ITx, doing well. NB: 15 cm of ileum and 30 cm of distal ileum and rectum.
Conclusion: In the setting of a living donor ITx, pre-transplant motility evaluation is essential to maximize the use of the NB given limited length of a graft and rescue the recipient in case the graft need to be removed due to rejection. In Japan, once national insurance approves ITx, the number of ITx will increase, hence specific management needs to be refined.

025 LIVER

O37*

RESULTS OF PILOT STUDY OF THE MESENCHYMAL STEM CELLS EFFICIENCY IN THE EARLY POST-TRANSPLANT PERIOD IN ORDER TO REPLACE IMMUNOSUPRESSIVE EFFECT OF TAC IN PATIENTS AFTER LT WITH AKI AND KIDNEY FAILURE

Sergey Korotkov, Aleksei Shcherba, Dmitry Fedoruk, Ekaterina Nazarova, Alla Koritko, Victoriya Smolnikova, Svetlana Krivenko, Oleg Rummo

Belorussian Centre of Organ and Tissue Transplantation

The aim of the study: To evaluate the efficiency and safety of CNI adverse effects minimization in the early postoperative period after liver transplantation by replacement of their immunosuppressive effect with allogenic MSC therapy.
Methods: This is a report of a pilot study designed for the purposes of subsecuent randomized controlled study of the superiority MSC over standard IS in regard of ACR rate and GFR imrovement. Inclusion criteria: Contraindications for the CNI administration - AKI during the LT, which required the delayed start of tacrolimus. Exclusion criteria and withdrawal from the study were: infectious and other complications, which treatment wasn't combined with the MSC introduction. MSC introduction was performed on 4–14 days after surgery in total dose of 4 million/kg in 4 infusions (2 million cells/kg, 1 million cells/kg, 0.5 million cells/kg and 0.5 million cells/kg) with 3–4 days intervals. The protocol liver biopsies were performed on the 7th (after 1st injection) and 14th day. IS therapy was reduced to a two-circuit (16 mg Medrol + Tac) with Tac concentration (up to 3 ng/ml).
Results: Frequency of ACR at 1st and 2nd protocol biopsy composed 20% and 0%. The severity of ACR was mild (RAI 4). Median GFR at the begining and end of protocol was 25 ± 4 and 53 ± 8 ml/min. Median trough level of tacrolimus was 0.9 ± 0.64 ng/ml with range of 0–2.4 ng/ml. There were no severe adverse effects of MSC therapy. After completion of protocol all patients were switched to 3 component IS composed of tacrolimus (5–8 ng/ml), MMF (1 g/day) and steroids (medrol 16 mg tapering after 28 POD). Patients with decreased GFR were managed by the minimizing of Tac and substitution of overall IS by prolonged steroids, increased MMF and addition of Everolimus.
Conclusions: The preliminary data shows that MSC therapy can effectively and securely substitute the Tac early after LT in order to allow renal function recover.

033 TISSUE ENGINEERING

O38 TISSUE ENGINEERING HUMAN KIDNEYS FOR COSTIMULATORY BLOCKADE

Lauren Brasile[1], Nicholas Henry[1], Giuseppe Orlando[2], Bart Stubenitsky[3]
[1]BREONICS Inc.; [2]Wake Forest Baptist Medical Center; [3]University Medical Center, Utretch

Body: Methods for preserving organs have employed hypothermic conditions, under which metabolism is substantially impaired. Tissue engineering of organs cannot be done without significant metabolism. New technologies only maintain organs at normothermic temperature for several hours. Such short periods limit the ability to adequately treat organs. We demonstrate the ability to tissue engineer renal allografts for costimulatory blockade during 24 h of warm (32°C) perfusion.
Methods: EMS acellular warm perfusion was used for tissue engineering. Human renal allografts ($n = 6$) were placed on EMS perfusion. Upon restored metabolism the passenger leukocytes (PL) trapped within the renal parenchyma migrated into the perfusate. The PL were collected, phenotyped as CD209+ and treated with antibodies to CD80, CD86 and CD40. Costimulation was evaluated using a Brdu proliferation assay.
Results: Histology confirmed the migration of PL from the parenchyma (Figure 1). A reduction in the number of resident PL was observed within the kidneys with migration into the perfusate ($12*10^6$ cells). Since the perfusion is acellular, cells found in the perfusate are of renal origin. Antibody treatment of PL prevented a proliferative response demonstrating potential for costimulatory blockade (Figure 2).
Conclusions: While using antibodies for costimulatory blockade has potential to prevent rejection, these therapies are associated with severe complications. Costimulatory blockade during ex vivo perfusion, rather than systemic administration, could eliminate systemic side effects and prevent direct antigen presentation. The vascular endothelium is a primary target of immune responses, strongly expresses CD86 along with MHC class II and functions as APCs. We are now testing the administration of antibody directly to the allograft. The ability to treat an allograft ex vivo could make multi-drug therapy feasible, while minimizing systemic side effects.

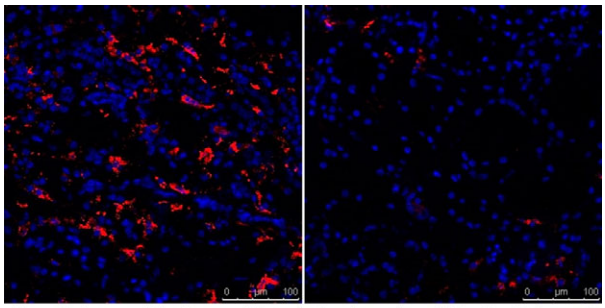


Figure 1. Representative image of the reduction in number of PLs after 24 hours of warm perfusion. Blue = DAPI nuclear stain, Red = Positive CD209 staining.

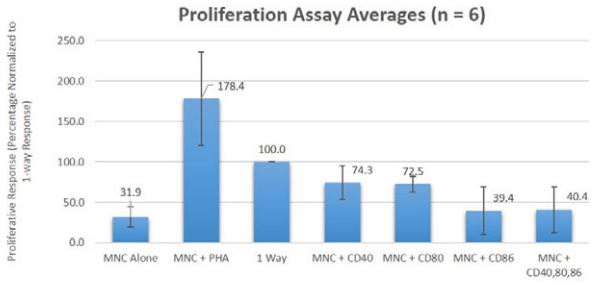


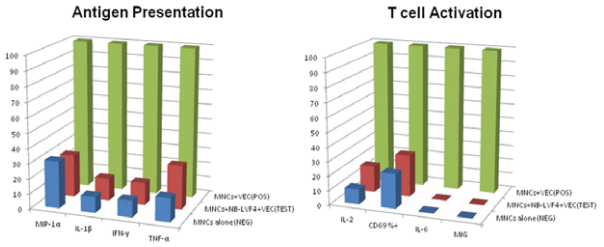
Figure 2. Results of proliferation assays. MNC alone = background proliferative response. MNC + PHA (phytohemagglutinin) = positive control. Kidney passenger leukocyte proliferative response measured against mitomycin C treated MNCs in mixed lymphocyte reactions. 1 Way = proliferative response of passenger leukocytes without antibody treatment. Average proliferative response of PLs treated with respective antibodies + MNCs shown. Responses from each assay are standardized against respective 1-way response.

O39 "IMMUNOCLOAKING" A STRATEGY TO ELIMINATE THE NEED FOR SYSTEMIC IMMUNOSUPPRESSION

Lauren Brasile[1], Bart Stubenitsky[2]
[1]BREONICS Inc.; [2]University Medical Center, Utretch

Background: Immunocloaking is an immunomodifying therapy that is organ-specific and prevents early allograft rejection without systemic immunosuppression. The therapy is a nano-barrier membrane, NB-LVF4, consisting of an extracellular matrix that is non-thrombogenic & non-immunogenic. NB-LVF4 is applied to the renal allograft vasculature using a near-normothermic perfusion technology. NB-LVF4 functions by interrupting the interface between donor vascular endothelial cells (VEC) lining the vasculature and the recipient's immune cells. NB-LVF4 therapy could provide the opportunity to introduce effective adjunct therapies within this 21-day window. We now report the underlying protective mechanisms involved in immunocloaking.
Methods: Assays ($n = 5$) were performed evaluating antigen presentation, T cell activation & proliferation using responding mononuclear cells (MNCs) stimulated with untreated confluent VEC (controls) and with confluent immunocloaked VEC (test). The cytokine & chemokine responses were evaluated using the Luminex platform. Early T cell activation was measured using CD4+, CD69+ detection by flow cytometry. Proliferation was determined using Bdru labeling. Transwell studies assessed chemotaxis using SDF-1.
Results: Immunocloaking resulted in significant inhibition of the cytokines/chemokines: IL-1β, IL-6, γ-IFN, IL-2, TNF-α, CD-69, MIG and MIP-1α ($p < 0.05$, Figure 1). The inhibition of the cytokines produced by antigen presenting cells; IL-1β, TNF-α, MIP-1α and γ-IFN show that antigen presentation was prevented. Similarly, the inhibition of markers of T cell activation, IL-6, IL-2, CD-69 and MIG suggest the blockade of T cell mediated responses. Immunocloaking VEC also resulted in a significant inhibition of mononuclear cell diapedesis (>95%).
Conclusions: Immunocloaking with NB-LVF4 provides a mechanism to eliminate the allo-recognition that normally occurs immediately upon reperfusion. The lack of early allo-responses could provide a window of opportunity to introduce adjunct therapies to support tolerance induction.

Figure 1. Inhibition of Antigen Presentation and the Prevention of Tcell Activation



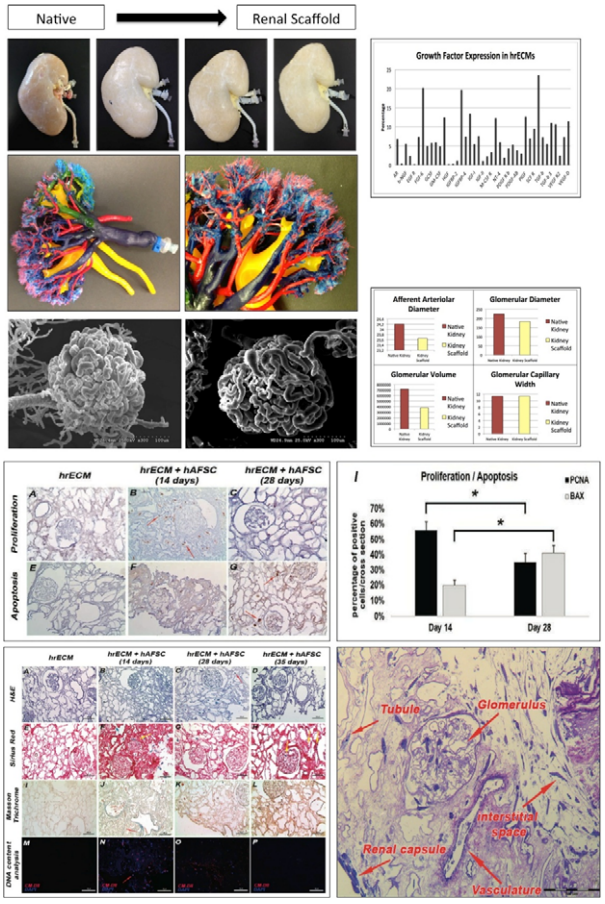
MNCs: Responding peripheral blood mononuclear cells
VEC: Confluent monolayers of vascular endothelial cells
NB-LVF4 VEC: NB-LVF4 treatment of confluent monolayers of vascular endothelial cells
Values represented as a percent of the positive controls tested in triplicate of n=5

O40 RENAL ECM SCAFFOLDS FROM DISCARDED KIDNEYS ARE BIOACTIVE, PRO-TOLEROGENIC, CYTO-COMPATIBLE AND FEATURE AN INTACT, PATENT AND RESILIENT VASCULAR FEATURE AN INTACT, PATENT AND RESILIENT VASCULATURE

Giuseppe Orlando[1], Andrea Peloso[2], Astgik Petrosyan[3], Stefano Da Sacco[3], Paolo Cravedi[4], Joao Paulo Zambon[5], Timothy O'Brien[6], Charles Aardema[6], John Robertson[6], Roger E. De Filippo[3], Shay Soker[5], Robert J. Stratta[1], Perin Laura[3]
[1]Wake Forest School of Medicine, Department of General Surgery, Section of Transplantation, Winston Salem, NC; [2]IRCCS Policlinico San Matteo – Department of General Surgery, University of Pavia, Pavia, Italy; [3]Saban Research Institute, Children's Hospital Los Angeles, University of Southern California, Los Angeles, CA, USA; [4]Renal Division, Department of Medicine, Icahn School of Medicine at Mount Sinai, NY, USA; [5]WFIRM - Wake Forest Institute for Regenerative Medicine; [6]Virginia Tech, Blacksburg, Virginia

Background: In the attempt to identify a potentially inexhaustible source of transplantable kidneys, we are using human renal ECM scaffolds (hrECMs) produced through the decellularization (Dec) of discarded kidneys as a platform for kidney bioengineering. This study aimed at: 1) assessing the status of the innate vasculature (IV) post Dec; 2) quantifying the growth factors (GFs) stored in the matrix; 3) studying the behavior of multipotent cells when seeded on hrECMs; 4) evaluating the immunogenicity of hrECMs.

Methods: For aim 1, we studied the morphometry and resilience of hrECMs' native vasculature with resin casting at scanning electron microscopy and pulse-wave measurements, respectively. For aim 2, we determined the fate of 40 critical GFs post Dec with a glass chip-based multiplex ELISA array and in vitro immunofluorescence. For aim 3, we established a 3D in vitro model where stem cells (SC) were seeded on hrECMs and studied with histology, analysis of secretomes, and PCR to assess gene expression. For aim 4, immunological studies were performed by CD4 isolation, in vitro cultures and flow cytometry assays.
Results: Resin casting and pulse-wave measurements, showed that hrECMs preserve the microvascular morphology, morphometry, and physiological function. Significant amount of GFs is retained within hrECMs compared with intact kidneys. SC attach, migrate, proliferate, remodel the matrix, express genes of kidney development and induce angiogenesis. hrECMs inhibit naive CD4+ T cell proliferation in response to anti-CD3/anti-Cd28 mAb and promote their conversion into regulatory T cells.
Conclusion: Our data demonstrate that hrECMs feature a well preserved and resilient innate vasculature, which is a critical finding in perspective of on in vivo implantation. Importantly, they maintain GFs, are cytocompatible, able to determine organ-specific phenotype and show also immunomodulatory properties.



O41 TISSUE ENGINEERED ESOPHAGUS WITH HUMAN STEM CELLS: A STEP TOWARDS ESOPHAGEAL REPLACEMENT

Nikhil Nayakawde, Malin Berg, Ketaki Methe, Goditha Premaratne, Hasse Ejnell, Dick Delbro, Michael Olausson
Sahlgrenska University Hospital Sahlgrenska Academy

Background: Esophageal carcinoma is the sixth leading cause of cancer related mortality and affects almost 500 000 patients every year globally. Esophageal replacement surgery is a preferred treatment in esophageal cancer, congenital esophageal diseases in pediatric patients (esophageal atresia), traumatic conditions, and various end stage benign esophageal diseases. A tissue engineered esophagus would be a promising alternative for esophageal replacement surgery over conventional esophageal substitute such as stomach, colon and small intestine autograft. Here we present in vitro generation of tissue engineered porcine esophagus with human stem cells for in vivo transplantation.
Methods: Pig esophageal tissues (n = 9) were decellularized with detergent-enzymatic (DE) protocol consisted of sodium deoxycholate and Deoxyribonuclease I. Acellular scaffolds further analyzed by histology, and quantitative analysis for DNA as well as structural and functional proteins of extracellular matrix (ECM). Human amnion derived mesenchymal stem cells and epithelial cells were seeded onto acellular esophagus in a perfusion-rotation bioreactor.
Results: Esophagi were decellularized in three DE cycles. The decellularization protocol effectively produced scaffold which resembled to the native tissue with preserved mucosal network attached to the basement membrane without cell nucleus. ECM proteins such as collagen, elastin, and glycosaminoglycans are found to be present after decellularization. Recellularized scaffold shows infiltration of mesenchymal stem cells from outer adventitia into the muscle layer. Very few epithelial cells were attached to the lumen of the esophagi.
Conclusion: This study demonstrates stem cell growth in the acellular esophagus after DE decellularization method. Hence, provides the basis for a preclinical approach of tissue engineered esophagi in a large animal model.

O42 TRANSPLANTABLED CELL ENGINEERING STRUCTURES, CONTAINING LIVER CELLS AND BONE MARROW STEM CELLS FOR CORRECTION OF LIVER FAILURE

Murat Shagidulin[1], Nina Onishchenko[2], Mikhail Krasheninnikov[3], Igor Iljinsky[1], Natalia Mogeiko[2], Pavel Avramov[2], Viktor Sevastjanov[2], Sergey Gautier[1]
[1]Federal V.Shumakov Research Center of Transplantology and Artificial Organs, I.M.Sechenov First Moscow Medical State University; [2]Federal V.Shumakov Research Center of Transplantology and Artificial Organs; [3]Sechenov First Moscow Medical State University

Background: Treatment of chronic liver failure (LF) by using of new technologies is an actual aim. For this reason, there is the great interest in cell transplantation as a method of liver supporting therapy at LF, especially in pretransplant period.
Materials/methods: Chronic LF was modeled on Wistar rats by means CCl4. Wistar rats were used as donor cells. Isolated liver cells (LC) and mesenchymal stem cells (MSC) of bone marrow were obtained by standard procedure. LC (2.5–4.0 × 106 cells/cm^3) and MSC (0.5–0.8 × 106 cells/cm^3) were immobilized on particles powderen matrix, prepared from decellularizated liver. Formed cell engineering structures (CES) were transplantated (TX) into rat liver. All animals (n = 40) were divided into 2 groups:control (1 g) without CES and study (2 g) with CES. Dynamics reduction of LF;recovery liver, CES morphology and cell viability in CES were investigated within 90 days after TX.
Results: In 1 g. LF was characterized by ALT, AST, ALP rising and decreasing of synthetic (albumin) liver function and cirrhosis formation. In 2 g. all biochemical indices returned to normal levels on the 30 days; it were detected viable hepatocytes, neogenic bile ducts in CES. Indices of liver damage were significant less in 2 g. than in control 1 g. Recovery of hepatic lobe structures was better in studied 2 g.
Conclusion: Our preliminary studies demonstrate that in CES transplantated into damage liver there are formation of morphologic structures of liver (viable hepatic cells, neogenic vessels, bile ducts);other words the used method of LF correction allows to carry out organotypic liver remodeling into CES and to support the function of damage liver. Thereby the suggested method is a perspective one and can be used as a technology of building intracorporeal CES for the long-term auxiliary supporting of damaged liver and also in the pretransplant period.

023 KIDNEY

O43 SOCIO-ECONOMIC AND DEMOGRAPHIC DIFFERENCES BETWEEN DIALYSIS, WAITLISTED AND TRANSPLANT PATIENTS IN THE UNITED KINGDOM: FINDINGS FROM THE ATTOM STUDY

Diana Wu[1], Matthew Robb[2], Rommel Ravanan[3], Charles Tomson[4], John Forsythe[1], Clare Bradley[5], John Cairns[6], Christopher Dudley[3], Christopher Watson[7], Heather Draper[8], Wendy Metcalfe[1], Damian Fogarty[9], Paul Roderick[10], Andrew Bradley[7], Gabriel Oniscu[1]

[1]Transplant Unit, Royal Infirmary of Edinburgh; [2]NHS Blood and Transplant, Bristol; [3]Richard Bright Renal Unit, Southmead Hospital, Bristol; [4]Department of Renal Medicine, Freeman Hospital, Newcastle upon Tyne; [5]Health Psychology Research Unit, Royal Holloway, University of London; [6]Department of Health Services Research and Policy, London School of Hygiene and Tropical Medicine; [7]Department of Surgery, University of Cambridge and the NIHR Cambridge Biomedical Research Centre; [8]School of Health and Population Sciences, University of Birmingham; [9]UK Renal Registry, Bristol; [10]Primary Care and Population Sciences, Faculty of Medicine, University of Southampton

Background: Access to renal Transplantation and Transplant Outcome Measures (ATTOM) is a prospective study involving all 72 UK renal units, investigating factors influencing access to and outcomes from transplantation in order to optimise equity of access, survival, quality of life and cost effectiveness.
Methods: 6842 patients aged 18–75 years were recruited to the study between 2011 and 2013, including 2621 incident dialysis (ID), 2262 incident transplant (IT) of whom 807 received living donor (LD) transplants and 1959 waitlisted matched control (MC) patients (matched for centre, age +/-5 years, time on waiting list, diabetes and kidney/simultaneous kidney-pancreas transplant). Baseline data were collected at time of enrolment and analysed using SAS®9.4, p-values <0.05 were considered significant.
Results: Compared to MC and IT patients, ID patients were older (58.4 vs 50.7 yrs), first seen by a nephrologist later in life, had a higher BMI, a higher prevalence of diabetes and lower levels of education, employment and car/house ownership.

Compared to IT patients, a higher proportion of MC patients had a previous transplant (25.3 vs 12.4%), were born outside the UK, were from minority ethnic groups (25 vs 17.1%), were divorced/separated, were smokers (14.7 vs 7.5%), had no qualifications and did not own a car/house.

Compared to deceased donor recipients, recipients of LD transplants were younger (46.0 vs 53.4 yrs), first seen by a nephrologist earlier, a higher proportion were born in the UK, of white ethnicity (87.1 vs 79.3%), had qualifications (85.1 vs 74.4%) and at a higher level, were employed (43.7 vs 31.3%), were car/house owners and fewer were divorced/separated.
Conclusion: There are significant socio-economic and demographic differences between patients on dialysis, transplant recipients and their waitlisted matched controls. Patients with more education and less deprivation appear to have better access to the waiting list and to living donor transplantation.

O44 OMISSION OF PROSPECTIVE CROSSMATCH IN DECEASED DONOR KIDNEY TRANSPLANTATION PERFORMED ON A MIXED EMERGENCY LIST CAN REDUCE ISCHAEMIC TIME AND DELAYED GRAFT FUNCTION

Claire Rutherford, Emma Aitken, Matthew Wickham, Anne Margaret Little, Mark Clancy

Greater Glasgow and Clyde NHS Trust

Background: Donation after circulatory death kidneys make up an ever increasing proportion of the donor pool, and tolerate cold ischemia (CIT) less than donation after brainstem death grafts. Omission of the traditionally performed prospective crossmatch is one approach to address this by means of virtual crossmatch (vXM). Our aim was to assess the impact of vXM on outcomes of deceased donor kidney transplantation. Methods

327 cadaveric transplants were performed in the West of Scotland renal transplant unit between January 2007 and April 2012. Clinical, demographic and laboratory data was prospectively collected in Strathclyde Electronic Patient Record (SERPR). Three separate analyses were performed: all patients who underwent kidney transplant prior to and following introduction of vXM; all patients transplanted with vXM versus all patients transplanted without vXM; and all patients transplanted with vXM versus those patients transplanted prior to the introduction of vXM who would have been eligible.
Results: Of the 144 in the post-vXM group, 75 underwent vXM transplantation. There was lower overall CIT between pre-vXM (16.3 +/- 3.2 h) and post-vXM cohorts (12.9 +/- 3.4 h) ($p = 0.21$), but this difference did not reach statistical significance. There was no significant difference in overall rates of DGF, length of hospital stay, graft survival or eGFR at 1 and 2 years. However, when comparing patients who would have been suitable for vXM prior to its introduction with those patients who actually underwent transplantation without prospective crossmatch, CIT was reduced from 16.2 +/- 3.1 h to 10.1 +/- 2.1 h ($p < 0.001$). The use of vXM proved safe with no early graft losses and no positive cross-matches when performed retrospectively.
Conclusion: For eligible recipients, selective omission of the prospective crossmatch has the potential to maintain or even improve outcomes in kidney transplantation in the context of progressively less favourable organ quality.

O45 DO WE DISCARD TOO MANY TRANSPLANTABLE KIDNEYS?

Silke Niederhaus[1], Konrad Famulski[2], Jeff Reeve[2], Steven Bartlett[1], Jonathan Bromberg[1], Philip Halloran[2]

[1]University of Maryland Medical Center; [2]University of Alberta

The molecular kidney injury-repair response (AKI score) in post-implantation core biopsies predicts future graft function better than histology. This could change the pattern of discarding many kidneys from donors >50 years old due to uncertainty caused by clinical or histologic features. We hypothesize that AKI scores in transplanted kidneys will be similar to some similar-quality discarded kidneys when decision to transplant is based on conventional features. Pre-implantation core biopsies from transplanted and discarded kidneys were obtained from brain dead donors over age 50 years. Decision to discard was based on real-time clinical or wedge biopsy findings. All core biopsies were examined for molecular AKI scores and global gene expression by microarrays. The molecular AKI scores in all kidneys were compared by principal component analysis (PCA). PCA defined two sets of kidneys: Group 1 and Group 2. Group 1 had low AKI scores, including 14 discarded kidneys and 5 transplanted kidneys. Thus, many discarded kidneys had low AKI scores similarly to transplanted kidneys and were potentially transplantable. Group 2 contained 14 discarded kidneys and 2 transplanted kidneys, all with high AKI scores. We identified 775 transcripts associated with PC1 of the AKI scores. Pathway analysis demonstrated that kidneys with high AKI scores had decreased expression of genes related to oxidative phosphorylation paralleled by activation of their negative regulator, RICTOR and suggestive of activation of mTORC2 pathway that regulates cellular metabolism. These kidneys also had increased expression of genes related to antigen presentation driven by IFNG and increased acute phase stress response. Thus discarded kidneys with high AKI scores were more stressed. Molecular features combined with the clinical and histological evaluation of older donor kidneys may improve kidney utilization and provide mechanistic insights into why some kidneys have impaired performance after transplant.

O46* SHORT UK DIALYSIS DOES NOT SIGNIFICANTLY IMPACT ON LIVE DONOR KIDNEY ALLOGRAFT SURVIVAL

Andrew Bentall[1], Mark Jesky[2], Richard Borrows[2], Rachel Johnson[3], Simon Ball[2]

[1]University of Birmingham; [2]University Hospital Birmingham; [3]NHSBT Bristol

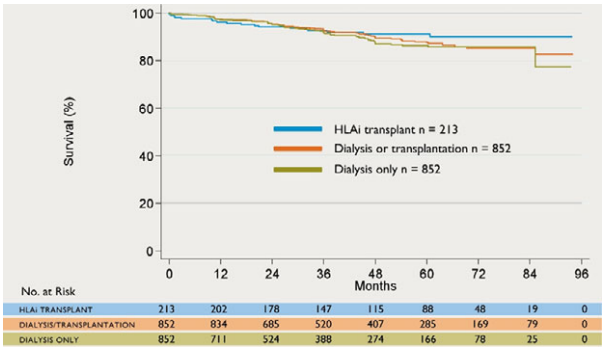
Background: The survival advantage of pre-emptive transplantation (Pre-empTx) in live donor kidney transplantation (LDKTx) was reported by Meier-Kreische (Kidney International, 2000). Dialysis practices differ internationally and immunosuppressive regimes have advanced. The aim of this study was to examine the effect of dialysis time on allograft survival in UK LDKTx, including an assessment of allograft survival (GS) between compatible (CTx) and antibody incompatible transplants (AiT) donors.
Methods: Data from NHSBT for LDKTx recipients in the UK were analysed between 2001 and 2013. Data with dialysis time were available for 7427/9755 (76.1%) transplants performed. These data were analysed for both GS and composite outcomes (combined death or graft failure). No meaningful differences were found in using a competing risk regression model. Dialysis time (DiT) was categorised into Pre-Tx, <1 yr, 1–2 yr, 2–4 yr and 4 yr dialysis. AiT ($n = 760$) were grouped as HLA incompatible (HLAi, $n = 390$) and blood group ABO incompatible (ABOi, $n = 370$). Paired Exchange recipients (PrEx, $n = 198$) and CTx ($n = 6469$) were also analysed in this study.
Results: Transplant groups differed significantly with respect to donor Age ($p < 0.001$), recipient Age ($p < 0.001$), calculated reaction frequency at transplant (median cRF%, $p < 0.05$), HLA mismatches ($p < 0.001$). The risk of graft failure (GF) of LDKTx compared to Pre-empTx increased with more DiT, but only after 1 year of dialysis in the whole cohort (<1 yr DiT HR 1.06.1–2 yr HR 1.32 $p = 0.01$, $p = 0.60$, 2–4 yr HR 1.76 $p < 0.01$ and 4 yr HR 2.12, $p < 0.01$). Overall Death Censored GS at 5 yr was Pre-empTx 92.7%, 0–1 yr 92.3%, 1–2 yr 90.0%, 2–4 yr 87.2% and >4 year 83.6%. Compared to CTKx, ABOi and HLAi were at increased risk of GF (HR 1.59, $p = 0.02$ and HR1.69, $p = <0.01$ respectively). Death Censored GS at 5 yr was ABOi 88.5%, HLAi 78.3% and CTx 91.1%.
Conclusion: A short time on dialysis does not reduce GS in LDKTx in the UK. Compared to CTx, AiT recipients have lower graft survival, however reduced dialysis time was associated with improved GS in ABOi and HLAi. These data help clinicians make informed decision on timing of LDKTx.

O47 DOES HLA-INCOMPATIBLE KIDNEY TRANSPLANTATION CONFER A SURVIVAL BENEFIT IN THE UK? A MATCHED COHORT ANALYSIS

Miriam Manook[1], Leonardo Koeser[2], Zubir Ahmed[3], Rachel Johnson[4], Olivia Shaw[3], Nicos Kessaris[3], Anthony Dorling[3], Nizam Mamode[3]
[1]Guy's Hospital; [2]KCL; [3]Guy's & St Thomas's Hospital; [4]NHSBT

Introduction: HLA-incompatible transplantation is perceived as being a 'high-risk' option with increased mortality and morbidity for transplantation of sensitized recipients of renal transplants. Data from the USA has indicated that HLA-incompatible transplantation confers an increased patient survival compared to 'dialysis only' (D) or 'dialysis or transplant' (DT). This study aims to determine whether this survival advantage applies in the UK, where options for deceased donor transplantation are different.
Methods: UK patients listed for transplantation at 1.1.2007, and all subsequent listings to 31.12.2013 were accessed from the NHSBT waiting list on 31.12.2014 Primary outcome measured was death on the waiting list, or after transplantation. Reference patients (213) were those receiving live-donor HLA-incompatible flow or CDC cross match positive (LD HLAi XM+) renal transplant and a cohort (852) of 'D' or 'DT' controls was matched for the following characteristics: calculated reaction frequency (cRF), age, gender, blood group, number of previous transplant & duration of end-stage renal disease.
Results: 38% of the matched controls were in the DT group. 5-year survival for HLAi XM+ patients was 93%, compared to 'dialysis or transplant' 88%, or 'dialysis alone' 86%. Log-rank test: 'dialysis or transplantation' vs. HLAi transplant: $\chi 2 = 0.77$, $p = 0.4$; 'dialysis only' vs. HLAi transplant: $\chi 2 = 1.35$, $p = 0.2$

Conclusions: Dialysis outcomes for highly sensitized patients on the waiting list for a transplant in the UK are excellent. For those with an incompatible living donor, LD HLAi XM+ transplantation offers a guaranteed transplant that does not adversely affect survival.



O48 PREEMPTIVE KIDNEY TRANSPLANTATION WITHOUT JAPANESE EXCELLENT HEMODIALYSIS THERAPY

Norihiko Goto, Kenta Ito, Kenta Futamura, Manabu Okada, Takayuki Yamamoto, Makoto Tsujita, Takahisa Hiramitsu, Shunji Narumi, Yoshihiko Watarai
Nagoya Daini Red Cross Hospital Renal Center

Purpose: Though survival rates among hemodialysis patients are much higher in Japan than in the US or Europe from DOPPS, we assessed the impact of preemptive kidney transplantation (PKT) on clinical events, which are defined as death with functioning graft (DWFG), graft loss, and posttransplant cardiovascular (CV) disease.
Methods: The investigation was a retrospective cohort of living kidney transplant recipients in a single center, Nagoya Daini Red Cross Hospital, Nagoya, Japan. Eligible recipients were 18 years of age or older and underwent consecutive kidney transplant from a living donor between November 2001 and December 2013 ($n = 786$). The study outcome was the occurrence of clinical events which had occurred at the end of 2014.
Results: PKT was performed in 239 (30.4%). Clinical events occurred in 78 (9.9%). Compared to without events, recipients with clinical events were older, male, had ABO incompatibility, were performed PKT (10.3% vs. 32.6%, $p < 0.001$), had long dialysis period (34.3 vs. 11.3 months, $p < 0.001$), diabetic (28.2% vs. 11.3%, $p < 0.001$), had pretransplant CV disease (14.1% vs. 7.1%, $p = 0.041$), had let ventricular mass index (LVMI). The incidence of clinical events were related to ABO incompatibility (indicates risk ratio (RR) = 2.98, $p < 0.001$), dialysis period (per year) (RR = 1.07, $p < 0.001$), diabetes (RR = 3.54, $p < 0.001$). Kaplan-Meier estimates show significant differences between PKT and non PKT in event free rate. ($p = 0.003$, log rank test) According to dialysis period of 0 year (PKT patients), <1 year, <2 years, <3 years, <4 years, <5 years, and ³5 years, clinical events were 3.3%, 10.8%, 11.1%, 10.4%, 10.2%, 16.7%, and 16.2%, respectively. ($p = 0.002$)

Conclusions: Even in Japan where long-term survival of hemodialysis patients are excellent, the patients who reach end-stage renal disease should receive a renal transplant as early as possible to reduce DWFG, graft loss, and posttransplant CV disease.

O49* ADVANTAGES OF CHAINS VS. CYCLES IN A KIDNEY PAIRED DONATION PROGRAM: A 7-YEAR ANALYSIS

Stanislaw Stepkowski[1], David E. Fumo[2], Laurie J. Reese[2], Jonathan E. Kopke[2], Alvin E. Roth[3], Alan B. Leichtman[4], Michael A. Rees[2]
[1]University of Toledo Collage of Medicine; [2]Alliance for Paired Donation; [3]Stanford University; [4]The University of Michigan

Failure to convert computer-identified possible exchanges into transplants limits Kidney Paired Donation (KPD) program. We have analyzed the efficacy of two methods in generating KPD transplants. The Alliance for Paired Donation (APD) utilizes an optimization algorithm selecting either simultaneous closed loop exchanges (Cycles) or Non-simultaneous Extended Altruistic Donor (NEAD) chains. NEAD chains are initiated by a non-directed donor (NDD) and end with a bridge donor, who enters back into the pool. This bridge donor can then initiate another NEAD chain. Computer-generated potential cycles and NEAD chains are presented to transplant centers for review, at which point they become a formal offer. We tracked the progress in moving from offer to completed transplants in order to calculate the success rate (SR). Between January 2007 and August 2014 the APD performed 225 transplants: 194 within the APD as well as 31 in collaboration with other KPD programs. Of the 194 APD transplants, 61 (31.4%) were performed through cycles (Figure 1a). In contrast, 133 out of 194 transplants (68.6%) were performed through chains (Figure 1b), suggesting that chains were two times more successful than cycles. The APD made 447 offers to transplant centers, of which 173 (38.7%) were cycles and 274 (61.3%) were chains. Consequently, the SR of cycles was 14.5%, while chains was 26.6%; the overall SR was 21.9%.

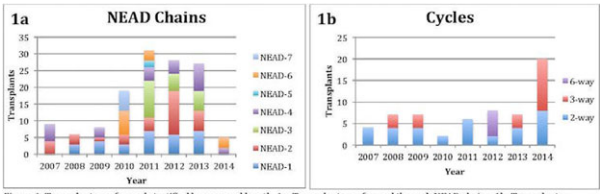


Figure 1: Transplants performed stratified by year and length. 1a: Transplants performed through NEAD chains. 1b: Transplants performed through cycles.

Although chains were not actively sought over cycles, the results show that chains were preferentially found by the optimization algorithm and more successfully progressed to completed transplants than cycles. Indeed, the concept of a bridge-donor introduces significant flexibility in generating transplants. We propose that NEAD chains are the most effective tool to increase the efficiency in KPD programs.

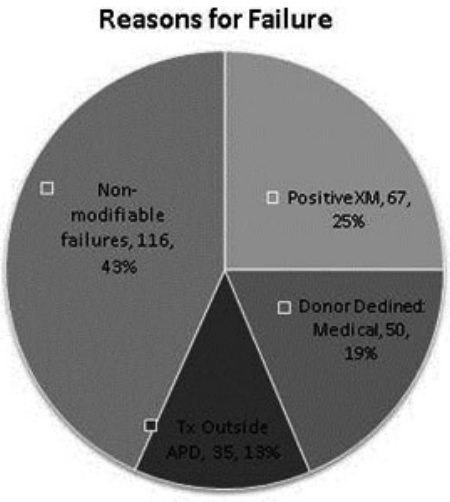
O50 REASONS FOR FAILING OFFERS IN A KIDNEY PAIRED DONATION PROGRAM: A 7-YEAR ANALYSIS

Stanislaw Stepkowski[1], David E. Fumo[1], Laurie J. Reese[2], Jonathan E. Kopke[2], Susan E. Rees[2], Alvin E. Roth[3], Alan B. Leichtman[4], Michael A. Rees[5]
[1]University of Toledo Collage of Medicine; [2]Alliance for Paired Donation; [3]Stanfor University; [4]University of Michigan; [5]University of Toledo

Computer-identified potential Kidney Paired Donation (KPD) exchanges are offered to participating transplant programs and, if accepted, are subsequently considered accepted transplants. We evaluated reasons why some offers failed to progress into accepted transplants. Each offer may be composed of one to multiple 1-way exchanges. The progress in moving from offers to transplants was tracked and recorded including the reason for failure. From January 2008 through August 2014, 981 possible 1-way KPD exchanges were offered to transplant centers. Of these possible 1-way exchanges, 181 resulted in transplants, while 268 failed for specific identifiable reasons (SIRs); the remaining 529 failed because they were dependent upon completion of these 268 failed exchanges. Several reasons for failure have been potentially preventable. The SIRs for failure include: positive crossmatch; competing offer outside the APD; and, donor declined by transplant center (Figure 1).

Overall, SIRs accounted for 57% of all failures. To decrease the frequency of transplant centers rejecting donors, the APD has begun presenting potential donors to transplant centers for initial review before a formal offer is made, thus improving the certitude of an accepted offer. While a centralized lab tests for crossmatch results, the APD pool has increased the number of highly sensitized patients. Indeed, the percentage of the pool with a PRA >80 has increased from 31% in 2008 to 47% in 2014. Many patients choose to enroll in multiple KPD registries resulting in futile exchanges with patients already

committed to alternative exchanges. In conclusion, many SIRs highlight the need to expand the donor pool to increase options for highly sensitized patients, as well as the need for better coordination with other KPD programs to avoid competing offers.



O51

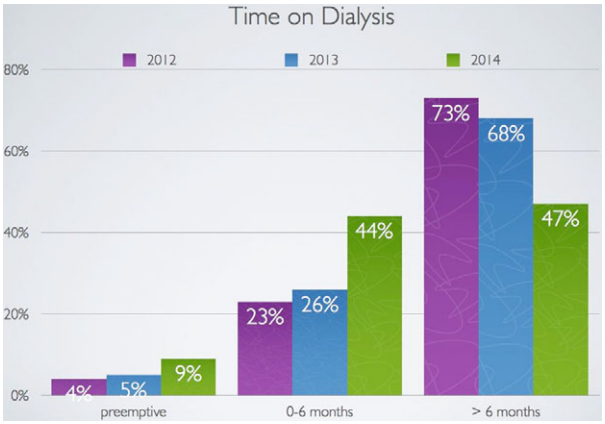
ONLINE PLATFORM TO REDUCE BARRIERS AND DISPARITIES IN ACCESS TO RENAL TRANSPLANT EVALUATION PROCESS ACROSS DIALYSIS FACILITIES: MAGNUS PROJECT

Gustavo Ferreira[1], *Ferreira Gustavo Fernanades*[2], *Bastos Kamille Vidon*[2], *De Freitas Elaine Barbeta*[1], *Vanelli Chislene Pereira*[1], *Sebastiao Ferreira*[1]
[1]*Santa Casa de Misericordia de Juiz de Fora;* [2]*Santa Casa de Juiz de Fora*

Background: Gaining access to kidney transplantation is complex and expensive. Despite the World Health Organization recommendations of equitable access to transplantation, studies demonstrate important inequalities in access to the cadaveric donor waiting list for patients. A longer time spent on dialysis has been associated with significantly poorer patient and graft survival after transplantation

Methods: We developed a platform that enables the dialysis centers refer and schedule for a kidney transplant evaluation at a single center. We first started using the platform on February 2012. Since than, 19 different dialysis facilities started using the platform online to refer and schedule their patients. We analyzed the time from dialysis initiation to refer to transplant evaluation in the first 3 years using the platform. Analyses of differences between the years were performed with chi-square test.

Results: Eight hundred and twenty four patients have been referred to transplant evaluation in our center (193 on 2012; 285 on 2013 and 346 on 2013) from 19 different dialysis facilities. Only 62 patients (7%) were referred to evaluation before dialysis initiation (4% 2012; 5% 2013 and 9% 2014). Patients referred to transplantation after dialysis initiation and before 6 months on dialysis rose from 23% to 26% to 44% on 2012, 2013 and 2014 respectively ($p < 0.05$). Patients referred to transplant evaluation after 6 months on dialysis declined form 73% to 68% to 47% on 2012, 2013 and 2014 respectively ($p < 0.05$) .



The median time on dialyses was 32.5 ± 44.1 months.

Conclusion: Our findings demonstrate that the online platform not only reduced time from dialysis initiation to pretransplant evaluation but also increased in 79% the numbers of patients referred do kidney transplantation evaluation in 3 years of evaluation.

025 LIVER

O52 LEDIPASVIR/SOFOSBUVIR WITH RIBAVIRIN IS SAFE IN >600 DECOMPENSATED AND POST LIVER TRANSPLANTATION PATIENTS WITH HCV INFECTION: AN INTEGRATED SAFETY ANALYSIS OF THE SOLAR 1 AND SOLAR 2 TRIALS

Didier Samuel[1], Michael Manns[2], Xavier Forns[3], Steven Flamm[4], Rajender K. Reddy[5], Jill Denning[6], Sarah Arterburn[6], Theo Brandt-Sarif[6], Phillip Pang[6], John Mchutchison[6], Nezam Afdhal[7], Michael Charlton[8], Edward Gane[9], David Mutimer[10], Gregory Everson[11]

[1]Université Paris-Sud; [2]Hannover Medical School; [3]Liver Unit, IDIBAPS, CIBEREHD Hospital Clinic; [4]Northwestern Feinberg School of Medicine; [5]University of Pennsylvania School of Medicine; [6]Gilead Sciences; [7]Beth Israel Deaconess Medical Center; [8]Intermountain Medical Center; [9]University of Auckland; [10]QE Hospital and University of Birmingham; [11]University of Colorado

Background: Patients with HCV who have decompensated liver disease or who have recurrent HCV post liver transplantation have substantial rates of morbidity and mortality. The safety of any HCV treatment regimen is therefore critical to evaluate in order to determine if, when and who to treat. The safety of ledipasvir/sofosbuvir (LDV/SOF) + ribavirin (RBV) was evaluated in a pooled analysis of two studies enrolling patients with advanced liver disease in the US (SOLAR 1, NCT01938430) and Europe, Canada, Australia and New Zealand (SOLAR 2, NCT02010255), the largest study of such patients to be evaluated to date.
Methods: Six groups of patients with HCV genotypes 1 or 4 and decompensated liver disease or who were post liver transplantation were randomized to receive 12 or 24 weeks of LDV/SOF + RBV treatment: patients without transplant and either 1) Child-Pugh-Turcotte (CPT) B cirrhosis, or 2) CPT C cirrhosis; or patients who have undergone transplantation and who were either 3) without cirrhosis (F0 to F3), 4) CPT A cirrhosis, 5) CPT B cirrhosis, or 6) CPT C cirrhosis. RBV was administered either at 600 mg/day and escalated as tolerated (decompensated) or weight-based (1000–1200 mg/day; F0-F3, CPT A).
Results: 658 patients were randomized. 392 had HCV genotype 1a, 224 had HCV genotype 1b, and 42 had HCV genotype 4. The major safety findings are included in the table below. Of 134 SAEs, only 20 were related to treatment, 11/20 (55%) were due to RBV-associated anemia. RBV-associated anemia was also the most common Grade 3 laboratory AE. There were 18 treatment-emergent (TE) deaths; sepsis (5), GI bleed (3), and myocardial infarct (3) occurred in more than 1 person and none were attributed to LDV/SOF treatment.
Conclusions: In these patient populations treatment with LDV/SOF + RBV was generally safe and well tolerated, irrespective of the degree of decompensation or whether patients were pre- or post-transplantation.

O53 EFFECTIVE PROPHYLAXIS FOR HEPATITIS B VIRUS (HBV) REINFECTION AFTER LIVER TRANSPLANTATION (TX) FOLLOWING SWITCH TO SUBCUTANEOUS (S.C.) HEPATITIS B IMMUNOGLOBULIN (HBIG) BY WEEK 2 POST-TRANSPLANT (TX)

Paulo De Simone[1], Mauro Salizoni[2], Antonio D. Pinna[3], Umberto Cillo[4], Fausto Zamboni[5], David Mutimer[6], Didier Samuel[7]

[1]General Surgery & Liver Transplantation, Azienda Ospedaliero-Universitaria Pisana, Pisa; [2]Liver Transplantation Centre, University Hospital Molinette, Turino; [3]School of Medicine and Surgery, University Hospital, Policlinico S. Orsola-Malpighi, Bologna; [4]Unità Operativa di Chirurgia Epatobiliare e Trapianto Epatico, Az. Osp. Uni. di Padova, Padova; [5]Centro Trapianti di Fegato e Pancreas, Azienda Ospedaliera "G. Brotzu" di Cagliari, Cagliari; [6]NIHR Liver BRU, QE Hospital and Birmingham University, Birmingham; [7]AP-HP Hôpital Paul Brousse Centre Hepato-Biliaire, Villejuif

Background: Subcutaneous HBIg (Zutectra®) maintains hepatitis B surface antibody (anti-HBs) serum trough level >100 IU/l, the minimum threshold for effective HBV reinfection prophylaxis, in maintenance liver tx patients (pts). The efficacy of early switch to s.c. administration is undocumented.
Methods: In a six-month prospective, open-label, single-arm study, HBV DNA-negative pts undergoing liver tx for HBV infection were switched at 8–18 days post-tx from i.v. to s.c. HBIg (500 or 1000 IU once a week or once every 2 weeks) if they were HBsAg negative at time of switch, combined with anti-HBV nucleoside therapy. After week 4, injection by the pt or a caregiver was permitted following training, if anti-HBs trough level was >100 IU/l. Primary endpoint was failure rate at month 6 (i.e. serum anti-HBs ≤100 IU/l or HBV re-infection with serum anti-HBs >100 IU/l).
Results: 49 patients were recruited, of whom 47 (95.9%) completed 6 months of treatment. By week 14, 47 pts were self-administering ($n = 35$) or being injected by a caregiver ($n = 12$). No treatment failures occurred i.e. serum HBs antibody concentrations remained ≥100 IU/l and all pts remained HBsAg negative. HBV DNA remained negative in 45/45 pts who were tested. Mean anti-HBs progressively declined to a protective titer of 250 IU/l at month 6 (figure). No pt developed clinical symptoms consistent with HBV re-infection. The only adverse event to be assessed as treatment-related was a mild injection site hematoma. All 44 pts who completed an end-of-study questionnaire reported that they were satisfied with s.c. HBIg.
Conclusions: Switching to s.c. HBIg early after liver tx (during days 8–18) maintained serum anti-HBs levels at an adequate level to prevent HBV re-infection in all pts, and was well-tolerated. Self-administration of s.c. HBIG with concurrent HBV virostatic therapy offers a successful and convenient strategy for preventing HBV re-infection after liver tx.

	Advanced Liver Disease CPTB	Advanced Liver Disease CPTC	Post-transplant F0-3	Post-transplant CPTA	Post-transplant CPTB	Post-transplant CPTC	Total
N	117	98	211	118	96	18	658 (100)
Any AE	113	94	203	107	91	17	625 (95)
>=Grade 3AE	17	33	43	25	22	6	146 (22)
Related AE	89	68	174	89	72	11	503 (76)
SAE	22	35	29	15	26	7	134 (20)
Related SAE	3	4	5	6	2	0	20 (3)
AE leading to D/C	3	4	2	2	3	1	15 (2)
OLT	4	7	0	0	0	0	11 (2)
Death	3	7	0	3	3	2	18 (3)
Hb < 10 g/dl	20	34	93	51	46	11	255 (39)
Hb < 8.5 g/dl	5	10	31	19	15	2	82 (12)

O54 MULTICENTER BELGIAN EXPERIENCE OF SOFOSBUVIR MEDICAL NEED PROGRAM IN PRE- AND POST LIVER TRANSPLANTATION PATIENTS: SAFETY AND EFFICACY RESULTS

Delphine Degré[1], Wim Laleman[2], Xavier Verhelst[3], Anne Lamproye[4], Thomas Vanwolleghem[5], Thierry Gustot[1], Peter Starkel[6], Nicolas Lanthier[6], Peter Michielsen[5], Jean Delwaide[4], Hans Van Vlierberghe[3], Frederik Nevens[2], Christophe Moreno[1]

[1]ULB Erasme Brussels; [2]UZ Leuven; [3]UZ Gent; [4]CHU Sart Tilman Liège; [5]UZ Antwerpen; [6]UCL Saint-Luc Brussels

Background: Severe hepatitis C (HCV) recurrence after liver transplantation (LT), and HCV in cirrhotic patients listed for LT have a negative impact on patient survival and current treatment options are clearly suboptimal. Sofosbuvir (SOF), Daclatasvir (DCV) and Simeprevir (SMV) have been recently approved in Europe but there are limited data on the use of these drugs in the treatment of these very difficult-to- treat patients. Methods

Multicenter Belgian retrospective analysis of patients with either severe HCV recurrence after LT or listed for LT receiving SOF with SMV, DCV, Peginterferon (PegIFN) + ribavirin (RBV) or RBV in compassionate use or medical need in Belgium. The aim of this study was to evaluate the safety and efficacy of the treatment.

Results: 35 patients were enrolled in this data collection, 14 cirrhotics listed for LT, and 21 LT recipients with severe recurrence. The majority of patients were male (77.1%) and median age was 55[50–67] years. Genotype distribution was: genotype 1 (n = 28), 2 (n = 1), 3 (n = 4), 4 (n = 1), 5 (n = 1). At baseline, median MELD and Child-Pugh scores were 12.9 [8–17.3] and 7[5–9.3], respectively. 15 patients have completed the treatment course and 18 are still on therapy. Two patients stopped prematurely the treatment, 1 patient because of pancytopenia and liver decompensation and 1 patient died but the death was not related to the treatment. W4 and W12 HCV RNA undetectable was 6/25 (24%) and 19/23 (82.6%). End of treatment response was 12/13 (92.3%) (2 viral loads are ongoing) and SVR12 was 92.3% (4/5). Final SVR results will be presented. Severe adverse events (SAE) were reported in 8 patients, 7 were not related to the treatment and 1 patient developed pancytopenia after 1 day of treatment. Conclusions: This preliminary experience in pre and post-LT patients shows that SOF in combination with DCV, SMV, RBV or PegIFN is safe and virological response seems to be promising.

O55 LEDIPASVIR/SOFOSBUVIR WITH RIBAVIRIN IS SAFE AND EFFICACIOUS IN DECOMPENSATED AND POST LIVER TRANSPLANTATION PATIENTS WITH HCV INFECTION: PRELIMINARY RESULTS OF THE PROSPECTIVE SOLAR 2 TRIAL

Michael Manns[1], Xavier Forns[2], Didier Samuel[3], Jill Denning[4], Sarah Arterburn[4], Theo Brandt-Sarif[4], Hadas Dvory-Sobol[4], Phillip S. Pang[4], John G. Mchutchison[4], Edward Gane[5], David Mutimer[6]

[1]Hannover Medical School; [2]Liver Unit, IDIBAPS, CIBEREHD Hospital Clinic; [3]Université Paris-Sud; [4]Gilead Sciences; [5]University of Auckland; [6]QE Hospital and University of Birmingham

Background: Treatment for patients with chronic hepatitis C (HCV) who have decompensated liver disease or who have undergone liver transplantation are limited. We evaluated the safety and efficacy of ledipasvir/sofosbuvir (LDV/SOF) fixed dose combination with ribavirin (RBV) in such patients in Europe, Canada, Australia and New Zealand.

Methods: We enrolled HCV genotype 1 or 4 treatment-naïve or treatment-experienced patients with decompensated liver disease or post-liver transplantation with recurrent HCV. Patients were randomized to receive 12 or 24 weeks of treatment stratified into 6 groups: patients pre transplant and either 1) Child-Pugh-Turcotte (CPT) B cirrhosis, or 2) CPT C cirrhosis; or patients with recurrent HCV after liver transplantation who were either 3) without cirrhosis (F0 to F3), 4) CPT A cirrhosis, 5) CPT B cirrhosis, or 6) CPT C cirrhosis.

Results: 327 patients were randomized. Most were male (76%), white (94%), and IL28B non-CC (80%). 159 (49%) had HCV genotype (GT) 1a, 131 (40%) GT 1b, and 37 (11%) GT 4. Mean baseline HCV RNA was 6.2 log10 IU/ml. 41 of 227 cirrhotic patients (18%) had a MELD score >15. 222 (68%) patients have completed study treatment and 171 (52%) have reached post treatment week 4, so far. 9 patients in the advanced liver disease group and 3 patients in the post-transplantation group have discontinued study treatment. 62 patients (19%) experienced serious adverse events (SAEs). Eight SAEs in 8 patients were considered related to study treatment; anemia (4), fall, diarrhea, malaise, and hyperbilirubemia. The most common adverse events were fatigue, anemia, nausea and headache. SVR4 by patient population are presented in table.

Conclusions: Administration of LDV/SOF + RBV in patients with decompensated cirrhosis and recurrent HCV post transplantation has been well tolerated and resulted in high SVR4 rates in these very difficult to treat populations treated with either 12 or 24 weeks of this regimen. SVR 12 will be presented.

O56 SOFOSBUVIR PLUS RIBAVIRIN BEFORE OR ACROSS LIVER TRANSPLANTATION TO PREVENT HCV RECURRENCE: A SINGLE CENTRE EXPERIENCE

Silvia Martini[1], Silvia Strona[1], Marco Sacco[1], Daniele Arese[1], Renato Romagnoli[2], Francesco Tandoi[2], Donatella Cocchis[2], Stefano Mirabella[2], Giorgia Rizza[2], Antonio Ottobrelli[1], Cerenzia Maria Rosaria Torrani[1], Federico Balzola[1], Mauro Salizzoni[2], Mario Rizzetto[1]

[1]Gastrohepatology Unit, AOU Città della Salute e della Scienza di Torino; [2]Liver Transplant Centre, AOU Città della Salute e della Scienza di Torino

Background: In Curry's* phase 2 study, Sofosbuvir (SOF) plus Ribavirin (RBV) prevented recurrence of HCV infection after liver transplantation (LT) in 25/26 (96%) of patients (pts) with undetectable viremia for >30 days (d) before LT, but only in 5/14 (36%) with undetectable viremia for <30 days. We administered SOF+RBV in HCV pts before or across LT.

Methods: We enrolled 43 HCV cirrhotics awaiting LT for HCC within Milan criteria (29 pts) and/or end-stage liver disease. Median MELD 11 (range 6–21); Child B 50% and C 19%; male 86%, mean age 55 years, mean BMI 26. Mean baseline HCVRNA 5.8 Log IU/ml (4.9–7.1 log IU/ml); GT1a 5%, GT1b 51%, GT2 2%, GT3 30%, GT4 12%; IL28B CC 17%; previous HCV therapy (Tx) 58%. Planned Tx: SOF 400 mg/die and RBV (weight-based) 48 weeks (w) or until LT; pts undergoing LT still viremic or with undetectable viremia for <30 d continued Tx across LT for an overall period of 24 w.

Results: On Tx, 16/36 (44%) pts at w4, 24/26 (92%) at w8 and 16/16 (100%) at w12 were HCVRNA negative. To date, 3 pts dropped off the waiting-list; 21 pts are still awaiting LT on Tx (median Tx duration: 74d, 5–227), while 19 underwent LT (median Tx duration: 66d, 9–207). 11 pts discontinued Tx at LT: 1 still viremic pt with multinodular HCC received the graft from HCVRNA positive donor, while 10 pts had HCVRNA <15 IU/ml for a median of 90d (31–187). All the pts are currently HCVRNA negative after a median follow-up of 71d (20–169). The other 8 transplanted pts continued Tx across LT: 4 underwent LT while still being HCVRNA positive, after <6 w of Tx, and 4 underwent LT with HCVRNA <15 UI/ml for <30 d. Since w2 on Tx after LT, HCVRNA is <15 IU/ml in all pts.

Conclusion: All oral Tx with SOF plus RBV is feasible not only before, but also immediately after LT. Bridging antiviral Tx from the pre to the post LT period may be a cost–effective strategy if HCVRNA undetectability for ≥30 day cannot be achieved before LT. *Curry MP et al. Gastroenterology 2015;148:100–7.

O57 A PHASE 3 STUDY (ALLY-1) OF DACLATASVIR, SOFOSBUVIR, AND RIBAVIRIN FOR TREATMENT OF HCV IN ADVANCED CIRRHOSIS OR POSTTRANSPLANT HCV RECURRENCE

Fred Poordad[1], Eugene Schiff[2], John Vierling[3], Charles Landis[4], Robert Fontana[5], Rong Yang[6], Fiona Mcphee[6], Eric Hughes[6], Stephanie Noviello[6], Eugene Swenson[6]

[1]Texas Liver Institute, University of Texas Health Science Center; [2]Schiff Center for Liver Diseases, University of Miami Miller School of Medicine; [3]Baylor College of Medicine; [4]University of Washington School of Medicine; [5]University of Michigan Medical Center; [6]Bristol-Myers Squibb

Background: The ALLY-1 study evaluated the pangenotypic regimen of daclatasvir (DCV) and sofosbuvir (SOF) with ribavirin (RBV) in two groups of HCV-infected patients in need of improved therapeutic options in the liver transplant setting.

	Pre-transplant CPT B 12 wks (N = 29)	Pre-transplant CPT B 24 wks (N = 29)	Pre-transplant CPT C 12 wks (N = 24)	Pre-transplant CPT C 24 wks (N = 25)	Post-transplant F0-F3 12 wks (N = 51)	Post-transplant F0-F3 24 wks (N = 49)	Post-transplant CPT A 12 wks (N = 34)	Post-transplant CPT A 24 wks (N = 33)	Post-transplant CPT B 12 wks (N = 22)	Post-transplant CPT B 24 wks (N = 22)	Post-transplant CPT C 12 wks (N = 3)	Post-transplant CPT C 24 wks (N = 6)
SVR4	24/28 (86)	11/11 (100)	14/16 (88)	3/6 (50)	29/31 (94)	8/8 (100)	31/32 (97)	11/12 (92)	17/17 (100)	6/6 (100)	2/2 (100)	1/2 (50)

Methods: This open-label study enrolled treatment-naive or experienced adults infected with any HCV genotype (GT) and either advanced cirrhosis or post-liver transplant HCV recurrence. Patients received 12 weeks of DCV 60 mg + SOF 400 mg (each once-daily) and RBV (600 mg/day, adjusted for haemoglobin and creatinine clearance). Cirrhosis patients who had liver transplants during treatment could receive 12 additional weeks of treatment immediately posttransplant. Immunosuppression with cyclosporine, tacrolimus, sirolimus, everolimus, corticosteroids, or mycophenolate mofetil was permitted. The primary endpoint was HCV-RNA <25 IU/ml at post-treatment Week 12 (SVR12) in patients with GT1.
Results: The posttransplant cohort ($N = 53$) was 42% naive, 77% GT1, without cholestatic recurrence or hepatic decompensation. The advanced cirrhosis cohort ($N = 60$) was 40% naive, 75% GT1, 20% Child-Pugh (CP)-A, 53% CP-B, 27% CP-C; MELD score range was 8–27. SVR12 was achieved in 95% of GT1 patients in the posttransplant cohort and 82% of GT1 patients with advanced cirrhosis; 12/13 SVR12 failures were relapses, of which 3 were in the posttransplant cohort. SVR12 rates were comparable irrespective of HCV genotype, prior treatment, or baseline demographics. Four cirrhotic patients with HCC had treatment interrupted for liver transplant; 3 received 12 additional weeks of treatment posttransplant, and all 4 achieved SVR12. There were no treatment-related serious AEs. One posttransplant patient discontinued at Day 31 due to headache and achieved SVR12.
Conclusions: DCV + SOF + RBV for 12 weeks was well tolerated and compatible with multiple immunosuppressive regimens without dose adjustments. SVR12 was achieved by 94% of liver transplant recipients with HCV recurrence.

	SVR12	
% (n/N)	Advanced Cirrhosis	Posttransplant
All patients	83 (50/60)[a,b]	94 (50/53)
GT 1 [95% CI]	**82 (37/45)[c]** [68-92]	**95 (39/41)[c]** [84-99]
GT 2	80 (4/5)	-
GT 3	83 (5/6)[a]	91 (10/11)
GT 4	100 (4/4)[b]	-
GT 6	-	100 (1/1)

[a]Includes 1 patient (GT3) who had SVR12 documented after database lock.
[b]Includes 1 patient who discontinued after 3 weeks for liver transplantation and achieved SVR12 off study.
[c]Primary endpoints.

O58 IMMUNOGENETIC FACTORS AFFECT SURVIVAL IN HCV-INFECTED LIVER TRANSPLANT RECIPIENTS

Renato Romagnoli[1], Francesco Tandoi[1], Paola Magistroni[2], Francesca Bertinetto[2], Ennia Dametto[2], Silvia Martini[3], Dominic Dell'olio[2], Mario Rizzetto[3], Mauro Salizzoni[1], Antonio Amoroso[2]
[1]Liver Transplant Centre - AOU Città della Salute e della Scienza di Torino; [2]Regional Transplant Centre - AOU Città della Salute e della Scienza di Torino; [3]Gastrohepatology Unit - AOU Città della Salute e della Scienza di Torino

Background: In viremic patients (pts) at liver transplantation (LT), HCV recurrence is universal. Host immune response is crucial in determining graft fate.
Methods: We studied HLA phenotypic frequencies in 1228 adult primary LT recipients and in their deceased donors. Genotype of IL28B rs12979860 polymorphism was determined in the 490 HCV + viremic pts and in their donors. Graft survival was considered as the sole endpoint and median follow-up was 10 years (range 5–16).
Results: HLA-DRB1*11 frequency was significantly lower in HCV+ (28%) compared to HCV- recipients (44%; $p < 0.001$) and donors (43%; $p < 0.001$). Other DRB1 antigens were proportionately increased in HCV+ recipients, in particular DRB1*07 ($p = 0.003$). IL28B C/C was rarer in HCV+ (27%) than in HCV- recipients (45%; $p < 0.001$) and HCV+ donors (49%, $p = 0.004$). In HCV+ recipients 10-year graft survival was lower than in HCV- ($p < 0.001$), but was better by 17% ($p = 0.018$) in HLA-DRB1*11 pts and by 13% ($p = 0.044$) in C/C pts. Concurrent absence of DRB1*11 and C/C in 228 HCV+ recipients constituted a significant risk for graft survival ($p = 0.0006$), evident since week 8 post-LT. These "at risk" HCV+ recipients presented at LT significantly higher plasma HCVRNA levels, greater prevalence of viral genotype 1 and positive crossmatch; post-LT higher HCV viremia at the third month, more common CMV infections and rejection episodes, while age, sex, MELD at LT and donor age were not different. Multivariate analysis showed that independent factors affecting graft survival in HCV+ were: MELD ≥25 (Hazard Ratio = 1.95), donor age ≥70 year (HR = 1.82), absence of both DRB1*11 and C/C (HR = 1.67), HCVRNA level at LT $>1 \times 10^6$ IU/ml (HR = 1.43).
Conclusions: A combination of genetic markers identifies a large population of HCV+ LT recipients who are at increased risk of graft loss from the very first weeks after LT. In this selected population every therapeutic effort should be made in the pre and peritransplant period to prevent HCV graft infection.

O59 SOFOSBUVIR PLUS RIBAVIRIN IN PATIENTS WITH SEVERE HCV RECURRENCE AFTER LIVER TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

Silvia Martini[1], Daniele Arese[1], Marco Sacco[1], Silvia Strona[1], Donatella Cocchis[2], Stefano Mirabella[2], Giorgia Rizza[2], Renato Romagnoli[2], Francesco Tandoi[2], Antonio Ottobrelli[1], Cerenzia Maria Rosaria Torrani[1], Federico Balzola[1], Mauro Salizzoni[2], Mario Rizzetto[1]
[1]Gastrohepatology Unit, AOU Città della Salute e della Scienza di Torino; [2]Liver Transplant Centre, AOU Città della Salute e della Scienza di Torino

Background: Recurrent HCV infection is almost universal in viremic recipients at liver transplantation (LT). We evaluated safety and efficacy of antiviral therapy (Tx) with Sofosbuvir (SOF) plus Ribavirin (RBV) in HCV patients (pts) with severe recurrent hepatitis after LT.
Methods: Among pts transplanted in our Centre since 2000, we enrolled 116 pts affected by HCV recurrent hepatitis with fibrosis score F3 (26 pts) or F4 (90 pts) according to Metavir. Mean age 61 years, mean BMI 24.8; 42% receiving cyclosporine, 37% tacrolimus, 44% mycophenolate. GT1 73%, GT1a 13%, GT2 5%, GT3 17%, GT4 5%; IL28B CC 23%; 69% experienced to previous antiviral Tx. According to compassionate use, pts received SOF 400 mg/die and RBV (weight-based) for 24 weeks (w). Median time since LT-Tx was 4.51 years (0.27–14.47). Mean baseline HCVRNA 6.62 log IU/ml (3.4–7.9 log IU/ml). Mean GFR 76.3 ml/min.
Results: After 1 w of Tx, median decrease in HCVRNA was 3.42 log IU/ml. By w4, 8 and 12 of Tx, HCVRNA was negative in 61/116 pts (53%), 113/116 (97%) and 113/113 (100%), respectively. To date, all pts have maintained undetected HCVRNA on Tx (96/96 pts at w20 and 66/66 at w24). Renal function, MELD and Child score were stable on Tx. Six out of 32 (19%) pts who reached 4 w of follow-up post Tx relapsed. All the relapsers are cirrhotic (Child A 2 pts and B 4 pts), GT1 (5 pts) and GT4 (1 pt), 50% IL28B CC and 50% HCVRNA positive at w4 on Tx. The most common adverse events were fatigue (31%), diarrhea (27%) and headache (26%). 24% pts received blood transfusions or epoetin and 15% needed RBV reduction. On Tx, 7 pts were hospitalized due to liver decompensation and 2 cirrhotics died of end-stage liver disease. Minimal immunosuppression dose adjustments were required on Tx and no rejections were recorded.
Conclusion: In pts with severe HCV recurrence post LT, all oral antiviral regimen with SOF plus RBV is well tolerated and easy to manage, allowing excellent on treatment virological response.

O60 SLOW ACHIEVEMENT OF HCV-RNA UNDETECTABILITY IN CIRRHOTIC PATIENTS TREATED WITH SOFOSBUVIR + RIBAVIRIN: POSSIBLE CLINICAL IMPLICATIONS IN THE LIVER TRANSPLANT LIST MANAGEMENT

Daniele Sforza[1], Ilaria Lenci[2], Matteo Manuelli[1], Valeria Cento[3], Maria Rendina[4], Francesca Donato Maria[5], Milana Martina[2], Aragri Marianna[3], Chiara Di Maio Velia[3], Abedrabbo Amal[2], Malinverno Federico[5], Monico Sara[5], Laura Ponti Maria[6], Canu Roberta[6], Ganga Roberto[6], Luciano Milanesi[7], Alfredo Di Leo[8], Carlo Federico Perno[9], Francesca Ceccherini-Silberstein[3], Massimo Colombo[5], Mario Angelico[2], Giuseppe Tisone[1]
[1]UOC Chirurgia dei Trapianti Policlinico Tor Vergata; [2]Hepatology Unit, Policlinico Tor Vergata, Tor Vergata University, Rome; [3]Virology Chair, Tor Vergata University, Rome; [4]Gastroenterology and Digestive Endoscopy, University Hospital, Policlinico Bari; [5]Gastroenterology and Liver Transplant Units, Cà Granda Maggiore Hospital Policlinico and University of Milan, Milan, Italy; [6]Gastroenterology and Hepatology Units, Brotzu Hospital, Cagliari, Italy; [7]Institute for Biomedical Technologies-CNR, Segrate, (Milano); [8]Gastroenterology and Digestive Endoscopy, University Hospital, Policlinico Bari.; [9]Virology Unit, Policlinico Tor Vergata, Tor Vergata University, Rome

Sofosbuvir (SOF) treatment, ±ribavirin (RBV) prior to LT has the potential to change the HCV recurrence after Liver Transplantation (LT). This approach has been reported to avoid graft reinfection in compensated patients with hepatocellular carcinoma (HCC), but only among those who reached and maintained undetectable HCV-RNA (TND) before LT. Since the time to obtain this result in decompensated cirrhotics is unknown, we sought to investigate the early HCV-RNA decay in this setting. Sixteen decompensated patients (M/F 12/4, median age 55.3, CPT score >B7), infected by HCV genotype 1a, 1b, 3 and 4 (2-8-4-2), 4 of whom with HCC, were treated with SOF 400 mg/day and RBV (200–1000 mg/day), except 2 who received SOF alone, for a median (IQR) of 12 (11–16) weeks awaiting LT. HCV-RNA levels were measured weekly and safety and clinical parameters were analyzed. No serious adverse events were reported. The median (IQR) RBV dose was 600 (400–800). Despite 11/16 patients had low baseline viremia (<600 000 IU/ml), HCV-RNA decay in the first 4 weeks of treatment was suboptimal (median[IQR] = −3.7 [−4.3; −3.3] LogIU/ml). Only 3/16 (18.7%) patients reached TND HCV-RNA at week 4 (rapid virological response, RVR), and 5/10 (50%) evaluable patients were still viremic at week-12. Median (IQR) MELD decreased from 15 (13–16)

at baseline to 13 (12–15) at week 4, when 14/16 (87.5%) patients returned in a compensated stage. One patient underwent LT after 6 weeks of treatment, while HCV-RNA was still positive (26 UI/ml). SOF was interrupted only during the first 4 perioperative days, obtaining TND HCV-RNA at week 10 of therapy. The kinetics of HCV-RNA decay in decompensated cirrhotic patients are slower compared to those obtained in non cirrhotic patients. As a practical implication, pre-LT treatment may need to be longer and/or based on more effective antiviral strategies. Alternatively, MELD-based prioritization to LT should be reassessed in light of the results of antiviral therapy.

013 IMMUNOBIOLOGY/BASIC SCIENCE

O61 RATIONAL DEVELOPMENT OF ALLOANTIGEN SPECIFIC REGULATORY T CELL THERAPY REQUIRES INSIGHT INTO LONGEVITY OF ALLOIMMUNE PATHWAYS

Jason Ali, Margaret Negus, Eleanor Bolton, Andrew Bradley, Gavin Pettigrew
University of Cambridge

Introduction: We have demonstrated indirect-pathway responses against alloantigens differ in strength and longevity; specifically, responses against MHC class I alloantigen are longlived whilst those against class II alloantigen are shortlived due to rapid clearance of donor APCs. Here we demonstrate how this knowledge may inform regulatory T cell (Treg) immunotherapy
Methods: An MHC-mismatched murine model of cardiac transplantation was used [bm12.Kd.IE to C57BL\6]. Polyclonal and antigen specific Treg were generated in vitro, utilising syngeneic C57BL/6 or T cell receptor transgenic CD4 T cells specific for self-restricted donor class I (TCR75) and class II (TEa) allopeptide. To limit donor class I expression to haematopoietic cells, bone marrow chimeric donors were incorporated (bm12.Kd.IE bone marrow to irradiated bm12.IE). Treg were administered at the time or 3 weeks after transplant
Results: When given on the day of transplant, although polyclonal Treg attenuated germinal centre allo and autoantibody responses and reduced vasculopathy, monoclonal populations of class I and II allopeptide-specific indirect pathway Treg were more effective. Moreover, when transferred late (3 weeks), only class I indirect Treg proved effective at ameliorating chronic rejection, presumably because presentation of alloantigen is limited at this stage to self-restricted MHC class I alloantigen. In support, class I specific Treg were ineffective at preventing progression of vasculopathy when administered late to recipients of heart allografts from bone marrow chimeric donors, in which indirect-pathway responses against MHC class I alloantigen were truncated due to restricted expression of the alloantigen on short-lived haematopoietic cells
Conclusion: Antigen specific Treg are more effective than polyclonal Treg at abrogating alloimmune responses. Their effectiveness when administered at late time points after transplantation is dependent upon ongoing presentation of target allopeptide

O62 REGULATORY T CELLS SUPPRESS NK CELLS TO INDUCE MIXED CHIMERISM

Benedikt Mahr[1], Nina Pilat[2], Karin Hock[1], Christoph Schwarz[1], Lukas Unger[1], Andreas M. Farkas[1]
[1]Medical University of Vienna/Section of Transplantation Immunology;
[2]Medical University of Vienna

Introduction: The adoptive transfer of in vitro activated regulatory T cells (Tregs) obviates the need for cytoreductive conditioning in a costimulation blockade based (C57BL/6 to Balb/c) bone marrow transplantation (BMT) model and induces long lasting tolerance to skin and heart allografts. We hypothesized that Tregs would enable BM engraftment by suppressing NK cells. To test our hypothesis we replaced Treg therapy by NK cell depletion or the use of F1 mice as BM donors (as recipient NK cells are not activated in this setting).
Methods: C57BL/6 mice received 20×10^6 unseparated Balb/c or CB6F1 (offspring of a cross between Balb/c females and C57BL/6 males) BM cells under costimulation blockade (anti-CD154mAb, CTLA4-Ig) and a short course of rapamycin. Mice transplanted with Balb/c bone marrow were additionally treated with NK cell depleting antibodies (anit-NK1.1) or co-injected with 1×10^6 in vitro activated Tregs. All BMT recipients received skin grafts and selected recipients also cardiac grafts.
Results: NK cell depletion at the time of BMT could be substituted for Treg therapy as all mice developed persistent mixed chimerism (7/7). Nevertheless, more than half of NK cell depleted recipients chronically rejected their skin grafts (4/7). In contrast, mice grafted with CB6F1 bone marrow developed stable mixed chimerism and retained their allografts indefinitely without histological signs of rejection (5/5), presumably due to the unresponsiveness of recipient NK cells to alloantigens. In line with this, the absence of chronic rejection in Treg treated mice was associated with the adaption of recipient NK cell receptors to donor antigens manifested by the modulation of their activating and inhibitory receptors.
Conclusion: Therefore we conclude that adoptively transferred Tregs prevent bone marrow rejection primarily by suppressing NK cell reactivity.

O63 ALLOGENEIC MATURE HUMAN MONOCYTE-DERIVED DENDRITIC CELLS GENERATE SUPERIOR ALLOREACTIVE REGULATORY T CELLS IN AN IL-2 INDEPENDENT MANNER

Nicolle Litjens[1], Karin Boer[1], Joke Zuijderwijk[1], Mariska Klepper[1], Annemiek Peeters[1], Errol Prens[2], Wenda Verschoor[1], Rens Kraaijeveld[1], Zeliha Ozgur[3], Vroonhoven Mirjam Van Den Hout-Van[3], Wilfred Van Ijcken[3], Carla Baan[1], Michiel Betjes[1]
[1]Erasmus Medical Center, Internal Medicine, Nephrology and Transplantation; [2]Erasmus Medical Center, Dermatology and Rheumatology; [3]Erasmus Medical Center, Erasmus Center for Biomics

Background: Alloantigen (alloAg)-specific expanded natural occurring regulatory T cells (nTregs) are an attractive source for targeted cellular immunotherapy to prevent or treat allograft rejection. However, optimal expansion protocols are needed to obtain the large number of stable, highly efficient alloAg-specific nTregs necessary for clinical use. In this study, the potential of different allogeneic stimuli to induce and expand functional alloAg-specific nTregs was studied.
Material/Methods: A highly enriched nTreg-fraction was alloAg-specific expanded using HLA-mismatched immature, mature monocyte-derived (mo) DC or PBMC. The different expanded nTregs were fully characterized by analysis of the demethylation status within the TSDR of *FOXP3* and the expression of FOXP3, HELIOS, CTLA4 and cytokines. In addition, the Ag-specific suppressive capacity was tested.
Results: Allogeneic mature moDC were superior in inducing nTreg-expansion compared to immature moDC or PBMC. Remarkably, the presence of exogenous IL-15, but not IL-2, was needed for optimal mature moDC-induced nTreg-expansion. Even at low ratios, allogeneic mature moDC- and to a lesser extent PBMC- but not immature moDC-expanded nTregs were potent suppressors of alloAg-induced proliferation. Alloreactive nTregs were highly demethylated at the TSDR within *FOXP3* and highly expressed FOXP3, HELIOS and CTLA4. In addition, hardly any expanded nTregs produced IL-17 whereas a minority of nTregs produced IL-10, IL-2, IFN-γ and TNF-α. Next generation sequencing of mRNA of moDC-expanded nTregs revealed a strong induction of Treg-associated mRNAs.
Conclusion: Human allogeneic mature moDC are highly efficient, IL-2-independent, stimulator cells for expansion of stable alloAg-specific nTregs with superior suppressive function. This opens a new avenue for using Tregs as source for cellular immunotherapy in kidney transplantation.

O64 MESENCHYMAL STEM CELL (MSC) MICROVESICLES (MV) INDUCE T REGULATORY (TREG) CELL DIFFERENTIATION TROUGH RNA TRANSFERT: ROLE IN T-CELL MEDIATED REJECTION

Sergio Dellepiane, Vincenzo Cantaluppi, Michela De Lena, Federica Civiletti, Caterina Dolla, Alessando D. Quercia, Antonio Amoroso, Giovanni Camussi, Luigi Biancone
University of Torino

Introduction: MSC attenuates renal injury trough release of several paracrine factors. MV are cell fragments shuttling cell specific protein, lipids and RNA. In this study we investigate the immunomodulatory role of MSC derived MV, focusing on TCMR related injury and Treg differentiation process.
Methods: MV were isolated from supernatants of cultured MSC and subsequently characterized (for RNA and proteic content). MV were then added to different culture experiments: 1) co-culture of deceased donor splenocytes and recipient T lymphocytes 2) renal tubular epithelial cells (TEC) cultured with TCMR related stimuli (perforin, granzyme B, Fas-ligand, TNF-alpha, IFN-gamma).
Results: MSC-MV have size of 60–150 nm and express several type of proteins involved in cell contact (i.e integrins). MV contain several mRNA and miRNA involved in immunomodulation (i.e. Foxp3, TIM-1, thrombospondin). MV are incorporated in T cells and reduce phytohemagglutinin/ionomycin induced proliferation as proliferation induced by donor splenocytes. MV transfer Foxp3 mRNA in activated T-lymphocytes and induce Treg differentiation. Moreover MV are internalized in TEC, reduce apoptosis and functional changes induced by TCMR related stimuli and preserved expression of TEC specific solute carriers. All the effect described are abrogated after treating MV with RNA-ase.
Conclusion: MSC derived MV inhibit TCMR renal injury trough several mechanisms including: preserving TEC, blocking T cell activation, inducing Treg differentiation.

O65 METABOLIC INTERFERENCES CAN EFFICIENTLY CONTROL PATHOGENIC CD8 T CELLS FROM KIDNEY TRANSPLANT RECIPIENTS

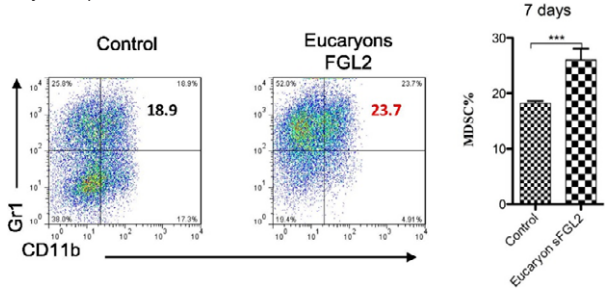
Gaëlle Tilly[1], Michelle Yap[1], Magali Giral[2], Sophie Brouard[1], Nicolas Degauque[1]
[1]INSERM UMR1064; [2]CHU de Nantes

Background: Naïve and memory CD8 T cells have been shown to exhibit different metabolic profiles, especially upon antigen stimulation. However, it is unknown whether prolong stimulation of the immune system such as allogeneic transplantation modifies the metabolic profiles of CD8 T cell subsets. In this report, we aimed to characterize the immune-metabolic profiles of CD8 T cells in kidney transplant recipients.
Method: ATP level, mitochondrial load, expression of pro-inflammatory cytokines (IL-2, TNF-a and IFN-g) and activation markers (CD25 and CD98) were monitored in purified CD8 subsets (naïve; effector memory, EM; TEMRA) from healthy volunteers (HV) and patients with stable kidney graft (TX) before and after polyclonal stimulation (aCD3 aCD28 mAb). 2-DG, oligomycin, or DON were used to inhibit glycolysis, mitochondrial respiration, and glutaminolysis respectively.
Results: Antigen-experienced CD8 (EM and TEMRA) from TX as compared to HV exhibit an activated metabolic profile with an increase of polarized mitochondria load, a higher basal use of ATP and an enhanced ability to reconstitute the ATP pool upon stimulation. This profile was associated with a high expression of CD98 upon stimulation. Inhibition of glycolysis or glutaminolysis prevents the reconstitution of ATP pool and efficiently blunts the pro-inflammatory cytokines secretion and the upregulation of activation markers. In contrast, the inhibition of mitochondrial respiration efficiently controls the effector function of antigen-experienced CD8 but does not prevent the reconstitution of the ATP pool.
Conclusion: The characterization of immune-metabolic profiles of pathogenic CD8 T cells fosters the design innovative therapies to improve the kidney graft survival.

O66 THE SOLUBLE FGL2 INDUCED MYELOID-DERIVED SUPPRESSION CELLS AND THEIR EXPRESSIONS IN KIDNEY TRANSPLANT RECIPIENTS

Ming Xu[1], Cheng Yang[2], Lingyan Wang[2]
[1]Zhongshan Hospital Fudan University; [2]Zhongshan Hospital, Fudan University

Background: Regulatory T cell (Treg) and myeloid-derived suppressor (MDSC) protect allografts against acute rejection (AR), while the full mechanisms have not been clarified yet. Soluble fibrinogen-like protein 2 (sFGL2), as a regulatory cytokine of Treg, plays an important role in Treg-mediated immunosuppression. In this study, we investigated the expressions of sFGL2 and MDSC in kidney transplant recipients, and the induction of MDSC in vitro by sFGL2.
Materials and Methods: MDSC was analyzed by flow cytometry, and sFGL2 was detected by ELISA and immunostaining in kidney recipients with stable renal function or biopsy-proved AR. Bone marrow (BM) cells were isolated from C57BL/6 mice and incubated with 10 ng/ml GM-CSF for MDSC induction with or without 10 μg/ml sFGL2. After 3 and 7 days incubation, the percentage of MDSC was analyzed by flow cytometry.
Results: sFGL2 significantly increased in the peripheral blood while decreased in the renal allograft in situ in renal transplant recipients with AR. The percentage of MDSC in the peripheral blood was significantly higher in the stable patients than that in the patients with AR. After 7 days incubation, sFGL2 significantly promoted the differentiation of MDSC from BM cells.
Conclusion: sFGL2 promoted the induction of MDSC. sFGL2 and MDSC were increased in kidney transplant recipients with stable renal function compared with AR. sFGL2 might be a novel target for diagnosis and therapy in kidney transplant AR.



O67 ALTERED CO-INHIBITORY MOLECULE EXPRESSION AND STAT SIGNALING OF MEMORY T CELLS IN KIDNEY TRANSPLANT PATIENTS

Carla Baan, Anne Bouvy, Mariska Klepper, Marcia Kho, Jan Ijzermans, Michiel Betjes, Willem Weimar
Erasmus MC

Background: T-cell depletion therapy is associated with diminished IL7/IL15 dependent homeostatic proliferation, resulting in incomplete T-cell repopulation and with T cell dysfunction. We hypothesized that this is the result of impaired cytokine responsiveness of T cells, through affected STAT5 phosphorylation and upregulation of co-inhibitory molecules.
Material and Methods: Patients were treated with T-cell depleting rATG (6 mg/kg, $n = 17$), or non-depleting, anti-CD25 antibody (basiliximab 2×40 mg, $n = 25$) induction therapy, in combination with tacrolimus, MMF and steroids. Before and the first year after transplantation, IL7 and IL2 induced STAT5 phosphorylation and the expression of co-inhibitory molecules PD-1, TIM-3, LAG-3, CTLA4, CD160 and CD244 was measured by flow cytometry.
Results: The first year after rATG, CD4+ and CD8+ T cells were affected in their IL7 dependent phosphorylation of STAT5 which was most outspoken in the CD8+ memory population. The capacity of CD4+ and CD8+ T cells to pSTAT5 in response to IL2 decreased after both rATG and basiliximab therapy. After kidney transplantation, the percentage of TIM-3+, PD-1+ and CD160+CD4+ T cells and CD160+ and CD244+CD8+ T cells increased, with no differences in expression between rATG and basiliximab treated patients. The decrease in pSTAT5 capacity CD8+ T cells and the increase in co-inhibitory molecules were correlated rATG: $r_s = -0.94$, $p < 0.05$ and basiliximab $r_s = -0.78$, $p < 0.05$).
Conclusion: We demonstrate that memory T cells in kidney transplant patients, in particular after rATG treatment, have decreased cytokine responsiveness by impaired phosphorylation of STAT5 and have increased expression of co-inhibitory molecules, processes which were correlated in CD8+ T cells.

O68 PERIPHERAL BLOOD DERIVED VIRUS-SPECIFIC MEMORY STEM T CELLS MATURE TO FUNCTIONAL EFFECTOR MEMORY SUBSETS WITH SELF-RENEWAL POTENCY

Michael Schmueck-Henneresse, Petra Reinke, Hans-Dieter Volk
Charite University

Memory T cells expressing stem cell-like properties (TSCM) have been described recently. The capacity of self-renewal and differentiation into various memory/effector subsets make them attractive for adoptive T cell therapy to combat severe virus infections and tumors. The very few reports on human TSCM are restricted to analyses on polyclonal T cells but extensive data on antigen-specific TSCM are missing. This might be due to their very low frequency limiting their enrichment and characterization. Here, we provide functional and phenotypic data on human virus-specific TSCM, defined as CD8+CD45RA+CCR7+CD127+CD95+. Whereas <1% of total T cells express the TSCM phenotype, e.g. human cytomegalovirus (HCMV)-specific TSCM can be detected at frequencies similar to those seen in other subsets, resulting in about 1/10 000 HCMV-specific TSCM. A new virus-specific expansion protocol of sort-purified TSCM reveals the up-regulation of various T cell subset markers and a significant proportion that preserves their stem cell phenotype indicating self-renewal and differentiation potency. Furthermore, we describe a simplified culture protocol that allows fast expansion of virus-specific TSCM starting from a mixed naïve T/TSCM pool of peripheral blood lymphocytes. Due to the clinical grade compatibility, this might be the basis for novel cell therapeutic options in life threatening courses of viral disease.

O69 CYTOTOXIC CD8 T CELL RECOGNITION OF ACQUIRED, INTACT MHC ALLOANTIGEN ON HOST DENDRITIC CELLS PROMOTES ACUTE ALLOGRAFT REJECTION

Simon Harper, Jason Ali, Ines Harper, Manu Chhabra, Eleanor Bolton, Andrew Bradley, Gavin Pettigrew
University of Cambridge

Introduction: Although there is evidence that recipient DCs can acquire intact MHC class I from parenchymal cells the functional relevance of 'semi-direct' presentation to CD8 T cells this permits to allograft rejection has not been demonstrated
Methods: Murine cardiac transplant models were utilised. Balb/c donors were lethally irradiated to eradicate haematopoietic cells (HPC) (Balb/c^{HPC-}) such that parenchymal cells were the only alloantigen source. Recipients included: 1) 2C transgenic mice (monoclonal CD8 T cells against Ld MHC class I) 2) Splenectomised aly/aly (aly/aly^{spl}) mice to investigate the importance of secondary lymphoid tissue (SLT) 3) CD11c-DTR transgenic mice in which host DCs can be depleted by diphtheria toxin

Results: 2C transgenic mice rejected Balb/c^{HPC-} grafts as rapidly as non-irradiated grafts suggesting a mechanism for parenchymal cell driven CD8 mediated rejection. Balb/c^{HPC-} allografts showed prolonged survival (>50 day) in aly/aly^{spl} mice given 2C CD8 T cells whereas in non-splenectomised controls all grafts rejected (MST = 17d; $p = 0.01$). In addition, when aly/aly^{spl} mice were given activated 2C CD8 T cells (from a 2C recipient of a Balb/c cardiac graft) they rapidly rejected Balb/c^{HPC-} allografts (MST = 7 d; $p = 0.01$) suggesting an essential role for SLT in this pathway. Wildtype recipient CD8 T alloreactivity acutely rejected Balb/c^{HPC-} allografts in CD11c-DTR recipients lacking B cells with rejection significantly attenuated if either CD4 T cells, or DCs were additionally depleted (MST = 12 vs >50 and 26 respectively; $p < 0.01$), highlighting an essential role for both recipient DCs and CD4 T cells in driving CD8 alloreactivity

Conclusion: These results provide support for the semi-direct pathway of allorecognition having the potential to contribute to allograft rejection. Recipient DCs can acquire intact MHC class I from donor parenchymal cells and upon transit to SLT receive indirect CD4 T cell help to activate directly alloreactive CD8 T cells.

003 CLINICAL IMMUNOSUPPRESSION & BIOLOGICALS

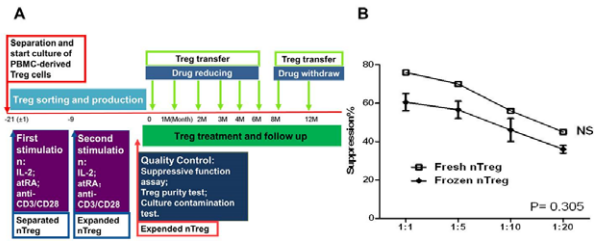
O70 THE PRELIMINARY DATA OF OPERATIONAL TOLERANCE BY TRANSFER EXPENDED RECIPENTS NATURE TREGS FOLLOWING LIVING DONOR LIVER TRANSPLANTATION

Jian Gu, Yunjie Lu, Kunpeng Wang, Jie Zhao, Xuehao Wang, Ling Lu
Nanjing Medical University

Purpose: We examined the efficacy and safety of the cell therapy using expended autologous nature regulatory T cells (nTregs) in living donor liver transplantation (LDLT).
Methods: Ten LDLT adult recipients who are stable in immune state were enrolled. CD4+CD25+CD127- Tregs were selected ex-vivo from liver recipient's PBMCs by MACS. Cells were stimulated and expanded with IL-2, Rapamycin and anti-CD3/CD28 beads under GMP facility and cultured for 3 weeks. Regulatory T cells were injected every month for the first 4 month and every two month for the next 4 month. During the injection, immunosuppression (IS) including Cyclophosphamide and FK506 was reduced step by step and finally terminated. IS reduction was determined by graft liver function tests, biopsy and immunological assessments. Ten patients were enrolled and have a good condition following LDLT.
Results: After cell expansion, more than 70% cells expressed Foxp3. Treg expanded for 200 fold which showed strong capacity proliferation with good suppression in vitro (Figure 1). Respectively, infusion of Tregs increased circulating Tregs in the periphery, while the cell-infusion did not cause any adverse events. Patients were tranfered with Treg, one have IS withdrawal without causing allograft rejection, and their graft function is well maintained.
Conclusion: Cell therapy by ex-vivo expanded autologus Tregs allows withdrawal and complete cessation of IS in LDLT patients. Nature regulatory T cells are effective for inducing operational tolerance in LDLT.

Figure Legend: Precultured nTreg were thawed and restimulated for 9–12 days in vitro. (A) Drug withdrew protocol for nTreg therapy. (B) The mean ± SEM percent suppression of cells expanded from nTreg before and after frozen.

Figure 1



O71 LOWER FREQUENCY OF REGULATORY T-CELLS AFTER CONVERSION FROM CNI OR MTORI TO BELATACEPT IN A PROSPECTIVE MATCHED CONTROLLED STUDY IN RENAL ALLOGRAFT RECIPIENTS

Michael Duerr, Juan Liu, Katharina Fabritius, Klemens Budde
Charité Universitätsmedizin Berlin

Regulatory T-cells (Tregs) are considered to play a crucial role in maintaining control of immune response following renal transplantation (Tx). So far, only limited data are available from prospective controlled trials on the time course and frequency of Tregs after conversion from either Calcineurin inhibitors (CNIs) or mammalian target of Rapamycin inhibitors (mTORi) to Belatacept, a co-stimulatory blocker of CD80/86. The aim of this study was to evaluate the influence of belatacept on T-cell subsets with focus on Tregs after withdrawal from CNI or mTORi.

20 renal transplant patients (pts) on either CNIs (n = 10) or mTORi (n = 10), who had intolerability of their maintenance regimen participated in a prospective controlled 12-month trial. Conversion from CNI or mTORi to belatacept was performed in a stepwise manner over a 4-week period. Characterization of T-cell subsets and Treg phenotype was performed at baseline, month (Mo) 1, 3 and 6, respectively. Peripheral blood mononuclear cells were analyzed with multi-color flow cytometry using antibodies against CD3/8/4/25 and CD127. Additionally, we isolated CD4CD25Tregs and co-cultured them with CD4 effector cells (Teffs) to evaluate their suppressive capacity.

Actually, 15 pts passed interim analysis 6 Mo after conversion to belatacept. At 3 Mo, frequency of CD4+CD25+CD127low Treg decreased in CNI and mTORi withdrawal groups. In addition, Tregs dropped to their minimum 6 Mo after conversion (CNI 2.9%; p = 0.003; mTORi 4%; p = 0.068, <IMAGE01>). Despite reduced Treg numbers after conversion to belatacept, the suppressive function of Tregs was not impaired when Teffs are co-cultured with Tregs 1:1 (CNI-group: BL = 33%, Mo3 = 31%, Mo6 = 20%; p = 0.11)(mTORi-group: BL = 27%, Mo3 = 24%, Mo6 = 19%; p = 0.4).

The present study demonstrated a decrease of Treg frequency after conversion from either CNI or mTORi to belatacept. Despite lower Treg frequencies, suppressive Treg function was not restricted after switch to belatacept maintenance therapy.

O72 ALEMTUZUMAB INDUCTION IN RENAL TRANSPLANT RECIPIENTS IS ASSOCIATED WITH LONG-TERM CHANGES IN IMMUNE PHENOTYPE, RESEMBLING THAT SEEN IN RTR DISPLAYING OPERATIONAL TOLERANCE: A PILOT STUDY

Matthew Bottomley[1], Paul Harden[2], Kathryn Wood[1]
[1]University of Oxford; [2]Oxford Transplant Centre

Introduction: Use of alemtuzumab (Campath), a pan-leucocyte depleting agent, at induction in renal transplant recipients (RTR) has been previously shown to result in alterations in the B cell and T cell compartment in the first two years post-transplant. Little is known of the long term effects of Campath in RTR. We hypothesised these changes would persist in long-term RTR and may be associated with previously described markers of operational tolerance.
Methods: We recruited 11 RTR who received Campath induction at transplant over nine years ago, and matched them for age, sex and duration of immunosuppression to 9 RTR who received basiliximab or no induction. Flow cytometry was performed on freshly isolated peripheral blood mononuclear cells. Quantitative RT-PCR was undertaken on RNA extracted from whole blood.
Results: RTR who received Campath had a marked increase in circulating $CD19^+$ B cell numbers, with an underrepresentation of $CD3^+$ T cells amongst lymphocytes. This was driven by an increase in naïve and transitional B cell numbers. Despite this, both number and percentage of $CD4^+CD25^+CD127^{lo}FoxP3^+$ (Treg) cells were increased in the Campath cohort. We examined these cohorts for the previously described multiplatform (RISET) and three-gene (ITN) tolerance signatures. RTR who received Campath induction had significantly increased expression of both reliably-amplifying ITN genes, and significantly greater CD19:CD3 ratio and reduced proportion of $CD4^+CD25^{intermediate}$ T cells, as described in the RISET signature. RTR receiving Campath had a trend towards an increased FoxP3/AMann ratio and a general trend in the RISET gene panel towards the tolerance signature phenotype in RTR who received Campath, with four of ten genes reaching significance.
Conclusion: Campath induction leads to long term changes in the immune phenotype that are consistent with signatures previously described in operationally tolerant RTR.

O73 IMMUNE MONITORING BY SINGLE CELL PHOSPHO SPECIFIC FLOWCYTOMETRY TO DEFINE THE FATE OF MONOCYTES IN KIDNEY TRANSPLANT PATIENTS

Nynke Kannegieter, Dennis Hesselink, Rens Kraaijeveld, Gretchen De Graav, Michiel Betjes, Willem Weimar, Carla Baan
Erasmus MC

Monocytes have been identified as key players driving rejection processes. Surprisingly, little is known about the effects of immunosuppressive drugs on activation cascades of monocytes during these responses. Here single-cell phospho-specific flow cytometry was used to explore the effects of immunosuppression on signaling pathways in monocytes of kidney transplant patients.

We measured the phosphorylation of the various signaling pathways NFκB, MAPK and mTOR in peripheral blood monocytes of kidney transplant patients (n = 13) in the first month after transplantation. Both in vivo phosphorylation levels and phosphorylation capacity after PMA/ionomycin stimulation were determined in CD14+ monocytes. Patients received maintenance therapy of tacrolimus, mycophenolate mofetil and prednisone in combination with basiliximab induction therapy.

Before transplantation in vivo phosphorylation levels of p38MAPK, ERK and AKT, but not of NFκB, are highly expressed by monocytes (MFI: 1917, 1791, 1102 and 958 respectively) compared to isotype controls (MFI: 653, 670, 647 and 905 respectively; $p < 0.001$ for p38MAPK, ERK and AKT). After transplantation lower levels of these phosphorylated signaling molecules were measured ($p < 0.05$ for p38MAPK, ERK and AKT). This inhibition was 51%, 42% and 44% respectively and has a negative correlation with tacrolimus predose levels ($p < 0.01$, $p < 0.05$ and $p < 0.01$ respectively). In the stimulated samples, phosphorylation levels of p38MAPK and AKT, but not of ERK, inversely correlated with tacrolimus predose concentrations (p = 0.03 and p = 0.004, respectively). No correlation was found between phosphorylated p38MAPK, ERK and AKT levels and kidney function, i.e. serum creatinine or eGFR levels.

Before kidney transplantation, monocytes of uremic patients are activated. The decreased phosphorylation levels of p38MAPK, ERK and AKT after transplantation demonstrate that currently prescribed immunosuppressive drugs also inhibit early monocyte activation.

O74 CD86-EXPRESSION ON MONOCYTES AS A TOOL FOR THERAPEUTIC DRUG MONITORING OF BELATACEPT

Gretchen De Graav[1], Dennis Hesselink[2], Wenda Verschoor[2], Marjolein Dieterich[2], Teun Van Gelder[2], Willem Weimar[2], Carla Baan[2]
[1]Erasmus MC; [2]Erasmus Medical Center

Belatacept blocks CD28-mediated T cell activation by binding CD86 on antigen presenting cells. No therapeutic drug monitoring of serum levels is recommended for belatacept, because of low inter-patient variability in pharmacokinetic parameters. We questioned whether the CD86 competition flow cytometry assay is useful as a tool for pharmacodynamic monitoring of belatacept. We hypothesized the degree of blockade of CD86 by belatacept might be associated with acute rejection after kidney transplantation. CD86-expression was assessed on monocytes of patients treated with tacrolimus or the Less-Intensive regimen of belatacept, in the stable situation, during rejection and after conversion of belatacept to tacrolimus. Before transplantation, flow cytometric analysis of whole blood samples showed that CD86 was expressed on monocytes: median 2029 molecules/cell [1179–4102]. After one dose of belatacept the numbers of free CD86-molecules per monocyte dropped by >85% in all patients ($n = 17$), $p = 0.0003$. Also in tacrolimus-treated patients ($n = 19$) the expression levels of this co-stimulatory molecule decreased by 33% ($p = 0.003$), but significantly less than in belatacept treated patients (p85% by belatacept. 5 months after conversion to tacrolimus, the expression of CD86-molecules returned to the same levels as those found in tacrolimus-treated patients. In short, the degree of belatacept-mediated blockade of peripheral CD86 expression is not different between rejecters and non-rejecters. Despite CD86-blockade, patients can still develop acute rejection, possibly due to insufficient inhibition of the CD28-CD80/86 pathway in lymph nodes and graft and/or redundant co-stimulatory pathways. Flow cytometric assessment of free CD86 molecules on monocytes in peripheral blood does not seem to be a promising tool to monitor belatacept.

O75 CD4+CD57+ CELLS ARE ASSOCIATED WITH AN INCREASE RISK REJECTION IN HUMAN RENAL TRANSPLANT: CARACTERISATION OF THEIR PHENOTPE AND BEHAVIOR IN ALLOGENIC CONDITIONS

Houria Chavez[1], Antoine Durrbach[2], Helene Francois[1], Florence Herr[3], Yassine Taoufik[1], Jaclyn Espinosa[4], Severine Beaudreuil[1], Bernard Charpentier[1], Allan Kirk[4]
[1]APHP, INSERM; [2]APHP; [3]INSERM; [4]Duke University

Belatacept treatment of non immunized patients is associated with the same rate of graft loss or death than cyclosporine A (CsA), but improved renal function and cardiovascular risk factors. However, an increased risk of acute cellular rejection was observed. In this study, we analyzed predictor risk factors of acute rejection present in patients treated with belatacept (BELA) at the day of the transplantation and we investigated function of BELA-treated memory CD4 T cells expressing senescence markers CD57. We determined the lymphocyte phenotype of 38 patients at day (D) 0, 15, 30, 90 and 180 post renal transplantation. Renal biopsy was performed systematically at D365 and when a 20% increase of serum creatinine was observed. Rejection was analyzed accordingly to the Banff classification. All patients received Simulect, cellcept (2 g/D) and steroids. Patients were randomized to receive BELA or CsA. Functional analysis of CD4 T lymphocytes was performed with PBMC from healthy donor.38 patients were included, 26 were receiving BELA and 12 CsA. 8 patients developed an acute rejection during the first year (7 treated with BELA and 1 with CsA). CD4, CD8, CD20, CD56 number was similar in the 2 groups during transplantation. For patients treated with BELA, the rate of CD4+ CD57+ cells (7.1% vs 1.9%; $p < 0.03$) at D0 were higher in patients who have developed an acute rejection. Other lymphocyte populations were comparable. Study of CD4+ CD57+ T cells behavior in MLR with BELA treatment revealed a strong and sustained proliferation of this population indicating the absence of senescent behavior and a decreased of CD25 expression even in proliferating cells. For transplant patients treated with BELA, the presence of CD4+ CD57+ is associated with higher rejection rate. These results suggest that CD4+CD57+ might participate to the rejection of kidney in the BELA approved regimen. Their presence at high levels may lead to modify treatment regimens associated with BELA.

O76 URINE-DERIVED CELLS AS NOVEL TOOLS FOR MONITORING ALLO-REACTIVITY IN KIDNEY-TRANSPLANT PATIENTS

Constantin Thieme[1], Benjamin Weist[1], Timm Westhoff[2], Petra Reinke[1], Nina Babel[3]
[1]BCRT (Berlin-Brandenburger Centrum für Regenerative Therapien); [2]Medical Clinical, Marien Hospital Herne, Ruhr University Bochum; [3]BCRT und Medical Clinic, Marien Hospital Herne, Ruhr University Bochum

Reactivity of the immune system against donor antigens determines transplant-rejection after transplantation, and analyzing the alloimmune response has been shown to predict acute rejection in previous studies. The available methods for assessing alloreactivity are based on measurements of reactivity of recipient peripheral blood mononuclear cells (PBMCs) upon stimulation by stimulator cells derived from donor spleen cells or artificial third party stimulator cell banks. Despite its potential utility alloreactivity testing in clinical routine is restricted mostly by either low quantity (limited amount of donor spleen cells) or quality (lack of sufficient matching between HLA-bank and donor HLA) of stimulator cells. The aim of this study was to establish a renewable source of donor-derived cells for alloimmunity analysis in kidney transplantation.

Urinary samples were successfully used for generation of induced pluripotent stem cells in a previous study. Taking advantage of this, we established a protocol for the generation of patient-specific donor-derived stimulator cell lines using recipient's urine. The urinary samples were centrifugated, washed and the cell-pellet was seeded and cultivated with daily medium changes. The cultivated cells showed an epithelial phenotype and HLA genotyping revealed that >50% were donor-derived. Alloreactivity was proven by proliferation assay. For this, cell lines were incubated with the recipient's PBMCs in the ratios 1:1 and 1:5 (epithelial cells:PBMC) to test for the direct alloresponse. Lysed cell lines presentated on recipients PBMCs were used for indirect alloimmunity testing. Multi-color flow-cytometry measurement showed a dose-dependent CD3+ Cells proliferation upon direct stimulation, and, at a lesser extent, after indirect stimulation.

In conclusion, we established a novel platform for monitoring allo-reactivity in kidney transplant patients. Further studies are required to assess clinical utility of this assay.

O77* IMMUNE PHENOTYPES AND MTORI-SENSITIVE TUMOR-SPECIFIC EFFECTOR T-CELL RESPONSES ARE KEY PLAYERS FOR PROMOTING SQUAMOUS SKIN CANCER IN KIDNEY TRANSPLANT PATIENTS

Elena Crespo[1], Loreto Fernandez[2], Marc Lucia[1], Segio Luque[1], Ricardo Lauzurica[2], Josep Maria Grinyó [3], Oriol Bestard[3]
[1]Experimental Nephrology Laboratory. IDIBELL. Barcelona. Spain; [2]Nephrology Department, Hospital germans Trias i Pujol, Barcelona, Spain; [3]Renal transplant Unit, Nephrology department. Bellvitge University Hospital. Barcelona University. Barcelona, Spain

Renal transplant patients are at higher risk of developing squamous skin cancer (SCC). Chronic immunosuppression promotes an imbalance between effector and regulatory tumor-specific cellular responses (TSCR), thus favoring cancer development.

Assessment of intratumoral and peripheral T CD8+, B CD20+, regulatory T-cells (Foxp3+CD4+CD25high) and NK cells CD56+ as well as functional TSCR was carried out in kidney transplant patients with SCC under CNI (KT-CNI-SCC) ($n = 42$) or mTORi-based immunosuppressive regimens (KT-mTORi-SCC) ($n = 17$), and were compared to non-transplanted patients developing SCC (no-KT-SCC) and healthy individuals. Moreover, immunophenotype evaluation and TSCR were re-assessed 12 months after conversion to mTORi in 15 patients who developed SCC under CNI.

No-KT-SCC patients showed significantly higher intratumor cellular infiltrates than KT-SCC. Intratumoral Treg infiltrates were significantly higher among KT-mTORi-SCC patients than KT-CNI-SCCs. Tumor relapses within KT-CNI-SCC patients positively correlated with the number of Tregs in the periphery and infiltrating the tumor, whereas it negatively correlated with NK-CD56+ cells in the periphery and within tumor infiltrates. Differently, the number of tumor relapses in KT-mTORi-SCC positively correlated with the percentage of Tregs and CD56dimCD16+ NK cells only in peripheral blood. One-year after conversion to mTORi, KT-CNI-SCC showed a significantly increase in Treg numbers in peripheral blood, reaching No-KT-SCC levels. To note, tumor-specific effector T-cell responses were significantly higher within No-KT-SCC than in KT-SCC. T-cell responses against MAGE-A1, MAGE-A3 and p53 antigens were significantly increased after mTORi conversion, reaching similar levels than No-KT-SCC.

Development and progression of SCC after KT under CNI-based regimens seems to be influenced by an increased activity of Tregs but decreased presence of NK-cells, as well as to the suppression of TSCR responsive to mTORi.

O78

THE EFFECT OF CYTOKINE RELEASE CAUSED BY INDUCTION IMMUNOSUPPRESSION ON NITRIC OXIDE IN RENAL TRANSPLANTATION

Rose Johns[1], Makis Laftsidis[2], Francis Edwards[2], Emma Burrows[2], Elijah Ablorsu[2], Phillip James[3], Aled Phillips[3], Argiris Asderakis[2]

[1]University Hospital of Wales.; [2]University Hospital of Wales, Cardiff; [3]Cardiff University

Background: Varying induction immunosuppression regimens have a differential impact on cytokine release. Pro-inflammatory cytokines stimulate endothelial cells causing secretion of nitric oxide (NO). Serum nitrite (NO2) sensitively reflects endothelial NO formation. We aimed to see if the change of NO2 in DCD renal transplants depends on factors associated with reperfusion injury and if differential induction influences this by effecting cytokine release.
Methods: In 35 DCD transplants we measured plasma levels of NO2 at 8 time points and analysed their change. We also measured cytokine levels and correlated them with the induction regime.
Results: ATG increases the release of TNF-a, IFN-g, IL-6, IL-10 and IL-17. IL-2, TNF-a and IL-10 are increased more by ATG compared to Campath ($p = 0.003$, 0.07, and 0.03 respectively). At regression analysis the change of NO2 at 8 h post perfusion was related to primary WIT ($p = 0.001$), recipient age ($p = 0.004$) and induction with Campath ($p = 0.04$). In order to see if the change in NO2 was attributable solely to the pro-inflammatory cytokine release caused by ATG or Campath, we checked NO2 in patients who received Simulect. In the Simulect group the change of NO2 was still dependent on primary WIT ($p = 0.008$) and recipient age ($p = 0.01$). Moreover in patients who received ATG the change of NO2 at 2 h post perfusion correlated with donor age ($p = 0.03$), primary WIT ($p = 0.04$), and secondary WIT.
Conclusion: NO2 levels post perfusion in DCD transplants are affected by warm ischemia and donor/recipient age. Despite up-regulation of cytokines by ATG and/or Campath this change of NO2 seems not to be dependent entirely on induction. This study suggests that NO2 levels post perfusion are linked to factors affecting reperfusion injury and differential induction regimes might be modifying those factors via their varying effect on cytokine release.

011 HEART

O79 COMPLEMENT-BINDING DONOR SPECIFIC ANTIBODIES IN HEART TRANSPLANTATION. CORRELATIONS WITH ANTIBODY MEDIATED REJECTION AND CLINICAL OUTCOMES

Michel Redonnet[1], Arnaud Gay[1], Annette Belin[2], Françoise Hau[1], Olivier Toutinais[2], Brigitte Le Manf[2], Arnaud François[1], Françoise Galateau[2], Catherine Nafeh[1], Katrien Blanchart[2], Gérard Babatasi[2], Yves Litzler Pierre[1], Paul Bessou Jean[1]

[1]Rouen University Hospital; [2]Caen University Hospital

Objectives: After heart transplantation (HTx), antibody-mediated rejection (AMR) is associated with a high risk of graft dysfunction, cardiac allograft vasculopathy (CAV) and death. We checked if de novo donor specific antibodies (dnDSA) binding with complement (C') was correlated with AMR and clinical events, using two different Luminex-based single antigen bed assays binding either the C' components C1q (One Lambda) or C3d (Immucor).
Methods: Out of 280 HTx pts, transplanted between 07/2008 and 09/2013, 33 pts (12%) developed dnDSA. The first sera with dnDSA (MFI ≥1000) were retrospectively studied for C1q- and C3d-binding. C1q- and C3d-binding DSA positivity (MFI ≥1000) was correlated with AMR (according ISHLT 2013 classification: positive if ≥pAMR1), graft dysfonction (echocardiographic and/or hemodynamic criteria) and CAV (any new angiographic abnormalitiy).
Results: In this series of 33 pts with a follow-up of 3.6 ± 1.8 years after appearance of dnDSA, 20 pts had ≥1 AMR episode, 17 developed CAV and 10 had graft dysfunction (2 deaths). DSA were mainly anti-HLA class II antibodies (92%), with anti-DQ specificities (70%). Irrespective to C' assay used, no correlation was found between C'-binding DSA positivity and AMR, CAV, graft dysfunction, as isolated parameters. However, positivity of C3d-binding DSA was associated with AMR and/or graft dysfunction (sensitivity = 64%, specificity = 77%, p = 0.05); and with AMR and/or CAV and/or graft dysfunction (sensitivity = 64%, specificity = 100%, p = 0.047). Moreover, if C3d-binding DSA positivity threshold is lowered from MFI ≥1000 to MFI ≥100, this last correlation is higher (sensitivity = 73%, specificity = 100%, p = 0.006).
Conclusion: C3d-binding dnDSA is associated with AMR and/or CAV and/or graft dysfunction in HTx, whereas C1q is not. Usual MFI cut-off value of 1000 seems to have to be lowered to 100 to enhance C3d-binding dnDSA sensitivity. A longitudinal study of C'-binding dnDSA with a larger cohort may be more informative.

O80 FROM ULTRASTRUCTURE TO MOLECULAR TESTING: ASSESSMENT OF ANTIBODY-MEDIATED TISSUE INJURY IN HUMAN CARDIAC ALLOGRAFT BIOPSIES

Bahman Afzali[1], Erin Chapman[2], Benjamin Adam[2], Fernando Riesgo Gil[3], Daniel Kim[3], Luis Hidalgo[2], Patricia Campbell[2], Banu Sis[2], Michael Mengel[2]

[1]Institute of Pathology University Duisburg-Essen Essen Germany;

[2]Department of Laboratory Medicine and Pathology, University of Alberta;

[3]Division of Cardiology, University of Alberta

Background: Despite continuous attempts to refine diagnostic consensus, accurate assessment of antibody-mediated rejection (AMR) in cardiac allograft biopsies is an ongoing unmet need. The microcirculation represents the primary target for donor-specific, anti HLA antibodies (DSA). Accordingly increased expression of endothelial, NK cell and inflammatory genes has been associated with AMR. This study evaluates a respective gene set for diagnosing AMR in formalin-fixed, paraffin-embedded (FFPE) cardiac allograft biopsies.
Methods: 107 archival FFPE biopsies were classified according to 2013 ISHLT consensus. In a subset of 42 cases endothelial swelling in capillaries was measured by transmission electron microscopy (TEM). A set of 34 endothelial, NK cell and inflammatory genes was quantified with NanoString nCounter system.
Results: AMR cases ($n = 70$) showed significantly higher gene set expression compared to ACR ($n = 22$, $p < 0.0001$) or normal controls ($n = 15$, $p < 0.0001$). While overall DSA was associated with higher gene set expression ($p = 0.001$), DSA-positivity did not result in higher AMR transcript counts within AMR ($p = 0.065$) or ACR diagnoses ($p = 0.182$, Figure 1A). C4d deposition tended to higher expression levels ($p = 0.052$). Ultrastructural quantification of endothelial swelling strongly correlated with gene set expression (Spearman's rho = 0.559, $p < 0.001$, Figure 1B) and was specific to AMR cases (spearman's rho = 0.616, $p = 0.002$). ROC analysis showed a higher diagnostic accuracy for gene set expression (AUC = 78.61) compared to DSA (AUC = 72.55) or C4d detection (AUC = 70.71). In 17 patients with sequential biopsies increasing gene set expression over the course of AMR was associated with allograft failure (Figure 1C).

Figure 1A

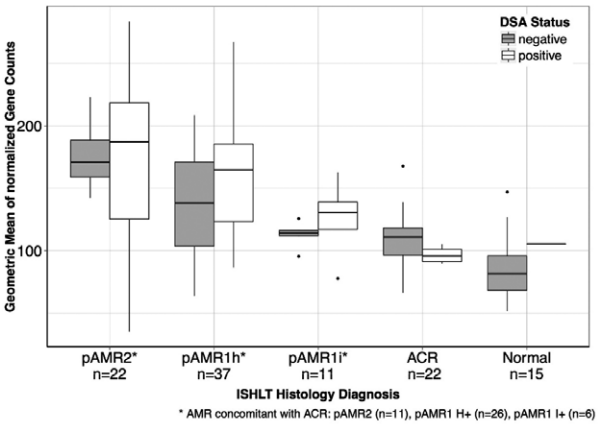


Figure 1B

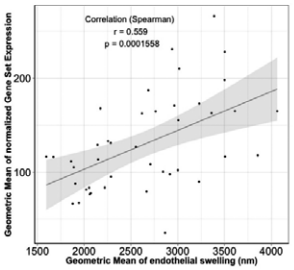
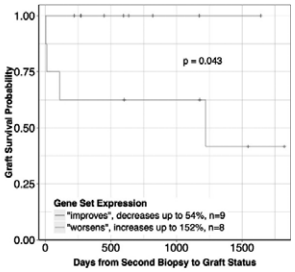


Figure 1C



Conclusion: Molecular diagnostics in FFPE allograft biopsies is feasible as routine diagnostic work-up. The gene set expression reflects ultrastructural and histopathological findings and can add diagnostic as well as prognostic value to the assessment of antibody-mediated tissue injury in human cardiac allograft biopsies.

O81 OVERCOMING THE NEGATIVE IMPACT OF PREFORMED DONOR-SPECIFIC ANTIBODIES (DSA) ON POST HEART TRANSPLANT OUTCOMES: POTENTIAL UTILITY OF ALLOCATION BASED ON VIRTUAL CROSSMATCH POLICY

Laura Borgese[1], Luciano Potena[2], Martina Chiavaroli[1], Andrea Bontadini[2], Sandra Iannelli[2], Fiorenza Fruet[2], Valentina Manfredini[1], Simonetta Capelli[2], Laura Righini[1], Paola Prestinenti[1], Gaia Magnani[2], Francesco Grigioni[1]
[1]University of Bologna; [2]S.Orsola-Malpighi Hospital

While pre-transplant HLA sensitization is known to represent a risk factor for post transplant outcomes, effectiveness of organ allocation based on virtual crossmatch (vXM) in heart transplant (HT) recipients is unknown.

Here we analyze the effect of vXM in our allocation policy by comparing the effect of DSA on post-transplant outcomes in historical controls – i.e. before the introduction of single antigen bead (SAB) assay – in whom we retested pre-transplant serum, vs. current patients population prospectively tested with SAB. Sensitized patients in this latter group were allocated according with HLA Ab profile, with a mean fluorescence intensity (MFI) ≥5000 identifying forbidden antigens.

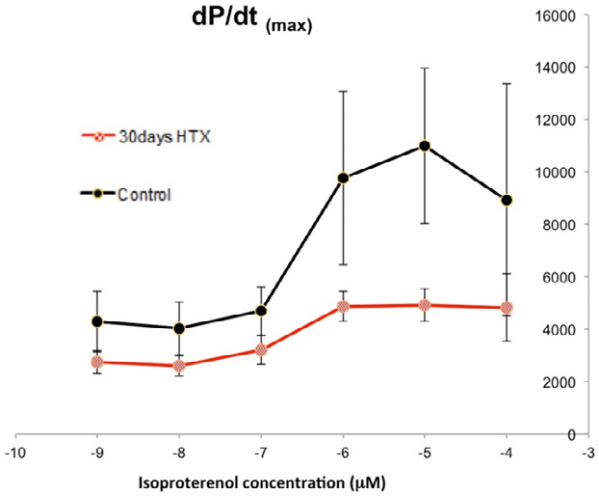
In the retrospective cohort, 15 (9%) patients received a graft they were pre-sensitized against, showing a significantly higher early mortality (38 vs. 13%) than the 14 who were sensitized but received a compatible graft. in these, mortality significantly rose at year 10 post-HT (70 vs 30% of the 147 who were non-sensitized; $p = 0.05$). Similarly, 58% of DSA group showed late cellular rejection, vs. 40% of HLA and 25% of non sensitized ($p < 0.01$). Assay of complement fixing ability of DSA did not improve the prognostic stratification. After 2010, 27 (16%) sensitized patients have been enlisted, and 11 received HT following allocation with vXM compatible donor, experiencing a post-transplant survival not different from the 72 non-sensitized recipients. Of note, 22% of sensitized patients died while on list vs. 16% of non-sensitized (p = n.s.).

Pre-transplant HLA sensitization appears a significant risk for post-transplant outcomes, in terms of late cellular rejection and survival. The introduction of vXM based allocation may void the risk of early mortality in sensitized recipients. The risk of increased waiting list mortality in sensitized patients should however be carefully monitored to keep offering these patients acceptable transplant opportunities.

O82 ATROPHIC REMODELING LIMITS CONTRACTILE FUNCTION OF LONG-TERM UNLOADED HEARTS ASSESSED VIA EX VIVO WHOLE HEART PERFUSION

Henriette Most, Xuebin Fu, Sarah Longnus, Adrian Segiser, Silvan Jungi, Thierry Carrel, Hendrik Tevaearai
Cardiac and Vascular Surgery, University of Berne

Objective: Substantial recovery of function was observed via ventricular unloading by left ventricular assist devices (LVADs). However, unloading confers not only beneficial remodeling of the failing myocardium, but also atrophic loss of myocardial mass. The consequences of unloading the heart are investigated in a small animal model of heterotopic heart transplantation (HTX) in isogenic rats via reproducible functional assessment of the remodeled heart on a cellular and organ level.
Methods: Normal rat hearts were unloaded by HTX for 30 days. Functional capacity of unloaded hearts (UN, $n = 10$) and age-matched control hearts (CTR, $n = 9$) was assessed in vitro and in an ex vivo working heart perfusion system. We analyzed sarcomere shortening, hemodynamic parameters, oxygen consumption and lactate production. Inotropic response to isoproterenol was tested.
Results: UN hearts showed significant atrophy with a diminution of heart weight by 46%, as well as sarcomere size by 8%. Contractile function was intact in isolated cells but diminished in the whole heart: developed pressure was 131 ± 22 mmHg in CTR vs 84 ± 16 mmHg in UN, cardiac output was 47 ml/min in CTR vs 15 ml/min in UN group. Also, contractile function was limited at baseline (dP/dt max of 4353 ± 377 vs 2594 ± 443 in UN) as well as under β-adrenergic receptor stimulation with isoproterenol. Inotropic response in the unloaded hearts showed an increase by maximally 87% regarding dP/dt max in contrast to 166% in CTR hearts. G_i-(alpha) Protein was enhanced in UN hearts, indicating inhibited adrenergic signaling.
Conclusion: This is the first successful ex vivo working heart perfusion in unloaded rat hearts assessing performance under loaded conditions. Diminished function of unloaded hearts at baseline and adrenergic stimulation implies that unloading-induced atrophic remodeling as such has detrimental effects that need to be adressed in view of a potential use of LVADs as a therapeutic option in heart failure patients.



O83 DEPRESSIVE SYMPTOMS AT 1 YEAR AFTER SURGERY INCREASE MORTALITY IN HEART TRANSPLANT PATIENTS: A PROSPECTIVE COHORT STUDY

Nathalie Duerinckx[1], Johan Vanhaecke[2], Sabina De Geest[3], Katrien Van Lommel[4], Steffi Lenaerts[4], Fabienne Dobbels[5]
[1]Department of Public Health and Primary Care, KU Leuven, Department of Cardiology, UZ Leuven; [2]Department of Cardiology, UZ Leuven; [3]Department of Public Health and Primary Care, KU Leuven, University of Basel; [4]UZ Leuven; [5]Department of Public Health and Primary Care, KU Leuven

Purpose: To study the impact of depressive symptoms at 1 year post-transplant on all-cause mortality in the long term after heart transplantation (HTx).
Methods: This is a secondary data analysis of an ongoing prospective cohort study assessing quality of life long-term after HTx. A consecutive sample of adult, Dutch speaking, literate HTx patients without cognitive or serious medical problems were included between February 2001 and 2014. After written informed consent, patients filled out the Beck Depression Inventory (BDI) to document presence and severity of depressive symptoms at 1 year post-transplant. Higher scores reflect more severe symptoms (range 0–63). BDI scores of 0–9 indicate no depression, scores of 10–15 indicate mild depression, and BDI scores of 16–63 indicate moderate to severe depression. All-cause mortality and survival time data were collected until April 4, 2014. Data was analyzed using Kaplan-Meier and cox-regression survival analysis (controlling for potential confounders).
Results: A total of 173 patients were included in the analysis (age, 54 years (43.5–16.0); 75.6% men; mean follow-up, 5.7 ± 3.9 years). Mild depressive symptoms occurred in 30 patients (17.3%) and moderate to severe depressive symptoms occurred in 11 patients (6.4%). A total of 18 patients (10.2%) died during follow-up. Kaplan-Meier survival analysis showed that depressive symptomatology at 1 year resulted in a significantly increased mortality risk (HR: 1.97; 95% CI: 1.09 – 3.56) [figure1] .

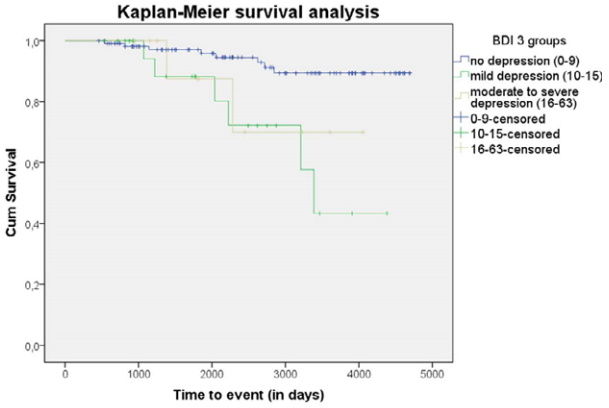


Figure 1: Kaplan-Meier survival curves for patients without (0-9), mild (10-15), and moderate to severe (16-63) depressive symptoms at 1 year after heart transplantation, Log Rank Test: $\chi^2 = 8.804$, df = 2; p=0.012.

Conclusion: Our results suggest that depressive symptoms at 1 year after HTx unfavorably impact mortality, highlighting the need for systematic screening of depressive symptoms in these HTx patients. Our cohort will allow us to further investigate if depression at different time points post-transplant impact mortality as well, taking into account other known risk factors.

O84 MULTILEVEL FACTORS ASSOCIATED WITH MEDICATION ADHERENCE IN HEART TRANSPLANTATION: A MULTI-CONTINENT CROSS-SECTIONAL STUDY (BRIGHT STUDY)

Sabina De Geest[1], Lut Berben[1], Cynthia L. Russell[2], Kris Denhaerynck[1], Fabienne Dobbels[3]

[1]University of Basel; [2]School of Nursing & Health Studies, University of Missouri-Kansas City; [3]Health Services and Nursing Research, KU Leuven, (on behalf of the BRIGHT study team)

Background: Medication non-adherence (NA) leads to poor outcomes and increased healthcare costs. Studies assessing NA have largely neglected healthcare system factors. We examined variability in immunosuppressive medication NA across heart transplant (HTx) centers in multiple countries and continents and assessed multilevel factors associated with NA.
Methods: The BRIGHT study is a 4 continent, 11 country (Australia, Belgium, Brazil, Canada, France, Germany, Italy, Spain, Switzerland, UK, US) and 37 HTx center cross-sectional study. A convenience sample of countries and centers was included followed by a random sample of adult HTx patients and clinicians within centers. NA was assessed by patient interview. Patients were classified as NA if they missed ≥1 dose of immunosuppressives (taking NA) and/or took it >2 h before/after the prescribed time (timing NA) over the past 4 weeks. Patient-level factors were derived from the Integrated Model of Behavioral Prediction. System-level factors were derived from the Ecological Model. Multilevel factors were assessed with established instruments or measures specifically developed for this study. Data were analyzed descriptively and by logistic regression analysis (Generalized Estimation Equations).
Results: Of the 1008 patients included in the study, 36.8% were NA (range: 17.7% Germany to 42.9% Australia). Factors independently associated with NA: Patient level: more routine-related barriers (OR: 12.1, 95%CI 7.0–27.7); Healthcare organization level: nurses reporting that NA patients were targeted with NA interventions (OR: 0.58, 95%CI 0.42–0.81); and Policy level: cost related NA (OR: 4.1, 95%CI 1.5–11.3) and higher out-of-pocket expenses (OR: 1.19, 95%CI 1.04–1.40).
Conclusion: In the BRIGHT study, multilevel risk factors are associated with NA. These modifiable factors can be translatranslated into interventions targeting the patient, healthcare provider, -organization and -policy levels.

O85 ACUTE KIDNEY INJURY AS A COMPLICATION OF CARDIAC TRANSPLANTATION: INCIDENCE, RISKFACTORS AND IMPACT ON 1-YEAR MORTALITY AND RENAL FUNCTION

Gijs Fortrie, Olivier Manintveld, Kadir Caliskan, Jos Bekkers, Michiel Betjes

Erasmus MC

Background: While chronic deterioration in renal function is a frequently seen complication after cardiac transplantation, which is partly explained by the prolonged use of calcineurin inhibitors (CNI), few data are present on the consequences of acute kidney injury (AKI) after cardiac transplantation. In the current study, incidence of AKI and its impact on mortality and renal function was evaluated.
Methods: Five hundred thirty-one cardiac transplant recipients (age ≥18 years) were evaluated for the incidence of AKI in the post-operative period as defined by the Kidney Disease Improving Global Outcome criteria. Secondary outcomes were renal function and mortality up to one year after transplantation.
Results: Overall 405 (76%) patients met the AKI criteria of which 211 (40%) had AKI stage I, 119 (22%) stage II, 75 (14%) stage III and 25 patients (5%) required renal replacement therapy (RRT). Independent risk factors for AKI and increase in AKI stage were body-mass index, renal function at baseline, diabetes mellitus and postoperative right ventricle failure. Protective factors were age and treatment with induction therapy compared to direct treatment with a CNI. One-year mortality rates in patients without AKI, stage I, II and III were 4.8%, 7.6%, 11.8% and 14.7%, respectively (log-rank test for trend $p = 0.008$). In patients that required RRT 1-year mortality was 28.2% (log-rank test $p = 0.001$). In multivariable analysis only AKI requiring RRT was an independent predictor of 1-year mortality (HR = 2.75, $p = 0.03$). General linear model analysis revealed that AKI stage I ($\beta = -5.77$, $p = 0.01$), II ($\beta = 6.76$, $p = 0.009$) and III ($\beta = -8.83$, $p = 0.004$) were independent predictors of renal function one year after transplantation.
Conclusions: AKI is highly frequent after cardiac transplantation and constitutes an independent risk factor for mortality and impaired renal function 1 year after transplantation.

O86 CLINICALLY RELEVANT WOUND EVENTS WITH EVEROLIMUS-BASED IMMUNOSUPPRESSIVE REGIMEN IN HEART TRANSPLANT RECIPIENTS: POST-HOC ANALYSIS BASED ON 12-MONTH RESULTS FROM THE A2310 STUDY

Andreas Zuckermann[1], Mauro Rinaldi[2], Nizar Yonan[3], Shoei-Shen Wang[4], Gaohong Dong[5], Patricia Lopez[6]

[1]Medical University of Vienna; [2]University of Turin; [3]Wythenshawe Hospital, Manchester; [4]National Taiwan University Hospital; [5]Novartis Pharmaceuticals; [6]Novartis Pharma AG

Background: Wound healing events (WHE), ranging from incision site pain to mediastinitis, were collected prospectively in de novo heart transplant (HTx) recipients treated with everolimus (EVR) or mycophenolate mofetil (MMF) from the A2310 study. We performed a post-hoc analysis to evaluate the possible increased risk for clinically relevant WHE (as identified by transplant surgical experts) with EVR regimen.
Methods: A2310 (NCT00300274) was a 24-month (M), open-label, multicenter study with 721 HTx recipients randomised to either EVR 1.5 mg or EVR 3.0 mg + reduced cyclosporine (CsA) or MMF 3.0 g + standard CsA; with steroids ± induction. Comprehensive data on WHE were collected using special case report forms with regard to location (sternal/non-sternal), degree, symptoms, diagnosis and intervention. A blinded review of reported WHE was performed subsequently by transplant surgical experts, and events considered not clinically relevant were excluded from this analysis. Any WHE reported as serious adverse event, or as mediastinitis event, and wound events requiring sternal rewiring, surgical debridement or vacuum assisted closure (VAC) were considered as critical wound events.
Results: Up to M12, clinically relevant WHE occurred in 23.7%, 24.6% and 20.5% of HTx recipients in EVR 1.5 mg, EVR 3.0 mg and MMF arms, respectively. Incidence of sternal and non-sternal WHE was similar across all arms. The incidence of critical WHE (including mediastinitis and wound dehiscence) was higher in EVR (3.0 mg) arm vs. EVR (1.5 mg) or MMF arms. The proportion of patients requiring treatment for sternal WHE (rewiring, surgical debridement or VAC) was not significantly different between the EVR (1.5 mg) and MMF treatment arms (Table).

Conclusions: Similar incidence of clinically relevant WHE was observed in HTx recipients treated with EVR (1.5 mg) and MMF. However, a higher dose of EVR (3.0 mg) may increase the incidence of wound complications.

Table: Wound healing events at Month 12 (safety population)

Parameters	EVR 1.5 mg N=279 n (%)	EVR 3 mg N=167 n (%)	MMF N=268 n (%)	p-value** (EVR 1.5 mg vs MMF)
Any patient with WHE*	66 (23.7)	41 (24.6)	55 (20.5)	-
Sternal WHE	52 (18.6)	29 (17.4)	39 (14.6)	-
Non-sternal WHE	20 (7.2)	16 (9.6)	19 (7.1)	-
Critical WHE	28 (10.0)	25 (15.0)	24 (9.0)	-
Mediastinitis	5 (1.8)	4 (2.4)	2 (0.7)	-
Wound dehiscence	2 (0.7)	2 (1.2)	1 (0.4)	-
Treatment for sternal WHE				
Rewiring	10 (3.6)	7 (4.2)	6 (2.2)	0.449
Surgical debridement	8 (2.9)	9 (5.4)	12 (4.5)	0.367
VAC	6 (2.2)	2 (1.2)	6 (2.2)	1.000

*Complication of device insertion, complication of device removal, crepitations, culture wound positive, device related infection, device related sepsis, dislocation of sternum, fracture nonunion, impaired healing, implant site discharge, implant site infection, incision site complication, incision site erythema, incision site infection; incisional drainage, incisional hernia, infected lymphocele, lymphocele, mediastinitis, pericarditis, post procedural discharge, post procedural fistula, post procedural infection, post procedural edema, postoperative thoracic procedure complication, postoperative wound complication, postoperative wound infection; wound complication; wound dehiscence; wound infection, wound infection staphylococcal, wound secretion and wound sepsis.

** Fisher's exact test
WHE, wound healing complications; VAC, vacuum assisted closure

O87 EVEROLIMUS (EVE) VERSUS MYCOPHENOLATE (MMF) DE NOVO AFTER HEART TRANSPLANTATION (HTX): DOES IT MATTER FOR LONG-TERM OUTCOMES?

Marco Masetti, Marcello Malossi, Mario Sabatino, Gaia Magnani, Laura Borgese, Valentina Manfredini, Antonio Russo, Claudio Rapezzi, Francesco Grigioni, Luciano Potena

Heart Transplant Unit, University of Bologna

Background: Several large prospective randomized trials (RTs) compare EVE and MMF de novo after HTX; however, long term comparison on clinically meaningful endpoints is lacking.
Methods/Matherials: In this study we included all patients enrolled in our Center in RTs about EVE de novo. We analyzed 5-years (yrs) incidence of fatal and non fatal major cardiovascular events (MACE), all-cause mortality, rejection, CMV infection, cancers, changes in GFR. Given the frequent cross-overs between the two drugs, we performed both an Intention to treat

(ITT) and an On-Treatment (OT) analysis, defined retrospectively as the drug taken for most of the time.
Results: 93 patients (80% males, 53 ± 11 years, HTX 2005–14) were enrolled: 57 randomized to EVE, 36 to MMF. 29 underwent at least one cross over, 10 for clinical reasons (cancers, renal failure, rejection), 19 for drug intolerance. Tolerability was lower in the EVE arm ($p = 0.05$), mostly due to pericardial effusions, but comparable to MMF after the first 3 months from HTx ($p = 0.42$). At ITT analysis, we found no differences about MACE, mortality, rejection, CMV infection, cancers, renal function (p all not significant). At OT analysis EVE group (42/93) had a lower incidence of MACE (2.6 ± 2.5% vs 19.0 ± 5.8%, $p = 0.01$) and of 1-yr CMV infection (28.8 ± 7.0% vs 44.0 ± 7.3%, $p = 0.02$), similar mortality ($p = 0.53$) and renal function ($p = 0.45$) and a promising lower 10-yrs estimated cancers incidence (9.5 ± 5.4% vs 38.7 ± 16.2%, $p = 0.33$).
Conclusion: This is a single-center post-hoc analysis of long-term outcomes in patients initially randomized in trials with different endpoints. While confirming problems in maintaining EVE therapy in the early post-HTX period, we found suggestive evidence of potential long-term benefits of EVE in reducing MACE, CMV infection and cancers. Although these data are only hypothesis generating, they suggest a delayed introduction of EVE to favour its tolerability, aiming to take better advantage of its possible long-term benefits.

029 PANCREAS

O88 DOES CARDIAC RISK QUANTIFICATION HAVE A ROLE IN ASSESSMENT FOR PANCREAS TRANSPLANTATION?

Hussein Khambalia[1], Daniel Doherty[2], Petros Yiannoullou[1], Emma Foster[1], Zia Moinuddin[1], Angela Summers[1], Titus Augustine[1], David Van Dellen[1]
[1]Manchester Royal Infirmary; [2]University of Manchester

Introduction: Pancreas transplantation (PT) is the gold-standard treatment for complicated insulin dependent diabetes mellitus. Perceptions of high cardiac risk persist, which currently mandate exhaustive cardiac investigations prior to listing. However, the validity of this approach is not verified and requires further examination.
Methods: Retrospective analysis was made of patients undergoing PT in a single centre, examining cardiac assessment and transplant outcomes. Patients were categorised by myocardial perfusion scan (MPS) into normal perfusion (NP), reversible ischaemia (RI) and permanent ischaemia (PI) groups.

Primary endpoints were cardiac death, patient and graft survival. Secondary endpoints were hospital length of stay (HLoS), reoperations and complications.
Results: 314 PTs were performed (01/01-03/14), with 152 MPS results available. (60.1% male; mean age 43.8, 82.9% SPK, 12% PAK, PTA 5.1%).

109 (71.7%) MPS showed NP, 24 (15.8%) RI and 12 (7.9%) PI. There was no difference in graft and patient survival between groups ($p = 0.31$, 0.33 (log rank test)). No significant difference was seen for HLoS (NP: 32.7; RI: 53.3 PI: 35.0), mean reoperation number (NP: 0.8, RI: 0.8, PI: 1.0) or complications (NP: 2.4, RI: 1.0, PI: 1.4) ($p = 0.45$, 0.12, 0.89 (ANOVA)).

Angiography was performed in 11.1%, 100% and 83.3% of patients in NP, RI and PI groups respectively since 2011 with no subsequent revascularisations as a result of investigations. Of patients with available MPS results, cardiac causes accounted for 6.5% ($n = 2$; NP: 1, RI: 1) of 31 deaths with median follow-up time from transplant was 1224 days (IQR = 2230).
Conclusion: MPS poorly stratifies outcome prediction for cardiovascular mortality and angiography appears unnecessary, ultimately resulting in a delay to listing. Post-operative cardiac mortality is minimal compared to waiting list, suggesting a requirement for expedited workup.

O89 USE OF HIGH PANCREAS DONOR RISK INDEX (PDRI) DONOR GRAFTS LEADS TO INCREASED RISK OF GRAFT THROMBOSIS FOLLOWING PANCREAS TRANSPLANTATION

Wouter Kopp, V.A.L. Huurman, J.W. De Fijter, A.F.M. Schaapherder, J. Ringers, A.E Braat
Leiden University Medical Center

Introduction: Despite improvements, outcome following pancreas transplantation is still endangered by a high risk of graft thrombosis. Scarcity of pancreatic grafts, demands use of high-risk donor grafts. High-risk donor grafts might lead to an increased number of graft thromboses.
Methods: All consecutive pancreas transplantations from May 2004 until December 2012 were analysed. Routine CT-scan was performed within the first week. Primary endpoint was graft loss due to thrombosis within 90 days. Influence of donor risk (measured as PDRI) on incidence of graft thrombosis was analysed. Patient received once daily and, as of 2007, twice daily, low molecular weight heparin (LMWH, nadroparin 2850 IE). Secondary endpoint was surgical reintervention for bleeding within 90 days.
Results: 163 transplantations were performed. Complete graft thrombosis occurred in 12 cases (7.4%). Complete graft thrombosis led to immediate graft failure in 11 cases (91.7%). Median PDRI was 1.25 (0.7–2.2). ROC curve revealed PDRI $\geq$1.30 as optimal cut-off point for graft thrombosis. High PDRI (PDRI $\geq$1.30) was associated with increased risk of graft thrombosis: complete thrombosis occurred in 9 cases (11.3%) vs. 3 cases (3.6%) in the low PDRI group ($p = 0.062$). Graft survival was 87.9% at 1-year and 77.7% at 5-years follow up. Numbers of both complete thrombosis ($p = 0.377$) as well as surgical reintervention for bleeding ($p = 0.537$) were equally distributed in both LMWH groups.
Conclusion: Graft survival was comparable to literature, despite the use of high PDRI organs, which have an increased risk of graft thrombosis. Our current regime of anticoagulation appears to be effective in the prevention of graft thrombosis in low PDRI grafts (3.6%). With the doubling of the dose of nadroparin, we did not observe a decrease in the number of thrombosis, nor an increase in bleeding complications requiring surgical reintervention. Higher PDRI (PDRI $\geq$1.30) grafts may warrant more aggressive anticoagulation therapy.

O90 EFFECT OF DONOR AGE ON PANCREAS TRANSPLANTATION OUTCOME

Muthusamy Anand Rathnasamy[1], Maria Irene Bellini[1], Giorgios Vrakas[1], Sanjay Sinha[1], Srikanth Reddy[1], Edward Sharples[1], Isabel Quiroga-Giraldez[1], Rutger Ploeg[2], Peter Friend[2]
[1]Oxford Transplant Centre; [2]University of Oxford

The global increase in the average donor age over the last decade has resulted in widespread acceptance of pancreases from older donors for transplantation. This abstract aims to review the effect of this cohort on pancreas graft and patient survival. Methods

Pancreases transplanted from 2004–2014 at a single centre was analysed, comparing pancreas & kidney, graft & patient survival, delayed graft function (DGF) and non-function (PNF) between all (DCD and DBD) donors aged >50 years (ED) and standard criteria donors (SD).
Results: 684 transplants occurred from 562 SD and 122 ED including 89 SD-DCD and 4 ED-DCD with median follow up of 32 months (SD) and 28 months (ED). There were 422 SPK, 55 PAK and 85 PTA from SD; 105 SPK, 1 PAK and 16 PTA from ED. ED had a median age of 55 yrs (51–67). Recipients of ED grafts were older (47 vs. 42, $p < 0.0001$) reflecting the intention to match donor and recipient age. Actuarial pancreas (90% SD vs. 87% ED, $p = 0.9$), kidney (87% SD vs. 88% ED, $p = 0.9$) and patient survival (90% SD vs. 87% ED, $p = 0.1$) was similar. Overall DGF of the pancreas (9% ED vs. 4% SD, $p = $ NS) and kidney (27% ED vs. 16% SD, $p = 0.06$) was similar, as was PNF rate for pancreas (4% ED vs. 1% SD, $p = $ NS) and kidney (4% ED vs. 1% SD). The Isolated pancreas transplants (PAK & PTA) had similar pancreas graft outcomes (76% ED vs. 71% SD, $p = 0.5$). However, when the DCD grafts were excluded, kidney DGF rates became significantly higher in ED (26% vs. 10%, $p = 0.0006$) whereas the pancreas DGF rate remained similar (3% ED vs. 2% SD).
Conclusion: Utilising older donors for pancreas transplantation appears to be safe, providing the increased risk of delayed graft function is duly considered in the risk-benefit analysis. Older donors for isolated pancreas transplant merit careful consideration.

O91 PANCREAS TRANSPLANTATION FROM CONTROLLED DCD: A SINGLE CENTRE EXPERIENCE

Muthusamy Anand Rathnasamy[1], Michiel Voskuil[1], Sanjay Sinha[1], James Gilbert[1], Srikanth Reddy[1], Isabel Quiroga-Giraldez[1], Rutger Ploeg[2], Edward Sharples[1], Giorgios Vrakas[1], Peter Friend[2]
[1]Oxford Transplant Centre; [2]University of Oxford

Background: The use of DCD pancreata is gaining popularity in the UK. Isolated pancreas transplantation (IP) is known to have inferior outcomes in comparison to SPK. We summarize a large single centre experience with transplanting pancreases from controlled DCD (Maastricht III).
Methods: Our pancreas transplant database analyzed data on 691 pancreas transplants from 2004 to 2014. DCD pancreata were accepted from 2007, based on donor age <60 years, BMI <30, cardiac arrest <1 hour.
Results: 43 SPK-DCD, 47 IP-DCD, 488 SPK-DBD and 113 IP-DBD were identified, resulting in more IP from DCD ($p = 0.0001$). DCD donors were younger (33 ± 12 vs 37 ± 14y, $p = 0.01$), had less vascular cause of death (35% vs 59%, $p < 0.0001$) and longer cold ischaemia (701 ± 15 vs. 1663 ± 156 min, $p = 0.02$). DCD had more graft thrombosis leading to early graft loss (8% vs. 1%, $p < 0.001$) despite frequent use of therapeutic anticoagulation (16% vs. 7%, $p = 0.008$); IP-DCD graft thrombosis required pancreatectomy more frequently (vs. IP-DBD, $p = 0.02$ vs. SPK-DCD, $p = 0.02$). In IP-DCD, thrombosis was predictive of the need for pancreatectomy ($p = 0.001$), delayed graft function ($p = 0.015$), and graft failure ($p = 0.05$). DCD grafts were also lost to pancreatitis (6%) and/or to rejection (9%). DCD kidneys had more frequent DGF than DBD (34% vs. 16%, $p = 0.02$). DCD pancreata had similar DGF (requiring insulin at discharge) (8% vs. 3%, $p = 0.06$). PNF incidence and patient survival was similar. DBD pancreas grafts had better survival (79% vs. 71%, $p = 0.01$) although graft survival of SPK and IP sub-groups was similar (82% SPK-DBD vs. 86% SDCD, $p = 0.8$; IP-DBD 70% vs. DCD 57%, $p = 0.2$) Conclusion

Excellent SPK-DCD results suggest that this cohort is a good additional source to expand the donor pool. IP-DCD pancreata are more at risk of early graft loss requiring pancreatectomy. IP-DCD grafts remain a feasible source for pancreases with comparable long-term survival, despite early graft loss risk.

O92 THE ROLE OF DIABETIC AUTOANTIBODIES IN PANCREAS TRANSPLANTATION

Shruti Mittal[1], Rachel Franklin[2], Srikanth Reddy[1], Isobel Quiroga[1], James Gilbert[1], Sanjay Sinha[1], Rutger Ploeg[1], Edward Sharples[1], Stephen Gough[2], Peter Friend[1]
[1]Oxford Transplant Centre; [2]OCDEM

Aim: Anti-islet cell (ICA) and anti-glutamic acid decarboxylase autoantibodies (GADA) are associated with beta-cell destruction and are known to emerge prior to the development of diabetes. Autoantibodies usually decrease following diabetes development but may persist in some individuals. The aim of this study was to examine the significance of autoantibody positivity in the context of pancreas transplantation.
Method: Pretransplant ICA and GADA results and graft outcomes were retrieved for all pancreas transplants performed at a single centre from 2002-2011. Graft failure was defined as a return to exogenous insulin. Kaplan Meier analysis was performed to assess associations between autoantibody positivity and graft outcomes.
Results: 471 pancreas transplant recipients were included (363 SPK, 108 IP transplants). Pretransplant ICA titres were available for 385/471 (81.7%) and were positive in 21/385 (5.5%). GADA titres were available for 386/471 (82.0%) and were positive in 72/386 (18.7%). Neither ICA nor GADA positivity pre-transplant was associated with graft survival in the SPK group. However, in the IP group, ICA and GADA were associated with poorer graft survival ($p = 0.024$ and 0.036 respectively). Pre-transplant autoantibody positivity was associated with shorter duration of diabetes and IP transplantation. Post-transplant GADA and ICA data were available and positive in 93/271 (40.3%) and 17/229 (7.4%), however no association with graft survival was found.
Conclusion: This is the largest study to examine the role of autoantibodies in pancreas transplantation and clearly shows an association between pre-transplant autoantibody positivity and graft failure in the IP group. The poorer outcomes in this group may be attributable to a hostile immune environment, and may benefit from immunosuppressive interventions.

O93 GRAFT SURVIVAL AFTER ISOLATED PANCREAS TRANSPLANTATION: IS THERE A DIFFERENCE BETWEEN ENTERIC VS. BLADDER DRAINAGE? RESULTS FROM A UK DATABASE OVER 10 YEARS

Georgios Vrakas, Rubens Macedo Arantes, Srikanth Reddy, Peter Friend, Anil Vaidya
Oxford University Hospitals

Background: Isolated pancreas transplant (IPT) is a procedure that is currently recommended for patients with Type 1 Diabetes with severe, life threatening hypoglycemic unawareness and preserved renal function. The current options for the exocrine secretion management are either enteric or bladder drainage of the transplanted pancreas. Furthermore IPT poses a unique scenario where there is an absence of a reliable biochemical marker to detect rejection apart from urinary amylase.
Methods: This is a retrospective study were we evaluate a total of 336 cadaveric IPT performed from January 2004 until October 2013. Graft and patient survival were available for 245 cases and pancreatic exocrine drainage was documented for 228 cases (183 Enteric drainage vs. 45 Bladder drainage).
Results: One-year, 3-year and 5-year pancreatic graft and patient survival were 66%, 53% and 44% and 96%, 90% and 85% for the enteric drainage group and 81%, 55% and 45% and 97%, 97% and 97% for the bladder drainage group respectively.
Conclusion: Our data shows that there is clearly a trend to better graft survival of bladder drainage pancreas in the first year. However this benefit is lost at 3 and 5 years. This data demonstrates that it may be crucial to have another marker to monitor the graft after it has been converted to enteric drainage and It would be imperative for the transplant community to find a more accurate marker, biochemical, radiological or remote (sentinel) with low complications to monitor rejection in isolated pancreas transplantation.

O94 FIVE YEARS EXPERIENCE OF DOUBLE INDUCTION REGIMEN AND DE NOVO USE OF EVEROLIMUS TO AVOID PANCREAS GRAFT LOSS IN SIMULTANEOUS PANCREAS-KIDNEY (SPK) TRANSPLANT PATIENTS

Jun Li, Katja Kloth, Kathrin Kramer, Martina Koch, Eike Achilles, Lutz Fischer, Silke Scheidat, Friedrich Thaiss, Franz Rinninger, Björn Nashan
University Medical Center Hamburg-Eppendorf

Background: In order to avoid graft loss as well as balancing the risk of rejection and/or infection after SPK, the following immunosuppressive regimen was applied: 1) double induction by using Thymoglobuline and Basiliximab; 2) steroid-free after week one; 3) mTOR inhibitor in combination with low dose CNI for maintenance.
Methods/Materials: Between 2009 and 2014, 21/26 patients underwent de novo SPK. Portal vein drainage of the pancreas graft was performed in all but 2 patients. Immunosuppression started before reperfusion using iv Thymoglobuline 1.5 mg/kg, Basiliximab 20 mg together with 500 mg MPS. Tacrolimus (C0 3–8 μg/l) and Everolimus (C0 3–8 μg/l) were started 6 h after reperfusion. All patients received CMV prophylaxis for 3 months. Postoperative graft function, surgical and infectious complications were studied according to the early (till POD 30) or late phase (follow-up till 02.2015).
Results: Postoperative surgical complications (≥Clavien-Dindo grade 3) were reported in 23.8% (5/21), with reoperation in 3/21. Non-rejection associated graft pancreatitis was diagnosed in 2/21 without the need for surgical intervention. Surgical site complications were reported in 3/21 while incisional hernia was found in 7/21 during the follow-up. Six patients demonstrated acute rejection (POD 17 – 44). Early CMV activation was noticed in 1/21 whereas no late reactivation was found. No patient had CMV syndrome or disease. Two patients developed a late BK viremia. One patient was lost to follow-up after 2 years. Another patient died of pulmonary infection during the first year with a functional pancreas graft. The 1, 3 and 5-year patient survival were 100%, 93.8%, 75% with pancreas graft survival rate of 95.2%, 92.9%, 75%. No PTLD were encountered.
Conclusion: The use of a double induction and the early start of everolimus is effective to reduce the risk of pancreas graft loss, with a very low rate of surgical site complications as well as CMV infections.

O95 SUPRA-PHYSIOLOGICAL HAEMODYNAMIC OPTIMISATION IMPROVES SHORT-TERM OUTCOMES FOLLOWING SIMULTANEOUS PANCREAS AND KIDNEY TRANSPLANTATION: A RANDOMISED CLINICAL TRIAL (NCT01619904)

Hussein Khambalia[1], Mahesh Nirmalan[1], David Van Dellen[1], Angela Summers[1], Zia Moinuddin[1], Giuseppe Giuffrida[1], Insiya Susnerwala[2], Simon Varley[1], Yvonne Alexander[3], Titus Augustine[1]
[1]Manchester Royal Infirmary; [2]Blackpool Victoria Hospital; [3]Manchester Metropolitan University

Background: Simultaneous pancreas and kidney transplantation (SPKT) is high-risk surgery, associated with significant peri-operative morbidity. Peri-operative supra-physiological optimisation (Goal-Directed Therapy, GDT) improves outcomes in other high-risk patients following major surgery. The aim of this study was to investigate the benefits of GDT in SPKT.

The primary outcome was hospital length of stay. Secondary outcomes were critical care length of stay, delayed graft function and objective markers of morbidity and recovery.
Methods: 60 SPKT recipients were randomly allocated to either GDT or Standard therapy (ST) cohorts. The GDT cohort underwent peri-operative supra-physiological optimisation, guided by lithium indicator dilution, to attain an indexed oxygen delivery of >600 ml/min/m^2. The optimisation protocol was initiated at the start of surgery and continued for 6 h post-operatively in the GDT arm. The ST cohort was managed according to current unit protocols.
Results: There were no differences in length of hospital stay between the two cohorts (18.0 days (IQR 14.0–31.0) and 15.0 days (IQR 13.0–22.3) in GDT and ST cohorts respectively; $p = 0.162$, Mann-Whitney U (MWU) test).

However, the GDT cohort had significantly lower critical care unit length of stay when compared to the ST cohort (4 days (IQR 3–5.5) and 8 days (IQR 6.0–9.3) respectively, $p < 0.001$, MWU test). In addition, the GDT cohort had significantly lower rates of renal delayed graft function, compared to the ST group (6.9% and 33.3% respectively, $p = 0.021$, Fisher's Exact). They also had shorter time to mobilisation out of bed (2.0 days (IQR 1.0–3.0) and 4.0 days (IQR 3.0–6.25) respectively; $p < 0.001$, MWU test) and shorter time to tolerating oral diet (5.0 days (IQR 4.0–8.0) and 8.0 days (IQR 6.75–10.0) respectively; $p < 0.001$, MWU test).
Conclusions: This study demonstrates improved short-term outcomes following protocolised physiological optimisation following SPKT.

O96 OUTCOMES AND RISK FACTORS IN PANCREAS RETRANSPLANTATION

Manuel Maglione, Silvia Gasteiger, Matthias Biebl, Claudia Bösmüller, Christian Margreiter, Franka Messner, Robert Öllinger, Dietmar Öfner-Velano, Stefan Schneeberger
Innsbruck Medical University

Despite recent data reporting that 5% of all pancreas transplantations performed per year are pancreas retransplantations (rePT), there is still a paucity of data describing outcomes following this demanding procedure. Retrospective data analysis identified 52 rePT performed at our institution from 1997 to 2013. Induction immunosuppression (anti-thymocyte globuline, alemtuzumab or IL-2R antagonist) was followed by tacrolimus based maintenance immunosuppression with MMF and tapered steroids. There were 20 simultaneous pancreas-kidney retransplantations (reSPK) and 30 pancreas alone retransplantations (rePTA). 6 procedures were 2nd rePT and one was a 3rd rePT. Graft loss was defined as the return to insulin therapy. Median recipient

age was 47 years (range 23–59), 31 recipients males and 21 females. Median BMI was 23.4 kg/m^2 (range 16.9 – 33.2). Median donor age was 31 years (range 12 – 51), 31 were males and 21 were females. Median donor BMI was 22.9 kg/m^2 (range 17.3 – 30.3). Waiting time for rePT ranged from 0 to 88 months. Median cold ischemia time was 14 h (range 8 – 22). Acute rejection occurred in 11 patients, in 6 of them resulting finally in graft loss. Morbidity and early reoperation rates were 71.1% and 36.5%, respectively. After a median follow up of 47 months (range 1 – 181) patient and graft survival was 86% and 55% at 5 years, respectively. There was a tendency towards better 5-year graft survival in reSPK, however, without reaching statistical significance. Multivariate analysis aimed at describing variables influencing graft survival identified two independent risk factors for graft loss: acute rejection ($p = 0.012$) and early surgical complications ($p = 0.021$). In contrast, patient survival was not affected. Pancreas re-transplantation is a valuable option for patients with failure of the previous graft. Centre experience and meticulous immunological monitoring play a crucial role in these highly selected patients.

035 TOLERANCE

O97* **A EUROPE-WIDE SURVEY OF OPERATIONAL TOLERANCE IN KIDNEY TRANSPLANTATION**

Annick Massart[1], Annaïck Pallier[2], Pierrick Guérif[3], Julio Pascual[4], Klemens Budde[5], Goce Spasovski[6], Mehmet Sukru Sever[7], Ondrej Viklicky[8], Søren Schwartz Sorensen[9], Hans De Fijter[10], Marian Klinger[11], Karine Hadaya[12], Chris Dudley[13], Alexander Yussim[14], Rainer Oberbauer[15], Daniel Baron[3], Umberto Maggiore[16], Magali Giral[3], Marc Abramowicz[17], Daniel Abramowicz[18], Sophie Brouard[3]

[1]Erasme Hospital - ULB, Brussels; [2]ITERT and Nantes University; [3]ITERT and Nantes Univesrity; [4]Hospital del Mar, Barcelona; [5]Charité Universitätsmedizin Berlin, Campus Mitte; [6]University Clinical Center, Skopje; [7]Istanbul School of Medicine; [8]Institute for Clinical and Experimental Medicine, Prague; [9]Rigshospitalet, University of Copenhagen; [10]Leiden University Medical Center; [11]Wroclaw Medical University; [12]Geneva University Hospitals; [13]Richard Bright Renal Unit, Bristol; [14]Rabin Medical Center, Petah Tikva; [15]KH Elisabethinen Linz and Medical University of Vienna; [16]Ospedale Maggiore, Parma; [17]Erasme Hospital – ULB; [18]Antwerp University Hospital

Background: Operational Tolerance (opTOL) is deemed to be extremely rare in kidney transplantation. We launched a Europe-wide survey to assess the frequency of opTOL.

Methods: 17 coordinators were recruited for 27 countries through the ERA-EDTA-DESCARTES working group. They sent a questionnaire to 247 transplant centres to identify tolerant (defined as having a S creat <1.7 mg/dL and U prot <1 g/d or L despite at least one year w/o any immunosuppressive drug) or almost tolerant patients (same criteria but with minimal immunosuppression: prednisone <10 mg/day). They also recorded the total number of kidney recipients ever followed at their center.

Results: 144 questionnaires covering 214 690 transplants were returned, which identified 67 tolerant and 51 almost tolerant patients. Hence, tolerance and almost tolerance were declared in 1/3204 and 4209 kidney transplantations, respectively. 27 of the 67 tolerant patients were previously reported. We had insufficient data in 5. Therefore, we describe 35 new tolerant patients who withdrew immunosuppressive medications for noncompliance (n = 32/35). The median number of HLA mismatches was 2.8 (IQR 0.25–3, n = 30). The mean follow-up was 204 $\pm$ 95 months (n = 33). The mean duration of tolerance, according to our strict criteria, was 77 $\pm$ 55 months (n = 32). The mean duration of a functioning transplant not requiring dialysis w/o any immunosuppressive drug, irrespective of creatinine, was 90 $\pm$ 62 months (n = 33). At the end of the observation period, 31/35 patients were alive and 22/31 still tolerant. For the remaining 9/31, 2 were restarted on immunosuppressive drugs and 7 had rising creatinine of which 3 resumed dialysis.

Conclusion: We confirm that the incidence of OpTOL in kidney transplantation is low (declared as1/3178 transplantations), but also that tolerance is only a metastable status with patients harboring graft dysfunction that can culminate in graft loss over time. * The two first and senior authors contributed equally.

009 ETHICS/LAW/PSYCHOSOCIAL/PUBLIC POLICY

O98* THE IMPACT OF CAREGIVER BURDEN IN LIVING DONATION

Hannah Maple, Nizam Mamode
Guy's Hospital London

Introduction: A large number of living kidney donors are also their recipient's primary caregiver. Whilst caregiver burden is a well-established concept, the impact of caregiver status on living donors has never been investigated. We hypothesized that primary caregivers would demonstrate increased psychopathology before donation, but that they would also demonstrate a greater improvement post-operatively due to the positive impact of transplantation on their own lives.
Methods: 100 living kidney donors completed questionnaires prior to surgery and at 3 and 12 months post-operatively. Questionnaires contained validated measures of wellbeing, mood, distress, stress and anxiety.
Results: 43 donors were primary caregivers (53.5% parents; 41.9% spouses). Primary caregivers experienced lower pre-operative scores for wellbeing (31.2 vs. 27.1; $p < 0.001$) and mood (0.81 vs. 0.33; $p = 0.11$) and higher scores for stress (5.5 vs. 3.9; $p = 0.006$), anxiety (12.4 vs. 9.8; $p < 0.001$) and distress (12.4 vs. 8.8; $p = 0.001$). At 3 months the 2 groups were no longer significantly different, principally due to improved scores in the primary caregiver group. By 12 months primary caregiver scores for distress, stress, anxiety and mood had all suffered a decline and therefore primary caregivers were again psychologically more distressed than their non-primary caregiver counterparts.
Discussion: This study has demonstrated that primary caregiver donors are psychologically more distressed before and after donation. The results demonstrated here may reflect a post-transplant euphoria at 3 months, which is no longer present by 12 months. 12 month scores may represent a realisation that transplantation is associated with its own stresses and complications, which in turn are a source of psychological distress. Pre-operative identification of primary caregiver status and interventions to improve psychological wellbeing prior to donation may be beneficial to donors.

O99* A SUCCESSFUL WAY OF INCREASING CONSENT RATE FOR ORGAN AND TISSUE DONATION: TELEPHONE ADVICE BY NEUROPSYCHOLOGIST

Sohal Ismail, E. Kums, B.J.J.M. Haase-Kromwijk, A. Hoitsma, N.E. Jansen
Dutch Transplant Foundation

Background: Family refusal is the main outcome after the donation request. Misconceptions and concerns regarding donation impede next of kin from making a well-considered decision. The donation request is the moment in which such concerns should be addressed by the requester. 'Telephone Advice (neuro)Psychologist' (TAP) is a direct telephone support for physicians who are just about to request the relatives for donation. Thus the aim of this study is to improve physician's communication skills and knowledge regarding the donation request and thereby increase the consent rate for organ and tissue donation.
Method: The study started on the 1st of April and lasted until 31st of December 2014. To determine the effects, the consent rates were compared between physicians who received the TAP intervention and those who did not. Physicians rated their self-confidence with regard to the donation request pre- and post-intervention on a likert scale (1–10) and were also asked to give feedback on this new intervention.
Results: The requestors who received the TAP intervention ($N = 141$) had a significant ($p < 0.001$) higher consent rate (58%) compared to the group ($N = 1563$) who did not receive the intervention (consent rate: 34%). More tissue donor requestors received the intervention (74%) and most interventions took place outside office hours (82%). Furthermore, the physician's confidence in requesting for donation increased by the intervention ($p < 0.001$) and is positively associated with consent to donation (OR: 4,078, 95% CI: 2.07–9.53). The intervention is unanimously experienced as positive and valuable by the participating requestors.
Discussion: Based on these results telephone advice is effective in increasing the consent rate for organ and tissue donation. The next step is 1) to reach more requestors by promoting the intervention and 2) to tailor the advice for the requestors in order to further improve self-confidence.

012 HISTOCOMPATIBILITY

O100* **QUANTIFICATION OF HLA CLASS II-SPECIFIC B CELLS IN SENSITIZED INDIVIDUALS**

Gonca Karahan[1], Yvonne J. H. De Vaal[1], Dave L. Roelen[1], Rico Buchli[2], Frans H.J Claas[1], Sebastiaan Heidt[1]

[1]Leiden University Medical Centre; [2]R&D, Pure Protein LLC

Background: Currently available methods for the quantification of HLA-specific memory B cells from peripheral blood of sensitized individuals are limited to detecting only HLA class I-specific memory B cells. Since the majority of donor specific antibodies formed after transplantation are directed against mismatched HLA class II antigens, we aimed at establishing an ELISPOT assay for detection of HLA class II specific memory B cells.
Methods: Polyclonally activated B cells were transferred into anti-human IgG coated wells after which HLA-specific B cells were detected by biotinylated HLA class II molecules. We first validated the assay using human B cell hybridomas that produce monoclonal antibodies directed at defined HLA class II molecules.
Results: The anti-HLA-DR11 antibody producing human B cell hybridoma was tested against biotinylated HLA-DRB1*11:01 and DRB1*13:03. Spots were detected when HLA-DRB1*11:01 was used as detection matrix whereas no spots were detected against HLA-DRB1*13:03. We found comparable spot numbers in total IgG ELISPOT and HLA class II-specific ELISPOT assays, showing that the HLA class II-specific B cell ELISPOT assay detects all antibody-producing cells of the right specificity. We next tested samples from pregnancy-immunized individuals ($n = 6$) and found memory B cell frequencies ranging from 25 to 756 spots per 106 B cells specific for immunizing HLA class II molecules. Against non-immunizing HLA molecules and in non-immunized males, no spot formation was detected.
Conclusions: The current data indicates that it is possible to quantify memory B cells directed at HLA class II molecules in an ELISPOT system. We are currently extending our observations to HLA class II-specific memory B cells in sensitized kidney transplant recipients. This HLA class II-specific ELISPOT assay, in addition to our previously developed HLA class I-specific assay is of potential value for pre- and post-transplant risk assessment of transplant recipients.

003 CLINICAL IMMUNOSUPPRESSION & BIOLOGICALS

O101*

A NOVEL NON-INVASIVE BLOOD TRANSCRIPTIONAL ASSAY, KSORT, MONITORS ALLOIMMUNE RESPONSE IN THE SAILOR RANDOMIZED MULTICENTER TRIAL

Per Lindner[1], Jana Ekberg[1], Bente Jespersen[2], Karin Skov[2], Sue Hsieh[3], Minnie Sarwal[3]

[1]Transplant Institute, Sahlgrenska University Hospital, Gothenburg; [2]Skejby Hospital, Aarhus; [3]University of California San Francisco

Background: Non-invasive prediction of acute rejection (AR) is a critical unmet need. A In a randomized multicenter trial of a target enrollment of 222 renal transplant recipients, a novel blood transcriptional assay, kSORT was evaluated for its accuracy in diagnosing and predicting biopsy confirmed AR.
Methods: Blood samples were drawn at day 0, 10, months 3, 6, 12 and at clinical graft dysfunction for blinded analysis of kSORT, a customized 17 gene assay (CFLAR, DUSP1, IFNGR1, ITGAX, MAPK9, NAMPT, NKTR, PSEN1, CEACAM4, EPOR, GZMK, RARA, RHEB, RXRA, SLC25A37, RNF130, RYBP) that provides AR high or low immune risk scores. Biopsies were done at engraftment, 12 months post-transplantation and when clinically indicated. The kSORT assay was run on 338 blood samples obtained from the first 79 patients. 22 patients had clinically suspected acute rejection (AR) and 18 were biopsy confirmed.
Results: Of the 18 biopsy confirmed AR episodes, all were cellular and DSA negative; 15 had definite kSORT scores and 3 were intermediate; 14/15 AR had high-risk KSORT scores. 11 AR episodes had prior blood samples collected per protocol in the previous 4 months; 8/11 of the pre-AR samples had high kSORT scores, in the absence of clinical graft dysfunction. Of the 80 biopsy matched blood samples without histological AR, 73 had definite kSORT scores, and 7 had intermediate calls. 67/73 blood samples matched with biopsies without AR, had low-risk kSORT scores.
Conclusion: The kSORT assay in a randomized prospective multicenter trial in renal transplantation, confirms that the assay has 93.3% sensitivity and 90% specificity and 98.6% NPV for the non-invasive diagnosis of AR, and is not confounded by BK viremia. 73% of AR could have been diagnosed by the kSORT assay days-months prior their current time-line for diagnosis based on the serum creatinine alone, supporting the use of this assay for serial monitoring of rejection risk and proactive immunosuppression customization.

025 LIVER

O102*

EUROPEAN ELITA ELTRE MULTICENTER SURVEY ON THE MANAGEMENT OF BILE DUCT DURING LIVER PROCUREMENT, PRESERVATION AND TRANSPLANTATION

Nicolas Meurisse[1], Diethard Monbaliu[2], Paolo Muiesan[3], Andreas Pascher[4], Martin Oliverius[5], Gabriela Berlakovich[6], Rene Adam[7], Ilse Senesael[2], Jacques Pirenne[2]

[1]CHU LiÃ¨ge; [2]Abdominal Transplant Surgery Department, University Leuven, Belgium; [3]Liver Unit, University of Birmingham, United Kingdom; [4]Department of Visceral & Transplantation Surgery, Humboldt University, Berlin, Germany; [5]Transplant Surgery Department, IKEM Prague, Czech Republic; [6]Department of Transplant Surgery, Medical University of Vienna, Austria; [7]Centre Hépato-Biliaire, University Paris Sud, France

Background: Only scarce data are described on what is the best practice to manage the bile duct during procurement/preservation/LTx.
Aim: To characterize the different techniques used among European transplant centers in terms of bile duct management in case of donation after brain death (DBD) and circulatory death (DCD).
Method: an anonymous European web-survey has been sent to surgeons procuring and/or transplanting livers.
Results: 44% responded ($N = 210/475$). 53% of respondent worked as procurement and transplant surgeon in large transplant centers (>50 procurements/year). 5% of surgeons never flush bile duct before cold preservation. If flushed, the bile duct is rinsed-out through both the common bile duct (CBD) and the gallbladder by only 21% and 25% of surgeons in case of DBD and DCD, respectively. The cystic duct is ligated during the procurement of DBD/DCD donors in 33%, whatever the decision concerning cholecystectomy. 46% of surgeons prefer to do a cholecystectomy before implantation in case of DBD/DCD. An arterial back table pressure perfusion is performed by 48% and 54% of surgeons in DBD and DCD LTx, respectively. 2% and 7% of surgeons prefer to perform a hepatic artery reperfusion first in case of DBD and DCD LTx, respectively. 16% do not shorten the CBD (until bleeding) before biliary anastomosis. Protective interventions as donor pretreatment with steroids, fibrinolytics or heparin, prostacyclin analogue in cold preservation solution and recipient treatment with fibrinolytics are described.
Conclusion: Obvious heterogeneity management of bile duct during procurement/preservation/LTx is observed among respondent surgeons in Europe. Internationally recognized guidelines with validated maneuvers to better preserve bile duct are urgently needed, especially with use of less-than optimal livers.

029 PANCREAS

O103* THE ROLE OF THE INCRETIN EFFECT IN PANCREAS TRANSPLANTATION

Shruti Mittal, Rachel Franklin, Edward Sharples, Peter Friend, Stephen Gough
Oxford Transplant Centre

Aim: The incretin effect, associated with prandial insulin secretion, is reduced in abnormal glucose tolerance and is likely to require an intact neuro-endocrine axis to exert its full effect. We have recently reported that 30% of pancreas transplant subjects have abnormal glucose tolerance post-operatively, despite insulin independence and that this is associated with later graft failure. The role of the incretin effect in people receiving a denervated pancreas transplant is unknown. This study aimed to assess the incretin effect after pancreas transplantation.

Method: The incretin effect was measured with extended frequently-sampled oral glucose tolerance tests and matched isoglycaemic intravenous glucose infusions in 10 pancreas transplant recipients and 10 kidney transplant recipients at 2 weeks and 3 months post-transplant, and in 10 healthy controls.
Results: The groups were comparable for demographics. Isoglycaemia was achieved in each group. The pancreas transplant group at 2 weeks post-transplant showed lower glucose disposal compared to the kidney only transplant group and healthy controls (16.0% vs 36.2% vs 52.5%), with an absent incretin effect (−1.5% vs 38.0% vs 48.0%, $p = 0.001$). However, by 3 months, glucose disposal and the incretin effect had improved (16.0%−50.4% and −1.5%−29.1% respectively). GLP-1 secretion was reduced at 2 weeks post-transplant in both kidney and pancreas transplant groups, with some improvement by 3 months.
Conclusion: The present data suggest, for the first time, that pancreas transplantation may be associated with a delay in establishing a full incretin effect, and that suppression of GLP-1 secretion may follow transplant surgery. Whilst we cannot attribute a causal role, future studies including an intervention trial are needed to determine whether incretin based therapies can improve pancreas function and long term pancreas transplant outcomes.

005 COMPOSITE TISSUES

O104* AUTOLOGOUS ADIPOSE-DERIVED STEM CELL INFUSION AS SALVAGE THERAPY FOR RELAPSING REJECTION OF VASCULARISED COMPOSITE ALLOTRANSPLANTATION: A FIRST CLINICAL CASE REPORT

Emmanuel Morelon[1], Jonathan Rodriguez[1], Palmina Petruzzo[1], Jean Kanitakis[1], Odile Damour[2], Lionel Badet[1], Aram Gazarian[3], Fanny Buron[1], Olivier Thaunat[1], Celine Auxenfans[2]

[1]Hospices Civils de Lyon; [2]Hospices Civils de Lyon/Banque de tissus et de cellules; [3]Clinique du Parc Lyon

Infusion of adipose-derived mesenchymal cells (ASCs) can control GvHD and prolong survival of experimental vascularised composite allotransplantations (VCAs), consequently they were used as salvage therapy for relapsing rejection of a VCA.

The patient, a 34 year-old woman, underwent bilateral hand transplantation on 19.02.2007 and received an immunosuppressive treatment (IST) based on steroids, tacrolimus and mycophenolate mofetil, and ATG. Her follow-up was characterized by 9 episodes of acute rejection (AR), which were successfully treated with IV steroids or ATG and two of them with alemtuzumab; Since 2010 the IST included low-dose prednisone, tacrolimus, sirolimus and MMF. Despite the IST change the patient further developed episodes of AR and some complications such diabetes mellitus. Because of the high number of AR episodes recipient ASCs were prepared in GMP facilities and considered as AR treatment.

530 mL of patient's abdominal adipose tissue were lipoaspirated. After collagenase digestion, the stromal vascular fraction contained 60 $\times$ 106 viable cells which were cultured. At passage 1, 2 doses of 180 $\times$ 106 ASC were cryopreserved. After quality and security controls, 108 $\times$ 106 ASCs were IV infused on October 13 and 117 $\times$ 106 ASC on October 20, 2014 after another AR episode, which was considered severe (grade III). IST remained unchanged the first 2 weeks after infusion. The skin biopsies performed one week after the first ASCs infusion showed a milder dermal infiltrate but persisting microvessel thrombosis. Two weeks after the first infusion clinical lesions were still present and corticosteroid doses were increased from 5 to 20 mg per day. 6 weeks after infusion, grafted skin was almost normal. Neither side-effects nor complications were reported.

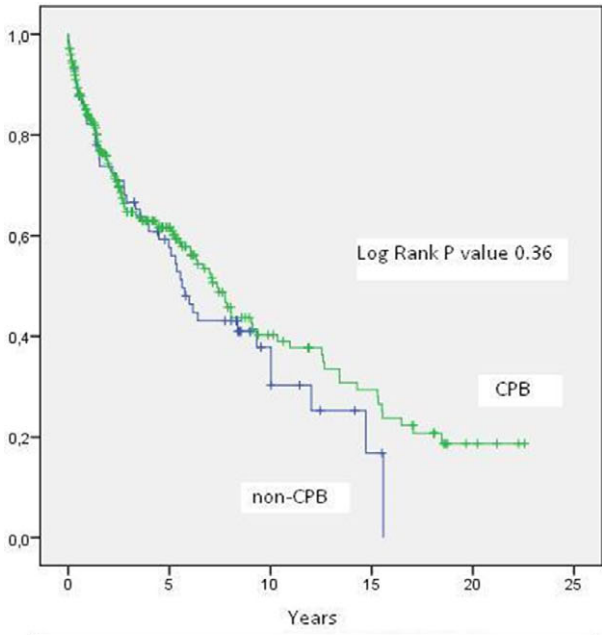
Conclusion: This report documents the favourable risk-benefit ratio of ASC infusion, suggesting that this therapy could be an interesting option for the treatment of refractory acute skin rejection in VCA.

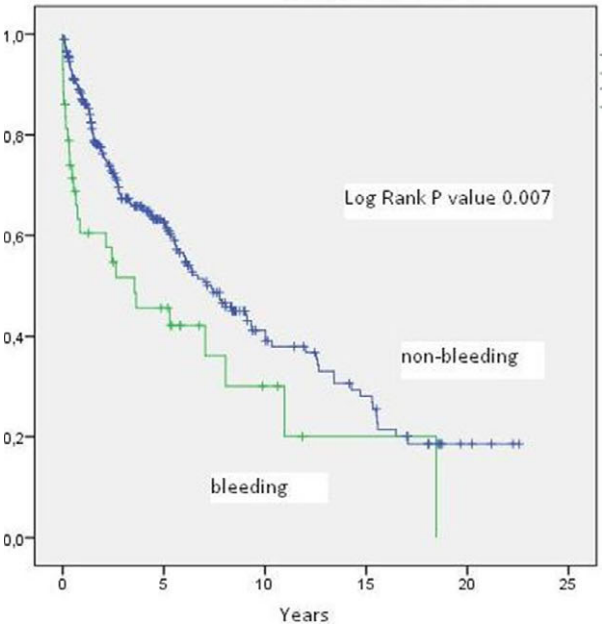
027 LUNG

O105* **SIMILAR SURVIVAL AFTER BILATERAL LUNG TRANSPLANTATION PERFORMED WITH OR WITHOUT CARDIOPULMONARY BYPASS**

Mikhail Zemtsovski, Michael Perch, Christian Møller, Pia Bredahl Jensen, Martin Iversen
Rigshospitalet

Background: Bilateral lung transplantation (BLT) can be performed with or without cardiopulmonary bypass (CPB) depending on recipient's condition and/or surgical ability. The impact of CPB on long-term survival after lung transplantation remains unidentified.
Methods/Materials: A retrospective review of all patients receiving BLT at Rigshospitalet, Denmark between June 1992 and December 2014.
Results: In our center, 323 BLT has been performed including 249 with and 74 without CPB. Main indications were cystic fibrosis (n = 99), alpha1-antitrypsin deficiency (n = 71), chronic obstructive pulmonary disease (n = 49), pulmonary fibrosis (n = 43), sarcoidosis (n = 21), primary pulmonary hypertension (n = 14), and other end-stage lung diseases (n = 26). Overall, 43 patients (13%) were re-operated due to postoperative bleeding. Of these, 40 patients were operated with CPB and 3 without CPB (p = 0.006). Median for survival time was 7.4 years after BLT with CPB and 5.6 years without CPB (p = 0.36). However, patients who experienced postoperative bleeding requiring re-operation had significant shorter survival compared with patients without bleeding (p = 0.007).
Conclusion: Survival after BLT performed with or without CPB was similar. The use of CPB increased the risk of postoperative bleeding. Postoperative bleeding was associated with a poorer overall survival rate.





033 TISSUE ENGINEERING

O106* **TARGETED TISSUE ENGINEERING OF ISCHEMICALLY DAMAGED KIDNEYS**

Lauren Brasile[1], *Nicholas Henry*[1], *Bart Stubenitsky*[2]
[1]*BREONICS Inc.*; [2]*University Medical Center, Utretch*

Introduction: The ability to tissue engineer repair of warm ischemically damaged renal tubule epithelium prior to transplantation would provide the potential to expand organ donor criteria to uncontrolled DCD (uDCD) patients. We have demonstrated that an ex vivo acellular, warm perfusion can resuscitate oxidative metabolism after warm ischemia, restoring cytoskeletal integrity. However, lethally injured epithelial cells are not replaced during a 24 h time frame. We now describe the ability to deliver human progenitor renal epithelial cells (REC) to the renal tubule epithelium with homing to the sites of damage.
Methods: Human progenitor REC were fluorescently labeled with PKH26 red fluorescent cell linker. Discarded ischemically damaged human kidneys were transitioned from cold storage to warm perfusion at 32°C for 24 h. Labeled REC (5.0×10^7) were infused into the renal artery at the rate of 0.5×10^6 per minute.
Results: Human REC were detected within the renal tubule epithelium. By using an ex vivo closed perfusion system we were able to determine the number of human REC that were introduced intra-arterially and the number of REC remaining in the perfusate post-perfusion. More than 90% of the fluorescently labeled human REC were taken up by the kidney and could be detected predominantly in the tubules of the outer medulla (Figure 1). The human REC were not found in the vascular compartment.
Conclusions: These results demonstrate the ability to target tissue engineering of the renal parenchyma during an ex vivo perfusion. The ability to target the delivery of progenitor renal cells to the site of damaged tubule epithelium, along with the corresponding ability to quantify the number of the progenitor cells within the renal tissue represents an advancement in regenerating damaged tissue. Such targeted tissue engineering approaches could provide novel opportunities to support expanding the donor pool with uDCD kidneys.

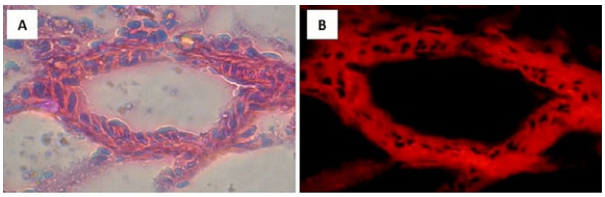


Figure 1. 1A, H&E of renal tubule after 24 hours warm perfusion with RECs. 1B, immunofluorescent image of the same tubule, red = PKH26 labeled RECs.

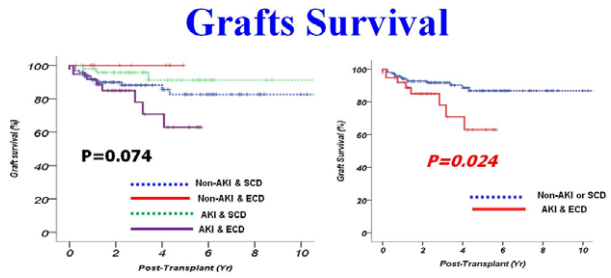
023 KIDNEY

O107 KIDNEY TRANSPLANTATION FROM EXPANDED CRITERIA DECEASED DONORS WITH TERMINAL ACUTE KIDNEY INJURY

Jeongkye Hwang[1], Mi-Hyeong Kim[2], Kang Woong Jun[2], Ahn Sang-Hyun[3], Keun Myoung Park[4], Sang Dong Kim[5], Sun Cheol Park[6], Ji Il Kim[2], Sang Il Kim[7], In Sung Moon[2]

[1]Daejeon St. Mary Hospital College of Medicine The Catholic Univers; [2]Seoul St. Mary Hospital College of Medicine The Catholic Univers; [3]Youido St. Mary Hospital College of Medicine The Catholic Univers; [4]College of Medicine The In-Ha Univers; [5]Incheon St. Mary Hospital College of Medicine The Catholic Univers; [6]Uijeongbu St. Mary Hospital College of Medicine The Catholic Univers; [7]Department of Internal Medicine, Seoul St. Mary Hospital College of Medicine The Catholic Univers

We investigated clinical outcomes of kidney transplantation (KT) from deceased donors with terminal acute kidney injury (AKI) defined acute kidney injury network (AKIN) criteria and expanded criteria donor (ECD). Between February 2000 and December 2013, we performed 202 deceased donor renal transplants from 159 patient defined brain dead in our institution. Of those 159 deceased donor, 38 patients (23.9%) were ECDs and 68 (42.8%) were diagnosed with AKI. According to the ECD and AKIN criteria, we divided 202 recipients into 4 groups: Group I: Non-AKI & SCD (n = 98, 48.5%), Group II: Non-AKI & ECD (n = 15, 7.4%), Group III: AKI & SCD (n = 51, 25.2%), Group IV: AKI & ECD (n = 38, 18.8%). Among four groups, the incidence of delayed graft function was significantly higher in patient with AKI group than in non-AKI group (6.1%, 0%, 25.5%, 23.7%, respectively, p = 0.015). The mean MDRD GFR level at 1 month, 6 months, 1 and 2 years after KT was significantly lower in group IV but MDRD GFR level after 3, 5, 7 10 years did not differ significantly (p = 0.122, 0.708, 0.296, 0.686, respectively). The incidence of acute rejection episodes, surgical complications and infectious complication did not differ significantly among four groups. Actual graft and patient survival rates were similar between groups with a mean follow-up of 40.3 months (p = 0.429, p = 0.221, respectively). There were no significant differences among four groups in graft survival (p = 0.074) and patient survival (p = 0.091). In our center, 42.8% of deceased donor was diagnosed with AKI and 23.8% was ECD. Allograft from AKI with ECD donor commonly showed a higher incidence of DGF and lower allograft function for 2 years after KT. Although there is no significant different in graft and patient survival rates, KT from ECD with terminal AKI have to be considered more careful.



O108 CAN WE REDUCE THE NUMBER OF KIDNEYS THAT ARE RETRIEVED BUT NOT TRANSPLANTED?

Louise Alechinsky[1], Geraldine Malaquin[2], Olivier Huot[2], Benoit Barrou[1]

[1]Hôpital Pitié-Salpêtrière; [2]AGENCE DE LA BIOMEDECINE

Objective: Despite a growing organ shortage, a significant number of deceased donor kidneys is retrieved from donors but not transplanted (RNTK). The aim of the study was to determine the main causes of refusal and analyze if it was justified or not.
Method: This multicenter national study included all RNTK from 2012. Data were collected from the national database Cristal managed by the Agence de la Biomedecine. Each case was retrospectively analyzed by an urologist expert in transplantation, to determine whether the refusal was justified or not.
Results: 252 retrieved kidneys (8.8%) were not transplanted. The main reasons for refusal were as follows: 107 vascular causes (artery (95) or vein (12); 48 tumor suspicion, within the graft (17) or at another site (31); pejorative graft biopsy findings (31); and multiple other factors (66). Among suspected tumors, one third were identified as benign. The refusal was due to iatrogenic lesions for 63 kidneys (25%): 29 complete artery section (main artery in 9 and polar artery in 20 cases), 11 venous injuries, 9 incident during cannulation, 6 kidney capsule lacerations, 5 complete ureteral section and 3 hematoma. Retrospective analysis of refusal causes suggested that 63 kidneys (25%) were probably improperly denied, 115 kidneys (46%) were duly turned down. The analysis was not feasible in 74 kidneys (29%).
Conclusion: A significant proportion of donated kidneys are unduly not transplanted. The solutions to reduce this unnecessary loss of grafts could be
1. the improvement of data collection and transmission
2. the evaluation by 2 experts in the field before turning down an organ
3. an uropathologist on duty for urgent histology

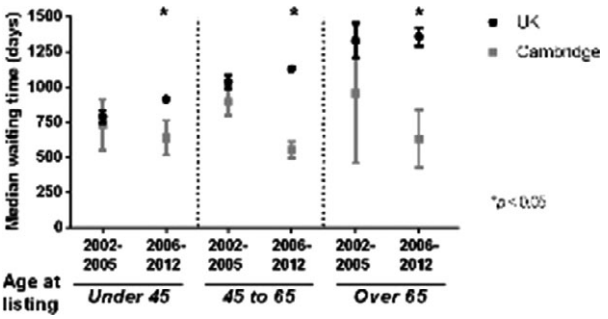
O110* LOCAL EXPANSION IN DCD KIDNEY TRANSPLANT ACTIVITY IMPROVES WAITING LIST OUTCOMES AND ADDRESSES AGE-RELATED INEQUITIES OF ACCESS TO TRANSPLANTATION

Bahar Mirshekar-Syahkal[1], Lisa Bradbury[2], Pamela Sun[1], Mohamed Aly[1], Miriam Berry[1], Joseph Norris[1], Andrew Bradley[1], Gavin Pettigrew[1]

[1]University of Cambridge Department of Surgery; [2]NHSBT

Introduction: An expansion in our circulatory death (DCD) transplant program has resulted in twice as many DCD as brainstem-dead (DBD) kidney transplants being performed in our centre. DBD kidneys are allocated via the national kidney allocation scheme, which strongly favours younger recipients, whereas until 2014 DCD kidneys were all allocated locally. We investigated how a large pool of locally-allocated DCD kidneys influences outcomes for waiting list populations of different ages.
Methods: A retrospective observational cohort study comparing outcomes for adults listed for renal transplantation between 2002 and 2012 according to age at listing: under 45 years (Grp A); 45 to 65 years (Grp B); and over 65 years (Grp C). Data on UK transplant activity was obtained from NHSBT and cross-referenced locally.
Results: Compared to UK data, listed patients of all ages in our centre waited significantly less time for a transplant (Figure). This effect was most apparent for group C patients, whose median time to transplant was much shorter than the national average and notably, comparable to waiting times for our Grp A and Grp B patients.

Proportions of living-donor, DBD and DCD kidneys were strikingly different between the recipient groups in our centre, with Grp A patients receiving an approximately equivalent proportion of each, whereas two-thirds of the kidneys transplanted to Grp C patients were from DCD donors (p < 0.001). The majority of DCD kidneys were, however, still transplanted to younger recipients (n = 131, 244, 46 for Grps A to C, respectively), because fewer elderly patients are listed. Transplantation was associated with a survival benefit from listing for Grps A and B, whereas survival for listed and transplanted patients in Grp C was similar.
Discussion: Local expansion in DCD kidney transplant activity improves survival outcomes from listing in younger patients and may be used to address inequity of access to transplantation for older patients.



O111 EXTENDED CRITERIA DONORS: POTENTIAL FOT TRANSPLANTATION AND EFFECT ON OUTCOMES

Lisa Bradbury, Laura Pankhurst

Organ Donation and Transplantation Directorate, NHS Blood and Transplant, Fox Den Road, Bristol

Background: In the United Kingdom (UK) over the last decade there has been an increase in the proportion of deceased donors (DD) aged ≥60 leading to the use of more marginal donors for kidney transplant. This study investigates whether recipients in the UK should accept an extended criteria donor (ECD) defined as DD aged ≥60 years at the time of death OR aged 50 to 59 years with at least two or three donor characteristics (hypertension, creatinine >130 μmol/l or death due to intracranial haemorrhage) or

remain on dialysis and wait for a possible standard criteria donor (SCD) transplant.
Methods: We analysed the effect of ECD on 5 year survival after first adult kidney only transplant from brain-dead donors between 2006 and 2013, by Cox regression modelling of data from the UK Transplant Registry. We estimated the effect of acceptance of kidneys from ECD on survival and compared it with the effect of remaining on the waiting list for a potential SCD transplant, by analysing all waiting-list registrations during the same period with a risk-adjusted sequentially stratified Cox regression model.
Results: Of the 11 173 kidney only transplants, 4482 (40%) were ECD. Recipients of such kidneys had poorer 5 year survival than those who received an SCD transplant (unadjusted hazard ratio (HR) 2.08, 95% CI 1.81–2.40; adjusted HR 1.29, 1.11–1.50). Patients receiving kidneys from ECD had a similar unadjusted hazard of death after registration than those who remained on the waiting list (HR 1.01, 0·90–1·14).
Conclusions: Although kidneys from ECD are associated with worse outcomes, the individual probability of survival is similar if they are accepted than if they are declined and the patient chooses to wait for a potential transplant from an SCD. Therefore there is no evidence to suggest patients should stop accepting these marginal kidneys which would result in a dramatic increase in waiting time to kidney transplant in the UK.

O112 DONATION AFTER CARDIAC DEATH (DCD): A SOLUTION TO THE SHORTAGE OF KIDNEY TRANSPLANTS?

Pierre-Olivier Delpech[1], Jérôme Tourret[2], Louise Alechinsky[2], Nadia Arzouk[2], Saida Ourahma[2], Benoit Barrou[2]
[1]CHU Poitiers; [2]CHU Pitié Salpêtrière

Faced with the shortage of kidney transplants, donation after cardiac death (DCD) is an alternative with a risk of delayed graft function (DGF) and primary nonfunction (PNF) more important but long-term results comparable to deceased donation brain death (DBD). The objective of the study was to evaluate the DCD and identify risk factors for graft loss.

This retrospective monocentric study from 2007 to 2013 compared the DCD and DBD with standard criteria donors (SCD) and expanded criteria donors (ECD). The characteristics of donors, transplant and follow-up until 60 months were compared. The survival curves were performed by Kaplan-Meier test, and univariate and multivariate analysis were performed.

476 grafts were studied including 78 DCD, 198 SCD and 141 ECD. The study of serum creatinine level did not show any difference between the DCD and SCD at 5 years but was better than EDC. In DCD group, the transplant waiting times were significantly shorter compared to the SCD ($p = 0.025$) and EDC groups ($p < 0.0001$) as well as waiting times on dialysis. The ischemia time, the DGF and HLA incompatibilities were significantly higher in the DCD group ($p < 0.0001$ respectively), but did not appear to be risk factors for graft loss. The donor age appeared to be a risk factor for graft loss ($p = 0.03$) and death of recipients ($p = 0.004$), as well as cardiovascular risk factors. Five-years graft survival was similar in the three groups, but patient survival was better in SCD and DCD groups than in the ECD group ($p = 0.0014$).

DCD kidney transplantatin is a promising source of grafts with better results than EDC, and could increase up to 16–40% the number of donors. It is necessary to respect the protocol criteria established for this type of donors.

O113 PREDICTIVE FACTORS FOR RENAL FUNCTION AFTER KIDNEY TRANSPLANTATION FROM DONORS AFTER UNCONTROLLED CIRCULATORY DEATH

Maureen Assayag, Arzouk Nadia, Sarah Drouin, Philippe Rouvier, Jerome Tourret, Emmanuel Vanglabeck, Saida Ourahma, Mathieu Raux, Jerome Parra, Bruno Riou, Benoit Barrou
Hôpital de la Pitié Salpétrière

Background: Recipients, donors selection, preservation technique, are now well codified in most centres for kidney transplantation from donors after circulatory death (Maastricht I-II DDAC), but the predictive factors for renal function remain to be determined.
Methods: This monocentric, prospective cohort study was designed to determine the predictive factors of a poor renal function at 3 months after kidney transplantation from uncontrolled DDAC. Low renal function was defined as an estimated glomerular filtration rate (eGFR) lower than 45 ml/min.
Results: From August 2008 and October 2014, 88 patients received a DDAC kidney transplant. Four of those were excluded (death or graft loss before 3 months), and 84 were included in the study: group 1 ($n = 40$) with eGFR <45 ml/min/1.73 m² and group 2 ($n = 44$) with eGFR ≥45 ml/min/1.73 m². Both groups were similar in terms of recipient and donor age, HLA mismatches and pretransplant hemodialysis duration. In the univariate analysis, significant predictive factors for eGFR <45 ml/mn/1;73 m^2 at 3 months were body mass index (BMI) ($p = 0.0170$), donor BMI ($p = 0.0009$), donor eGFR ($p = 0.0095$), number of CMV infections ($p = 0.0056$) and local procurement ($p = 0.004$) were associated with a low renal function. In the multivariate analysis, significant factors were recipient BMI (OR = 1,212 (95% CI, 1,034–1,420)), donor BMI (OR = 1,176 (95% CI, 1,018–1,358), donor eGFR (OR: 0,961 (95% CI 0,927–0,997), the use of normothermic reperfusion (nRP) in the donor (OR = 0,226, 95%CI 0,063–0,804). Cold Ischaemic time, delayed graft function and histological patterns in preimplantation biopsies were not significatively different between the 2 groups.
Conclusion: Recipient BMI, donor BMI, donor eGFR, and the use of nRP circuit are predictive factors for eGFR >45 at 3 months after transplantation from uncontrolled DDAC.

O114 PEDIATRIC EN BLOC KIDNEY TRANSPLANTATION FROM DONATION AFTER CARDIAC DEATH (DCD) DONORS VS. BRAIN-DEAD (BD) DONORS: MATCHED-PAIR ANALYSIS OF 130 EN BLOC TRANSPLANTS FROM VERY SMALL (≤10 KG) DONORS

Christoph Troppmann[1], Chandrasekar Santhanakrishnan[1], Ghaneh Fananapazir[2], Kathrin Troppmann[1], Richard Perez[1]
[1]Department of Surgery, University of California, Davis, Medical School;
[2]Department of Radiology, University of California, Davis, Medical School

En bloc kidneys (EBKs) from very small BD pediatric donors are increasingly considered for transplantation (Tx) to help address the donor organ shortage. There has been reluctance to apply the same approach to very small DCD donors, in part because of the perceived added risk that results from the warm ischemia time associated with DCD. The impact of DCD on short- and long-term outcomes of small pediatric EBKs has therefore not been systematically studied.
Methods: We reviewed our single center experience from 12/2007 to 12/2014 with 65 consecutive EBK Txs from DCD donors ≤10 kg (median age, 2 months [range, 0.03–24]; $n = 37$ [57%] weighed ≤5 kg). We pair-matched the 65 EBK DCD donors with 65 EBK BD donors based on donor weight, terminal donor creatinine (Creat), and cold ischemia time. DCD and BD grafts were placed on pulsatile hypothermic perfusion pre-Tx. All recipients were given a 5-day induction with Thymoglobulin® followed by steroid-free Tacrolimus-MMF maintenance. Pairwise comparisons of continuous variables were performed with the Wilcoxon test for paired samples.
Results: For DCD donors, median warm ischemia time was 34 min (range, 10–84).
Conclusions: Kidney graft function (as measured by Creat) and long-term graft survival were similar for DCD vs. BD donor EBK Txs. Against the background of a median cold ischemia time >24 h for both groups, overall thrombotic complication rate was slightly higher, but not statistically significantly different, for Txs from DCD vs. BD donors.

The results of our controlled study suggest that DCD—as compared to donation after BD—does not impart a higher risk for adverse outcomes for grafts from donors ≤10 kg. Our favorable long-term results suggest that small pediatric en bloc DCD donors should be considered as an important option to augment the deceased donor pool.

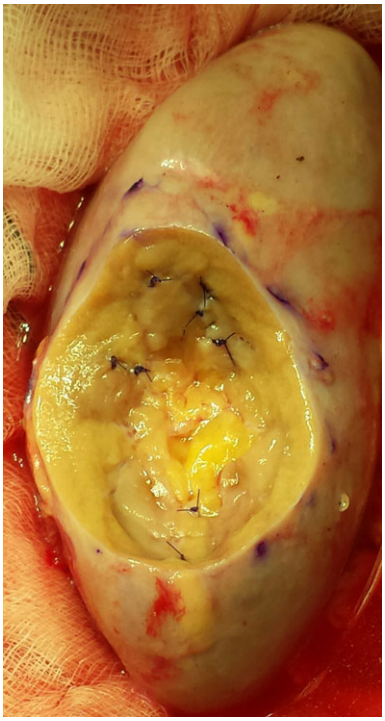
	DCD Group* ($n = 65$)	BD Group* ($n = 65$)
Donors and grafts		
Median donor weight (range) [kg]	5.0 (1.9–10.0)	5.0 (2.6–10)
Median terminal donor Creat (range) [mg/dL]	0.3 (0.1–1.9)	0.3 (0.1–1.6)
Median cold ischemia time (range) [min]	1483 (525–1616)	1502 (685–3002)
Recipients		
Median recipient weight (range) [kg]	60 (44–83)	63 (32–96)
Delayed graft function, *n*	17 (26%)	8 (12%)
Thrombosis event (of one or both kidneys)	7 (11%)	6 (9%)
Median Creat at 1 mo (range) [mg/dL]	2.8 (0.9–9.1)	2.4 (1.05–9.2)
Median Creat at 6 mo (range) [mg/dL]	1.1 (0.62–3.2)	1.1 (0.55–3.13)
Median Creat at 3 year (range) [mg/dL]	0.67 (0.39–1.19)	0.78 (0.4–2.4)
1-year Graft survival	89%	87%
3-year Graft survival	87%	87%

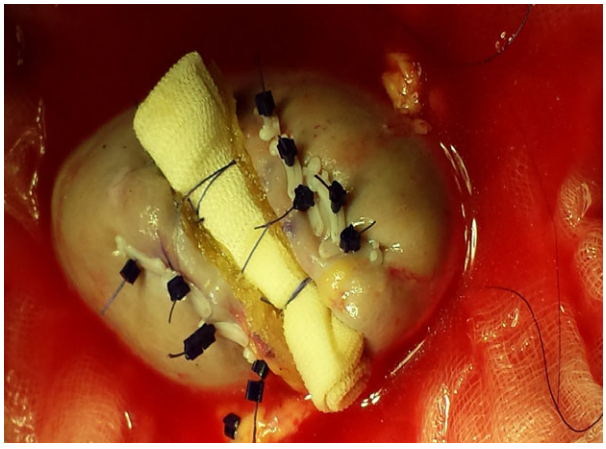
*p = *not significant (≥0.05) for all DCD vs. BD comparisons*

O115 IMPLANTATION OF KIDNEYS FOLLOWING TUMOUR EXCISION FOR MALIGNANCY; COMPLICATIONS AND EXPERIENCE: A CASE SERIES

Rachael Coates[1], Faryal Afridi[1], Muhammad Khurram[1], Carter Noel[2], Naeem Soomro[1], David Rix[1], David Talbot[1]
[1]Freeman Hospital; [2]Sunderland University

Introduction: Annual incidence of T1a renal cel carcinoma is approximately 4500 which is mostly treated with radical nephrectomy with the kidney being discarded. There is potential for use of these discarded organs particularly in high risk recipients with limited chances of qualifying for a deceased donor transplant. This stems from reports of accident and intentional kidneys with RCC transplanted with low recurrence rates. This series reports our initial experience of using kidneys with tumours after partial nephrectomy and tumour resection.
Methods: Donors identified from staging CT following independent decision to undergo radical nephrectomy. Elderly high risk and poor HLA match recipients ($n = 4$) identified from local transplant waiting list. Technique: Tumour were excised under direct vision with or without US guidance. Calyces are then oversewn and subsequently surgicel and/or tachosil is sewn into the defect. After preparation, kidneys were transplanted using standard transplant techniques.
Results: Mean recipient age was 72. There was 1 early graft loss from renal vein thrombosis, one urinary leak treated conservatively and one case of AV malformation managed with angio-embolisation. No tumour recurrence is seen to date.
Discussion: Using kidneys after partial nephrectomy for RCC has the potential for improving quality and quantity of life in marginal recipients otherwise unlikely to receive a transplant. There has been no timor recurrence in this series however longer followup is required.

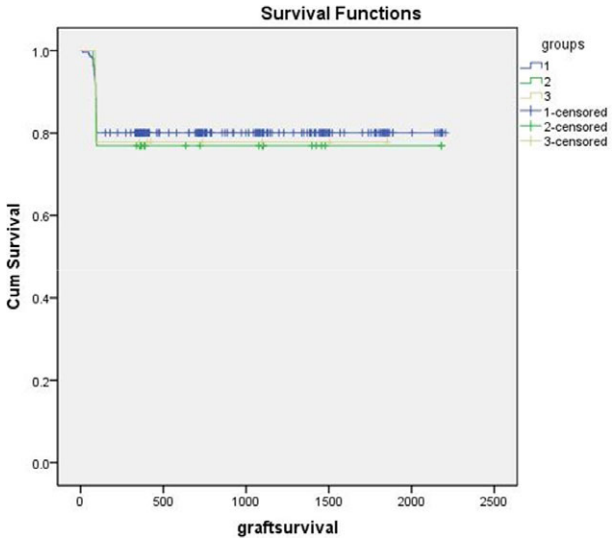




O116 ACUTE KIDNEY INJURY IN DECEASED ORGAN DONORS: OUTCOME ANALYSIS OF RENAL TRANSPLANTS USING RIFLE CRITERIA.

Amro Elboushi[1], Eman Alkizwini[2], Qurat Hussain[2], Madiha Abbas[3], Tamer Ghatwary[1], Atul Bagul[2], Mohamed Morsy[2], Abbas Ghazanfar[2]
[1]Vascular surgery department, Zagazig university; [2]St Georges university hospitals foundation trust; [3]Croydon university hospital NHS trust

Correspondence author: Mr Abbas Ghazanfar; Consultant Transplant Surgeon & Honorary Senior Lecturer St George's University Hospitals NHS Foundation Trust London United Kingdom. Email: abbas.ghazanfar@stgeorges.nhs.uk
Background: Deceased donor kidneys (DDK) are often subjected to acute kidney injury (AKI) before procurement. This AKI is secondary to both ischaemic-hypoxic-insult and nephrotoxic injury. This injury is mostly reversible if the patient survives through the critical phase. However use of such kidneys can have an impact on the outcome of renal transplant (RTx). In this study we have analysed the outcome of RTx from DDK with AKI. We used Risk, Injury, Failure, Loss and End stage kidney disease (RIFLE) criteria developed by ADQI (Acute Dialysis Quality Initiative) to classify DDK.
Material and Methods: In this retrospective study we analysed our RTx data between 2008 and 2013. We recorded donor and recipient demographics and analysed outcome of transplants in terms of allograft survival at 6, 12 and 24-months. Statistical Package for the Social Sciences (SPSS 19) was used for data analysis. Differences between the characteristics of groups were compared using one-way ANOVA test and chi-square for categorical variables. Kaplan Meier was used for graft survival analysis and difference in groups analysed using log rank test. p value of <0.05 was considered statistically significant.
Results: There were a total of 332 deceased donor RTx during this period. We divided them into; Control group ($n = 294$), risk group ($n = 28$) and injury group ($n = 10$). There were no DDK in failure or loss groups. Graft outcomes in *table 1* and Kaplan Meier graft survival is shown in *figure 1*.



Conclusion: DDK with AKI have similar long-term outcome as for DDK without AKI. Therefore they can safely be an addition to the deceased donor pool.

Mean S/creat	Control [95% CI] (n = 294)	Risk [95% CI] (n = 28)	Injury [95% CI] (n = 10)	Sig p value
3-months	148.5 [139.7–157.4]	156.3 [130.1–182.6]	136.4 [104.6–168.2]	0.755
12-months	138.2 [128.6–147.7]	139.8 [117.2–162.1]	120.7 [98.0–142]	0.764
24-months	140.4 [127.2–153.5]	161.4 [118.4–204.5]	122.4 [95–155]	0.555

O117 EARLY RESULTS OF DUAL KIDNEY TRANSPLANTATION – EXPANDING THE DONOR POOL

Tahawar Rana[1], Usman Khalid[1], Laszlo Szabo[1], Argiris Asderakis[1], Jack Jones[2], Elijah Ablorsu[1]

[1]University Hospital of Wales Cardiff; [2]Cardiff University School of Medicine

Background: The most common reason for declining potential kidney donors is age coupled with Diabetes Mellitus (DM) and/or Hypertension (HTN). The implantation of both kidneys from such donors into a single patient can provide a positive result. Materials & Methods

Donors considered for DKT included: 1) DBDs older than 70 with DM, HTN or both, 2) DCDs older than 65 with DM, HTN or both, and 2) all DCD donors older than 70. Recipient exclusion criteria included: history of DM, Adult Polycystic Kidney Disease, severe Cardiovascular Disease, Clopidogrel/ Warfarin therapy and BMI >31. Both kidneys were implanted on the same side. We compared outcomes of consecutive DKT performed between 6/2010 and 5/2014 with single kidney transplants from matched donors. Data was collected prospectively in a computerised database and function, survival and complication rates were calculated.

Results: 34 recipients received DKTs (88% DCDs) and 51 ECDs were transplanted over that period. The median recipient age for DKTs was 67.5 (52 – 80) compared to 65 (38 – 75) in the control (p = 0.02). Mean eGFR was significantly higher at six months (44.6 vs 35.4, p = 0.005) and one year (46.7 vs 34.9, p = 0.0009). This difference increases when comparing the donors over 70 years of age (at 6 months 46.4 vs 35.6, p = 0.006, & at 12 months 46.5 vs 34.3, p = 0.0005). The DKT group had lower Delayed Graft Function rate (79% vs 82%, p = 0.73), though Primary non-function had a higher incidence (9% vs 2%, p = 0.14). One-year graft survivals for the DKT and matched groups was 88% and 96%, whereas 4-year graft survival 88% and 87% (p = 0.47). One-year patient survival 93% and 98%, while 4-year survival was 75% and 86% (p = 0.13).

Conclusion: Function of grafts from older donors with HTN and DM, which are still considered 'not suitable for transplant' is significantly superior if performed as DKT. Graft function and survival are also improved.

O118 INCREASED RISK OF INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY IN CONTROLLED DONATION AFTER CIRCULATORY DEATH KIDNEY TRANSPLANTATION

Laurent Weekers[1], Hieu Le Dinh[2], Catherine Bonvoisin[3], Martina Milicevic[3], Stephanie Grosch[3], Olivier Detry[4], J-P Squifflet[4], Michel Meurisse[4]

[1]CHU Liege; [2]Department of Urology University of Medicine PHAM NGOC THACH; [3]Department of Nephrology, University of Liege, CHU Liege; [4]Department of Surgery & Transplantation, University of Liege, CHU Liege

Introduction: Comparable transplant outcomes between controlled donation after circulatory death (cDCD) and donation after brain death (DBD) kidney transplantation (KT) have been confirmed. However, few data describes the histology of cDCD-KT which is subjected to prolonged procurement warm ischemia. This study aimed to evaluate the rate of interstitial fibrosis (IF) and tubular atrophy (TA) on the surveillance biopsy performed in our unit between the 2 and 6 months post KT. Acute rejection was considered as secondary endpoint.

Patients and Methods: 330 KT (226 DBD and 104 DCD) have been performed between 2008 and 2014. Surveillance or per-cause biopsy was performed in 272 recipients. Among them, the rate of adequate (≥8 glomeruli and ≥1 large-sized artery) was 76.8%.

Results: IFTA was found in 11.5% and 25.7% of DBD and cDCD-KT, respectively (p = 0.004). Considering IF and TA separately, the corresponding rates were 20.4% vs 32% (p = 0.04) and 23% vs 36% (p = 0.03), respectively. If acute rejection before routine biopsy was excluded, either IF or TA rate was significantly higher in cDCD- than DBD-KT (12.6% vs 27.1%, p = 0.006; 17.6% vs 31.4%, p = 0.016; and 20.9% vs 35.7%, p = 0.015 in case of IF-TA, IF, and TA, respectively). A cDCD-KT compared to a DBD-KT was 3.11 (95%CI 1.51–6.43, p = 0.002), 2.34 (95%CI 1.21–4.53, p = 0.011) and 2.29 (95%CI 1.23–4.27, p = 0.009) times more likely to have IFTA, IF, and TA, respectively. Extended criteria donor (ECD) vs standard criteria donor (SCD) was also an independent risk factor for IFTA (OR = 3.11, 95%CI 1.51–6.43, p = 0.002), IF (OR = 4.86, 95%CI 1.96–12.05, p = 0.001), and TA (OR = 4.09, 95%CI 1.68–9.93, p = 0.002). The rate of acute rejection diagnosed by SB was 7.1% and 8.9% in DBD and cDCD kidney grafts (p = ns), respectively.

Conclusion: KT from cDCD increased the risk of IF-TA between 3 and 6 months post-transplant. Further studies are warranted to investigate the evolution of this phenomenon over time and its effect on graft function.

O119 CAPILLARY C4D PREDICTS ADVERSE KIDNEY TRANSPLANT PERFORMANCE INDEPENDENTLY OF MORPHOLOGICAL LESIONS SUGGESTIVE OF ANTIBODY-MEDIATED REJECTION

Zeljko Kikic[1], Alexander Kainz[2], Nicolas Kozakowski[3], Rainer Oberbauer[2], Heinz Regele[3], Gregor Bond[4], Georg Boehmig[2]

[1]Medical University of Vienna; [2]Internal medicine III, Division of Nephrology and Dialysis, Medical University of Vienna; [3]Institute of Clinical pathology, Medical University of Vienna; [4]gregor.bond@meduniwien.ac.at

Background: Recent data supporting a role of C4d-negative antibody-mediated rejection (AMR) have questioned the diagnostic significance of C4d staining as an independent rejection marker. Nevertheless, considering the presumed role of complement as an important effector of humoral rejection, C4d staining, in addition to a histomorphological biopsy work-up, could help identify a more severe form of AMR.

Methods: This large retrospective clinico-pathological study sought to assess the predictive value of C4d staining on graft survival and function in relation to AMR morphology. Overall, 885 renal transplant recipients subjected to one or more indication biopsies (n = 1976) were re-evaluated for linear capillary C4d staining and the presence of distinct morphological lesions suggestive of AMR, icluding glomerulitis, peritubular capillaritis, capillary microthrombi, transplant glomerulopathy, and severe intimal arteritis.

Results: C4d-positive patients, with or without AMR features, had worse death-censored eight-year graft survival (53% or 67%) than C4d-negative patients (67% or 81%; p < 0.001). In Cox regression analysis, C4d posed a risk of graft loss independently of baseline confounders and AMR morphology [hazard ratio: 1.85 (95% confidence interval: 1.34–2.57), p < 0.001]. Moreover, in a mixed model, C4d was independently associated with a steeper decline of estimated glomerular filtration rate (slope per year: −8.23 ± 3.97 ml/min/1.73 m2, p < 0.001). As shown in a multivariable spline interaction model, C4d conferred a particular risk of graft loss, additively to the effects of AMR morphology.

Conclusions: Our study supports the concept that detection of intragraft complement activation represents a specific AMR marker indicating adverse kidney transplant outcomes.

O120 DIFFUSE PERITUBULAR CAPILLARITIS IN RENAL ALLOGRAFT REJECTION: AN INDEPENDENT RISK FACTOR FOR GRAFT LOSS

Nicolas Kozakowski[1], Harald Herkner[2], Georg Boehmig[3], Gregor Bond[3], Heinz Regele[1], Christoph Kornauth[1], Zeljko Kikic[3]

[1]Institute of Clinical pathology, Medical University of Vienna; [2]Department of Emergency Medicine, Medical University of Vienna; [3]Internal medicine III, Division of Nephrology and Dialysis, Medical University of Vienna

Background: According to the Banff classification the score of peritubular capillaritis (ptc), its extent and its cellular composition should be routinely reported in renal allograft pathology. While ptc score represents an important diagnostic and prognostic variable, the clinical value of ptc extent or composition has yet to be determined.

Methods: This retrospective study included 749 renal transplant recipients subjected to 1322 indication biopsies. The effect of ptc and its qualities on graft loss was estimated using proportional hazards Cox regression models. Potential confounders for multivariate analysis were: baseline immunosuppression, C4d positive graft dysfunction, acute T-cell mediated rejection = Banff ≥Ia, re-transplantation, HLA mismatch and pre-sensitization (CDC PRA >10%).

Results: The prevalence of ptc scores 1, 2 or 3 in biopsy specimens was 10.7%, 11.6% and 2.6%, while focal and diffuse ptc (inflammation of >50% of cortical PTC in the biopsy core) was diagnosed in 10.5% vs. 14.4%, respectively. Mononuclear, granulocytic and mixed ptc was present in 13.1%, 3.3% and 8.5%, respectively. While ptc without further sub-classification was not related to higher allograft loss rates, ptc 3 [HR = 2.57 (CI: 1.25–5.28), p

= 0.01] and diffuse ptc [HR = 1.67 (CI: 1.1–2.54), p = 0.015] were independent risk factors for allograft loss. In contrast to ptc 3, diffuse ptc remained an independent risk factor for graft loss even after adjustment for multiple rejection episodes or glomerulitis. Moreover, diffuse ptc was independently associated with features of chronic antibody mediated rejection and with greater eGFR decline after 3 years. In contrast, detailed report of leukocytic composition in ptc did not confer additional prognostic information.
Conclusions: Hence, in contrast to typing its infiltrating inflammatory cells, it seems that indicating score and extent of ptc in kidney allograft pathology is compulsory for the assessment of transplant prognosis.

O121 DOES TOTAL INFLAMMATION IN EARLY KIDNEY GRAFT BIOPSIES PREDICT PROGRESSION OF FIBROSIS AND TRANSPLANT FUNCTION AT 1 YEAR POST-TRANSPLANT?

Christina Dörje[1], Dag Olav Dahle[1], Geir Mjøen[2], Karsten Midtvedt[1], Ole Øyen[1], Christian Naper[3], Trygve Syversveen[4], Johnsen Linda Flaa[1], Hallvard Holldaas[1], Anders Hartmann[1], Trond Jenssen[1], Helge Scott[5], Finn Per Reinholt[5], Anna Varberg Reisæter[1]
[1]Oslo University Hospital, Rikshospitalet, Department of Transplant Medicine, Oslo; [2]Oslo University Hospital, Ullevål, Department of Nephrology, Oslo; [3]Oslo University Hospital, Rikshospitalet, Institute of Immunology, Oslo; [4]Oslo University Hospital, Rikshospitalet, Department of Radiology, Oslo; [5]University of Oslo and Oslo University Hospital, Rikshospitalet, Department of Pathology, Oslo

Background: The future impact of inflammation, especially in fibrosis, detected in kidney graft biopsies early after transplantation has not been settled. We investigated whether inflammation (within and outside fibrotic areas) at week 6 could predict progression of fibrosis at 1 year and also influence graft function at year one. A 10% step score was applied to potentially improve sensitivity compared to the Banff classification.
Methods: Renal graft recipients during 2010 with adequate 6 week and 1 year transplant biopsies were included. Standard immunosuppression: Basiliximab, CNI, MMF and steroids. Biopsies were scored by two experienced renal pathologists according to current Banff criteria. Additionally inflammation inside and outside fibrotic areas and fibrosis were scored in a 10-graded semi-quantitative eyeballing system 0–100%. The chronic allograft damage index (CADI) was calculated. Inflammation parameters at week 6 as riskfactors for progression of fibrosis were assessed in linear or logistic regression models for continuous change scores or dichotomous progression scores as appropriate.
Results: 312 biopsies (156 recipients) were included. 114 (73%) were males, mean age donor /recipient 50.6/ 54.1. Sixteen recipients were DSA positive at transplantation, 12 developed de novo DSA and 48 experienced acute rejection within one year. Fibrosis progressed significantly from week 6 to 1 year (Table). No significant positive association was found between any inflammation parameter at week 6 and change in fibrosis evaluated by Banff, in 10% steps or with CADI. Δ eGFR increased by 3.6 ml/min at 1 year. Change in kidney function was not associated with inflammation at week 6.
Conclusion: Inflammation in kidney transplant biopsies at week 6 did not predict progression of fibrosis or graft function at 1 year post-transplant. Scoring in 10% steps did not change these results.

BIOPSYFINDINGS	6 WEEKS	1 YEAR	p-value
Banff interstitial fibrosis (ci), mean ± SD	0.81 (±0.65)	1.13 (±0.87)	<0.001*
Banff tubular atrophy (ct), mean ± SD	1.01 (±0.45)	1.18 (±0.67)	0.04*
ci + ct (IFTA), mean ± SD	1.81 (±0.97)	2.31 (±1.49)	0.002*
10% fibrosis (ci), mean ± SD	0.68 (±1.1)	1.5 (±1.9)	0.001*
10% interstitial inflammation outside fibrosis, mean ± SD	0.3 (±0.8)	0.3 (±1.0)	0.3*
10% interstitial inflammation inside fibrosis, mean ± SD	2.0 (±2.2)	2.0 (±2.2)	0.8*
10% total interstitial inflammation (inside + outside fibrosis), mean ± SD	2.3 (±2.8)	2.3 (±2.7)	0.4*
CADI, mean ± SD	3.4 (±2.1)	3.8 (±2.6)	0.5*
LINEAR REGRESSIONANALYSIS of change in 10% Fibrosis from 6 weeks to 1 year	beta	95% CI	p-value
10% interstitial inflammation outside fibrosis	−0.06	−0.55 to 0.25	0.4
10% interstitial inflammation inside fibrosis	−0.06	−0.18 to 0.08	0.5
10% total interstitial inflammation	−0.07	−0.15 to 0.06	0.4

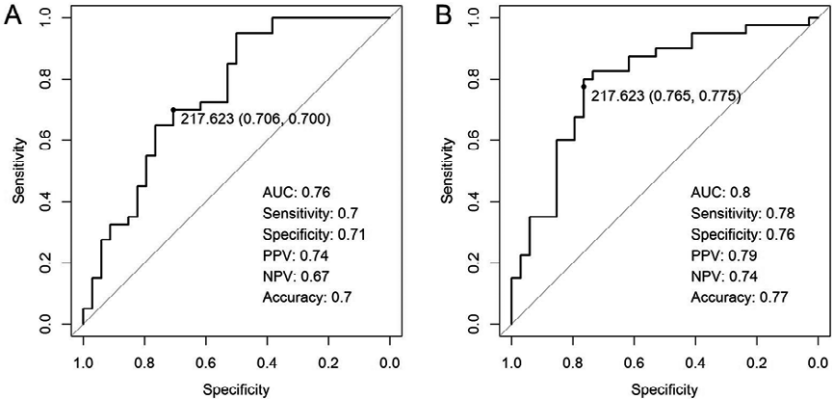
*Wilcoxon matched pair signed rank test.

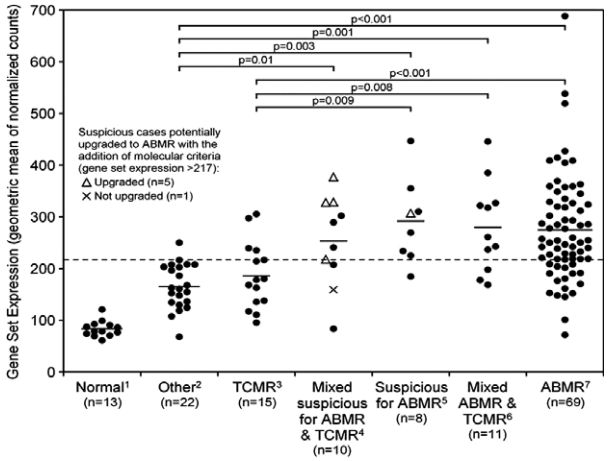
O122* VALIDATION OF A MOLECULAR DIAGNOSTIC FOR ANTIBODY-MEDIATED REJECTION IN FORMALIN-FIXED PARAFFIN-EMBEDDED HUMAN RENAL ALLOGRAFT BIOPSIES

Benjamin Adam, Bahman Afzali, Nikhil Shah, Reeda Gill, Luis Hidalgo, Patricia Campbell, Michael Mengel, Banu Sis
University of Alberta

Background: In 2013, the Banff classification adopted molecular diagnostics as an adjunct for the diagnosis of antibody-mediated rejection (ABMR) in renal allografts. The NanoString gene expression platform is unique in its ability to use formalin-fixed paraffin-embedded (FFPE) tissue samples. We aimed to utilize this method to assess the validity of molecular ABMR diagnostics in routine renal allograft biopsies.
Methods/Materials: NanoString was used to quantify expression of a literature-derived 34-gene set in 148 archival FFPE renal allograft biopsies. Gene set expression was correlated with serologic and histologic data. A diagnostic threshold for ABMR was derived from receiver operating characteristic (ROC) analysis of a 74-biopsy training cohort and applied to a balanced 74-biopsy validation cohort.
Results: Gene set expression correlated with the presence of donor-specific antibodies and histologic lesions of ABMR, but not T-cell mediated rejection (TCMR; Table 1).

Serologic/histologic feature	Correlation coefficient (r)	p-value
Donor specific antibodies		
• Class I or II	0.47	<0.001
• Class II	0.36	<0.001
• Class I	0.22	0.006
ABMR-related lesions		
• Peritubular capillary margination (ptc)	0.38	<0.001
• Transplant glomerulopathy (cg)	0.36	<0.001
• Glomerulitis (g)	0.32	<0.001
TCMR-related lesions		
• Interstitial inflammation (i)	0.15	0.063
• Tubulitis (t)	0.03	0.712
ABMR and TCMR-related lesion		
• Intimal arteritis (v)	−0.03	0.744
Scarring/atrophy-related lesions		
• Mesangial matrix increase (mm)	0.35	<0.001
• Interstitial fibrosis (ci)	0.33	<0.001
• Tubular atrophy (ct)	0.26	0.002
• Arteriolar hyalinosis (ah)	0.24	0.003
• Total interstitial inflammation (ti)	0.29	0.023
• Arterial fibrous intimal thickening (cv)	0.11	0.201
Immunopathology		
• C4d-positive	0.03	0.726
Electron microscopy		
• Peritubular capillary basement membrane multilayering	0.42	<0.001





Increased gene set expression was seen in cases classified by Banff 2013 serohistologic criteria as ABMR, suspicious for ABMR, and mixed ABMR/TCMR, compared with those called TCMR only, normal, or other. Five cases classified as 'suspicious for ABMR' by Banff 2013 would be upgraded to 'ABMR' with the addition of gene set testing (Fig. 1).

Fig. 1: Gene set expression vs. Banff 2013 diagnostic categories. Using the diagnostic threshold derived from the training set (Fig. 2A), gene set diagnosis of ABMR in the validation cohort showed the following performance parameters compared with Banff 2013: sensitivity 0.78, specificity 0.76, PPV 0.79, NPV 0.74, and accuracy 0.77 (Fig. 2B).

Fig. 2: ROC curves for training (A) and validation (B) cohorts.

Conclusion: Our results demonstrate the feasibility of multiplexed gene expression quantification from FFPE renal allograft biopsies. These data suggest a method for molecular ABMR diagnostics to be introduced into routine clinical transplantation pathology.

O123 VALIDATION OF THE NANOSTRING GENE EXPRESSION PLATFORM FOR MOLECULAR REJECTION DIAGNOSTICS IN FORMALIN-FIXED PARAFFIN-EMBEDDED HUMAN RENAL ALLOGRAFT TISSUE

Benjamin Adam[1], Bahman Afzali[1], Katherine Dominy[2], Reeda Gill[1], Candice Roufosse[2], Banu Sis[1], Michael Mengel[1]
[1]University of Alberta; [2]Imperial College London

Background: In 2013, molecular diagnostics were added to the Banff classification with the aim of improving the diagnostic accuracy of standard histology-based assessment in transplantation pathology. The NanoString gene expression platform can be used with formalin-fixed, paraffin-embedded (FFPE) samples and thus has the potential to be integrated into routine pathology workflows. We aimed to evaluate the methodological robustness and feasibility of this platform for molecular transplantation pathology applications.

Methods/Materials: NanoString was used to quantify expression of 34 antibody-mediated rejection-related genes in archival FFPE tissue from 12 allograft nephrectomies and 3 routine renal allograft biopsies (Fig. 1). Six technical replicates were performed with each set of specimens, including 3 different reagent lots, 3 assay operators of varying expertise, and 4 RNA input quantities. Forty-five additional renal allograft biopsy FFPE blocks were tested that had quantitative reverse transcription polymerase chain reaction (qRT-PCR) data available for 11 overlapping genes from corresponding fresh tissue samples. Correlation and ANOVA statistics were performed between technical and platform replicates.

Figure 1: NanoString FFPE workflow.

Results: NanoString gene expression was reproducible across a range of RNA input quantities ($r = 0.99$, $p < 0.001$, Fig. 2A), with different operators of varying technical expertise ($r = 0.99$, $p < 0.001$, Fig. 2B), between different lots of reagents ($r = 0.98$, $p < 0.001$), and using different normalization procedures ($r = 0.99$, $p < 0.001$). Two-way ANOVA confirmed that slight differences in gene set expression are not due to changes in reagent lots or replicate assay runs (inter-lot: $F = 0.0002$, $p = 0.99$; inter-run; $F = 0.02$, $p = 0.88$; combined: $F = 0.004$, $p = 0.99$). Weak correlation was observed between NanoString on FFPE tissue and qRT-PCR on fresh tissue ($r = 0.25$, $p < 0.001$), likely related to challenges with qRT-PCR that include technical difficulty, variability of the reverse transcription step, and lack of histologic confirmation of tissue submitted for molecular testing.

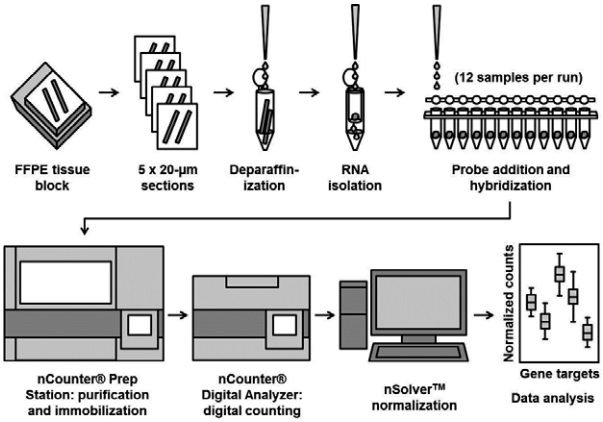
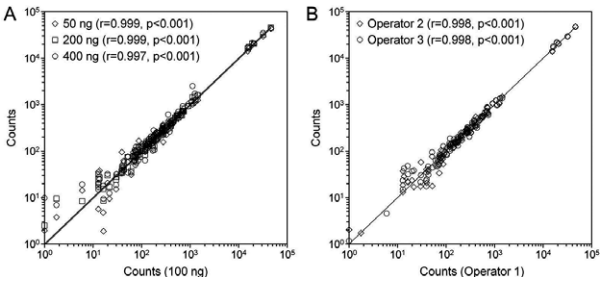


Figure 2: NanoString gene expression correlation between different RNA input quantities (A) and different operators of varying technical expertise (B).

Conclusion: Our results demonstrate the feasibility of multiplexed gene expression quantification from FFPE renal allograft tissue. The NanoString platform produces robust results with routine transplantation pathology specimens and is appropriate for multicenter applications. These data suggest a method for molecular diagnostics to be introduced into existing clinical transplantation pathology workflows.



O124* A MOLECULAR CLASSIFIER FOR CALCINEURIN-INHIBITOR EFFECTS IN KIDNEY TRANSPLANTS IDENTIFIES PATIENTS WITH UNDER-IMMUNOSUPPRESSION AND AT RISK FOR GRAFT LOSS

Gunilla Einecke[1], Jeff Reeve[2], Philip F. Halloran[2]
[1]Department of Nephrology, Hannover Medical School; [2]Alberta Transplant Applied Genomics Centre, University of Alberta

We examined the molecular changes associated with arteriolar hyalinosis (ah) in kidney transplants as a measure of the effect of calcineurin inhibitors (CNIs). Molecular ah (MAH) was estimated by a molecular classifier built in indication biopsies from 562 patients, taken 3 days to 35 years post-transplant, using linear discriminant analysis with 10-fold cross-validation. MAH correlated with histologic ah ($r = 0.50$, $p < 10E-15$). Both increased with time, but histologic ah remained low for the first 18 months and then increased rapidly. In contrast, MAH increased linearly with (log)time starting from the time of transplantation ($r = 0.86$, $p < 10E-15$), indicating that the histologic lesions only start to appear after the underlying molecular changes have reached a certain threshold. MAH correlated with interstitial fibrosis ($r = 0.38$), tubular atrophy ($r = 0.35$), and transplant glomerulopathy ($r = 0.40$) and was inversely related to interstitial inflammation ($r = -0.24$) and tubulitis ($r = -0.28$). MAH was increased in biopsies with antibody-mediated rejection (ABMR) vs no ABMR (0.7 vs 0.56, $p = 7E-07$) and low in biopsies with T cell mediated rejection (TCMR) vs no TCMR (0.3 vs 0.61, $p = 2E-13$) or BK nephropathy (0.35 vs 0.59, $p = 0.0007$). Despite the linear relationship with time, some biopsies had much less MAH than expected (Fig. 1a). These biopsies had a high incidence of TCMR, non-adherence, and increased risk for graft loss (Fig. 1b), indicating that diversion from the MAH-/time relationship is a sign of lack of exposure to CNI. We conclude that MAH in indication biopsies reflects two dimensions: time post transplant and exposure to CNI. Measurement of MAH can identify patients not receiving adequate doses of CNI immunosuppression and provides an estimate of risk which is not captured by the observed histologic hyalinosis lesion changes.

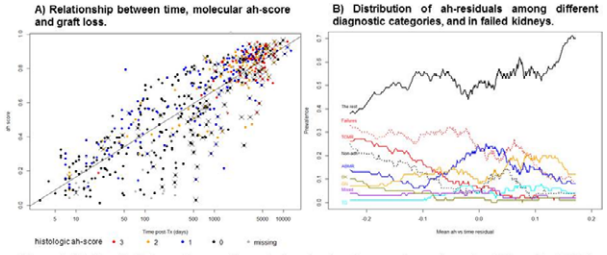


Figure 1. Relationship between time post transplant, molecular ah-score, diagnosis, and graft loss. Panel **A)** The molecular ah-score is plotted against time of biopsy post transplant, illustrating the increasing degree of arteriolar hyalinosis with time. Biopsies from grafts that failed during follow-up are marked with "x". Panel B) While there is a strong linear relationship between the molecular hyalinosis-score and time post transplant, multiple biopsies diverge from the degree of molecular hyalinosis-ness that is to be expected given the time of biopsy post transplant. We assessed whether the diversion from the expected level of arteriolar hyalinosis was associated with specific features of the clinical or histologic presentation at the time of biopsy. The diversion from the expected molecular change (i.e. the regression line) can be quantitated and is called "residual". The graph shows the distribution of ah-residuals in different diagnostic categories and in failed kidneys.

O125 PERIOPERATIVE B CELL ACTIVATING FACTOR (BAFF) LEVEL AS A BIOMARKER FOR PREDICTION OF ANTIBODY MEDIATED REJECTION IN KIDNEY TRANSPLANTATION RECIPIENTS

Wannarat Pongpirul[1], Natavudh Townamchai[2], Wiwat Chancharoenthana[2], Krit Pongpirul[2], Asada Leelahavanichkul[2], Kriang Tungsanga[2], Somchai Eiam-Ong[2], Kearkiat Praditpornsilpa[2], Yingyos Avihingsanon[2]
[1]Bamrasnaradura Infectious Diseases Institute; [2]Faculty of Medicine, Chulalongkorn University

Background: The prediction of antibody mediated rejection (ABMR) has depended mainly on the donor specific antibody (DSA) but only 30–40% of recipients with DSA developed ABMR. As a crucial factor for B-cell activation, differentiation, and antibody production, the B Cell Activating Factor (BAFF) is a potential candidate biomarker. Herein, we explored the association between perioperative BAFF level and ABMR.
Method: This prospective cohort study was conducted in all new kidney transplant (KT) recipients at King Chulalongkorn Memorial Hospital during June 2013 and December 2014. BAFF was measured at the 7th and 180th day post-KT, using ELISA method. Pre-KT DSA was measured by solid phase Luminex® platform. The transplanted kidney biopsy was performed at 180th day for detection of ABMR. Recipients were stratified by pre-KT DSA and BAFF (low vs high, using 500 pg/ml cut point). The risks of ABMR between high and low BAFF KT recipients were compared using Cox proportional hazard ratio (HR).
Results: Seventy-six KT recipients were included with no loss to follow up. The 6-month incidence of ABMR by surveillance biopsy was 17.1%. Overall mean BAFF level at day 7 was 392.3 ± 318.8 pg/ml. Eighteen recipients with high BAFF level at 7th day have significantly higher incidence of ABMR than 58 recipients with low BAFF level (44.4% and 8.62%, respectively, $p < 0.05$). Thirty-nine percent of recipients with positive DSA developed ABMR. High BAFF recipients had 2.07 times higher risk of ABMR than low BAFF recipients, given similar pre-KT DSA. The rates of ABMR among DSA-negative/BAFF-low, DSA-negative/BAFF-high, DSA-positive/BAFF-low, and DSA-positive/BAFF-high recipients were 0, 17.9, 16.7 and 41.7%, respectively ($p < 0.05$).
Conclusion: BAFF can be used as a biomarker and a noninvasive immune monitoring for ABMR in KT. The high BAFF level on day 7th post-KT was significantly correlated with pathological diagnosis of ABMR in both positive and negative pre-KT DSA status.

O126 ANTI-DONOR T-CELL IMMUNITY IS A CONTINUUM IMMUNE PROCESS FAVOURING SUBCLINICAL REJECTION AFTER KIDNEY TRANSPLANTATION

Elena Crespo[1], Marc Lucia[1], Josep Maria Cruzado[2], Segio Luque[1], Edoardo Melilli[2], Josep Maria Grinyó[2], Oriol Bestard[2]
[1]Experimental Nephrology Laboratory, IDIBELL, Barcelona, Spain; [2]Renal Transplant Unit, Nephrology Department, Bellvitge University Hospital, Barcelona University, Barcelona, Spain

Despite current potent immunosuppression (IS), subclinical T-cell mediated rejection (sc-TCR) still unexpectedly occurs in an important number of patients remaining as a main cause of kidney allograft loss. We here prospectively immune-monitored the anti-donor humoral and cellular effector immune responses by means of circulating alloantibodies (DSA) by Luminex and alloreactive T cells (DSTc) using the IFN-g ELispot assay in 135 kidney transplant patients prior to transplantation, at 3 and at the time of 6-mo protocol biopsy in order to investigate their dynamics and association to basic histological allograft lesions. An initial discovery set of 45 patients was used to assess the most sensitive and specific IFN-g Elispot value discriminating sc-TCMR. An independent consecutive cohort of 86 recipients was used as a validation set. The impact on 12 and 24-month allograft function evolution was also analyzed. Using a frequency of 20 IFN-g-producing DSTc as cut-off value (AUC = 0.77; 95% CI 0.56–0.95; Sensitivity 75%, Specificity 83%), 6-mo DSTc were significantly associated to sc-TCR when acute Banff score lesions were classified as IA or higher (13/16 vs 3/16; $p = 0.003$), whereas they were not related to either BL changes (p = NS) or presence of antibody-mediated lesions (p = NS). Interestingly, the absence of circulating DSTc 3 months prior to the protocol biopsy, could also rule out sc-TCR with high accuracy (Sensitivity 80% and NPV = 92.3%). In the multivariate analysis, 3-mo DSTc was revealed to be an independent correlate predicting sc-TCR (RR = 0.115; CI95% 0.018–0.731; $p = 0.022$). In addition, 3 and 6-months DSTc alloreactivity discriminated patients with progressive worse 12 and 24-month allograft function. In summary, DSTc represents a continuum alloimmune process after kidney transplantation in some patients, yielding to subclinical allograft damage and worse allograft function over time.

O127 ASSESSMENT OF CIRCULATING HLA-SPECIFIC ALLOREACTIVE MEMORY B CELLS REVEALS SURREPTITIOUS IMMUNIZED KIDNEY TRANSPLANT RECIPIENTS AT HIGH RISK OF ANTIBODY-MEDIATED REJECTION

Marc Lucia[1], Segio Luque[1], Elena Crespo[1], Josep Maria Cruzado[2], Edoardo Melilli[2], Jaume Martorell[3], Josep Maria Grinyó[2], Oriol Bestard[2]
[1]Experimental Nephrology Laboratory, IDIBELL, Barcelona, Spain; [2]Renal transplant Unit, Nephrology Department, Bellvitge University Hospital, Barcelona University, Barcelona, Spain; [3]Histocompatibility Unit, Immunology Department, Hospital Clinic, Barcelona, Spain

Even though current bead-based multiplex techniques enable an accurate assessment of circulating HLA-specific antibodies, a more comprehensive study of the humoral alloimmune response through the evaluation of circulating HLA-specific memory B cells would allow a better identification of immunized transplant patients at increased risk of ABMR.

Using a novel IgG B-cell Elispot assay we evaluated the impact of circulating alloreactive memory B cells among kidney transplants recipients and compared it to the presence of circulating HLA-specific antibodies in different clinical settings. For this purpose, both class I and II HLA-immunized and non-immunized patients on the waiting list for kidney transplantation as well as patients undergoing acute ABMR were evaluated at time of event and prior to transplantation.

A wide spectrum of both class I and class II alloreactive memory B-cell frequencies was consistently identified in highly HLA-immunized patients in the waiting list for a kidney transplant, whereas none were observed among non-immunized individuals. A more stable detection of alloreactive memory B cells was observed when assessed over time as compared to circulating HLA-specific antibodies. Intriguingly, while some HLA-specific antibodies could not be detected using current Luminex platform in some patients, detection of circulating IgG-ASC against the same target HLA antigen revealed persistence

of humoral immunization. Moreover, high frequencies of alloreactive memory B cells were also observed in kidney transplant patients undergoing acute ABMR at the time of rejection and prior to transplantation. Of note, the higher HLA-specific memory B-cell immune response the more severe ABMR was observed.

Assessment of donor-specific alloreactive memory B-cell frequencies may be of relevance to improve patient alloimmune-risk stratification, and provides new insight into the mechanisms of the adaptive humoral alloimmune response that takes place after transplantation

O128 SOLUBLE CASK, A NEW FACTOR IMPLICATED IN THE RECURRENCE OF FSGS AFTER RENAL TRANSPLANTATION

Severine Beaudreuil[1], Helene Francois[1], Bernard Charpentier[1], Antoine Durrbach[2], Zhang Xiaomeng[3], Hans Lorenzo[3], Jean Jacques Candelier[3], Francis Harper[4]

[1]APHP, INSERM; [2]APHP; [3]INSERM; [4]CNRS

Background: Focal segmental glomerulosclerosis (FSGS) account for 20% of all cases of nephrotic syndrome, both in children and adults. Its recurrence after renal transplantation has suggested the presence of a soluble factor of permeability (sFP) which has not be clearly characterized. In case of recurrent FSGS, the reduction of proteinuria with immunoadsorption on protein A columns (IA) suggest that the sFP can bind to this columns and cad be eluted from them. In order to characterize the sFP, we have analyze the elute from these columns.
Methods: Elutes of IA used for the treatment of recurrent FSGS have been compared to elutes of IA used for the treatment of antibody mediated autoimmune disease on SDS page and different proteins (bands) have been analyze by mass spectrometry. We have identified one molecule cask (calcium/calmodulin-dependent serine-threonine kinase). Recombinant CASK has been produced in *E. coli* (recCASK) to test its effect in vitro on podocyte culture and in vivo in mice.
Results: We have observed a protein of 85 kDa which has been identifed as a serine Threonine Kinase, CASK to be eluted from IA of patients with recurrent FSGS. CASK can be immunoprecipited in sera of those patients but not in healthy donor or patients having a diabetes nephropathy. recCASK impairs the morphology of podocytes in vitro with a redistribution of actin stress fibers, ZO-1, synaptopodin, vinculin and induces albumin permeability of a podocyte monolayer in a transwell assay. The intravenous injection of cask induces proteinuria in mice and electron microscopy analysis of kidneys shows podocyte foot process effacement in treated animal with CASK.
Conclusion: A new soluble form of CASK has been detected in sera from patients with recurrent FSGS. Our preliminary data suggest that CASK would be implicated in the pathogenesis of recurrent FSGS after renal transplantation.

O129 T-CELL MEDIATED VASCULAR REJECTION CAN BE IDENTIFIED BY THE COMBINED MEASUREMENT OF SPECIFIC MICRORNAS IN BLOOD

Mareen Matz[1], Christine Lorkowski[1], Katharina Fabritius[1], Jens Gaedeke[1], Michael Dürr[1], Pawel Durek[2], Joachim Gruen[2], Anne-Kathrin Goestemeyer[1], Friederike Bachmann[1], Kaiyin Wu[3], Birgit Rudolph[3], Ulrike Weber[1], Claudia Haftmann[2], Nadine Unterwalder[4], Nils Lachmann[5], Andreas Radbruch[2], Hans-H Neumayer[1], Mir-Farzin Mashreghi[2], Klemens Budde[1]

[1]Nephrology Charité Berlin; [2]DRFZ; [3]Pathology Charité Berlin; [4]Labor Berlin; [5]Transfusion Medicine Charité Berlin

MicroRNAs hold important roles in the regulation of gene expression. Their function has been correlated with kidney disease and they might represent a new class of biomarkers for frequently evaluating renal graft status. We analyzed their potential in identifying severe T-cell mediated vascular rejection (TCMVR) in kidney transplanted patients. Microarray experiments and real-time RT PCR were performed with RNA from blood cells. Initial microarray analysis revealed 23 differentially expressed microRNAs distinguishing patients with TCMVR from patients with stable grafts. After validation we determined the expression of 6 strongly deregulated microRNAs and two control microRNAs in samples from patients with T-cell mediated rejection (borderline, BANFF I-III), antibody mediated rejection, IFTA, in samples from stable patients and in samples from patients with urinary tract infection. The panel of 6 microRNAs were significantly down-regulated in blood of TCMVR patients compared to the other groups and displayed high sensitivities and specificities for diagnosing vascular rejection by receiver operating characteristics analysis. A subsequent multivariate logistic regression showed the diagnostic value by combining five candidate microRNAs thereby obtaining high AUC, sensitivity and specificity. The combined measurement of five specific microRNAs may help to better identify TCMVR after renal transplantation in a precise and clinically applicable way.

O130 INCREASED PROTEINURIA AND URINARY EXCRETION OF C5B-9 MEMBRANE ATTACK COMPLEXES IN KIDNEY TRANSPLANT RECIPIENTS DEVELOPING ANTIBODY-MEDIATED REJECTION

Miha Arnol, Manca Oblak, Gregor Mlinšek, Jadranka Buturović-Ponikvar, Aljoša Kandus

University Medical Centre Ljubljana, Department of Nephrology

Background: In antibody-mediated rejection (AMR), antibody binding to endothelial cells can lead to complement activation, formation of complement C5b-9 membrane attack complexes and glomerular injury. The relationship between urinary excretion of proteins, complement C5b-9 membrane attack complexes and podocalyxin (PCX)-positive glomerular epithelial cells, and graft histology was explored in kidney transplant recipients with proteinuria.
Methods/Materials: In 2013, 190 patients with proteinuria (spot urine protein/creatinine ratio >20 mg/mmol) and stable graft function were identified from 572 prevalent kidney transplant recipients (33%). We measured 24-h proteinuria and urinary excretion of C5-b9 membrane attack complexes (by ELISA) and PCX-positive cells (by immunofluorescence using anti-human PCX antibody) in 168 patients who consented for the study. In 69 patients (41%) with significant proteinuria (>1 g/day) or subsequent graft dysfunction (increase in serum creatinine >15% from the baseline), biopsy was performed and presence of donor-specific antibodies (DSA) was determined.
Results: Patients in whom biopsy was performed had significantly greater 24-h proteinuria and lower 24-h creatinine clearance as compared with control patients (1126 $\pm$ 818 vs. 508 $\pm$ 377 mg/day; $p < 0.001$, and 47 $\pm$ 19 vs. 56 $\pm$ 19 ml/min; $p = 0.006$, respectively). C5-b9 membrane attack complexes were detected in 19/29 patients with AMR (66%), 6/26 patients with T-cell mediated rejection (TCR) (23%), 3/14 (21%) with no signs of rejection, and in 4/99 control patients (4%) ($p < 0.001$). The detection rate of PCX-positive cells was similar in all patient groups (79%, 81%, 71%, and 77%, respectively; $p = 0.91$). AMR was associated with significant increase in 24-h proteinuria and urinary C5-b9 levels relative to patients with TCR, no rejection or control patients.

Patients with AMR and DSA (21/29) had greater median C5-b9 levels than those with no detectable DSA (130 (0–281) and 11 (0–35) ng/ml; $p = 0.028$). Urine excretion of PCX-positive cells was increased in all patient groups; however, the number of PCX-positive cells did not distinguish between patients with AMR and other patients.
Conclusion: In kidney transplant recipients with proteinuria, greater urinary excretion of proteins and complement C5-b9 membrane attack complexes may be useful noninvasive markers for prediction of active AMR.

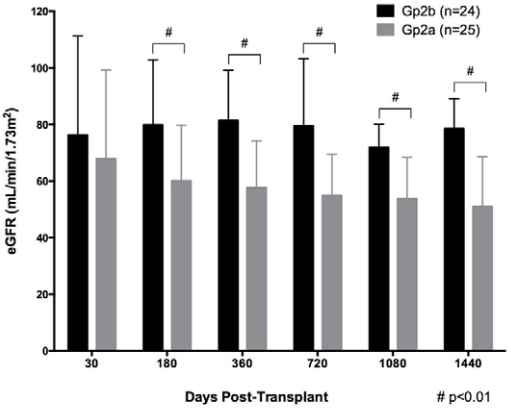
Parameter (median, IQR)	AMR ($n = 29$)	TCR (n=26)	No rejection ($n = 14$)	Control patients ($n = 99$)	p value
24-h proteinuria (mg/day)	148 0(740–2080)	500 (308–740)	450 (308–925)	400 (220–680)	<0.001
C5-b9 complexes (ng/ml)	68.0 (5.5–208.0)	0 (0–36.9)	0 (0–9.0)	0 (0–0)	<0.001
PCX-positive cells (number/ml)	0.20 (0.10–0.80)	0.1 5(0.18–0.51)	0.21 (0–0.53)	0.16 (0.03–0.5)	0.62

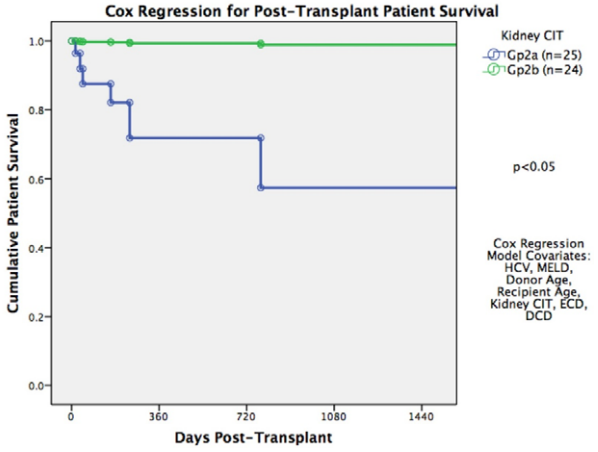
025 LIVER

O131 THE POSTREPERFUSION SYNDROME IS ASSOCIATED WITH DEVELOPMENT OF ACUTE KIDNEY INJURY AFTER DONATION AFTER BRAIN DEATH LIVER TRANSPLANTATION

Marit Kalisvaart, Jubi E. De Haan , Jan N. M. Ijzermans, Frank Grune, Wojciech G. Polak, Herold J. Metselaar, Jeroen De Jonge
Erasmus University Medical Center

Introduction: Up to one third of the donation after brain death (DBD) recipients develop acute kidney injury (AKI) after liver transplantation (LT) and this complication is associated with impaired short- and long-term survival rates. The increasing use of higher risk grafts is associated with more severe hepatic ischemia/reperfusion injury and in this group more AKI is observed. The postreperfusion syndrome (PRS), which is characterized by hemodynamic instability directly after reperfusion, is a probable first manifestation of hepatic IRI. Our objective was (1) to explore the impact of PRS on the development of AKI after DBD LT and (2) to investigate the relationship between PRS and hepatic IRI.
Methods: Development of AKI in the first week after LT, according to AKIN criteria, was retrospectively evaluated for patients who underwent LT from 2008 until 2014 in our hospital. PRS was defined as a >30% decrease of mean arterial pressure (MAP) ≥1 min in the first 5 min after reperfusion. The peak serum AST level in the first 72 postoperative hours was used to measure the severity of hepatic IRI.
Results: 155 recipients were included, of whom 61 (39%) developed AKI. PRS occurred more in the AKI group (AKI 46%; no-AKI 26%; p = 0.013). After multivariable logistic regression analysis with all clinical relevant donor-, recipient-, and intraoperative factors, PRS was independently associated with development of AKI (OR 2.28; 95% CI 1.02–5.08; p = 0.044). The decrease in MAP after reperfusion correlated well with both severity of AKI (p = 0.012) and postoperative AST levels, as marker of hepatic IRI (p < 0.001) (figure 1).
Conclusion: Recipients experiencing PRS during DBD liver transplantation, have an increased likelihood to develop AKI in the first week. Furthermore, the decrease in MAP after reperfusion correlated well with both AKI severity and peak AST levels. Our findings suggest that hepatic IRI plays an important role in the development of AKI after DBD liver transplantation.





O132 DELAYED KIDNEY TRANSPLANT IN COMBINED LIVER-KIDNEY TRANSPLANTATION IMPROVES GFR, GRAFT AND PATIENT SURVIVAL

Burcin Ekser, Richard S. Mangus, Shunji Nagai, Chandrashekhar A. Kubal, Jonathan A. Fridell, John A. Powelson, William A. Goggins, A. Joseph Tector
Transplant Surgery Indiana University School of Medicine

Background: Patients requiring combined liver-kidney transplantation (LKTx) are at risk for worse outcomes compared to LTx and KTx alone due to severity of illness and complexity of the procedure. Delayed function of the renal graft (DGF) can result from hypotension and pressor use related to the LTx procedure. Delayed KTx post-LTx may allow stabilization of hemodynamics and coagulopathy.
Methods: 118 LKTx were performed between 2002 and 2014 at our center. All kidneys underwent continuous hypothermic pulsatile perfusion until Tx; 69 with simultaneous KTx (at time of LTx, Gp1) and 49 with delayed KTx (Gp2) (performed at a later time as a second operation). In each case, the kidney was transplanted through a separate Gibson incision in the left pelvis. All patients received same immunosuppression and continuous veno-venous hemodialysis during the LTx which was continued until KTx. DGF was defined as the need for dialysis in the 1st week after KTx.
Results: Recipient and donor characteristics were comparable in both groups, except more ECD (p < 0.05) and DCD (p < 0.05) donors were used in Gp2. The mean MELD score was 26.3. Mean liver cold ischemia time (CIT) was comparable (<7 h) in both groups, while kidney CIT was 10 ± 3 (range 5–19) in Gp1 and 47 ± 15 (range 20–77) hours in Gp2 (p < 0.001). DGF rate was 7% in Gp1, however no DGF was seen in Gp2 (p < 0.05). Subgroup analyses in delayed KTx group (kidney CIT <48 h [Gp2a, *n* = 25], and >48 h [Gp2b, *n* = 24]) showed better GFR, patient and graft survivals in Gp2b [figure1]. On multivariate analysis, hepatitis C (HCV) (hazard ratio, [HR] = 2.6) and DGF (HR = 10) were significant independent risk factors for patient survival.
Conclusion: Delayed Tx of the kidney post-LTx (especially if it is delayed >48 h) allows; (i) the use of more ECD and DCD kidneys, (ii) better GFR rates starting from 6 months post-KTx with no DGF, (iii) better patient and graft survival in the long-term, and (iv) flexibility for the management of patients and personnel at a high-volume center.

O133 IMPACT OF DYNAMIC CHANGES IN MELD SCORE ON POSTTRANSPLANT SURVIVAL AFTER LIVER TRANSPLANTATION – A EUROTRANSPLANT REGISTRY ANALYSIS

Georg Györi[1], Gerd Silberhumer[1], Axel Rahmel[2], Erwin De Vries[2], Thomas Soliman[1], Sonja Zehetmayer[3], Xavier Rogiers[4], Gabriela A. Berlakovich[1]
[1]Medical University of Vienna, Deot. of Transplantation; [2]Eurotransplant Foundation, Leiden; [3]Center for Medical Statistics, Medical University of Vienna; [4]Department of Surgery and Transplantation, University of Gent

Background: With restricted numbers of available organs futility in liver transplantation has to be avoided. The concept of dynamic changes in MELD score (DeltaMELD) has been shown to be a tool to identify patients with the greatest risk of death after transplantation. Aim of this study was to validate this concept with the Eurotransplant (ET) Database.
Methods: A retrospective registry analysis was performed on all patients listed for liver transplantation (oLT) within ET from 2006 to 2011. Patients <18 y of age, acute liver failure, malignancy and listing for re-transplantation were excluded. From 16821 patients listed for oLT, 9107 met the inclusion criteria. Influence of MELD at listing (MELDon), MELD at transplantation (MELDoff), DeltaMELD, age, sex, underlying disease and time on the waiting list on survival after oLT were evaluated.
Results: Overall the median MELDon was 17 and MELDoff was 20. Waiting list mortality was 25% and mean waiting time 115 days. Age, MELDon and DeltaMELD (p < 0.001) had significant impact on survival on the list. The 1 y and 5 y post-oLT survival were 85% and 69% respectively. Only age and DeltaMELD had influence on the outcome (p < 0.001) with DeltaMELD>10 showing a 1.6× increased risk of death. Generally MELD increase is associated with a poorer post-transplant survival (HR 1.032, p = 0.001). However, patients with DeltaMELD10 did worse compared to those with a DeltaMELD5 or DeltaMELD3 (p = 0.03). Waiting time had no influence on survival post-oLT.
Conclusion: The concept of DeltaMELD validated from the ET data base confirms our previous results that patients with a deltaMELD >10 at oLT have the highest risk of post transplant death. The clinical implementation of this concept would necessitate to place patients with a DeltaMELD >10 temporarily

on hold. This measure would provide no disadvantage to the patients as waiting time has no impact on survival after oLT and might save scarce donor organs and maximize the benefit from oLT.

O134 INTENTION TO SPLIT POLICY: A SUCCESSFUL STRATEGY IN A COMBINED PAEDIATRIC AND ADULT LIVER TRANSPLANT CENTRE

Narendra Battula[1], Marco Platto[2], Khalid Sharif[3], Neto Benhur Ferraz[1], Garrett Roll[1], Hynek Mergental[1], Thamara Perera[1], John Isaac[1], Paolo Muiesan[1], Darius Mirza[1]

[1]Queen Elizabeth Hospital; [2]Liver Unit, Birmingham; [3]Birmingham childrens Hospital

Background: Split liver transplantation (SLT) is an established procedure to expand the organ pool and reduce wait list mortality. Technical and logistic issues are limiting the wider application of this effective technique. The primary aim of this study is to evaluate the role of SLT in a combined paediatric and adult liver transplant centre. The secondary aim is to reflect on our clinical practice and discuss strategies to build a successful split program using an intention to split policy.
Methods: Retrospective analysis of all deceased SLT procedures performed between November 1992 &March 2014.
Results: 3449 liver transplantation procedures were performed. Of them 516 were SLT in 266 children and 226 adult recipients. An ex-situ technique was used with an exception of 4 cases and majority were classical splits. Median donor age was 25 years (7–63 years) and the weight was 70 kg (22–111 kg). 66 were extended criteria donors (>40 years or >90 kg). SLT was used for re-transplant in 24 children and 11 adults. The indication was acute liver failure (ALF) in 24 children and 4 adults. The median waiting time for liver transplantation was 5 days (0–22) for ALF and 59 days (1–636) for end stage liver disease in children. The recipient demographics and complications are summarised in Table 1. The incidence of biliary complications were significantly higher in right lobe recipients ($p = 0.0001$). The overall 1, 5, 10-year patient and graft survival for paediatric recipients was 90%,87%,86% and 87%,83%,82%. For adults it was 83%,76%,62% and 78%,71%,58% respectively. These results were comparable to whole grafts. In the last decade SLT was the main source of organs for the paediatric liver transplant program (70%).
Conclusion: SLT is an acceptable treatment for both adult and paediatric recipients with acute liver failure and end stage liver disease. Splitting a well selected donor is an effective strategy to cope with the increasing demands of a paediatric liver transplant program.

TABLE 02

	Left ($n = 289$)	Right ($n = 219$)
Mean age (years)	4.1 ± 4	45.9 ± 16.1
Mean weight (Kg)	16.3 ± 12.4	68.6 ± 16.6
Cold ischemia (hours)	10.5 ± 26	9.9 ± 2.2
Warm ischemia (min)	40.2 ± 13.7	41.6 ± 10.3
Artery thrombosis	20 (7%)	19 (9%)
Portal vein thrombosis	8 (3%)	10 (5%)
Portal vein stricture	3 (1%)	2 (1%)
IVC stenosis	11 (4%)	1 (0.4%)
Bile Leak	11 (4%)	44 (20%)
Bile stenosis	26 (9%)	21 (12%)
PNF	12 (4%)	3 (1%)

O135 RENAL FUNCTION OUTCOMES WITH PROLONGED-RELEASE TACROLIMUS ACCORDING TO DONOR AGE AFTER DE NOVO LIVER TRANSPLANTATION: A POST HOC ANALYSIS FROM THE DIAMOND RANDOMIZED, CONTROLLED TRIAL

Pavel Trunečka[1], Frank Lehner[2], Wolf O. Bechstein[3], Jacques Pirenne[4], William Bennet[5], Alexey Zhao[6], Helena Isoniemi[7], Lionel Rostaing[8], Utz Settmacher[9], Christian Mönch[10], Malcolm Brown[11], Gbenga Kazeem[12], Nasrullah Undre[12], Giuseppe Tisone[13]

[1]Institute for Clinical and Experimental Medicine; [2]Hannover Medical School; [3]Goethe University Hospital and Clinics; [4]University Hospitals Leuven; [5]Sahlgrenska University Hospital; [6]A.V. Vishnevsky Institute of Surgery; [7]Helsinki University Hospital; [8]Toulouse University Hospital; [9]Jena University Hospital; [10]Westpfalz-Klinikum Hospital; [11]Astellas Pharma Inc; [12]Astellas Pharma Europe Ltd; [13]Policlinico di Tor Vergata

Submitted on behalf of the DIAMOND study group.
Background: To investigate the effect of prolonged-release tacrolimus on glomerular filtration rate (GFR) post-transplant in patient sub-groups stratified by donor age.
Methods: Arm 1: prolonged-release tacrolimus (initial dose: 0.2 mg/kg/day); Arm 2: prolonged-release tacrolimus (0.15–0.175 mg/kg/day) + basiliximab; Arm 3: prolonged-release tacrolimus (0.2 mg/kg/day delayed until Day 5) + basiliximab. All patients received MMF + 1 bolus steroid. Primary endpoint: GFR estimated by Modification of Diet in Renal Disease-4 (MDRD4); sub-analysis: eGFR (MDRD4) by donor age ≥50 and <50 years. All analyses: full analysis set (FAS: $n = 283, 287, 274$) at Week 24.
Results: Baseline characteristics were comparable; mean tacrolimus trough levels were lower in Arm 2 versus 1 for the first 2 weeks post-transplant (Day 14: 7.36 vs 8.62 ng/mL) and remained marginally lower until Week 4 (Day 28: 8.43 vs 8.74 ng/mL). By Day 35, trough levels were comparable in Arms 1–3. Least-square (LS) mean of eGFR in Arms 2 and 3 versus 1 were 76.4 and 73.3 versus 67.4 mL/min/1.73 m^2 ($p = 0.001$, $p = 0.047$). LS mean of eGFR for donor age ≥50 years was higher in Arm 2 versus 1 and 3 ($p = 0.019$, $p = 0.037$) and comparable between Arm 1 versus 3 ($p = 0.830$). For donor age <50 years see table.
Conclusion: At Week 24 in patients with organs from donors ≥50 years, an initial lower dose of prolonged-release tacrolimus (0.15–0.175 mg/kg/day) + MMF + basiliximab (without maintenance steroids) resulted in improved eGFR versus a higher dose of prolonged-release tacrolimus (initial dose: 0.2 mg/kg/day) initiated immediately post-transplant or delayed until Day 5. However, for recipients with organs from donors <50 years, delaying prolonged-release tacrolimus until Day 5 (Arm 3) versus immediate administration post-transplantation (Arm 1) was associated with improved eGFR at Week 24. Discussions regarding potential reasons and the role of confounding factors will be presented.

Table

Renal function (eGFR; MDRD4) at Week 24 stratified by donor age at baseline (FAS)

				P value and treatment difference (95% CI)		
Parameters	**Arm 1**	**Arm 2**	**Arm 3**	**Arm 1 vs Arm 2**	**Arm 1 vs Arm 3**	**Arm 2 vs Arm 3**
eGFR, LS mean in mL/min/1.73m²						
≥50 years	69.9 (n=159)	79.5 (n=161)	71.8 (n=155)	0.019 9.65 (1.41; 17.90)	0.830 1.89 (–6.39; 10.18)	0.037 7.76 (0.49; 15.03)
<50 years	65.4 (n=120)	73.5 (n=124)	76.7 (n=117)	0.079 8.10 (–0.76; 16.97)	0.010 11.29 (2.41; 20.17)	0.419 -3.19 (–10.94; 4.57)

Analysis performed using ANCOVA of eGFR by MDRD4 formula at 24 Weeks after transplantation in patients with donor age of ≥50 or <50 years.

O136 IMPACT OF WAITLIST FRAILTY ON LIVER TRANSPLANT OUTCOMES

Jeffrey Friedman, Joseph Fakhoury, David Cron, Jordan Derck, Jennifer Klei, Michael Englesbe, Christopher Sonnenday
University of Michigan Transplant Surgery

Background: Frailty is associated with decreased waitlist survival and QoL in liver transplant candidates. Yet, it is unknown if pre-TRANSPLANT frailty is associated with post-transplant survival. In a large prospective study of liver transplant CANDIDATES, we studied the association of frailty and post-transplant OUTCOMES.
Methods: 160 of 843 liver transplant candidates in the study have undergone liver transplantation at UMHS. Pre-transplant frailty was assessed using 5 Fried frailty score criteria: grip strength, gait speed, unintentional weight loss, physical activity, and self-reported exhaustion. Composite patient scores ranged from 0 to 5, with scores >2 considered frail. The relationship between frailty and survival was analyzed using Kaplan-Meier curves, log-rank tests, and multivariate Cox regression. Comorbidities and complications were examined using Fisher's exact tests.
Results: The median frailty score in this cohort was 2, mean frailty was 2.4 (SD + 1.3), and 46.9% of patients were frail. Frail patients WERE more likely to have encephalopathy (47.5% v. 26.2%, $p = 0.01$), undergo reoperation due to bleeding (31% v. 7.8%, $p = 0.001$), develop renal failure requiring dialysis (27.6% v. 10.9%, $p = 0.02$), and suffer bacterial or fungal infection (58.6% v. 38.5%, $p = 0.03$; 20.7% v. 7.8%, $p = 0.04$). Survival was significantly lower for frail patients than for non-frail patients (74.7% v. 88.2%, $p = 0.03$, Figure). Frailty WAS associated with increased risk of post-transplant mortality in multivariate analysis (HR = 2.59, 95%CI: 1.12–5.99, $p = 0.03$).
Conclusion: Frail patients have decreased survival following liver transplantation WHEN ADJUSTED FOR OTHER RECIPIENT CHARACTERISTICS and have a lower survival rate than national benchmarks (~88% PER UNOS data). Identifying frail patients prior to transplant may improve recipient selection and enable the creation of prehabilitation programs to ameliorate frailty and improve outcomes.

O137* WAITING TIME IMPACTS ON INTENTION-TO-TREAT SURVIVAL IN PATIENTS WITH HEPATOCELLULAR CANCER WAITING FOR LIVER TRANSPLANTATION: A MULTICENTRE EUROPEAN STUDY GROUP

Quirino Lai[1], Gerd Otto[2], Ivo Graziadei[3], Massimo Rossi[4], Giuseppe Tisone[5], Alfonso W. Avolio[6], Pierre Goffette[1], Michael B. Pitton[2], Wolfgang Vogel[3], Jan Lerut[1]

[1]UCL; [2]Mainz; [3]Innsbruck; [4]Roma Sapienza; [5]Tor Vergata Rome; [6]Rome Cattolica

Growing interest has been observed regarding to the role of the time-to-liver transplant (LT) as predictor of recurrence in patients with hepatocellular cancer (HCC). Many studies on this argument are from USA, but European studies are lacking. The aim of the present analysis was to investigate the role of rapid waiting time (RWT: <120 days) in relation to HCC-related drop-out, post-LT HCC recurrence and post-LT patient death. During the period 1985–2012, 891 HCC patients were enlisted for LT in 6 different European Centers (EurHeCaLT study group: Brussels, Innsbruck, Mainz, Rome Cattolica-Sapienza-Tor Vergata). RWT patients were 338 (37.9%), whilst the slower counterparts (SWT) were 553 (62.1%). Median FU time was 3.6 years (IQR:1.3–7.2). Median waiting time was 5.4 months (IQR:2.2–10.0). Total drop-out rate was 12.8% (n = 114). Total mortality during the waiting list period was 5.4%. Total recurrence rate after LT was 12.4%. Comparing the two groups, a higher mortality rate during the waiting time was observed in RWT patients (7.4 vs. 4.2%; p = 0.03). Post-LT recurrence rates were similar (13.6 vs. 11.6%; p = 0.24). Pathological data from explanted livers (n = 777; 87.2%) (Milan Criteria status, poor grading, microvascular invasion) were similar in the two groups. One- and 5-year intention-to-treat (ITT) survivals were 79.0 and 61.1% in RWT group vs. 90.7 and 68.7% in SWT group (p = 0.009). Post-LT tumor-free (p = 0.449) and patient (p = 0.524) survivals were similar in the two groups. At multivariable Cox regression analysis, only waiting time <120 days was an independent risk factor for ITT patient death (OR = 1.33, 95% CI = 1.04–1.68; p = 0.21). Waiting time <120 days looks to play a selective role during the waiting time period, but it does not affect post-LT survival nor tumor recurrence. More aggressive tumors were not observed in explanted livers of RWT patients. The reported data are partially not in line with results from USA.

O138 THE IMPACT OF ROUTINE ANTERIOR SECTOR DRAINAGE IN RIGHT LOBE LIVING DONOR LIVER TRANSPLANTATION

Murat Dayangac, Sema Baghaki, Barkin Malkoc, Altan Alim, Yalcin Erdogan, Sencan Acar, Arzu Oezcelik, Murat Akyildiz, Refik Killi, Yildiray Yuzer, Yaman Tokat

ISTANBUL BILIM UNIVERSITY FLORENCE NIGHTINGALE HOSPITAL

In right lobe (RL) living donor liver transplantation (LDLT), a good hepatic venous outflow constitutes one of the basic principles of a technically successful LDLT procedure. However, the issue of whether the anterior sector (AS) of the RL graft should or should not be routinely drained is still controversial. We designed this retrospective cohort study to evaluate the impact of routine AS drainage strategy on recipient outcomes.

Between January 2010 and December 2014, we performed 396 primary RL LDLT procedures at our institution. After May 2014, we changed our operative protocol to perform AS drainage whenever possible, either by harvesting the middle hepatic vein (MHV) partially, or by individual drainage of all sizeable (>4 mm) AS veins, which were anastomosed to the middle-left HV stump using interposition polyester (Dacron®) grafts. We also started performing splenic artery ligation (SAL) in all RL grafts with post reperfusion portal flow of ≥250 ml/min/100 g liver tissue (Group 1 = 45). We compared the early outcomes of this group with that of 351 patients who have undergone RL-LDLT between January 2010 and May 2014 (Group 2).

There was no statistically significant difference between the groups in terms of the donor and recipient age, MELD score, and graft-to-recipient weight ratio (GRWR). Graft ischemia time, as well as the rate of AS drainage and the frequency of SAL were significantly higher in Group 1. In 17 patients with SAL in Group 1, mean portal flow significantly decreased from 2520 ± 753 to 1674 ± 536 ml/min. Most importantly, compared to 7.7% in-hospital mortality rate in Group 2 (13/218), there was no mortality in Group 1 in a median follow-up of 5 (3.5–7.0) months.

	Group 1 (n = 45)	Group 2 (n = 351)	p
Donor age	32.6 ± 9.4	31.9 ± 8.8	0.6
Recipient age	51.6 ± 9.9	51.1 ± 11.3	0.7
MELD score	16.0 ± 5.1	16.5 ± 6.5	0.6
Graft-to-recipient weight ratio	1.1 ± 0.2	1.2 ± 0.7	0.7
Graft ischemia time (min)	96.1 ± 34.8	83.7 ± 31.8	0.02
Anterior sector drainage (%)	75.6	39.6	<0.001
Splenic artery ligation (%)	37.8	3.1	<0.001
Perioperative mortality (%)	0	7.7	0.03
3-month survival (%)	100	91.4	0.02

We are convinced that in LDLT, the AS of the RL graft should be routinely drained whenever possible. In RL grafts with a portal flow of ≥250 ml/min/100 g liver tissue, SAL is an effective strategy for decreasing portal flow to prevent graft injury.

O139* INTENTION-TO-TREAT SURVIVAL BENEFIT OF LIVER TRANSPLANTATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA: A MULTICENTER COHORT STUDY. ON BEHALF OF THE ITA.LI.CA STUDY GROUP

Umberto Cillo[1], Giacomo Zanus[1], Fabio Farinati[1], Patrizia Burra[1], Paolo Angeli[1], Franco Trevisani[2], Alessandro Vitale[1]

[1]Liver Transplantation Unit Padua University Hosptital; [2]Department of Medical and Surgical Sciences, Division of Semeiotics, Alma Mater Studiorum – University of Bologna

Introduction: A recent simulation study showed that the survival benefit of liver transplantation (LT) increased in patients with hepatocellular carcinoma (HCC) as their BCLC stage increased. There are no "real life" studies evaluating the intention-to-treat (ITT)– i.e. from the day of waiting list inclusion-LT survival benefit in HCC patients.
Methods: The overall design of the study involved a real cohort of transplantable Italian HCC patients (n = 1177 selected from the ITA.LI.CA database) undergoing non-transplant therapies in different Italian hepatologic units and a real cohort of 227 HCC patients listed for LT in a Italian liver transplant unit. Both the cohorts were enrolled between 2000 and 2010 with a minimum follow-up period of 48 months. A propensity score analysis was performed to match the two groups in terms of patient, liver function, and tumor characteristics.
Results: After propensity score analysis, two homogeneous groups of 227 HCC patients were compared in terms of ITT survival. 5-year survival was significantly higher in the LT group, 65% vs. 19% (p < 0.01) with an overall gain of 9.4 life months. The gain in life expectancy was 2.6, 18.1, and 24.0 life months in BCLC stages 0-A, B-C, and D respectively. The gain in life expectancy for each organ used was 6, 25, and 41 life months in BCLC stages 0-A, B-C, and D respectively.
Conclusions: Using real cohorts of patients, we validated the results obtained with a previous simulation study. In particular we confirmed that LT could result in survival benefit for patients with HCC an advanced liver cirrhosis (BCLC stage D) and in those with intermediate tumours (BCLC stages B–C), regardless of the nodule number–size criteria (ie, Milan criteria), provided that macroscopic vascular invasion and extra-hepatic disease are absent.

O140 TREATMENT OF SEVERE (F3/F4) HCV-RECURRENCE AFTER LIVER TRANSPLANTATION USING SOFOSBUVIR-BASED REGIMENS: THE ANRS CO23 CUPILT STUDY

Jerome Dumortier[1], Danielle Botta-Fridlund[2], Audrey Coilly[3], Vincent Leroy[3], Claire Fougerou-Leurent[3], Sylvie Radenne[3], Francois Durand[3], Christine Silvain[3], Pascal Lebray[3], Pauline Houssel-Debry[3], Nassim Kamar[3], Lucien D'alteroche[3], Christophe Duvoux[3], Filomena Conti[3], Alpha Diallo[3], Inga Bertucci[3], Jean-Charles Duclos-Vallée[3], Georges-Philippe Pageaux[3]

[1]Hôpital Edouard Herriot; [2]ANRS CUPILT CO23; [3]ANRS CO23 CUPILT Study

Recurrence of HCV after liver transplantation (LT) can rapidly lead to liver graft cirrhosis, and therefore graft failure and re-transplantation or death. Study aim was to assess efficacy and tolerance of sofosbuvir (SOF)-based regimens for the treatment of HCV recurrence in patients with severe fibrosis after LT.
Methods: The CUPILT study is a prospective nationwide cohort including patients with HCV-recurrence following LT treated by second-wave direct antivirals. The present study focused on patients included between Oct 2013 and Sept 2014 and diagnosed with HCV recurrence and liver graft extensive fibrosis (METAVIR F3/F4).
Results: A sofosbuvir (SOF)-based regimen was administered to 62 patients fulfilling inclusion criteria. The median delay from LT was 76 months [31–117]. The characteristics of patients were: median age: 59 years [53–64]; male: 84%, G1: 82%, G2: 2%, G3: 8%, G4: 6%; G5: 2%, bilirubin: 15.8 micromol/L [10–23], albumin: 37.4 g/l [32.6–40.5], median HCV RNA: 6.2 log IU/ml [5.9–6.5]. Ascites was present in 9 (16%) patients. Fifty-eight (95%) failed to previous antiviral therapy containing first generation protease inhibitors in 10 (16%) cases. The following regimens were used: Peg-IFNa + SOF + ribavirin (n = 2), SOF + ribavirin (n = 4), SOF + daclatasvir (n = 37) and SOF + daclatasvir + ribavirin (n = 19). All patients were alive without re-transplantation at W24. At W12, 11 (18%) patients had HCV RNA <15 IU/ml and 50 (82%) were not detectable. At EOT, HCV RNA was not detectable in all the patients (n = 59). Median bilirubin serum level decreased from 15.8 to 12 at W24. Albumin level increased from 37.4 to 39.8 g/l at W24. Ascites was Present at W24 in 4 patients. Severe adverse-events occurred in 16 (26%) patients before EOT.
Conclusion: SOF-based regimens show very promising results in patients with severe HCV recurrence after LT. Sustained virological response rates will be presented during the meeting.

O141* A CLINICAL TRIAL OF CELL THERAPY-BASED TOLERANCE INDUCTION IN LIVING DONOR LIVER TRANSPLANTATION: AN UPDATE

Kenichiro Yamashita[1], Ryoichi Goto[2], Masaaki Zaitsu[2], Akihisa Nagatsu[2], Tetsu Oura[2], Masaaki Watanabe[2], Takeshi Aoyagi[2], Tomomi Suzuki[2], Tsuyoshi Shimamura[3], Toshiya Kamiyama[2], Norihiro Sato[4], Junichi Sugita[5], Kanako Hatanaka[6], Anthony Demetris[7], Hisashi Bashuda[8], Ko Okumura[8], Satoru Todo[9]

[1]Department of Transplant Surgery, Hokkaido University; [2]Department of Gastroenterological Surgery I, Hokkaido University; [3]Division of Organ Transplantation, Hokkaido University Hospital; [4]Clinical Research and Medical Innovation Center, Hokkaido University Hospital; [5]Department of Hematology, Hokkaido University; [6]Division of Pathology, Hokkaido University Hospital; [7]Department of Pathology, University of Pittsburgh; [8]Department of Immunology, Juntendo University; [9]Research Institute, St Mary's Hospital

Background: Induction of tolerance is an ultimate goal in organ transplantation. In this clinical study, we examined the effect of the cell-therapy using *ex vivo* derived antigen-specific regulatory T cells (Tregs) on tolerance induction in living donor liver transplantation (LDLT).
Methods: Ten adult liver recipients (HBV; 1, HCV; 1, Alcoholic: 2, NASH; 3, PBC; 2, PSC; 1) were enrolled in this study. They all received a left-lobe liver allograft from living donors. Antigen-specific Tregs were generated *ex-vivo* by co-culturing recipients' PBMCs (+splenocytes) and irradiated donor-PBMCs under αCD80+αCD86 mAbs for 2-weeks. LDLT recipients received steroid, MMF and calcineurin inhibitor (CNI) for immunosuppression (IS). Cyclophosphamide was given on day 4. The generated cells were infused on day 13. Steroid and MMF was stopped within a month. CNI was space-weaned every 3 months starting from 6 months post-LT, and was finally stopped.
Results: After *ex vivo* cell culture, the $CD4^+CD25^+Foxp3^+$ T cells increased from 6.7 ± 3.8% to 28.1 ± 17.7%. The cultured cells inhibited MLR against donor-antigens in a cell number dependent fashion. The mean cell number of the infused $CD4^+CD25^+Foxp3^+$ T cells was 2.3×10^8 cells/body. No adverse event was noted related to the cell infusion. The mean follow-up time after LDLT was 42 (33–52) months. IS was successfully withdrawn in 7 (70%) LT recipients, whereas a mild ACR occurred at IS-weaning in the other 3, who were replaced on regular IS. All IS-off patients are maintaining a good liver graft function without an episode of rejection for 23.1 (13–30) months. Among the IS-free patients, 3 developed DSA, while graft histology did not show any sign of rejection or fibrosis progression. Immunological assays revealed a hyporesponsiveness state against the donor-antigens.
Conclusion: The cell therapy using the *ex-vivo* generated antigen-specific Tregs is an attractive strategy to induce operational tolerance in LDLT.

O142 UK REGISTRY OF COMBINED LIVER-KIDNEY TRANSPLANTATION VERSUS LIVER TRANSPLANT ALONE: PATIENT AND GRAFT SURVIVAL BY GLOMERULAR FILTRATION RATE STRATIFICATION

Francesca Tinti[1], Anna Paola Mitterhofer[2], James Hodson[1], James Ferguson[1], Paolo Muiesan[1], Tamara Perera[1]

[1]The Liver Unit, Queen Elizabeth Hospital Birmingham; [2]Sapienza University of Rome

Aims: To compare overall and graft survival for patients receiving liver transplant alone (LTA) and combined liver-kidney transplant (CLKT) and identify predictive factors of survival.
Methods: Data analysis of 6035 patients from NHSBT UK Transplant Registry (01/2001-12/2012). Survival outcomes were compared between CLKT and LT using Kaplan-Meier curves with log-rank tests, and Cox regression models. Subgroup analyses were performed with stratification on the basis of glomerular filtration rate (GFR) at transplant and treatment with renal replacement therapy (RRT).
Results: 5912 patients (98.0%) underwent liver transplant alone (LTA) and 123 (2.0%) received a CLKT. 305 (5.2%) of the LTA group were on RRT at the time of transplantation, compared to 72 (58.5%) of the CKLT group. No patient with a MELD score <20 received RRT before transplant. LTA and CLKT patients demonstrated a significantly different MELD (median:15[quartiles:12–20] and 21[20–25], respectively; $p < 0.0001$). Patients in CLKT only received DBD transplants, whilst LTA patients also received organs from DCD (11.0%) and living donors (0.4%).

In patients on RRT at time of transplantation, those who received a CLKT showed significantly improved graft ($p = 0.030$) and patient ($p = 0.038$) survival compared to those receiving a LTA. No significant differences between the groups were detected in patient or graft survival for other GFR stratifications.20 (HR 1.77; 95% CI 1.50–2.72, $p = 0.010$), HCV (HR 1.32; 95% CI 1.02–1.72, $p = 0.035$), diabetes (HR 1.42; 95% CI 1.11–1.82, $p = 0.006$) and increasing donor age (HR 1.38; 95% CI 1.09–1.83, $p = 0.008$). None of these factors were significant in CLKT patients, although the statistical power of the latter analysis was lower, due to the small sample size.

In a multivariable Cox regression model including all clinically relevant variables simultaneously, the independent predictors of mortality in patients undergoing LTA were RRT in patients with MELD >20 (HR 1.77; 95% CI 1.50–2.72, $p = 0.010$), HCV (HR 1.32; 95% CI 1.02–1.72, $p = 0.035$), diabetes (HR 1.42; 95% CI 1.11–1.82, $p = 0.006$) and increasing donor age (HR 1.38; 95% CI 1.09–1.83, $p = 0.008$). None of these factors were significant in CLKT patients, although the statistical power of the latter analysis was lower, due to the small sample size.
Conclusions: This is the first study of its kind from a European registry. Beside the recognized risk factors for mortality in LTA patients, renal replacement therapy seems to be associated with reduced survival in this group.

013 IMMUNOBIOLOGY/BASIC SCIENCE

O143 CD4+ T CELL HELP IS MANDATORY FOR NAÏVE AND MEMORY B CELLS TO GENERATE DONOR-SPECIFIC ANTIBODIES

Chien-Chia Chen[1], Alice Koenig[2], Dimitri Chartoire[1], Thierry Defrance[1], Emmanuel Morelon[3], Olivier Thaunat[3]
[1]INSERM U1111, Lyon; [2]INSERM U1111, Hospices Civils de Lyon, Lyon; [3]INSERM U1111, Hospices Civils de Lyon, Université de Lyon, Lyon

Background: Antibody-mediated rejection (AMR) is the leading cause of kidney transplant failure. Current immunologic dogma predicts that B cells respond to protein antigen (such as HLA molecules) only with the help of CD4+ T cells. Yet, despite progress in T cell immunosuppression, about 10% of patients develop de novo anti-donor HLA antibodies (DSA) during the 1st year post-transplantation, a time when immunosuppressant is maximal and compliance usually good. In the present project we aimed at determining whether DSA can be generated in the absence of CD4+ T cell help.
Methods and results: We first investigated the importance of CD4+ T cell for naive humoral allogeneic response. B6 mice genetically deficient in CD4+ T cells (MHC II KO mice) or wild type (WT) were used as recipient of a skin graft (alloantigen drainage to lymph node) or a heterotopic heart transplant (drainage to spleen). Donors were HLA A2 transgenic mice, i.e. B6 mice that express human HLA A2 molecule under the murine MHC I promoter. This trick allowed for the monitoring of DSA response with assays routinely used in the clinic. While WT recipients, all developed circulating anti-HLA A2 antibodies, no MHCII KO recipients did whatever the location of alloantigen drainage. We then determined whether CD4+ T cell help was dispensable for memory B cells to respond to a second challenge with the alloantigen. Balb- (3rd party) or A2-specific memory B cells were purified from the spleen of recipient WT mice, 45 days after heterotopic heart transplantation. 5×10^6 purified splenic B cells were transferred intravenously to RAG KO mice that were then transplanted with A2 heart. None of the RAG recipients developed circulating anti-A2 antibodies.
Conclusion: Our results demonstrate that CD4+ T cell help is mandatory for the generation of DSA by naïve and memory B cells. Monitoring the activation state of circulating CD4+ T cells could allow identifying patients at risk for DSA generation.

O144 FOLLICULAR T HELPER CELLS AND HUMORAL REACTIVITY IN KIDNEY TRANSPLANT PATIENTS

Gretchen De Graav[1], Marjolein Dieterich[1], Dennis Hesselink[1], Karin Boer[1], Marian Clahsen-Van Groningen[1], Rens Kraaijeveld[1], Nicolle Litjens[1], Rachida Bouamar[1], Joris Vanderlocht[2], Marcel Tilanus[2], Ilse Houba[2], Dave Roelen[3], Frans Claas[3], Michiel Betjes[1], Willem Weimar[1], Carla Baan[1]
[1]Erasmus MC; [2]Maastricht University Medical Center; [3]Leiden University Medical Center

Memory B cells play a pivotal role in alloreactivity in kidney transplantation. Follicular T helper (Tfh) cells play an important role in the differentiation of cells into immunoglobulin-producing plasmablasts (through interleukin (IL)-21). It is unclear to what extent this T cell subset regulates humoral alloreactivity in kidney transplant patients. Therefore we investigated the absolute numbers and function of peripheral Tfh cells (CD4+CXCR5+ T cells) in patients before and after transplantation. In addition, we studied their relationship with the presence of donor-specific anti-human leucocyte antigen (HLA) antibodies (DSA), and the presence of Tfh cells in rejection biopsies. After transplantation peripheral Tfh cell numbers remained stable, while their IL-21-producing capacity decreased under immunosuppression. When isolated after transplantation, peripheral Tfh cells still had the capacity to induce B cell differentiation and immunoglobulin production, which could be inhibited by an IL-21-receptor-antagonist. After transplantation the quantity of Tfh cells was the highest in patients with pre-existent DSA. In kidney biopsies taken during rejection, Tfh cells co-localized with B cells and immunoglobulins in follicular-like structures. Our data on Tfh cells in kidney transplantation demonstrate that Tfh cells may mediate humoral alloreactivity, which is also seen in the immunosuppressed milieu.

O145 DIVERSITY OF DONOR-SPECIFIC ANTIBODIES INCLUDES MHC I-SPECIFIC IGE

Andreas M. Farkas[1], Ulrike Baranyi[1], Lukas Unger[1], Christoph Schwarz[1], Benedikt Mahr[1], Karin Hock[1], Nina Pilat[1], Rudolf Valenta[2], Thomas Wekerle[1]
[1]Section of Transplantation Immunology, Department of Surgery, Medical University of Vienna, Vienna, Austria; [2]Department of Pathophysiology & Allergy Research, Center of Physiology & Pathophysiology, Med.Uni. Vienna

Background: The pleiotropic functions of donor-specific antibodies (DSA) are still debated controversially. The presence of DSA is an adverse marker in most, but not all settings. Additionally the function and diversity of DSA-isotypes is insufficiently understood. In particular it is unknown if IgE is induced, which is capable of mediating unique effector mechanisms. Recently we developed a non-MHC antigen-mismatched transgenic mouse model (expressing the antigen Phl p 5 on the cell-surface), in which we found high levels of mismatch-specific IgE after rejection of heart and skin grafts. Here, we studied if donor-specific IgE is induced in an MHC-mismatched mouse model.
Methods: Tail skin or hearts of Balb/c (H-2^d) or C3H (H-2^k) mice was grafted in an onto naïve B6 (H-2^b) or C3H or Balb/c mice ($n = 6$ skin, $n = 4$ hearts). Serum samples were taken pre and post transplantation (TX) at several timepoints to analyze H-2D^d-, H-2K^d-, H-2D^k-, H-2K^k-, H-2I-E^d and H-2I-E^k specific (murine MHC-I and MHC-II antigens) IgE levels were measured via ELISA by using recombinant MHC monomers provided by the NIH tetramer facility. Serum samples were also used for an in vitro basophil degranulation assay (RBL-assay) to assess if IgE is functional.
Results: Via utilization of this novel ELISA we revealed an induction of MHC-I-specific IgE (α-H-2D^d and K^d or α-H-2D^k andK^k, respectively) detectable upon rejection of MHC-mismatched skin and heart grafts grafts in all strain combinations tested. In contrast to MHC-I-specific IgE MHC-II-specific IgE (αI-E^d and αI-E^k, respectively) remained undetectable. Additionally we were able to detect basophil degranulation upon cross-linking with recombinant MHC in the in vitro RBL-assay.
Conclusion: To the best of our knowledge, this is the first report of MHC I-specific IgE developing upon graft rejection. IgE is functional at the effector cell level in vitro, but whether this isotype plays a pathophysiological role role in vivo remains to be assesed.

O146 THE ROLE OF IL-21 IN CHRONIC ALLOGRAFT REJECTION

Paul Schroder, Stanislaw Stepkowski, Caitlin Baum, Wenhao Chen, Mithun Khattar
University of Toledo College of Medicine

Background: IL-21 is a cytokine expressed almost exclusively by cells of the adaptive immune system. This makes it an attractive target for reducing chronic allograft rejection while avoiding innate immune side effects. Our work has shown that IL-21 signaling is critical for maintaining T-cell survival under IL-2 deprivation conditions as well as in sustaining autoimmune responses to islets and cardiac allografts. Methods

We performed heart transplants in IL21-/-, BATF-/-, and wild-type B6 mouse recipients with or without transient therapy with an IL-21 receptor fusion protein (IL21R.Fc).
Results: Donor class-II mismatched Bm12 heart allografts were protected from chronic rejection in IL21-/- mice (>100 days) but not in control B6 mice (56.3 $\pm$ 22.9 days; p100 days), which lack this regulator of IL-21 production, and in IL21R.Fc treated B6 recipients of Bm12 heart allografts (>100 days). The figure below shows the overall impact of these components of the IL-21 signaling pathway with respect to heart allograft survival. These data indicating protection from chronic rejection were further confirmed by significant reductions in graft arterial disease (GAD) scores and decreased vasculopathy. Allografts in IL21-/- mice develop significantly fewer tertiary lymphoid organs (TLOs) than do allografts in control B6 mice. Furthermore, the presence of IL-21 or its signaling components influenced the architecture of TLOs with respect to size and composition.
Conclusion: In summary, IL-21 signaling promotes T cell survival in chronic allograft rejection and regulates the creation and maintenance of TLOs within the allograft tissue.

O147 PROGRESSION OF ALLOGRAFT VASCULOPATHY IS BLOCKED BY TARGETING THE T FOLLICULAR HELPER CELL SUBSET

Manu Chhabra[1], R. Motalleb-Zadeh[1], J. Alsughayyir[1], Mc Negus[1], J. Ali[1], Sm Qureshi[1], M. Mallik[1], M. Aly[1], M. Goddard[2], Em Bolton[1], Ja Bradley[1], Gavin Pettigrew[1]
[1]Department of Surgery, University of Cambridge; [2]Papworth Hospital

Background: Development of long-lived alloantibody is closely linked with chronic rejection and graft failure. We examined in a murine model of antibody-

mediated rejection (AMR) if targeting T follicular helper (Tfh) cell development prevents chronic rejection by blocking germinal centre (GC) activity.
Methods: T-cell deficient CB57BL/6 recipients of a BALB/c heart were reconstituted with monoclonal TCR75 CD4 T cells (with indirect specificity to donor Class I H-2K^d) or with TCR75 CD4 T cells genetically deficient for the adaptor molecule SAP. SAP signalling is essential for Tfh development but does not influence extrafollicular antibody production.
Results: Adoptive transfer of 10^3 TCR75 T cells generated persistent anti-H-2K^d antibody responses, characterised by K^d-binding GC B cells within the spleen, and long-lived anti-K^d-secreting plasma cells in the bone marrow. This was associated with endothelial complement deposition and activation; resulting in chronic allograft vasculopathy, and ultimately, graft rejection (median survival time (MST) = 50 d, n = 10). In contrast, transfer of 10^3 SAP-deficient TCR75 T cells failed to initiate GC responses, with substantial reduction in anti-K^d IgG production. Grafts in this group survived indefinitely (n = 5), without development of allograft vasculopathy. Transfer of large numbers (10^5) of SAP-deficient TCR75 T cells likewise did not initiate GC responses, but did provoke strong and immediate extrafollicular responses, which precipitated acute graft loss (MST = 13 d, n = 4), with histological hallmarks of acute AMR.
Conclusions: The demonstration that GC alloantibody responses are essential for allograft vasculopathy highlights the potential for targeting the Tfh subset for improving clinical transplant outcomes. High T helper cell precursor frequency may however provoke acute graft rejection through extrafollicular antibody production.

O148 GERMINAL CENTRE AUTOIMMUNITY MEDIATES PROGRESSION OF ALLOGRAFT VASCULOPATHY, WITH ESSENTIAL HELP BEING PROVIDED BY RECIPIENT FOLLICULAR HELPER T CELLS

Muhammad Saeed Qureshi , R. Motallebzadeh, T. Conlon, M. Negus, S. Rehakova, M. Chhabra, E. Bolton, Ja Bradley, Gj Pettigrew
University of Cambridge

Introduction: In our previous work we have shown that donor CD4 T cells within heart grafts initiate autoantibody responses. Here we clarify the contribution of host CD4 T cells to progression and maintenance of the response.
Methods: Bm12 heart grafts were transplanted into wild type (WT), T-cell deficient (TCR$^{-/-}$) or SAP$^{-/-}$ B6 recipients that lack T follicular helper (T_{FH}) cells.
Results: Bm12 heart allografts developed progressive allograft vasculopathy (AV) when transplanted into WT recipients and provoked long lasting GC (68 $\pm$ 3% PNA^{+ve} splenic B cell follicles) autoantibody responses, with late generation of anti-vimentin autoantibody (relative anti-vimentin IgG levels were 77 $\pm$ 3 at week 7 vs 287 $\pm$ 2 at week 15, p = 0.007) evident. Depleting CD4 T cells in the donor abrogated autoantibody production and resulted in minimal AV. In contrast, transplantation into TCR$^{-/-}$ recipient triggered autoantibody responses, but GC activity was not observed, and heart grafts survived indefinitely (MST >100 d) and ameliorated AV (1 $\pm$ 2% luminal stenosis). Critically, heart grafts transplanted into SAP$^{-/-}$ recipients triggered autoantibody generation, but neither GC activity nor late anti-vimentin responses were detectable and grafts developed only minimal AV (% luminal stenosis was 10 $\pm$ 8 in SAP$^{-/-}$ recipients vs 74 $\pm$ 1 in WT, p = 0.01) and survived significantly longer (MST was 96d in SAP$^{-/-}$ vs 56d in WT, p = 0.006). In support of the role of GC autoantibody responses in mediating allograft vasculopathy, in a wound-induced endothelial cell (EC) migration assay, cultured bm12 ECs showed significantly lower migration upon addition of serum from SAP$^{-/-}$ recipients than when serum from WT recipients was added (p = 0.01).
Conclusion: Our results demonstrate that donor CD4 T cells within an allograft can trigger recipient autoantibody responses, but graft rejection is dependent upon progression to a GC response, with essential help for its development provided by host T_{FH}.

O149 NK CELLS PROMOTE KIDNEY GRAFT REJECTION INDEPENDENT OF CYCLOSPORINE A THERAPY

Resch Thomas[1], Muhammad Imtiaz Ashraf[1], Cornelia Fabritius[1], Paul Ritschl[1], Susanne Ebner[1], Julia Guenther[1], Vanessa Mellitzer[1], Felix Aigner[2], Johann Pratschke[2], Katja Kotsch[1]
[1]Department of Surgery Medical University Innsbruck; [2]Department of Surgery, Charité-Universitaetsmedizin

Background: NK cells have recently been recognized as key players in chronic allograft failure. Consequently, comprehensive studies are required to address whether NK cells can escape conventional immunosuppressive regimens.
Methods: We characterized the effects of cyclosporine A (CsA) *in vitro* and *ex vivo* on human and murine NK cells and further assessed its functional influence on NK cells after murine KTX *in vivo*.
Results: *In vitro*, human NK cells treated with CsA concentrations (5–1000 ng/ml over 1–3 days) were insensitive concerning viability, expression of activation markers (CD16, NKp30, NKp44, NKp46), pattern of differentiation (CD65^{dim}/CD56^{bright}) and IFNγ production. Concordantly, *ex vivo*, CD3$^-$NKp46$^+$ NK cells isolated from CsA treated C57BL/6 mice (10 mg/kg for 7 days) revealed normal function regarding degranulation and IFNγ production, whereas CD8$^+$ T cells were functionally impaired. *In vivo*, application of CsA to C57BL/6 recipients of fully allogeneic Balb/C kidneys resulted in a significant reduction of creatinine levels at day 7 (KTX: 35.6 $\pm$ 4 vs. KTX+CsA: 19.8 $\pm$ 4 mmol/l; p <0.05). Flow cytometric analysis revealed a CsA mediated reduction of intragraft CD4$^+$ and CD8$^+$ T cells by halve, whereas intragraft NK cell frequencies significantly increased (KTX: 10 $\pm$ 0.4 vs. 17.5 $\pm$ 1.5; p < 0.01) and remained unaffected within the spleen or liver. Importantly, the additional depletion of NK cells (anti-NK1.1 Ab) resulted in a further improvement of kidney function (12 $\pm$ 0.5 mmol/l; p < 0.01) associated with reduced intragraft and splenic IFNγ expression levels (p < 0.05, respectively).
Conclusion: CsA insufficiently targets human and murine NK cell numbers and function. We show for the first time that NK cell depletion combined with CsA synergistically improves graft function in an acute transplantation setting, suggesting that selective NK cell targeting might constitute a novel approach to ameliorate KTX outcomes in the long-term.

O150 ANTIBODY-DEPENDENT NATURAL KILLER CELL RESPONSES ASSOCIATE WITH CHRONIC KIDNEY ALLOGRAFT DYSFUNCTION AND COMPLEMENT INDEPENDENT MECHANISMS OF DSA CYTOTOXICITY

Tristan Legris[1], Valérie Moal[1], Christophe Picard[2], Dilyana Todorova[3], Laurent Daniel[4], Luc Lyonnet[5], Henri Vacher-Coponat[1], Bertrand Dussol[1], Françoise Dignat-George[3], Yvon Berland[1], Stéphane Burtey[1], Pascale Paul[3]
[1]Centre de Néphrologie et Transplantation Rénale, Assistance Publique des Hopitaux de Marseille, Hopital de la Conception; [2]Établissement Français du Sang Alpes Méditerranée; [3]Aix Marseille University, Inserm, UMR 1076, Vascular Research Center of Marseille; [4]Laboratory for Anatomy, Pathology, Neuropathology, Hôpital de la Timone, Aix-Marseille University; [5]Hematology unit, Assistance Publique des Hopitaux de Marseille, Hopital de la Conception

Background: Complement-independent mechanisms that lead to Antibody Mediated Rejection (ABMR) remain largely unexplored and may contribute to heterogeneous clinical outcome of patients with donor-specific antibodies (DSA). We investigated whether Natural Killer (NK) antibody dependent cell cytotoxic activity (ADCC) of recipients can be associated to long-term kidney transplant function and be identified as an effector mechanism contributing to chronic allograft injury
Methods: Phenotypic evaluation of peripheral blood NK cells cytotoxic activity was conducted in 148 Kidney transplant recipients (KTRs, mean 6 years post graft). A flow cytometry cellular humoral activation test (NK-CHAT) was designed to index CD16-Fc receptor mediated NK-cell recognition of allogeneic target cells exposed to decomplemented DSA+ and DSA- sera or Rituximab. In a pilot study, we tested whether NK-CHAT indexing of seric DSA could be associated with histological scoring of their pathogenic effects towards graft vasculature.
Results: NK cytotoxic responses exhibit high inter-individual variability in KTRs. Analysis of Rituximab induced NK cell activation reveals that high ADCC responsiveness was associated with late graft dysfunction and constitutes an independent factor associated to further risk of degradation of kidney graft function. We show that NK-CHAT evaluation of CD16 down-regulation (CD16DRI) in response to DSA coated allogeneic B-cell targets constitutes a sensitive and specific index of host ADCC responsiveness. Evaluation of sera at time of graft histology showed that CD16DRI levels associate with conventional features of ABMR diagnostic such as DSA associated microcirculation lesions. We further provide evidence that NK-CHAT can be used to monitor complement-independent reactivity of DSA towards donor, spleen and endothelial allogeneic cells.
Conclusion: Collectively these data support that DSA have variable capacities to induce FcR-mediated immune activation which may condition

O151 HIGHLY-MATURATED CD27LOW NK CELLS PROLONG ALLOGRAFT SURVIVAL IN MICE BY CONTROLLING CD8+ T CELL RESPONSES AND GRAFT DC-DERIVED IL-15 AVAILABILITY

Lantow Margareta[1], Elke Eggenhofer[1], Manije Sabet-Baktach[1], Philipp Renner[1], Jordi Rovira[1], Gudrun E. Koehl[1], Hans J Schlitt[1], Edward K Geissler[1], Alexander Kroemer[2]
[1]University Hospital Regensburg Experimental Surgery; [2]Georgetown Transplant Institute

Background: The underlying mechanisms by which distinct NK cell subsets promote either allograft rejection or survival remains unclear. Therefore, we tested the hypothesis that mature NK cell subsets control alloreactive T cell responses through their interactions with graft-derived donor DCs.

Material and Methods: We used BALB/c.Rag-T-bet double-KO (DKO, lack highly-differentiated CD27low NK cells) to study the role of mature CD27low NK cells in a model of T cell-mediated allograft rejection. Thus, DKO and Rag-KO recipients that received fully mismatched B6 skin allografts (STx, graft from C57Bl.6 WT, IL-15-KO or CD11c-DTR donors) were adoptively transferred with CD3+ T cells from BALB/c.Foxp3-gfp mice and treated with costimulatory blockade (CTLA4Ig and MR-1). Alloimmune responses were analyzed by histology, ELISA, RT-PCR and flow cytometry.
Results: Costimulatory blockade-treated Rag-KO mice (with CD27low NK cells) showed significantly prolonged allograft survival (MST 35 $\pm$ 3d, $p < 0.001$) and reduced alloreactive CD8+ T cell responses (ELISA, flow cytometry) vs DKO mice (28 $\pm$ 1d). Moreover, Rag-KO recipients showed significantly lower IL-15 serum levels (2.24 fold) vs DKO recipients (ELISA), suggesting that CD27low NK cells control graft-derived IL-15, thereby inhibiting CD8+ T cell responses and promoting allograft survival. To test this we grafted DKO recipients with STx from IL-15-KO donors and found significantly prolonged allograft survival (39 $\pm$ 2d vs 28 $\pm$ 1d, $p < 0.05$) and reduced CD8+ T cell expansion. Moreover, when we grafted DKO recipients with STx from CD11c-DTR mice that were depleted from CD11c+ DCs via diphtheria toxin, we found significantly prolonged allograft survival (50 $\pm$ 4d vs 28 $\pm$ 1d) and reduced CD8+ T cell alloresponses in DKO recipients.
Conclusion: Under costimulatory blockade conditions mature CD27low NK cells control IL-15-expressing graft-derived donor DCs and inhibit alloreactive CD8+ T cell responses thus promoting allograft survival.

O152 ENDOTHELIAL CELLS POLARIZES INTRAVASCULAR MACROPHAGES INTO PROINFLAMMATORY M1 VIA NOTCH SIGNALING, DLL4 AND IL-6 IN CARDIAC TRANSPLANTS DURING ANTIBODY-MEDIATED REJECTION

Sylvain Pagie[1], Angelique Pabois[2], Claire Toquet[3], Nathalie Gerrard[1], Christian Laboisse[3], Sabine Pattier[3], Beatrice Charreau[1]
[1]INSERM U1064; [2]LUDWIG Center; [3]CHU DE NANTES

Rationale: Antibody-mediated rejection (AMR) of heart transplant is a clinical complication associated with progression to cardiac allograft vasculopathy and poor graft outcome. AMR is caused by the deposition of donor specific alloantibodies donor targeting HLA molecules expressed on graft endothelial cells (ECs) that trigger activation of complement and inflammatory responses. Mechanisms of AMR remain unclear and treatment for AMR is still limited. Objective- This study aimed to better understand molecular and signaling mechanisms underlying vascular and inflammatory cell network involved in AMR. Methods and Results- We investigated Notch signaling in human cardiac transplants upon AMR at endothelial and macrophage levels on endomyocardial transplant biopsies and using EC cultures from transplant donors. Here, we provide unique in vivo evidence that Notch signaling contributes to the pathogenesis of AMR. We established that regulation of the Notch pathway occurs in graft's ECs leading to a loss of Notch4 and a gain of the Dll4 ligand. We show that endothelial Dll4 induces macrophage polarization into a pro-inflammatory M1 fate in a Notch-dependent manner. Dll4 expression on ECs also triggers IL-6 release. Dll4 and IL-6 are key coregulators of macrophage polarization and cooperatively shape M1 phenotype. Dll4 and IL-6 selectively down and up regulates M2 and M1 markers, respectively.
Conclusions: Overall, our findings provide novel insights into the impact of the graft's endothelium on macrophage recruitment and differentiation upon AMR and identify Dll4 and IL-6 as novel molecular targets for therapy in cardiac transplantation.
Keywords: Cardiac transplant, endothelial cell, Notch signaling, M1 macrophages, Dll4.

O153 ENDOTHELIAL CELL ACTIVATION BY DONOR SPECIFIC ANTIBODIES AGAINST HLA CLASS II PROMOTES AN IL-6-DEPENDENT PATHWAY OF TH17 EXPANSION AND REDUCES REGULATORY T CELLS AMPLIFICATION

Julien Lion[1], Cécile Taflin[2], Caroline Suberbielle Boissel[3], Maryvonnick Carmagnat[3], Dominique Charron[4], Denis Glotz[5], Nuala Mooney[6]
[1]INSERM U1160; [2]Service de Néphrologie; [3]Laboratoire d'histocompatibilité/INSERM U1160; [4]Laboratoire d'histocompatibilité/Université Paris Diderot; [5]Service de Néphrologie et Transplantation/INSERM U1160/Université Paris Diderot; [6]INSERM U1160/Université Paris Diderot

Background: Chronic antibody mediated rejection, strongly associated with HLA II Donor Specific Antibodies (DSA), remains a major problem in renal transplantation. HLA class II molecules which are constitutively present on microvascular endothelial cells (EC), are highly increased under inflammatory conditions. EC is therefore a target for DSA and an activator of the $CD4^+$T response. We have demonstrated that $HLA\text{-}DR^+$EC mediated expansion of allogeneic regulatory $FoxP3^+$T and pro-inflammatory Th17. We here report the outcome of EC activation by HLA-DR DSA on EC activation of $CD4^+$T.
Methods: HLA-DR expressing primary renal glomerular ECs or HMEC-1 cells were co-cultured with PBMC or $CD4^+$T from healthy donors. HLA-DR monoclonal antibody (mAb), Panel Reactive Antibody (PRA) containing relevant HLA-DR Ab and purified HLA-DR DSA were used to activate ECs prior to co-culture.
Results: Activation of $HLA\text{-}DR^+$ECs by HLA-DR Ab (mAb, PRA or DSA) before co-culture with allogeneic PBMC or $CD4^+$T cells was necessary to increase IL-6 production by EC beyond the level of IFNg-activated EC. The pathological significance was underlined by the increased in IL-6 when EC were pre-activated with either PRA or purified DSA. Proteomic studies revealed Akt and MEK activation and use of specific inhibitors showed that IL-6 production was Akt dependent. The $CD4^+$T cell response was altered due to heightened IL-6-dependent amplification of Th17 and significantly lower Treg expansion. The F (ab')2 fragment was as efficient as the full length antibody.
Conclusion: These data establish a new pathway of EC activation mediated by DSA without either complement activation or FcR^+ effector cell intervention, that leads to increased IL-6 and amplification of pro-inflammatory Th17 previously implicated in renal allograft damage. Transplant glomerulopathy in chronic rejection is associated with microvascular endothelial damage. These data reveal a novel mecanism by which DSA may disrupt the allograft endothelium.

O154 IMPORTANT CONTRIBUTION OF BASOPHILS TO DEVELOPMENT OF CARDIAC ALLOGRAFT FIBROSIS

Gabriela Schiechl[1], Fabian Hermann[1], Manuel Rodriguez Gomez[1], Simone Kutzi[1], Kathrin Schmidbauer[1], Yvonne Talke[1], Sophia Neumayer[1], Nicole Goebel[1], Hajime Karasuyama[2], Kazushige Obata-Ninomiya[2], Hilke Bruehl[3], Edward Geissler[4], Stefan Fichtner-Feigl[4], Matthias Mack[1]
[1]University Regensburg/Medical Center/Nephrology; [2]Tokyo Medical and Dental University Graduate School; [3]University Regensburg/Medical Center/Internal Medicine I; [4]University Regensburg/Medical Center/Surgery

Introduction: Fibrosis is a major cause of chronic cardiac allograft failure. Although several cell types are able to produce collagen, resident (donor-derived) fibroblasts are thought to be mainly responsible for excessive production of extracellular matrix proteins. So far, it is unclear which cells contribute to production of connective tissue elements in chronic allograft fibrosis and how basophils, as producers of pro-fibrotic cytokines, are involved this process.
Method: We have studied this question in a fully MHC mismatched model of heart transplantation with transient depletion of $CD4^+$ T cells to prevent acute rejection. The model is characterized by myocardial infiltration of leukocytes and development of fibrosis within 20 days. Before transplantation basophils were depleted with mAb against FcεR1, the high affinity IgE receptor, or with antibody against anti-CD200R3. Fibroblasts were identified by flow cytometry ($CD45^-$ Collagen-type-1^+ cells) and via immunohistochemistry (by expression of αSMA).
Results: Using depletion of basophils, IL-4 deficient recipients and IL-4 receptor deficient grafts we show that basophils and IL-4 play a crucial role for activation of fibroblasts and production of matrix proteins. We could demonstrate that fibrotic remodelling of chronically rejected allografts is a two-stage process, involving the infiltration of basophils into the allograft with production of IL-4 and the subsequent IL-4 dependent activation of resident fibroblasts. This results in deposition of various connective-tissue elements like collagen type I and fibronectin leading to destruction of the normal tissue architecture.
Conclusion: Our results indicate that depletion of basophils reduces the potential of myofibroblasts to produce various connective tissue elements and further induce organ fibrosis. Our data show for the first time the importance of basophils on activation and function of myofibroblasts and their role in regulation of tissue fibrosis.

019 ISCHEMIA/REPERFUSION INJURY/PRESERVATION

O155 ALVEOLAR AND CIRCULATING MICROPARTICLES ARE MARKERS OF LUNG ISCHEMIA REPERFUSION INJURY IN A RODENT MODEL FOR EX VIVO LUNG PERFUSION

Anne Olland[1], Olland Anne[2], Burban Mélanie[3], Toti Florence[3], Auger Cyril[3], Kessler Laurence[4], Schini-Kerth Valérie[3], Massard Gilbert[5]
[1]University Hospital Strasbourg; [2]EA 7293 Stress Vasculaire et Tissulaire en Transplantation; [3]UMR CNRS 7213; [4]EA7293 Strass Vasculaire et Tossulaire en Transplantation; [5]Thoracic SUrgery Department, Lung Transplantation Group, University Hospital Strasbourg

Introduction: Post-operatively to lung transplantation, lung ischemia reperfusion injury is only graded by means of radiological and oxymetric characteristics. There is no actual relevant biomarker of ischemic insult in lungs. Microparticles (MPs) are sub-micronic fragments shed from stimulated and stressed cells measured as biomarkers of cell injury. We aimed at the assessment of MPs released during ischemic lung injury in an ex vivo rat lung perfusion model (EVrLP).
Method: We used an ex vivo rat lung perfusion and ventilation model. Following anesthesia, lungs were harvested from adult male Wistar rats. Lungs were placed in the EVrLP model either immediately (no ischemia [NI]; $n = 6$) either following 1 h cold ischemia ([IC1]; $n = 6$), or following 20 h cold ischemia ([IC20]; $n = 6$). We used acellular Perfadex® as a perfusate. Lungs were ventilated and perfused for 1 h. Lung function was assessed with hemodynamic and oxymetric criteria. Alveolar and circulating MPs were assessed in the broncho-alveolar lavage and in the perfusate respectively.
Results: In [NI], the relative oxygenation ratio was significantly higher in comparison to [IC1] and [IC20] ($p < 0.05$). In [IC20], mean pulmonary artery pressure and wet to dry weight ratio were significantly increased in comparison to [NI] and [IC1] ($p < 0.01$ both). Besides, in [IC20], alveolar MPs were significantly higher after 10 min of perfusion in comparison to alveolar MPs in [NI] and [IC1] ($p < 0.05$). After 60 min of perfusion, alveolar MPs were significantly higher in [IC1] and in [IC20] in comparison to [NI] ($p < 0.05$). In [IC20] circulating MPs were significantly higher after 10 and 60' of perfusion in comparison to [NI] and [IC1] ($p < 0.01$). The rate of circulating MPs significantly incresaed in [IC20] along reperfusion ($p < 0.05$).
Conclusion: Alveolar and circulating MPs do reveal as relevant biomarkers for lung ischemia related injury in an experimental model of rodent ex vivo perfusion.

O156 ANGIOTENSIN II TYPE 1 RECEPTOR INHIBITION AND SIRT1 ACTIVATION IN RAT REDUCED- SIZE ORTHOTOPIC LIVER TRANSPLANTATION

Eirini Pantazi[1], Mohamed Bejaoui[1], Emma Folch-Puy[1], Anabela Pinto Rolo[2], Carlos Marques Palmeira[2], Marina Gerousi[1], Joan Roselló-Catafau[1]
[1]Intsitute of Biomedical research of Barcelona (IIBB-CSIC); [2]Department of Life Sciences, Faculty of Science and Technology, University of Coimbra

Background: Ischemia-reperfusion injury (IRI) remains an important limitation for transplantation outcome and the study of more effective strategies is an urgent need. Silent Information Regulator 1 (SIRT1) is a histone deacetylase related with protective actions against IRI, but its role in liver transplantation has been poorly investigated. Moreover, inhibition of angiotensin II receptors has been found to protect livers against IRI, but the underlying mechanisms are unclear. The aim of this study is to examine the possible SIRT1 implication in rat reduced-size orthotopic liver transplantation (ROLT), as well as a potential link between SIRT1 and losartan, an antagonist of angiotensin II type I receptor.
Methods/Materials: Livers of male Sprague-Dawley rats were preserved in University of Wisconsin (UW) storage solution for 1 h at 4 C and then subjected to ROLT. In an additional group, losartan was orally administered (5 mg/kg) 24 h and 1 h before the surgical procedure to both the donor and the recipient rats. Liver injury (transaminases), SIRT1 protein levels and activity, SIRT3 protein and mRNA expression, endoplasmic reticulum stress (ERS) parameters (GRP78, IRE1a and p-elf2), heat shock proteins expression (HSPs; HO-1, HSP70) and apoptosis parameters (Caspase 12 and Caspase 3) were measured 24 h after reperfusion.
Results: Losartan pretreatment diminished hepatic injury in ROLT. This was accompanied by enhanced SIRT1 protein expression and activity, which can be attributed to the elevated NAD+ (SIRT1 co-factor) levels. No changes reported in SIRT3 mRNA and protein levels between the two transplantation groups. Furthermore, losartan treatment resulted in decreases in the ERS parameters and in liver apoptosis. Losartan administration also modulated HSPs expression. Conclusions: SIRT1 is a downstream target of angiotensin II and SIRT1 up-regulation might be an up-coming strategy in order to lessen hepatic IRI related with ROLT.

O157 BACK-TABLE PORTAL AND ARTERIAL LIVER PERFUSION WITH CUSTODIOL AND TACROLIMUS REDUCE THE INCIDENCE AND SEVERITY OF GRAFT DYSFUNCTION

Aliaksei Shcherba, Sergey Korotkov, Andrew Minou, Denis Efimov, Evgeni Santotski, Alla Karitka, Alexander Dzyadzko, Oleg Rummo
RSP Center for Organ and Tissues Transplantation

Pharmacological liver protection is one of possible ways to reduce EAD in LTx. It was shown that tacrolimus can suppress inflammation and immune response involved in liver IRI (Kristo I., Transpl Int.2011). We hypothesize that back-table arterial and portal liver perfusion with Tac can influence the incidence and severity of EAD. A prospective randomized study was conducted (ClinicalTrials.gov Identifier: NCT01887171). 80% power estimation required a 41 pt sample size. 43 pts were assigned to each group. Including criteria: 1st LT from standard criteria DBD donor with sequential portal-arterial reperfusion. During back table, portal vein and hepatic artery were cannulated and perfused each with 500 ml of HTK solution containing 20 ng/ml Tac during 10–15 min followed by portal flushing with 200 ml 5% solution of Albumin containing 20 ng/ml Tac and by resting of liver in the effluent. No Tac was added in the control group. Primary Outcome: EAD (Olthoff KM, et al. Liver Transpl. 2010) and severe EAD (PR Salvalaggio, et al. Transpl. Proceed. 2012). No difference was found between groups (main vs control) in terms of MELD (16 vs 16), steatosis (10 vs 10%), ballooning (45 vs 40%) of liver grafts, recipient age (50 vs 50 y), warm ischemia time (50 vs 50 min) and total ischemia time (482,5 vs 485 min). Median donor age was higher in the main group (44.5 vs 39 year). The overall rate of EAD was 27.9%. EAD rate was significantly lower in the main group (6/43 vs 18/43; $p = 0.003$). The rate of moderate-to-severe EAD was lower in the main group (1/43 vs 10/43; $p = 0.009$) too. There was one case of PNF in each group (NS). The median levels of AST and ALT 24 h after reperfusion were significantly lower in the intervention group (1004 vs 1596; $p = 0.03$ and 449 vs 759; $p = 0.057$). There was no difference in RRT rate (9/43 vs 6/43) and hospital mortality, 2/43 (4.6%) vs 3/43 (6.9%) in main vs control groups. Conclusion. Back-table liver perfusion with HTK+tacrolimus can reduce incdence and severety of EAD after LTx.

O158* MODULATION OF LEUKOCYTES AND ENDOTHELIUM BY ANTI-THYMOCYTE GLOBULIN CONFERS PROTECTION AGAINST RENAL ISCHEMIA REPERFUSION INJURY IN RATS

Prabhu Nesargikar[1], Brad Spiller[2], Robert Jenkins[2], Usman Khalid[3], Donald Fraser[2], Rafael Chavez[3]
[1]Department of Transplant Surgery Cardiff and Vale University Hospital; [2]Cardiff University; [3]Cardiff and Vale University Hospital

Introduction: Renal ischemia reperfusion (IR) injury is a complex mechanism involving inflammatory and immune cells, with the endothelium driving the IR process. Anti-thymocyte antibody (ATG) is a polyclonal antibody that is predominantly used in renal transplantation as an immunological agent, and its effect on IRI in clinical and animal studies have been equivocal. The aim of this study was to define the role of rat specific ATG (Genzyme Corporation) in a unilateral renal IRI model.
Methods: Adult Lewis rats were subject to unilateral left renal ischemia ($n = 10$) for 40 min, followed by 48 h of reperfusion. The ATG group ($n = 8$) received rat specific ATG (10 mg/kg BW) prior to the laparotomy, and then underwent 48 h of renal IR. The sham group ($n = 6$) and ATG Isotype group ($n = 8$) served as controls. CD3 lymphocyte counts from blood samples were used to check ATG efficacy. The kidneys retrieved at 48 h were analysed for histopathology, immunohistochemistry and qPCR studies.
Results: The IRI group showed significant damage compared to the sham group ($p = 0.002$). The ATG treated group showed significant histological protection compared to IR group ($p < 0.0001$). Immunohistochemistry revealed marked reduction in inflammatory cells (CD3, CD4, CD8, CD15, CD68) and complement deposits (C3, C9) in the ATG group compared to IR and ATG IgG groups. Thrombomodulin was still evident on the endothelium in ATG treated rats compared to the IR group, indicating endothelial protection. KIM-1 and NGAL-1 were also significantly reduced in the ATG treated rats on qPCR studies.
Conclusion: In this study, ATG protects against renal IRI via its anti-inflammatory properties and more interestingly, through protection of the vascular endothelial cells. Decrease in complement deposits may be a reflection of decreased activation of the complement system from the protected endothelial cells. Further clinical studies are needed to define the role of ATG as an IRI attenuating agent.

O159 DONOR BRAIN DEATH LEADS TO DIFFERENTIAL IMMUNE ACTIVATION IN SOLID ORGANS BUT DOES NOT ACCELERATE ISCHEMIA REPERFUSION INJURY

Paul Ritschl[1], Muhammad Imtiaz Ashraf[1], Rupert Oberhuber[1], Cornelia Fabritius[1], Thomas Resch[1], Susanne Ebner[1], Martina Sauter[2], Karin Klingel[2], Johann Pratschke[3], Katja Kotsch[1]

[1]Center of Operative Medicine, Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University; [2]Department of Molecular Pathology, University Hospital Tübingen; [3]Department of Visceral-, Abdominal – and Transplantation Surgery, Charité-Universitätsmedizin Berlin

Brain death (BD) has been proposed to influence graft quality and accelerates ischemia reperfusion injury (IRI). A comparative analysis of inflammation between solid organs following BD is still missing and the detailed influence of BD accelerating IRI still needs to be comprehensively addressed. Applying a murine model of BD we demonstrated, that organs following 4 h of BD were characterized by distinct inflammatory expression patterns. For instance, Lipocalin 2 (LCN2), a marker of acute kidney injury, was selectively induced in BD livers but not in kidneys ($p < 0.01$). BD resulted further in significantly reduced frequencies of CD3+CD4+, CD3+CD8 + T cells and NKp46+ NK cells in the liver ($p < 0.01$, $p < 0.01$ and $p < 0.05$ respectively), whereas BD kidneys and hearts were characterized by significantly lower frequencies of conventional dendritic cells ($p < 0.01$ and $p < 0.05$). Impact of donor BD was further tested in syngeneic models of kidney (KTx) and heart transplantation (HTx) illustrating that organs derived from BD or sham donors display comparable gene expression levels, intragraft lymphocyte frequencies, and graft function 20 h post transplantation. Moreover, the deposition of the complement factor C3d detected in small vessels and capillaries in cardiac syngrafts was not significantly different between BD and sham transplanted groups. Solely NK cell numbers derived from BD syngrafts demonstrated organ specific variation (increased in KTx and decreased in HTx, $p < 0.01$ respectively). No influence of donor BD on graft survival was detected in an allogeneic heart transplantation setting (C57BL/6 grafts into Balb/C recipients). We showed for the first time that solid organs are characterized by a varying inflammatory profile following BD characterized by cytokine and lymphocyte expression patterns. However, BD does not accelerate IRI in syngeneic KTx and HTx.

O160 HIGH-DOSE ATORVASTATIN REDUCE OXIDATIVE STRESS OF ISCHEMIA/REPERFUSION INJURY AFTER ISOGENEIC KIDNEY TRANSPLANTATION IN RATS

Edoardo Cola[1], Giacomo Cusumano[2], Jacopo Romagnoli[1], Giorgia Copponi[3], Franco Citterio[1], Maria Emiliana Caristo[4], Melissa Manchi[3], Anna Severino[3], Giovanna Liuzzo[3], Filippo Crea[3], Arnaldo Capelli[5]

[1]Department of Surgical Science, Renal Transplantation Unit, Catholic University, Rome; [2]Vittorio Emanuele Policlinico Hospital Catania; [3]Department of Cardiovascular Medicine, Catholic University, Rome; [4]Experimental Animal Care, Catholic University of Rome; [5]Department of Histopathology, Catholic University

Background: Renal ischemia/reperfusion injury is an unavoidable event in transplantation in which free radical-mediated injury determines release of pro-inflammatory cytokines and activation of innate immunity. In addition to their cholesterol-lowering action statins have shown dose-dependent pleiotropic effects on inflammatory pathways and oxidative stress. Aim of the study. We investigated the effects of high-dose atorvastatin (HATA, Atorvastatin 40 mg/kg) in preventing ischemia/reperfusion injury in a model of kidney transplant in the rat.
Methods: Forty female rats underwent left nephrectomy and subsequent orhtotopic autotransplantation. The animals were divided in four groups: A = Transplant only; B = HATA + Transplant; C = Rigth nephrectomy + Transplant; D = HATA + Right nephrectomy + Transplant. Twenty-four hours post-transplant all animals underwent bilateral nephrectomy. Oxidative stress was assessed by measuring malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and Myeloperoxidases (MPO) activity on renal tissue, ischemia/reperfusion injury was evaluated also by renal histology.
Results: Donor pretreatment with HATA improved oxidative stress. MDA levels were lower in group B vs group A ($p = 0.002$) and vs group D ($p = 0.004$). HATA pre-treated rats displayed significantly higher GPx activitiy in Group B vs Group A ($p = 0.009$) and Group D ($p = 0.005$). SOD scavenger activity was also higher in Group B vs Group A ($p < 0.001$) Group D ($p < 0.001$) and Group C ($p = 0.003$). MPO activity was lower in group B vs group A ($p = 0.02$), group C ($p = 0.007$) and group D ($p = 0.03$). Histology revealed a significantly lower rate of intratubular cast formation and luminal congestion in Group D vs Group C ($p = 0.02$ and $p = 0.008$, respectively). Conclusions. Treatment with HATA is associated with a protective effect against oxidative stress and inflammation in a model of kidney transplant in the rat.

O161* SINGLE DOSE SCR1 ATTENUATES RENAL ISCHEMIA REPERFUSION INJURY IN RATS DESPITE EARLY RECONSTITUTION OF THE COMPLEMENT SYSTEM

Prabhu Nesargikar[1], Gilda Pino-Chavez[2], Brad Spiller[3], Donald Fraser[4], Robert Jenkins[3], Rafael Chavez[2]

[1]Department of Transplant Surgery Cardiff and Vale University Hospital; [2]Cardiff and Vale University Hospital; [3]Cardiff University; [4]University Hospital of Wales

Introduction: The complement system is one of the key mediators in mediating renal ischemia-reperfusion injury (IRI). Soluble complement receptor-1 (sCR1) accelerates the decay of C3/C5 convertase and aids inactivation of C3b and C4b of the complement pathways. There is limited evidence regarding its role in renal ischemia reperfusion injury.
Methods: Adult Lewis rats ($n = 10$) underwent unilateral left renal ischemia (40 min) and reperfusion (48 h). Sham group ($n = 6$) underwent laparotomy without renal ischemia. Single dose sCR1 (25 mg/kg BW) was administered intravenously just prior to the laparotomy. Blood samples were retrieved for CH50 assay. Kidneys harvested at 48 h were analysed for histopathology. Immunohistochemistry (IHC) and real-time PCR were used to assess complement deposits and cellular infiltration.
Results: CH50 assay showed complete ablation of complement activity at time of reperfusion (40 min), with return to normality at 24 h. Histological scoring showed significant difference between the IRI vs sham group ($p = 0.002$), with sCR1 treated rats showing protection from renal injury compared to the IR group ($p = 0.002$). IHC showed decreased C3 and C9 deposits in endothelium and the soft tissues in the sCR1 group, along with significant reduction of inflammatory cells (CD3, CD4, CD8, CD68, CD15 cells) compared to the IR group. CD59 was decreased in the IR group compared to sCR1, indicating protection ($p = 0.04$). Specific endothelial markers, P-Selectin and thrombomodulin were also protected by sCR1. qPCR showed down-regulation of renal injury molecules – KIM1 and NGAL in the sCR1 treated group.
Conclusion: In this study, single dose sCR1 offers protection from renal IRI as evident by decreased complement deposits. The complement system was ablated at the time of reperfusion and was reconstituted by 24 h, thus indicating that suppression of complement system prior during the phase of IR provides as avenue for mitigating IRI.

O162 ROLE OF COMPLEMENT IN MEDIATING PERICYTE - MYOFIBROBLAST TRANSITION IN RENAL ISCHEMIA/ REPERFUSION (I/R) INJURY

Giuseppe Castellano[1], Rossana Franzin[1], Chiara Divella[1], Alessandra Stasi[1], Angelica Intini[1], Margherita Gigante[1], Giuseppe Lucarelli[2], Pasquale Ditonno[2], Michele Battaglia[2], Antonio Crovace[3], Francesco Staffieri[3], Giovanni Stallone[4], Giovanni Batttista Pertosa[1], Beatrijs Oortwijn[5], Edwin Van Amersfoort[5], Giuseppe Grandaliano[6], Loreto Gesualdo[1]

[1]University of Bari, Department of Emergency and Organ Transplantation, Renal, Dialysis and Transplantation Unit; [2]University of Bari, Department of Emergency and Organ Transplantation, Urology, Andrology Unit; [3]University of Bari, Department of Emergency and Organ Transplantation, Veterinary Surgery Unit; [4]University of Foggia, Department of Biomedical Sciences, Section of Nephrology; [5]Pharming Technologies NV, Leiden; [6]University of Foggia, Department of Medical and Surgical Sciences

Background: Preservation of endothelium-pericyte interaction could be critical to counteract vascular rarefaction and fibrosis during renal I/R injury. Pericytes regulate medullary and cortical blood flow in kidney. We investigated whether Complement may be able to modulate pericytes differentiation in renal I/R injury.
Methods: Ten pigs underwent to 30 min of renal warm Ischemia, followed by 24 h of Reperfusion. Five pigs were treated with C1-Inhibitor (C1-Inh). Biopsies were analyzed by IHC for PDGFRβand Caspase3. Detection of apoptosis (Facs Analysis by AnnV-IP), MTT test and IF were performed on human pericytes stimulated with C5a (1×10^{-7} M) and TGFβ (10 ng/ml, as positive control of transition) for 24 h.
Results: I/R injury led to pericytes dedifferentiation as indicated by a significant reduction of PDGFRβ expression in peritubular capillaries ($p < 0.05$), without induction of apoptosis (PDGFRβ+/Caspase3-cells). Pericytes activation was accompanied by a significant decrease in capillary lumen area (Fig. Capillary Area Fraction %T0:11.3 ± 2.1; T24:3.96 ± 2.3; $p < 0.05$). Interestingly, Complement inhibition preserved PDGFRβ expression and restored basal capillary area fraction (T24C1-Inh:12.06 ± 3.5 vs T24). In vitro, C5a did not affect endothelial cells or pericytes proliferation, and did not increase apoptosis (*data not showed,* $p < 0.05$). C5a significantly induced pericyte dysfunction causing down-regulation of PDGFRβ (Fig 2. PDGFRβ Area Fraction %Bas: 15.22 ± 3.63; C5a:3.66 ± 2.35; TGFβ:2.08 ± 1.04, $p < 0.05$) and remodeling of αSMA-stress fibers, indicating the acquirement of a contractile phenotype.

Conclusions: Our study suggests that, during early phase of renal I/R injury, Complement caused pericytes-myofibroblasts transition, leading to a reduction in the density of peritubular capillaries. C1-Inh may be an effective therapeutic strategy to preserve endothelial-pericytes cross-talk and to prevent fibrosis development in transplanted kidney.

Fig.1

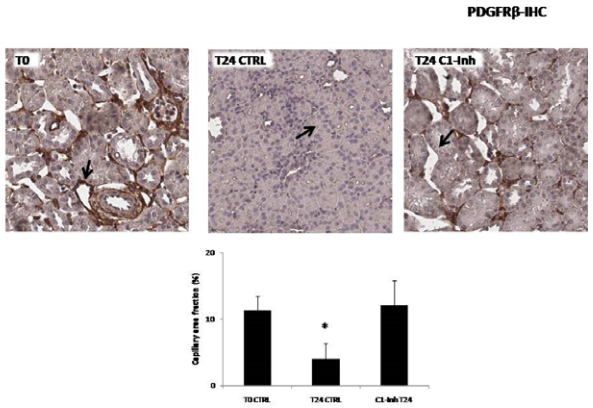
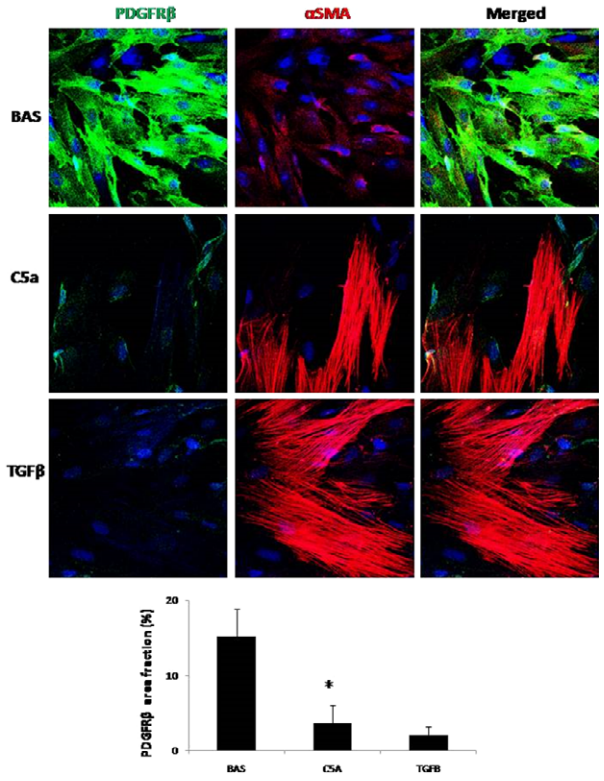


Fig.2



O163* THE HOT STUDY: A RANDOMISED, PLACEBO-CONTROLLED, BLINDED, PHASE IIB TRIAL TO DETERMINE IF HEMIN CAN UPREGULATE HEME-OXYGENASE 1 (HO-1) AND PROTECT RENAL TRANSPLANT RECIPIENTS

Rachel Thomas[1], *Alicja Czopek*[1], *David Kluth*[1], *Stephen Mcnally*[2], *Chris Bellamy*[1], *Lorna Marson*[1]

[1]*University of Edinburgh;* [2]*Newcastle Freeman Hospital*

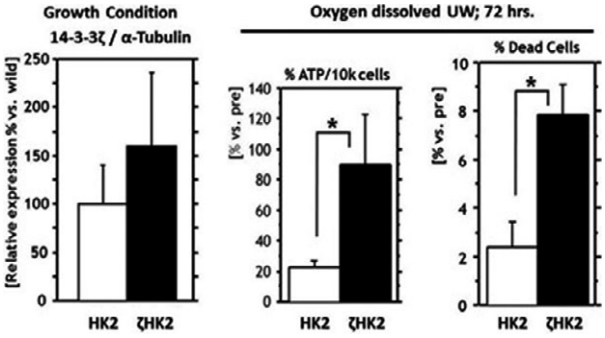
Aims: There are few proven therapies that can protect against the inevitable ischaemia reperfusion injury (IRI) that occurs during transplantation. IRI increases the likelihood of delayed graft function (DGF), which negatively impacts on the long-term survival of a transplanted kidney. One enzyme of interest, heme oxygenase-1 (HO-1), degrades heme and protects against IRI oxidative stress. Heme arginate (HA), a form of hemin, can safely induce HO-1 in humans. Clinical renal recipients with higher HO-1 levels have improved graft function. The HOT study aimed to evaluate whether HA could safely upregulate HO-1 and protect recipients of deceased donor kidneys.
Methods: 40 recipients were randomised to either active (two doses 3 mg kg^{-1} HA: pre-operatively, day 2) or placebo (NaCl: same schedule). Recipient blood was taken daily for peripheral blood mononuclear cells (PBMC) extraction. Urine was also collected. Graft biopsies were taken pre-op and day 5. DGF was calculated.
Results: HA upregulated PBMC HO-1 protein at 24 h more than placebo: HA 11.1 ng/ml [1.0–37.0] vs. placebo 0.14 ng/ml [-0.7–0.3] (p = <0.0001). PBMC HO-1 mRNA was also increased: HA 2.73 fold [1.8–3.2] vs. placebo 1.41 fold [1.2–2.2] (p = 0.02). HA increased HO-1 protein immunopositivity in day 5 renal tissue compared with placebo: HA 0.21 [−24–0.7] vs. placebo −0.03 [−76–0.15] (p = 0.02) and HO-1 positive renal macrophages were also increased: HA 50.8 cells per hpf [40.0–59.8] vs. placebo 22.3 [0–34.8] (p = 0.012). Urinary biomarkers were reduced after HA but not significantly so. Histological injury and DGF rates were similar but the study was not powered to these endpoints. Adverse events were equivalent between groups.
Conclusion: The primary outcome was achieved and demonstrated for the first time that HA safely induces HO-1 in renal transplant recipients. Larger studies are planned to determine the impact of HO-1 upregulation on clinical outcomes and evaluate the benefit to patients at risk.

O164* TIME COURSE OF AUTOPHAGY AND OXIDATIVE PHOSPHORYLATION DURING HYPOTHERMIC OXYGENATED CONDITION AND SUBSEQUENT RE-WARMING IN HUMAN RENAL TUBULAR CELLS (HK-2)

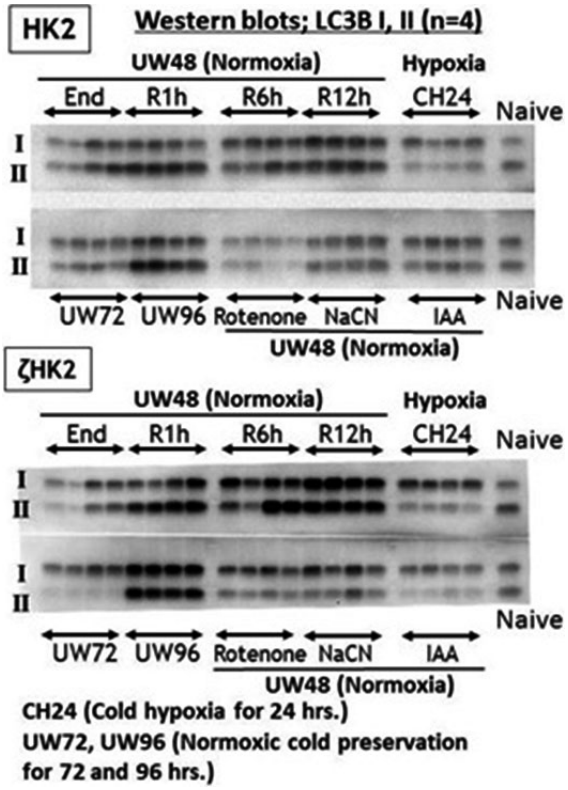
Moto Fukai[1], *Takahisa Ishikawa*[2], *Kenji Wakayama*[2], *Shingo Shimada*[2], *Masato Fujiyoshi*[2], *Taichi Kimura*[3], *Kenichiro Yamashita*[1], *Tsuyoshi Shimamura*[4], *Akinobu Taketomi*[2]

[1]*Transplant Surgery, Hokkaido University Graduate School of Medicine;* [2]*Gastroenterological Surgery I, Hokkaido University Graduate School of Medicine, Sapporo;* [3]*Pathology, Laboratory of Cancer Research, Graduate School of Medicine, Hokkaido University, Sapporo;* [4]*Organ Transplantation, Hokkaido University, Sapporo*

Background: 14-3-3ζ is a phosphoserine-binding protein that regulates many pathophysiological activities including anti-apoptosis, survival signals, and energy production. To develop machine perfusion of sub-optimal graft as a safe technique, precise understanding of oxidative phosphorylation related cellular injury is necessary.
Aims: The aims of the present study were to assess whether 14-3-3ζ protect cells from injury due to the hypothermic oxygenated condition, and to clarify its role on the energy production and cell death.
Methods: Normal human renal tubular cell line (HK-2) or 14-3-3ζ overexpressed cells (ζHK-2) were subjected to 48–96 h of hypothermic oxygenated storage (OxS) in UW solution with or without oxidative stress inducers (H2O2, t-BuOOH), antioxidant (Deferoxamine), GAPDH inhibitor (IAA), mitochondrial complex1 and 4 inhibitors (Rotenone, NaCN). Cells were collected at the end of OxS or after subsequent growth culture for 1, 6, and 12 h. Cellular death, ATP content, and viability were assessed. Time course of 14-3-3ζ and LC3B (II/I ratio) was assessed by western blots.
Results: In normal growth condition, ATP content was higher in ζHK-2. In 72-hour OxS, ζHK-2 showed higher ATP content, MTT assay with higher cell death rate, unexpectedly. This augmented cell death was canceled by antioxidant and exacerbated by hydroperoxides. Western blots revealed that 14-3-3ζ was higher in ζHK-2 throughout the experiment. Cytosolic 14-3-3ζ was reduced in both groups by hypoxia or by inhibition of GAPDH, suggesting rapid translocation to anywhere. LC3B II/I ratio, possible autophagy, was lower in ζHK-2 during OxS, and identical during re-warming.



Conclusions: 14-3-3ζ stimulated oxidative phosphorylation in normal condition as well as in cold oxygenated condition, leading to the oxidative stress and cell death. Control of ATP production and autophagy via oxygen tension, and 14-3-3ζ and GAPDH activities would help safe machine perfusion.



O165 USE OF PROTEOMICS TO IDENTIFY MOLECULAR PATHWAYS RELEVANT FOR REMOTE ISCHAEMIC CONDITIONING IN KIDNEY TRANSPLANTATION

Honglei Huang[1], Roman Fischer[2], Niels Secher[3], Kristian Ravlo[3], Peter Soendergaard[3], Rikke Norregaard[3], Zeeshan M. Akhtar[4], Maria Kaisar[4], Benedikt Kessler[2], Rutger Ploeg[4], Bente Jespersen[3]

[1]University of Oxford; [2]Central Proteomics Facility, Targeted Drug Institute, University of Oxford; [3]Department of Renal Medicine, Aarhus University Hospital; [4]Nuffield Department of Surgical Sciences, University of Oxford

Graft function and survival with 'higher risk' donor kidneys are compromised. To simulate the clinical condition a brain death (BD) and remote Ischaemic Conditioning (rIC) model was established in the pig. After tx, kidneys exposed to rIC showed improved function vs controls, but the underlying mechanism remains unknown. In this study we have used proteomics to evaluate molecular signatures enhancing function in rIC tx recipients.

Material: rIC was induced in recipients (clamping of the aorta: 4 × 5 min) ($n = 8$); non-rIC pigs ($n = 8$) served as sham controls. BD was induced using an inflated balloon catheter in the epidural space ($n = 8$). DBD kidneys were then transplanted into rIC and non-rIC recipients in a paired design. Kidney tx tissue was collected after 10 h reperfusion.

Methods: A LC-MS/MS based unbiased proteomics approach was applied to measure tryptic digested tissue lysates. The central proteomics facilities pipeline (CPFP) developed in-house was used to identify proteins differentially expressed between rIC and non-rIC groups. Ingenuity pathway analysis (IPA) was performed to elucidate proteins associated with rIC.

Results: In total, 3263 proteins were identified (1% false discovery rate); 463 proteins were differentially expressed with at least 2 fold changes between groups. Relevant proteins could be assigned to caveolar-mediated endocytosis signalling ($N = 7$) and coagulation system ($N = 5$), cell death and survival ($N = 9$), organ injury ($N = 13$) and renal disease ($N = 10$). In addition, one specific protein, poly (ADP-ribose) polymerase 1 (PARP1) was found to be significantly upregulated in non-rIC vs rIC.

Summary: Proteomics determination appears helpful to identify key pathways of injury and repair. Bioinformatic analysis reveals a complex interplay between coagulation, necrosis and organ injury. In particular, the specific PARP1 is related to ischaemic condition leading to poly ADP-ribosylation and reduced activity of key glycolytic enzyme GAPDH. Reduced GAPDH exacerbates.

O166 HIGH THROUGHPUT PROTEOMIC EXPLORATION OF COLD ISCHEMIA MECHANISMS

Sebastien Giraud[1], Raphael Thuillier[1], Sophie Lamarre[2], Solenne Tillet[3], Michel Eugene[4], Nicolas Bourmeyster[5], Thierry Hauet[6]

[1]INSERM U1082 IRTOMIT/CHU de Poitiers, service Biochimie/Faculte de medecine-Pharmacie, Poitiers; [2]Université de Toulouse, INSA, UPS, INP, LISBP, Toulouse; [3]INSERM U1082 IRTOMIT/Faculte de medecine-Pharmacie, Poitiers; [4]INSERM U1082 IRTOMIT/CHU de Poitiers/Faculte de medecine-Pharmacie, Poitiers; [5]Plate-forme de Proteomique « PROTEOME UP », Universite de Poitiers, CNRS, Poitiers; [6]INSERM U1082/CHU Poitiers, Biochimie/Faculte de medecine/IBiSA INRA Surgeres/FHU SUPPORT

Background: Extended criteria organs use is increasing. Since these are more sensitive to ischemia reperfusion injuries, it is of paramount importance to better understand the underlying mechanism of this pathology in order to design optimized organ preservation strategies.

Methods: We used high-throughput screening methods on two models: 1- in vitro human endothelial cells subjected to preservation in UW solution, analyzed by proteomics (nano LC MS MS); 2-a preclinical pig kidney model subjected to warm (60 min) followed by cold ischemia (24 h in UW 4°C), analyzed by transcriptomics.

Results: Proteomics: Differential analysis between ischemic and healthy cells showed more than 500 modifications. Clustering analysis revealed alterations in the energy metabolism pathways (glycolysis, mitochondrial structure), cell structure (cytoskeleton, vesicle transport), and response to stress (endoplasmic reticulum stress, oxidative stress, red/ox equilibrium, proteolysis). Transcriptomics: Differential analysis between ischemic and healthy kidneys showed statistical differences in the expression profile of 43 genes ($p < 0.05$). Functional enrichment showed 77 upregulated Gene Ontology pathways including: RNA splicing, inflammatory cytokine production, regulation of lipoprotein oxidation, response to stress stimulus, regulation of T cells migration…) and 5 downregulated Gene Ontology pathways: nucleoside transport, cellular adhesion regulation, regulation of small GTPases ARF, RAS et KRAS ($p < 0.05$).

Conclusion: Our data shows that ischemia is a key step during the transplantation process with important proteomic and transcriptional modifications on major cellular pathways. We highlight that ischemia is not a static period characterized by a simple slowing of the metabolism, but a time of deep changes. Our data shows several pathways which could be critical for the discovery of mechanism-backed biomarkers and targets to better manage organs during preservation and improve quality.

O167 RECOMMENDATIONS ON PAEDIATRIC DECEASED DONATION: A REPORT OF THE TRANSPLANTATION SOCIETY GENEVA MEETING

Marion Johanna Siebelink[1], Dominique Martin[2], Thomas Nakagawa[3], Katrina Bramstedt[4], Joe Brierley[5], Fabienne Dobbels[6], James Rodrigue[7], Minnie Sarwal[8], Ron Shapiro[9], Beatriz Dominquez[10], Gabriel Danovitch[11], Stuart Sweet[12], Richard Trompeter[13], Farhat Moazam[14], Michael Bos[15], Francis Delmonico[16]

[1]University Medical Center Groningen; [2]University of Melbourne; [3]Wake Forrst University School; [4]Bond University; [5]Great Ormond Street Hospital; [6]University of Leuven; [7]Beth Israel Deaconess Medical Centre; [8]University of Californie San Francisco; [9]Thomas E Strarzl Transplantation Institute; [10]Organisation Nacional de Transplantes; [11]David Geffen School of Medicine, University of California Los Angeles; [12]Department of Pediatrics, Washington University; [13]UCL Centre for Nephrology; [14]Center for Biomedical Ethics and Culture; [15]Ethics Committee, The Transplantation Society; [16]The Transplantation Society, Harvard Medical School

Purpose: To provide ethically informed practical recommendations for health professionals and policy makers seeking to establish or improve existing paediatric organ donation programs globally, and identify neglected opportunities for research in this field.

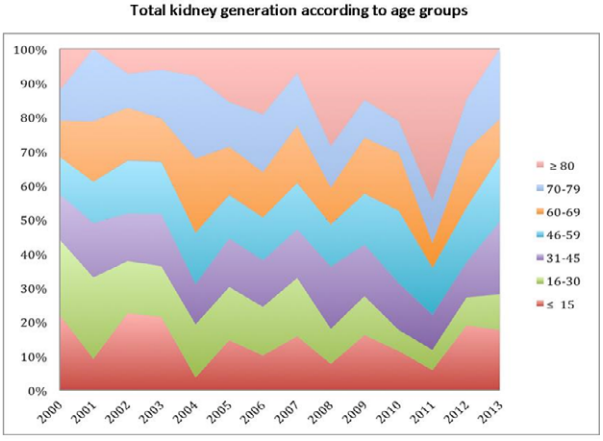
Methods: An international meeting was convened in Geneva, Switzerland, on March 21 and 22, 2014 by the Ethics Committee of The Transplantation Society. The intent was to explore practical and ethical issues pertaining to paediatric organ donation. 34 experts from Africa, Asia, the Middle East, Oceania, Europe and North and South America, representing paediatric intensive care, internal medicine, surgery, nursing, ethics, organ donation and procurement, psychology, law, and sociology participated in this meeting.

Results: Recommendations based on available literature, expert opinion, and consensus highlight the need for multidisciplinary research, dedicated training, and education in the field of paediatric deceased donation among public and healthcare professionals, to preserve and provide opportunities for donation where possible for children and their families. Priority interventions should aim to promote public and professional awareness of paediatric deceased donation; improve public and professional understanding and support for donation through education; expand paediatric donation research; improve organ allocation and implementation of policies and protocols to increase authorization rates for donation and organs recovered and utilised for transplantation.
Conclusion: The report of the Geneva meeting is an international call to action for development of evidence based and best practice resources to globally increase awareness, enhance opportunities, and promote research for deceased donation in neonates and children.

O168* ONE SIMPLE STRATEGY TO DECREASE KIDNEY DISCARD RATE FROM EXPANDED CRITERIA DONORS AFTER BRAIN DEATH: IMPACT OF THE HYPOTERMIC PULSATILE PRESERVATION MACHINE

Zapata David Paredes[1], *Angel Ruiz-Arranz*[1], *Camino Rodriguez-Villar*[1], *Rebeca Roque*[1], *Ramon Adalia*[1], *Lluis Peri-Cusi*[2], *Federico Oppenheimer*[3]
[1]*Donation and Transplant Coordination Section. Hospital Clinic - University of Barcelona;* [2]*Urology Department-Hospital Clinic, University of Barcelona;* [3]*Kidney Transplant Unit-Hospital Clinic, University of Barcelona*

Introduction: Expanded Criteria Donors Donors after Brain Death (DBD), have become 46.8% of our current donor activity, with higher risk of Discard Rate (DR). Assessment of kidney suitability requires a careful protocol of complimentary strategies to decide organ suitability: macroscopic evaluation, Kidney Biopsy Score (KBS) and renal hemodynamic evaluation with Pulsatile Perfusion Machine (PPM).
Methodology: Analysis of a descriptive, cross-sectional, comparative study of kidneys procured and DR, comparing three time periods: 2000-June 2004 when only KBS were used; July 2004–2008 (introduction of PPM and learning period) and 2009–2013 (experienced use and acceptance of PPM). Accepted transplantation criteria were KBS <4; Renal Resistance <0.04 mmHg/ml/min and Renal Blood Flow 70 ml/min.



Donors older 60 years after brain death: Kidney procurement and Discard Rate according to period of time and viability assessment strategy

	2000-June 2004	July 2004-2008	2009-2013	p
Kidney Procurement - N	832	711	492	< 0,05
Kidneys > 60 yo - N (%)	279 (33,5%)	307 (43,2%)	230 (46,8%)	< 0,05
Kidney > 60 yo Discard Rate - %	25,40%	35,20%	38,20%	< 0,05
Kidney preserved in PPM - N		75	56	
PPM Discard Rate - %		29,30%	21,40%	0.06
Kidney preserved in CS - N	279	232	174	
CS Discard Rate - %	25,40%	43,10%	43,70%	<0,05

Results: The figure and table showed a 59.2% reduction in DBD procured kidneys as well as a reduction in the procured older kidneys. However, the relative proportion of older kidneys has increased from 33.5% to 46.8%. The DR has increased comparing first to third period from 25.4% to 38.3%. However, the DR was lower when kidneys were evaluated with PPM than those evaluated only with KBS and preserved in CS (21.4% vs 43.7%). No differences were founded between both preservation groups, regarding age, gender, cardiovascular risk factors and cause of death. Only significant difference was found in cold ischemia time, because CS kidney was grafted before PPM kidneys. During third period, more kidneys with KBS equal or >4 were assigned to PPM.
Conclusions: Nevertheless the decrease in DBD procured kidneys and the increase in older kidneys during last period, the use of PPM has allowed low DR compared with CS. A bias in the results of PPM could be generated when kidneys with higher KBS are excluded from PPM. The only use of KBS to decide acceptance, could preclude to offer the kidney an additional tool to evaluate suitability.

O169 EFFECT OF DURATION OF BRAIN DEATH ON OUTCOMES IN ABDOMINAL ORGAN TRANSPLANTATION: RUSH AND RETRIEVE OR RELAX AND REPAIR?

Catherine Boffa[1], *Elinor Curnow*[2], *Kate Martin*[2], *Rachel Johnson*[2], *Rutger Ploeg*[1]
[1]*Oxford Transplant Unit and Nuffield, Department of Surgical Sciences;* [2]*NHS Blood and Transplant*

Background: Brain death (BD) induces an inflammatory and pro-coagulatory response that leads to injury in donor organs. This raises the clinical dilemma of whether to retrieve organs ASAP to minimise the effects of this hostile environment or allow time to optimise the donor. Limited data are available with regards to this issue, so we assessed the effect of BD duration on outcomes following abdominal organ transplantation in the UK.
Methods: In a retrospective analysis, UK DBD donors during 2008–2012 were evaluated. Cox regression was used to investigate the relationship between BD duration and graft survival (GS) at 90d, 1 and 3 y.
Results: 1881 UK adult donors after brain death (DBD) who donated at least one abdominal organ were included in the analysis. Median BD duration was 33 h in 2008 increasing to 36 h in 2012 ($p = 0.03$). Longer BD duration did not have a detrimental effect on GS, and there is evidence of increasing transplant survival after first adult kidney-only transplant as BD duration increases ($p = 0.01$). There was no significant difference in transplant survival across BD duration groups for liver or pancreas ($p = 0.47$ and $p = 0.11$). Risk-adjusted Cox regression analyses of GS after kidney transplant suggest an interaction between BD duration and CIT, with longer brain death durations tending towards improved survival when the CIT is >12 h. There was a significant interaction between BD duration and year of donation post pancreas transplant, with survival improving in the latter years of the study. No interactions were found in the liver model.
Discussion: Our study demonstrates that prolonged BD is not detrimental to outcomes in abdominal organ transplantation and may benefit transplant survival following kidney transplantation when CIT is >12 h. This finding supports that time is required to adequately optimise organ donors and renounces the need for a rush and retrieve policy.

O170 RECIPIENTS DO NOT BENEFIT FROM DBD ORGAN PROCUREMENT DURING NIGHTTIME

Willemijn Nijboer[1], *Jeroen De Jonge*[2], *Christina Krikke*[1], *Jan Ijzermans*[2]
[1]*University Medical Center Groningen;* [2]*Erasmus Medical Center Rotterdam*

Background: Many organ procurement procedures are scheduled to take place during nighttime, which is known to affect workers' health negatively. This is done partly for logistical reasons (empty operating rooms) but also because it is widely felt that organ donation 'can't wait' because organ quality will deteriorate. Earlier, we reported that a prolonged duration of brain death in the donor does not negatively affect graft and recipient survival after abdominal organ transplantation, suggesting organ procurement can be safely scheduled at daytime. However, it is not known whether scheduling organ procurement at daytime does affect outcome after transplantation.
Methods: Databases from both OPTN (2006–2012) and Eurotransplant (2002–2012) were used. Graft and patient survival of transplanted abdominal organs were studied using Kaplan Meier curves. As start and end times of procurement operations were not recorded, we used 'start of cold perfusion' (SCP) as a surrogate. Operations with SCP from 9.00 am till 19.00 pm were considered as daytime surgery and compared with SCP 19.00 pm-9.00 am.
Results: For kidney transplantation, pancreas transplantation and combined pancreas/kidney transplantation, moment of organ procurement did not influence outcome. For liver transplantation however, patient survival was better when the liver had been procured during daytime both in the OPTN (Log Rank patient survival $p = 0.014$) and the Eurotransplant cohort ($p = 0.007$); for graft survival, the same trend was not statistically significant.
Conclusion: Organ retrieval during nighttime does not have advantages for transplantation outcome. For liver transplantation, nighttime organ procurement even seems to influence transplantation results negatively, however further analyses are needed to elucidate those factors negatively affecting

outcome during nighttime procedures. Based on our data it may be advocated to schedule procurement procedures for abdominal organs preferably at daytime.

O171 HEMODYNAMIC MONITORING DURING ORGAN HARVESTING FROM BRAIN DEAD DONORS. USE OF OESOPHAGEAL DOPPLER IS SUPERIOR TO CLINICAL ASSESSMENT AND IMPROVES HEMODYNAMIC MANAGEMENT

Jan Frederik Bugge, Viesturs Kerans, Hans Christian Nyrerød
Dep of Anesthesiology Rikshospitalet Oslo University Hospital

Background: Keeping brain dead donors circulatory stable is challenging and requires adequate fluid resuscitation and vasoactive medication. Clinical judgement of global haemodynamics was compared to oesophageal Doppler measurements and the latter was then used to improve management during organ procurement. Methods/materials: In 60 brain dead donors clinical assessments were performed, an oesophageal Doppler probe was inserted, and the haemodynamic variables derived, were compared to the clinical assessment. The Doppler variables were used to guide therapy aiming at mean arterial pressure (MAP) ≥65 mmHg, cardiac index (CI) ≥2.5 l/min, systemic vascular resistance index (SVRI) = 1300–1700 dyne*s/cm5*m^2, and flow time corrected (FTc) ≥350 ms.
Results: Of the 60 donors. 47 were clinically assessed to be haemodynamically stable. Of the other 13, five was assessed to be hypovolemic and vasodilated. Only 2 of these were hypovolemic and 4 were vasodilated. Their CI was between 3.2 and 7.1 l/min. Two donors were assessed to be hypovolemic and vasoconstricted, and this was confirmed for one of them. Doppler guided therapy normalized his haemodynamic variables. The other was normovolemic and vasodilated with CI = 6.5 l/min. Four donors were clinically assessed to be hypoperfused and was confirmed for two of them (CI = 2.2 l/min and SVRI >3200 dyne*s/cm5*m2). Subsequent Doppler guided therapy normalized their CI and SVRI. The other two had CI of 4.7 and 5.0 l/min, and SVRI between 1000 and 1100 dyne*s/cm5*m2. The CardioQ measurements revealed 10 donors as hypoperfused, with CI ≤2.2 l/min. Of these, eight were clinically assessed to be haemodynamically stable. Doppler guided therapy normalized CI in eight of them and improved CI in the others. Conclusions: Clinical assessment of global haemodynamics in donors is difficult and often wrong, and may lead to inadequate therapy and suboptimal organ quality. Oesophageal Doppler improves haemodymamic management.

O172 NEW PERSPECTIVES AND ADVANCES IN THE COORDINATION PROCESS AND IN THE PROCUREMENT PHASE FOR OPTIMIZING QUALITY IN RENAL TRANSPLANTATION

Constantinos S. Mammas[1], Andreas Lazaris[2], Georgia Kostopanagiotou[3], Chrysoula Lemonidou[4], Achilleas Chatzioannou[5], Eustratios Patsouris[6]
[1]National and Kapodistrian University of Athens Scholarship State Foundation; [2]National and Kapodistrian University of Athens Medical School Department of Pathology; [3]Attikon University Hospital; [4]National and Kapodistrian University of Athes Nursing School; [5]National and Kapodistrian University of Athens Aretaieion University Hospital Department of Radiology; [6]National and Kapodistrian University of Athens Department of Pathology

Back-round: Damaged Renal Grafts (RG) in Renal Transplantation (RT) include pre-existing donor organ disease and damage during retrieval or bench preparation. It is searched the feasibility and reliability of Tele-medicine Systems (TS) in the Coordination Process (CP), of ergonomics of Tele-radiology (TRE) before grafting and of Macroscopic (Mac) and Microscopic (Mic) Tele-pathology (TPE) evaluation after grafting for remote evaluation and minimization of damaged/diseased RG.
Material and Methods: A. Experimental simulation of the CP and of the procurement phase of RT by applying TS, TRE and TPE:1. Forty participants (n = 40) who were divided in two groups, the specialists and the non-specialists and comparatively simulated the telephone/fax and the TS based CP upon a RT scenario, B. A simulation of TRE before grafting between radiologists in the donor hospital (DH) and the coming grafting team (GT) of RG based on 30 MR series of images and by simulating: C. The mac. TPE after grafting by 46 specialists in DH and the Transplant Team in the Recipient Hospital based on 130 macroscopic graft images assessing all possible RG damages and diseases and D. The mic. TPE by two specialists based on 238 microscopic renal tissue images assessing only inflammatory and neoplastic diseases.
Results: A. Specialists found TS significantly more reliable in the fulfillment of all medico-legal requirements of OT ($p < 0.001$). B.TRE and mac.TPE showed in total: Sensitivity = 96.7%, Specificity = 100% Accuracy = 97.6%, C.TRE and macroscopic and mic.TPE for only inflammatory and neoplastic diseases showed: Sensitivity = 100%, Specificity = 100% Accuracy = 100%.
Conclusion: On a clinical simulation level: 1.TS seem feasible and more reliable than fax and telephone in the CP of RT. 2. TRE integrated with TPE seems feasible, reliable and optimum for remote identification and minimization of damaged/diseased RG and for pre-grafting and pre-transplant decision support and planning.

007 DONATION/RETRIEVAL

O173 THE ANALYSIS OF DISCARDED DECEASED ORGANS IN KSA

Besher Al-Attar[1], Faissal A.M. Shaheen[1], Michael Abeleda[1], Abdullah A. Al-Sayyari[2], Mohammad Kamal[1]
[1]Saudi Center for Organ Transplantation; [2]KIng Abdulaziz Medical City and National Guard

Objective: To evaluate the rate and causes of unused organs in ten-year period.
Methods: A retrospective study was done during the period of 2000–2009, comprising the eligible, the actual, the utilized deceased organ donor cases and consented not harvested cases. Organs involve were kidneys, livers and hearts.
Results: From the total of 4227 reported cases as Possible Deceased Donors (DD), 2162 (51.1%) were approached for organ donation after declaration of Death, and 702 (32.5%) were consented for organ donation with 635 (90.5%) harvested, while 67 (9.5%) were rejected. From the 702 total consented cases, 98% were consented for kidney donation. There were 1066 kidneys retrieved locally, 1009 (94.7%) of them were utilized, and 57 (5.3%) were not used. All in all, 653 (93%) cases were eligible for liver donation, 249 (38.1%) of which were rejected for utilizing mainly due to hypernatremia and elevated liver enzymes, 422 (64.6%) cases were harvested while 323 (76.5% from the harvested cases) were utilized with 99 (23.5%) unused. There were 596 (84.9%) eligible for heart donation with only 84 (14.1%) were used as whole heart and 296 used as a source for valves.
Conclusion: The rate of rejection to harvest were 10.13% for kidneys, 34.41% for liver and 36.92% for heart, mainly as a result of the donor qualities, while the rate of unused organ after harvesting is around 19.14%, usually due to characteristics itself. Still, we could lessen the rate of rejection by preventing technical reasons and the much needed improvement in the area of donor management.

Keywords: Deceased Donors, Unused Organs, Rejection Rate, Saudi Arabia

O174* USE OF PROTEOMIC SIGNATURES IN DONOR SERUM AND URINE TO DIFFERENTIATE BETWEEN IMMEDIATE FUNCTION AND DELAYED GRAFT FUNCTION AFTER KIDNEY TRANSPLANTATION

Maria Kaisar[1], Honglei Huang[1], Zeeshan Akhtar[1], Letizia Lo Faro[1], Henri Leuvenink[2], Benedikt Kessler[1], Rutger Ploeg[1]
[1]University of Oxford; [2]University Medical Centre Groningen

Introduction: Despite the persistent shortage of donor organs, paradoxically, many organs obtained from older and 'higher risk' donors are deemed unsuitable for transplantation and discarded. The ability to better assess quality of donor organs prior to transplantation and reduce uncertainty is vital. We tested the hypothesis that donor serum and urine proteomic signatures can discriminate between immediate and delayed graft kidney function after transplantation.
Method: Serum and urine samples from brain dead donors (DBD) and donors after circulatory arrest (DCD) were grouped according to the incidence of delayed graft function (DGF) or immediate function (IF) with living donors (LD) as controls. Samples were analysed using a label free quantitative (LFQ) proteomic approach.
Results: Without "a priori" assumptions and based only on serum proteomic signatures in the donor at time of kidney retrieval we could differentiate donors with kidneys that will develop DGF post-transplant (Fig 1). Discriminatory proteomic signatures were confirmed in donor urine. 137 serum proteins were either upregulated or uniquely expressed in DBDs associated with DGF (DGF vs IF >2 fold, ANOVA $p < 0.05$). A parallel activation of apoptotic, metabolic, inflammatory and cytoprotective pathways was demonstrated. Markers of kidney inflammation and fibrosis were also significantly increased in urine of donors that developed DGF when compared to IF (Fig 2). Interrogation of the proteomic signatures revealed that at the time of sample collection in the donor, a systemic state of inflammation was more pronounced in DBD than DCD reflecting pathophysiological changes due to brain death.
Conclusion: This is the first study that describes proteomic signatures in donor serum and urine reflecting the onset of donor kidney injury and classifying donors on the basis of outcomes in kidney transplantation. Further analysis is done to validate and develop diagnostic tools to assist clinical decisions at time of offering.

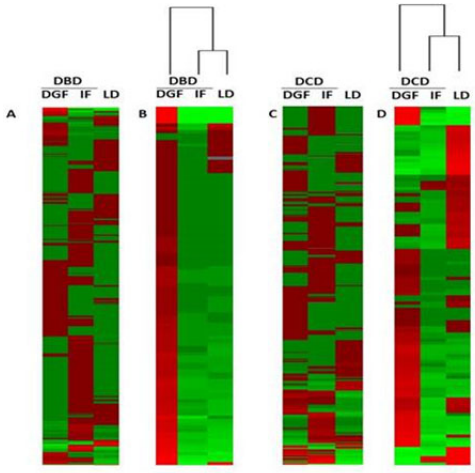


Figure 1. Donor serum proteomic signatures discriminatory of DGF in kidney recipients. (A) Unsupervised hierarchical clustering analysis of all the proteins identified in the proteomic analysis of DBD subgroups (DGF and IF) and LDs. (B) Supervised hierarchical clustering analysis of all significantly regulated proteins in DBD subgroups (DGF and IF) and LDs. (C) Unsupervised clustering of all the proteins identified. in the proteomic analysis of DCD subgroups (DGF and IF) and LDs. (D) Supervised hierarchical clustering analysis of all significantly regulated proteins in DCD subgroups (DGF and IF) and LDs. Red represents high and green low abundance of the identified proteins. (p<0.05, ANOVA)

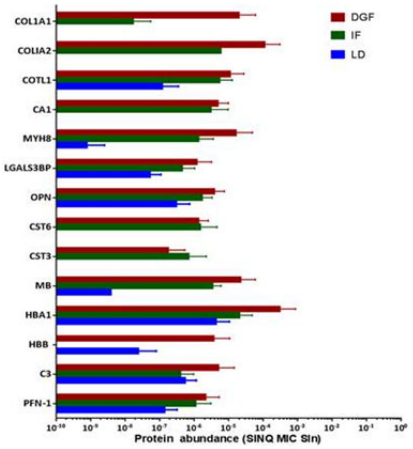


Figure 2. Donor urine protein regulation between DBD (DGF vs IF) and LD among individual samples. Protein abundance is expressed in spectra index normalised quantitation (SINQ). COL1A1: collagen alpha 1 chain, COL1A2: collagen alpha 2 chain,COTL1: Coactosin-like protein 1, CA1: carbonic anhydrase 1, MYH8: myosin-8, LGALS3BP:Galectin-3-binding protein, OPN: osteopontin, CST6: cystatin-M, CST3: cystatin-C, MB: myoglobin, HBA1: hemoglobin subunit alpha, HBB: Hemoglobin subunit beta, C3: complement 3, PFN-1: profilin-1.

O175 PSA POSITIVE ORGAN DONOR – STANDARD OR UNACCEPTABLE RISK FOR RECIPIENT

Krzysztof Pabisiak[1], Marek Ostrowski[2], Andrzej Kram[3], Jerzy SieńKo[2], Tomasz Huzarski[4], Marek MyśLak[5], Kazimierz Ciechanowski[5]
[1]Department Nephrology Transplantation and Internal Medicine/ Pomeranian Medical University; [2]Department of General Surgery and Transplantation/ Pomeranian Medical University; [3]West Pomeranian Oncology Center/ Szczecin; [4]Department of Genetics and Pathology/ Pomeranian Medical University; [5]Depatment Nephrology Transplantation and Internal Medicine

Background: Demographic prognosis estimates that in 2030, 24% of the population in the Eurotransplant region will be over 65 years of age, compared to the current 17%. Prostate cancer (CaP) is the most commonly diagnosed cancer among men in Europe. In 2009, Poland introduced a recommendation to determine tumor markers in potential organ donors. In the first year after implementation, 10% of 42 donors referred to the regional transplant center were disqualified due to elevated PSA levels (above 10 ng/ml), regardless of the PSA level. Taking into account demographic, epidemiological projections and not having an algorithm in the case of a "PSA-positive donor", the loss of organ recovery may occur more frequently due to the lack of clear procedures.
Aim: To avoid reducing the organ donor pool, since Jan 2010 to Dec 2014 each donor ($n = 52$) reported to regional transplant center during the selection

median value	No CaP/PIN	CaP/PIN	p-value	CaP	p-value
Donor Age (years)	53	60	0.0004	64	0.000002
ICU (days)	4	4	0.24	3	0.062
PSA (mg/ml)	3.9	6.08	0.09	5.89	0.35
Donor Cr^ (mg/dl)	1.1	1.1	0.61	0.92	0.77
BD CVA/T/A*	15/20/4 38%/52%/10%	2/4/1 46%/46%/8%	ns	4/2/0 66%/36%/0%	ns

*CranioVascular Accident/ Trauma/ Asphyxia ®Donor Creatinine

procedure undergoes a routine histological evaluation of the prostate to establish risk for transmission neoplastic disease.
Results: Clinical data compared with histopathological findings
PIN Prostate Intraepithelial Neoplasia CaP Prostate Carcinoma PSA prostate specific antigen
Conclusion: Elevated PSA levels in organ donors under 60 years of age are mostly associated with non-cancer prostate involvement. The age of donors is the basic criterion in the current diagnostic algorithm for PSA-positive donors implemented in our center. A rectal examination is performed in men over 60 years of age and PSA >4 ng/ml, and in those over 50 years of age and PSA >10 ng/ml. If the DRE is positive, they are subjected to histological verification; if the DRE is negative, they are qualified for organs recovery. Histological verification is conducted in subjects >60 years of age, despite DRE (-) and/or PSA >15 ng/ml, regardless of age, in order to carry out a prospective analysis of the decision tree.

O176 BIRTH WEIGHT AS A MARKER OF NEPHRON NUMBER: PREDICTING KIDNEY DONOR OUTCOMES

Thomas Schachtner, Petra Reinke
Charite Campus Virchow Clinic - Department of Nephrology and Internal Intensive Care

Background: It has been demonstrated that low birth weights give rise to a reduction in nephron number and compensatory glomerular hypertrophy with an increased risk for hypertension and renal disease. Its impact on renal function in kidney donors, however, hasn't been addressed so far.
Methods: To investigate the impact of birth weight, kidney weight, and volume on kidney function, we collected data from 91 living kidney donor/recipient pairs between 2003 and 2012. Samples were collected before nephrectomy, +12, +36, and +60 months to assess eGFR and proteinuria. Birth weight was retrieved from donors' birth records. Kidney volume was measured from contrast-enhanced CT-scans. Kidney weights were collected immediately before grafting.
Results: Donors remaining kidney function showed a strong positive correlation with birth weight at +12, +36, and +60 months after nephrectomy (p0.05). Donor birth weight showed a positive correlation with allograft function ($R = 0.236$, $p = 0.031$) and negative correlation with the number of antihypertensive drugs in the recipient ($p < 0.05$).
Discussion: Low donor birth weight predisposes donors to inferior remaining kidney function, hypertension, and proteinuria. The strong correlation in elderly donors may be attributed to a reduced renal functional reserve due to the natural decline of renal function with age.

O177 BRAIN DEAD DONORS ON EXTRACORPOREAL MEMBRANE OXYGENATION: AN ANALYSIS OF SUITABILITY FOR ORGAN DONATION AND ORGANS AND RECIPIENTS OUTCOME

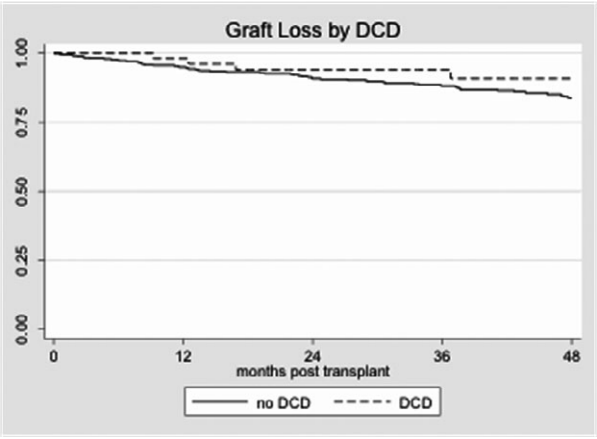
Regis Bronchard, Louise Durand, Camille Legeai, Patrice Guerrini, Olivier Bastien
Agence de la Biomedecine

Introduction: There is a lack of data concerning brain-dead donors (BDD) on extra corporeal membrane oxygenation (ECMO). The objectives of this study were to describe BDD on ECMO, to assess their suitability for organ donation and to analyze the outcome of organs transplanted from BDD on ECMO.
Method: Using the CRISTAL database (a national database of all donors and recipients in France maintained by the Agence de la Biomedecine), we identified BDD with ongoing ECMO from 2007 to 2013. We analyzed the characteristics of donors and transplant outcomes. These findings were compared to those of BDD without ECMO using Chi-square, Fisher or Wilcoxon procedures as appropriate. Survival rates were estimated using the Kaplan-Meier method and compared using the log-rank test. A $p < 0.05$ was considered significant.
Results: Between 2007 to 2013, there were 147 BDD on ECMO, 56 of whom procured at least one organ (BDD-ECMO group) compared to 10 813 BDD who procured at least one organ while not on ECMO (BDD-non ECMO group). Compared to BDD-non ECMO, BDD-ECMO were younger (39 years vs 56 years, $p < 0.0001$), had significantly fewer comorbidities (arterial hypertension, previous medication use, etc) and were significantly more severe (lower mean arterial pressure, lower hourly diuresis, higher arterial lactate, higher hepatic cytolysis, higher serum creatinine peak, higher transfusion requirements). Ninety-five kidneys were transplanted from 49 BDD-ECMOs (87.5% vs 89.4% of BDD-non ECMO; ns) and 31 livers were transplanted from BDD-ECMOs (55.4% vs. 66.6% of BDD-non ECMO; $p = 0.051$). One-year kidney survival rate was 93.7%[86.5%–97.1%] for BDD-ECMO vs 91.4%[90.9%–91.8%] for BDD-non ECMO. One-year liver allograft survival rate was 87.9% [70.9%–95.3%] for BDD-ECMO vs 80.6%[79.7%–81.5%] for BDD-non ECMO.
Discussion and conclusion: Despite the severity of their medical conditions, organs procured from BDD with ongoing ECMO appear to experience favorable one-year survival rates.

O178 LONG-TERM OUTCOMES OF RENAL TRANSPLANTATION UTILIZING DONATION AFTER CIRCULATORY DEATH (DCD) GRAFTS

Hemangshu Podder, Ana Islam, Larry Teeter, Samir Patel, Osama Gaber, Richard Knight
Houston Methodist Hospital

Background: The goal of this study was to compare outcomes of DCD and donation after brain death donor (DBD) transplants.
Methods: This was a single-center retrospective review of all deceased donor renal transplants performed between Jan 2008 and Jan 2014. Dual kidney and multi-organ transplants were excluded. Only Maastricht category III DCD donors were included in this review. All kidneys were preserved by pulsatile hypothermic perfusion. Immunosuppression included antibody induction with tacrolimus, mycophenolate mofetil, and prednisone. Donor specific antibodies (DSAs) and BK virus serum PCR testing occurred at 1, 2, 3, 6, 9, and 12 months post-transplantation.
Results: We compared 53 DCD and 460 DBD kidneys. Median follow-up for all recipients was 36.2 months (IQR 18.9–48.8). The median cold ischemia time for DCD versus DBD donors was 23 (IQR 19–28) and 19 h (IQR 14–26, $p < 0.001$) respectively. The terminal donor serum creatinine for DCD versus DBD donors was 0.8 (IQR 0.6–1.1) and 1.1 mg/dl (IQR 0.8–1.6, $p < 0.001$) respectively. There were no other differences in baseline characteristics between groups. The DCD and DBD cohorts had similar incidences of delayed graft function (9 versus 8%, $p = 0.814$) and acute rejection (17 versus 22% respectively, $p = 0.385$). There were no differences between the DCD and DBD groups in de novo DSA development (28 versus 32%, $p = 0.566$) or incidence of BK virus (15 versus 23% respectively, $p = 0.198$). Importantly, there was no difference in graft survival between the 2 groups ($p = 0.308$; Figure 1). DCD and DBD organ recipients had equivalent long-term renal function. Median eGFR for the DCD and DBD groups at 1, 2, and 3 years post-transplant was 63.2 versus 58.2, 62.3 versus 58.2, and 60.8 versus 58.2/min/1.73 m^2, respectively (p = ns at all time points). Conclusions: Utilization of DCD organs increases the donor pool while providing equivalent short and long-term outcomes to DBD organs.



023 KIDNEY

O179 TELEMEDICAL SUPPORTED AFTERCARE AFTER LIVING KIDNEY TRANSPLANTATION – A SINGLE CENTER EXPERIENCE

Silvia Hils[1], Anja Schmid[1], Lioudmila Bogatyreva[2], Dieter Hauschke[2], Albrecht Kramer-Zucker[3], Przemyslaw Pisarski[1]

[1]Medical Center - University of Freiburg, Department of Surgery, Transplantation-Center; [2]Institute for Medical Biometry and Statistics; [3]Medical Center – University of Freiburg, Department of Medicine IV

Background: The constant high number of patients on the German kidney waiting list and the permanent shortage of donor organs, forces some new solutions. The Transplantation Center Freiburg has always been innovative and telemedicine has been an upcoming part of the German health care system. The benefit of telemedical support, e.g. for chronic diseases has been reported in many studies.
Method: A prospectiv, randomized, controlled and open Project-Study with 50* living kidney transplanted patients (*4 drop outs) was initiated. 23 patients with standard aftercare, 23 patients with standard aftercare and additional webbased telemonitor with videocamera at home. Observation period: Oct.2011-Apr.2014. Repeated measure analysis at time points 0, 3, 6, 12 months post-tx via medical reports and standardized Interviews/Questionnaires (BAASIS, ESRD-SCL, BSI-18, ALL) about: course of their medical condition, adherence concerning the intake of immunosuppressive medication, psychosocial factors and economic factors.
Results: 12 months after living kidney transplantation (LKTx) significant results were reported. In the telemedicine group, at the onset of diseases, serious complications can be avoided because of an early diagnosis of infections and of acute renal failure. Significantly reduced duration (67%/ $p = 0.005$) and frequency (60%/$p = 0.002$) of unplanned hospital-readmissions in the first year after transplantation. Significant higher adherence to immunosuppressant medication ($p = 0.003$). Higher quality of life because of less cortisone side effects ($p = 0.004$) and less cardiac & renal dysfunction ($p = 0.050$).
Conclusion: The results of this innovative and new aftercare procedure confirm, that telemedical supported patients experience longer periods of being healthy during the first year after LKTx. It guarantees the patients an optimized follow-up and brings to the medical also economic benefit. It creates a win-win-situation for all participants and may help to prevent graft loss.

O180 LYMPHOPROLIFERATIVE DISORDERS AFTER RENAL TRANSPLANTATION: A LARGE LONGITUDINAL STUDY OF 21546 RECIPIENTS

Antonio Franco[1], Domingo Hernandez[2], Angel Alonso[3], Dolores Burgos[2], Sofia Zarraga[4], Marta Crespo[5], Ana Sanchez[6]

[1]Hospital Alicante; [2]Hospital Carlos Haya Malaga; [3]Hospital Juan Canalejo Coruña; [4]Hospital Cruces Bilbao; [5]Hospital Del Mar. Barcelona; [6]Hospital Clinico, Madrid

Post-transplant lymphoproliferative disorders (PTLD) are lymphoid proliferations. Epstein Barr Virus (EBV) seronegativity in the recipient, use of antilymphocyte antibodies (UAB), acute rejection and CMV infection are classical risk factors. We studied,in a longuitudinal study, the cumulative incidence of PTLD, its relationship with EBV, classical risk factors and outcome in 21.546 simple adult renal recipients from cadaveric and living donors, transplanted in 21 hospitals from 1990 to 2009. The follow up was at least of 3 years. A total of 243 recipients,173 males (71.4%), aged 50.6 (14.7)years, developed PTLD (1.1%), 8.18 cases (8.16;8.19)/10.000 patients/year. Cumulative incidence was 1.4%,5.1% and 9.8% at 1, 5 and 10 years post-transplant. Two hundred fourteen (88%) were 1st recipients and 238 (98%) from cadaveric donors. EBV in the tissue was reported in 89 out of 138 studied recipients (64.5%). One hundred sixty-four recipients (66.7%) had any classical risk factor, UAB the most frequent. The 86.0%of proliferations were B lymphocytes. PTLD median appearance after transplant were 42 months (25–75; 12,77,5), 81.7 in recipients with EBV presence in tissue (25–75;25;129) and 82.8 (25–75;41;109)with absence ($p = 0.725$). During the follow-up,160 patients (65.8%) died and 83 (34.2%)had a total remission, but 18 of them lost their grafts (7.4%). The main cause of death was PTLD progression ($n = 84$, 51.8%),followed by sepsis ($n = 22$, 13.5%). Patient survival after diagnosis was 51%, 44% and 39% and graft survival 48%, 39% and 33% at 1, 2 and 5 years. In conclusion, most of the PTLD are B lymphocytes and seem to have close relationship with EBV. PTLD can develop in the absence of classical risk factors, UAB the most frequent. There is no relationship between presence of EBV and appeareance of PTLD after transplant. The prognosis is poor at 1 year due to PTLD progression,but later the survivors can even keep their grafts.

O181 FROM PHARMACOGENETICS TO CLINICAL PRACTICE: WHICH SNPS ARE ASSOCIATED WITH MAJOR LONG TERM GRAFT COMPLICATIONS OF KIDNEY TRANSPLANTATION

Claudio Musetti, Marco Quaglia, Salvatore Terrazzino, Guido Merlotti, Piero Stratta, Armando Genazzani

Università del Piemonte Orientale

Introduction: Single-point gene variants (SNP) involved in the IS metabolism are able to predict drug efficacy and side effects, but it is uncertain if they are associated with long-term complications of kidney transplantation (KTx). Aim of this study was to evaluate the association between major SNPs involved in IS metabolism and long term complications of KTx.
Methods: We included 436 kidney transplant recipients (age 52.7 ± 12.4 years, 65.6% male) and evaluated the SNPs CYP3A5 *1/*3, MDR1 3435C>T and 1236C>T, TCF7L2 rs7903146C>T, and CYP3A4 rs35599367 C>T. An association study was performed with viral reactivation (CMV and BKV), post-transplant lymphoprolipherative disorders (PTLD), malignancies, cardiovascular events (CVE), and graft failure.
Results: The CYP3A5*1 allele ($n = 52$) was associated with more BKV nephropathies (5.8% vs. 1.0%; OR = 5.8, $p = 0.03$), but less CMV reactivations (1.9% vs. 18.8%; OR = 0.08; $p = 0.005$). Patients with a CYP3A5 *1 allele did not develop any virus-associated malignancy (0% vs. 8.3%, $p = 0.03$). The TCF7L2 T allele ($n = 274$) is associated with CVE (25.2% vs. 12.8% of TCF7L2 CC patients, OR = 2.3; $p = 0.003$), particularly for arterial atherosclerotic events (9.1% vs. 1.9%, OR = 4.8; $p < 0.001$).

PTLD are associated in our cohort with the MDR1-1236 TT genotype (6/112 = 5.4% vs. 0.7%, OR = 8.5, $p < 0.01$), TCF7L2 TT genotype (3/55 = 5.5% vs. 1.4%, OR = 4.1, $p = 0.04$), and an EBV negative serology (1/13 = 7.7% vs. 1.7%, OR = 4.7, $p = 0.09$). Patients with both homozygous mutations ($n = 14$) had 5-year risk of PTLD of 14.5%, patients with either homozygous mutation or EBV negative ($n = 142$) of 5.3%, while none of other patients ($n = 215$) developed a PTLD (risk <1.7%). Graft failure was not independently associated with any of the study SNPs.
Conclusions: SNPs CYP3A5 *1/*3, MDR1 1236 C>T and TCF7L2 rs7903146 C>T are able to well stratify the risk of viral-associated diseases, CVEs and PTLD. As they may be known before surgery, specific strategies might be adopted in high risk patients.

O182 5-YEAR PROGNOSTIC VALUE OF THE ESTIMATED GFR AND ITS SLOPE OF EVOLUTION DURING THE FIRST YEAR POST-TRANSPLANTATION IN KIDNEY ALLOGRAFT RECIPIENTS

Benjamin Taton[1], Karine Moreau[1], Karen Leffondre[2], Christian Combe[1], Pierre Merville[1], Lionel Couzi[1]

[1]CHU de Bordeaux - Néphrologie & Transplantation Rénale; [2]Institut de Santé Publique et d'Epidémiologie et de Développement de Bordeaux

Introduction: The 1-year estimated glomerular filtration rate (eGFR) and its evolution during the first year after the transplantation are prognostic markers associated with subsequent graft loss. The goal of our study was to evaluate their performance to predict 5-year allograft loss.
Patients and Methods: The characteristics of kidney donors and recipients, their 1-year eGFR and its slope during the first year computed by linear regression were analysed for each patient who received a first kidney allograft in our center between 1999-01-01 and 2011-08-31. Fine & Gray statistics and ROC curves were used to analyse the prognostic impact of all available variables, and the factors that incluenced the slope of eGFR were determined using a linear mixed model.
Results: During a mean follow-up period of 65 ± 41.3 months, 114 out of 961 patients lost their graft and 51 died. The only identified independant prognostic factors were the 1-year eGFR ($p = 7 \times 10^{-7}$) and its slope over the first year ($p = 3.5 \times 10^{-4}$). However these markers had poor predictive performances for 5-year graft loss: area under ROC curves were 0.68 and 0.66 respectively. Between 1 and 5 year post-transplantation, the sign of the eGFR slope changed for 40.5% of the analysed patients: 27.2% had a negative 1-year eGFR slope but a positive 5-year slope, and 13.3% exhibited a symmetrical pattern. Marginal donors and class 2 allo-immunisation were associated with more negative eGFR slopes. A lower 1-year eGFR and a steeper decreasing eGFR slope were associated with an increased mortality.
Discussion: One-year eGFR and its slope during the 1st year post transplantation are the strongest prognostic factors for kidney allograft. Nevertheless, none of them is reliable to predict 5-year allograft survival. The analysis of eGFR trajectories suggests that long-term allograft outcome depends on events which occur later than 1 year post-transplantation and change the eGFR trajectory in 40.5% of patients

O183* HISTOLOGY AND NOT DONOR SPECIFIC ANTIBODY HELPS PREDICT 5-YEAR KIDNEY TRANSPLANT FAILURE USING 1 YEAR POST-TRANSPLANT DATA IN BOTH LOW IMMUNE AND HIGH IMMUNE RISK PATIENTS

Andrew Bentall[1], Manuel Moreno Gonzales[2], Mark Stegall[2], Richard Borrows[3]
[1]University of Birmingham; [2]Mayo Clinic, MN; [3]University Hospital Birmingham

Introduction: The objective of this study was to validate the RISK score (Am J Kid D 2014; 63[4]:643) for renal allograft failure using a different cohort and to assess if protocol biopsy data and anti-donor HLA antibody improved the model's ability to predict 5-year graft failure using 12 month data.
Methods: The validation cohort included solitary kidney transplant (Tx) recipients at Mayo Clinic, Rochester, MN between January 1999 and December 2008. Negative crossmatch Tx recipients ($n = 1465$) and Positive crossmatch ($n = 170$) were assessed for death-censored failure 5 years post-Tx. Risk scores were evaluated for prognostic utility (discrimination, calibration, and risk reclassification). Weighted regression coefficients for baseline, 12-month demographic and clinical predictor characteristics were used.
Results: Multivariate analysis identified glomerulitis and interstitial fibrosis and Class II DSA as associated with graft failure. Histology (g and ci scores) improved discrimination for death censored Tx failure (C statistic = 0.90 vs 0.84 without histology). Class II DSA did not improve predictability (C-statistic = 0.83). Adding histology to the RISK score resulted in statistically significant risk reclassification for death-censored Tx failure (net reclassification improvement [NRI], 29.0%, $p < 0.001$ vs. NRI, 1.2%, $p = 0.90$). Class II DSA had no effect on reclassification. The new model with histology, in the positive crossmatch cohort demonstrated increased C-statistic (0.86 from 0.82) and NRI compared to the Birmingham risk score (32.1%, $p < 0.001$). Importantly DSA at 1 year was not a risk factor in univariate or multivariate analysis.
Conclusions: The RISK score was validated in a large cohort accurately identifying at-risk transplants. DSA did not improve classification in either risk group, but the addition of 1 year protocol biopsy data benefitted in the assessment of risk, improving the classification in 30% of patients into high or low risk populations.

O184 POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER FOLLOWING KIDNEY TRANSPLANTATION: A POPULATION-BASED COHORT STUDY

Eva Futtrup Maksten[1], Maja Ølholm Vase[2], Jan Kampmann[3], Francesco D'amore[2], Michael Boe Møller[4], Charlotte Strandhave[1], Knud Bendix[5], Claus Bistrup[3], Helle Thiesson[3], Esben Søndergaard[6], Stephen Hamilton-Dutoit[5], Bente Jespersen[1]
[1]Department of Renal Medicine Aarhus University Hospital; [2]Department of Haematology Aarhus University Hospital; [3]Department of Renal Medicine Odense University Hospital; [4]Department of Pathology Odense University Hospital; [5]Institute of Pathology Aarhus University Hospital; [6]Department of Endocrinology

Introduction: Post-transplant lymphoproliferative disorder (PTLD) incidence is difficult to determine, mainly because both early and other lesions often go unrecognised and unregistered. Few studies have included systematic pathology review to identify all cases and decide the true incidence with long term follow up after transplantation.
Methods: This retrospective cohort study included all kidney recipients transplanted at two Danish centres between 1990 and 2011 (2175 transplantations in 1906 patients). Pathology reports were reviewed for all patient biopsies to identify and classify all possible PTLDs. When a possible PTLD was identified, the tissue sample was reviewed.
Results: 70 cases of PTLD were identified in 2175 transplantations (3.2%). Incidence after first transplantation was 5.3 cases per 1000 patient-years (95% CI, 3.9–7.2). The majority of cases were monomorphic (58.5%), followed by early lesions (21.5%). The mortality rate ratio for PTLD-patients compared with PTLD-free patients was 2.7 (95% CI, 1.6–4.7). There was no difference in graft survival (incidence rate ratio 0.6 (95% CI, 0.3–1.2)).
Conclusions: The incidence of PTLD was higher than expected, probably reflecting our extensive review and the detection of both early non-malignant lesions and late cases. Graft survival was not significantly poorer in PTLD patients compared to other renal transplant recipients.

O185 PREVALENCE OF DNDSA IN THE WEST OF SCOTLAND RENAL TRANSPLANT RECIPIENTS

Vannesa Teng, Marc Clancy, Kathryn Stevens, Ann-Margaret Little, Julie Glen
University of Glasgow

de novo Donor Specific Antibodies (dnDSA) is associated with late graft rejection. As studies have shown that it can be detected before graft dysfunction, it is a potential prognostic biomarker for graft failure. It may be feasible for future monitoring of DSA post-transplant in the West of Scotland (WOS) whereby the prevalence of dnDSA in this population is not yet established. Current literature has reported dnDSA prevalence to be highly variable, between 4 and 27%. Hence, we aim to establish the true prevalence of dnDSA in the WOS renal transplant recipients. This study includes 299 consecutive adult renal transplant recipients in the WOS between January 2007 and December 2011. Prospective data was collected from the Manzen tissue-typing and renal unit database. Patients' serum samples were collected and screened for Class I and II HLA IgG antibodies, using LABScreen PRA and Single Antigen Bead test. dnDSA is defined as antibody specificity that corresponded to current HLA mismatch and was absent pre-transplant, with a cutoff of MFI ≥500. A total of 28 out of 299 patients (9.4%) developed dnDSA in the WOS with a mean follow-up of 4.0 ± 1.3 years post-transplant. As compared to patients without dnDSA, dnDSA patients were younger (41 vs 47 years, $p = 0.028$), more likely to have previous late acute rejections (35% vs 7%, $p < 0.001$) and had higher tacrolimus variability (21% vs 15%, $p = 0.003$). 6 patients (21.4%) had HLA-DP antibodies, which may contribute to inferior graft outcomes. Out of 28 patients with dnDSA, 7 patients (25%) had allograft rejection and 1 patient was deceased. Patients with dnDSA had poorer graft function with lower eGFR (30 vs 48 ml/min/1.73 m^2, $p < 0.001$) and poorer allograft survival rate (HR = 13.8; $p < 0.001$). The prevalence of dnDSA (9.4%) in the WOS is lower than that in other published studies. Thus, any likely benefit of routine post-transplant antibody monitoring in this population may not justify the manpower and cost associated.

O186 POST-KIDNEY ALLOGRAFT NEPHRECTOMY ANTI-HLA IMMUNIZATION IS NOT DUE TO A RELEASE OF ANTI-HLA ANTIBODIES FROM THE FAILED KIDNEY

David Milongo[1], Nicolas Congy[2], Arnaud Del Bello[3], Federico Sallusto[4], Celine Guilbeau-Frugier[5], Lionel Rostaing[3], Nassim Kamar[3]
[1]Department of Nephrology Dialysis and Organ Transplantation; [2]Department of Immunology, Centre Hospitalier et Universitaire de Toulouse; [3]Department of Nephrology, Dialysis and Organ Transplantation, Centre Hospitalier et Universitaire Rangueil; [4]Department of Urology and Kidney Transplantation, Centre Hospitalier et Universitaire Rangueil; [5]Department of Histopathology, Centre Hospitalier et Universitaire Rangueil

Background and aims: The reasons for the increased incidence of de novo anti-HLA donor specific antibodies (DSAs) that is observed after kidney allograft nephrectomy (NTx) are not fully known. Two mechanisms have been advocated: (i) at graft loss, DSAs are not detected in the serum because they are fixed on the non-functional transplant, and are released after NTx; (ii) NTx itself is responsible for de novo anti-HLA immunization. The aim of the present study was to test these two hypotheses.
Methods: Seventeen patients have undergone NTx, 4 (3–33) months after graft loss. Immunosuppression had been stopped in all patients at least three months before NTx. Anti-HLA antibodies were assessed in the serum before, and 1, 5, 30, and 90 days after NTx. In addition, fragments of the removed kidney allograft were eluted to characterized intra-graft anti-HLA Abs. Anti-HLA antibodies were analyzed using the Luminex Single antigen assay.
Results: At NTx, anti-HLA antibodies in the serum were detected in 13 patients. At that time, anti-HLA antibodies that were fixed in the kidney allograft were detected in 11 patients (85%) and positive C4d staining was positive in 9 of the last 11 patients. 22% of reactivity found in graft eluates matched with the donor serological HLA typing. However, this percentage increased to 90% when cross-reactivity against donor's HLA was assessed by epitope analysis. Anti-HLA antibodies are on average 79 times more concentrated in the graft than in sera by standardizing on total IgG antibodies concentration. After Ntx, de novo DSAs occurred in 70% of patients. All de novo DSAs were detected ≥1 month after NTx. Interestingly, these de novo DSAs were not detected previously in kidney allograft eluates.
Conclusion: Our data suggest that anti-HLA sensitization after NTx is related to the NTx itself rather than to a release of fixed anti-HLA antibodies from the failed kidney.

O187 WHICH PREFORMED DONOR-SPECIFIC ANTI-HLA ANTIBODIES ARE CLINICALLY RELEVANT?

Igor Salvade, Vincent Aubert, J.P. Venetz, Dela Golshayan, Rocco Sugamele, Stephanie Gregoretti, Giuseppe Pantaleo, Manuel Pascual
CHUV

Background: Pretransplant anti-HLA donor-specific antibodies (DSA) are considered a risk factor for acute antibody-mediated rejection (AMR) in kidney transplantation. Previous studies could not demonstrate a predictive value of C4d-fixing capability by DSA or of IgG DSA subclasses for acute AMR. However, DSA strength assesed by MFI may improve risk stratification. Cumulative mean fluorescence intensity (MFI) values above 5000 are predictive of a positive flow cytometric crossmatch (FCXM). We thus aimed to analyze the relevance of preformed DSA and of DSA MFI values.

Methods: We studied 280 consecutive kidney recipients, with negative complement-dependent cytotoxicity crossmatches, who received a transplant between 01/08 and 03/14. Pretransplant sera were screened for the presence of anti-HLA antibodies and DSA with the Luminex assay [positive if MFI ≥500], and the results were correlated with biopsy-proven acute AMR in the first year and graft survival.
Results: Pretransplant anti-HLA antibodies were present in 72 patients (25.7%), and 24 (8.6%) had DSA. There were 46 (16.4%) acute rejection episodes, 32 (11.4%) being cellular and 14 (5.0%) AMR. The incidence of acute AMR was higher in patients with DSA (41.7%) than in those without DSA (1.6%) ($p < 0.001$). The median (IQR) cMFI of the group DSA+/AMR+ was 5680 (2289–9641) vs 2208 (1347–3920) in DSA+/AMR- ($p = 0.058$). With univariate logistic regression a threshold value of 5276 cMFI was predictive for acute AMR. DSA cMFI's ability to predict AMR was also explored by ROC analysis: AUC was 0.728 and the best threshold was a cMFI of 4338 with a specificity of 0.93 and a sensitivity of 0.70. Pretransplant DSA >5000 cMFI had a strong detrimental effect on 5-year graft survival.

Conclusions: These results indicate that preformed DSA cMFI values are clinically relevant to predict acute AMR and graft survival in kidney transplantation. A threshold of 4000–5000 cMFI appears to be a determinant outcome predictor. Whether this is due to an association with a positive FCXM remains to be prospectively studied.

O188* DE NOVO DONOR-SPECIFIC HLA ANTIBODIES IN PEDIATRIC RECIPIENTS OF FIRST KIDNEY TRANSPLANTATION PREDICT GRAFT OUTCOME IRRESPECTIVE OF OCCURRENCE TIME

Arcangelo Nocera[1], Michela Cioni[2], Augusto Tagliamacco[1], Patrizia Comoli[3], Massimo Cardillo[4], Angela Nocco[4], Viviana Sioli[4], Antonella Trivelli[2], Alberto Magnasco[2], Enrico Verrina[2], Gian Marco Ghiggeri[2], Fabrizio Ginevri[2]
[1]Lab. di Ricerca Immunologia dei Trapianti, DIMI, Università di Genova; [2]UOC Nefrologia, Dialisi e Trapianto, IRCCS Ist. G. Gaslini, Genova; [3]Lab di Immunologia dei Trapianti, IRCCS Ospedale S.Matteo, Pavia; [4]Immunologia dei Trapianti, IRCCS Ospedale Maggiore Policlinico, Milano

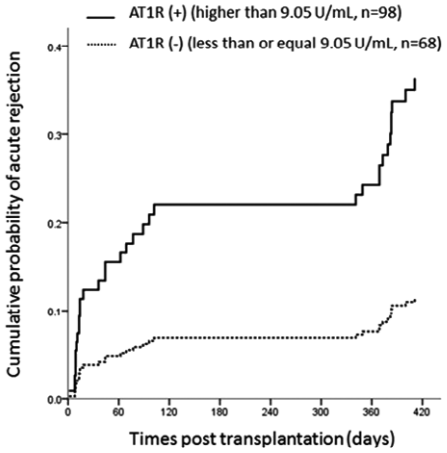
Background: The emerging role of de novo post-transplant donor-specific HLA antibodies (DSA) in the pathogenesis of late allograft damage has prompted recommendations on post-transplant HLA Ab monitoring as a tool to identify patients at risk for antibody mediated rejection (AMR) and graft loss. It is still unclear if time lapse from transplant to DSA occurrence (early or late) is a parameter associated with graft outcome.
Methods/Materials: Utilizing a source of sera collected three-monthly in the first year post-transplant, and annually thereafter, we evaluated 110 consecutive, non sensitized, pediatric recipients of first kidney transplant, grafted between 2003 and 2013, for de novo occurrence of DSA by Luminex platform (screening and single-antigen assay). The median time of follow up was 5.7 years (range 1.0–11.6). AMR was evaluated in kidney allograft biopsies according to Banff 2013 criteria.
Results: Thirty-six patients developed DSA (33%) at a median onset time of 2.1 years (range 0.2–9.6). Cumulative incidence of DSA at 10 years was 52%, with 6% of mean yearly seroconversion rate. We could evaluate bioptic data from 25 of the 36 DSA positive patients, 15/17 from patients developing DSA within 2 years post-transplant (early group), and 10/19 from patients who seroconverted beyond the second year post-transplant (late group). AMR was diagnosed in 53% (8/15) of the early group and in 90% (9/10) of the late group patients (p = ns). Graft loss occurred in 17% of the patients in both groups.
Conclusion: In non sensitized pediatric kidney recipients, de novo DSA predict AMR and graft loss irrespective of post-transplant occurence time. Long term post-transplant HLA ab monitoring is effective for identifying patients at risk of graft loss and possibly guiding pre-emptive intervention.

O189 PRETRANSPLANT ANTIBODIES AGAINST ANGIOTENSIN II TYPE 1 RECEPTOR IN RENAL TRANSPLANTATION: PRELIMINARY DATA FROM THE KNOW-KT STUDY

Juhan Lee[1], Kyu Ha Huh[1], Yongjung Park[1], Yu Seun Kim[1], Curie Ahn[2]
[1]Yonsei University College of Medicine; [2]Seoul National University College of Medicine

Background: The antibodies (Abs) against angiotensin II type 1 receptor (AT1R) have been suggested as a risk factor for graft failure and acute rejection. However, incidence and importance of anti-AT1R Abs had not been evaluated in Asia. The aim of our study was to evaluate the incidence of anti-AT1R Abs and their impact on post- transplant outcomes.
Methods: In this multicenter, observational cohort study, we tested anti-AT1R Abs by using AT1R assay kits (One Lambda, CA, USA) in pre-transplant sera from 166 consecutive kidney recipients. A threshold of anti-AT1R antibody levels was statistically determined at 9.05 U/mL based on the time to acute rejection. Serum creatinine and glomerular filtration rates (GFR) at 1 year and were evaluated to compare graft function.
Results: Anti-AT1R Abs were observed in 98/166 (59.0%) of the analyzed recipients. During the 12 month observation period, no graft failure was reported. AT1R (+) patients showed significantly higher incidence of biopsy-proven acute rejection than in AT1R (-) patients (27.6% vs. 10.3%, $p = 0.007$). Multivariate analysis showed that anti-AT1R Abs was an independent risk factor for acute rejection (HR = 3.18, 95% CI = [1.37, 7.39], $p = 0.007$). Mean serum creatinine after 1 year showed a significant difference (1.13 ± 0.34 mg/dl in AT1R (-) vs. 1.27 ± 0.51 mg/dl in AT1R (+), $p = 0.047$). However, there was no significant difference in GFR between groups.
Conclusion: Pretransplant anti-AT1R Abs are associated with acute rejection. Detection of anti-AT1R Abs could be helpful for assessment of immunologic risk for acute rejection.

Figure 1. Kaplan-Meier analysis of acute rejection episodes according to pretransplant anti-AT1R Abs



O190 ROLE OF DE NOVO DONOR-SPECIFIC ANTI-HLA ANTIBODIES IN KIDNEY GRAFT FAILURE: A CASE-CONTROL STUDY

Jorge Malheiro[1], La Salete Martins[1], Andreia Campos[1], Rui Abreu[2], Sandra Tafulo[3], Isabel Fonseca[1], Leonidio Dias[1], António Castro Henriques[1]
[1]Centro Hospitalar do Porto; [2]Centro Hospitalar de Vila Real; [3]Centro do Sangue e da Transplantação do Porto

Aims: Data on the impact of de novo donor-specific anti-HLA antibodies (dnDSA) within the pathways leading to graft failure are still scarce.
Methods: We investigated 56 patients transplanted between 2000 and 2010 with kidney graft failure (cases) for a possible association of development of dnDSA with graft failure. All patients were transplanted with a negative cytotoxic crossmatch and without presence of preformed DSA. The 56 patients with failed transplants were matched with 56 patients with a functioning graft at present for the variables deceased or living donor, transplant number, transplant year, recipient age and gender, donor age and gender, dialysis vintage time, transplant induction therapy. All patients with a failed graft had at least one serum collected 1 year before failure.
Results: Mean kidney graft survival years were 9.6 and 4.7 for controls and cases respectively.

Comparative data of dnDSA prevelance and characteristics, and post-transplant events between cases and controls.

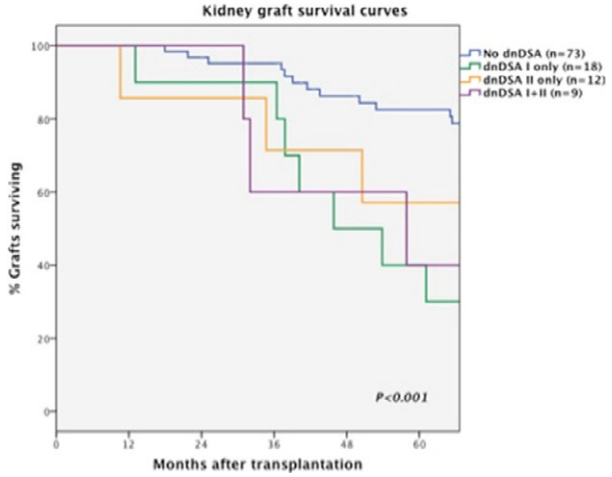
	Graft functioning ($n = 56$)	Graft failed ($n = 56$)	p
Anti-HLA+ antibodies, %	23	40	0.006
DSA+, %	16	54	<0.001
DSA against class I, %	7	41	<0.001
DSA against class II, %	11	27	0.029
Sum of all DSA MFI, median	10571	15427	0.215
Delayed graft function, %	21	48	0.003
Acute rejection during 1st year, %	9	21	0.065
Patients with graft biopsy after 1st year, %	20	75	<0.001
- Active antibody-mediated rejection, %	0	23	<0.001
- Transplant glomerulopathy, %	11	20	0.003

Considering only controls (n = 56), 9 patients showed presence of dnDSA. Graft function at last visit was significantly lower in dnDSA+ (34 ml/min) than in dnDSA- (52 ml/min) patients (p = 0.041). Proteinuria >0.5 g/g occurrence was similar between groups (dnDSA- 28%, dnDSA+ 39%, p = 0.504).

Kidney graft survival curves according to DSA presence and type are shown. At 5-years, 82.5%, 40%, 57.1% and 40% of grafts survived in patients with no DSA, DSA-I only, DSA-II only and DSA I + II, respectively.

Multivariable Cox regression showed that delayed graft function (HR 2.734, p = 0.014) and dnDSA (HR 2.610, P0.006) were independent predictors of graft failure, adjusted for confounding factors.

Conclusions: Posttransplant presence of dnDSA was clearly associated with graft loss, independently from HLA class. dnDSA was also associated with worse graft function in control patients. dnDSA pathological pathway in graft failure is foreseen in our results.



003 CLINICAL IMMUNOSUPPRESSION & BIOLOGICALS

O191 RAPID REMOVAL OF ANTI-HLA ANTIBODIES IN IMMUNIZED PATIENTS AWAITING RENAL TRANSPLANTATION – A DOSE FINDING STUDY OF THE IGG DEGRADING ENZYME IDES

Tomas Lorant[1], Mats Bengtsson[2], Alireza Biglarnia[1], Torsten Eich[2], Britt-Marie Eriksson[3], Lena Winstedt[4], Lars Bäckman[1], Erik Larsson[5], Christian Kjellman[4], Gunnar Tufveson[1]

[1]Department of Surgical Sciences, Section of Transplantation Surgery, Uppsala University; [2]Department of Immunology, Genetics and Pathology, Section of Clinical Immunology, Uppsala University; [3]Department of Medical Sciences, Section of Infectious Diseases, Uppsala University; [4]Hansa Medical AB, Lund; [5]Department of Immunology, Genetics and Pathology, Section of Molecular and Morphological Pathology, Uppsala University

Background: Approximately one third of the patients waiting for kidney transplantation are sensitized to human leukocyte antigen (HLA) that may deny patients from transplantation. The immunoglobulin G (IgG)-degrading enzyme of Streptococcus pyogenes (IdeS) has the characteristics of cleaving IgG.
Aim: To evaluate the efficacy, safety, tolerability, and pharmacokinetics of intravenous IdeS after administration of ascending doses in sensitized patients with chronic kidney disease (CKD).
Materials: A phase II single arm, ascending dose study was conducted at the Uppsala University Hospital in Sweden. Patients with CKD with antibodies against at least two HLA and at least one antibody with mean fluorescence intensity (MFI) >3000 were included. The patients were given 0.12 mg/kg BW ($n = 3$) or 0.25 mg/kg BW ($n = 4$) of IdeS. Based on MFI data the protocol allowed a second dose within 48 h. All patients at 0.12 mg/kg and two patients at 0.25 mg/kg received a second dose. The primary objective was to find an IdeS dosing scheme, which in the majority of the patients result in HLA antibody levels acceptable for transplantation, measured as an MFI <1100 within 24 h from dosing. The follow up period was 64 days.
Results: The MFI of HLA antibodies were significantly reduced in all patients treated with IdeS. At 0.25 mg/kg three out of four patients reached the primary endpoint. One patient with donor specific antibodies was successfully transplanted after having received 0.12 + 0.12 mg/kg BW of IdeS. Positive cytotoxic and flow cytometry crossmatch against the donor were converted to negative by IdeS treatment. The kidney is functioning with no signs of antibody-mediated rejection. Four serious adverse events were reported in the study, 3 patients with infections and 1 patient with myalgia.
Conclusions: Treatment with IdeS significantly reduced the levels of HLA antibodies. Furthermore, IdeS was considered to have an acceptable safety and tolerability profile.

O192 SYMPHONY IN REAL LIFE, OUTCOMES WITH LOW-TARGETED TACROLIMUS IN DE NOVO STANDARD RISK RENAL TRANSPLANTS IN A SINGLE CENTER

Anders Åsberg, Elisabet Størset, Anders Hartmann, Anna V. Reisæter, Hallvard Holdaas, Morten Skauby, Stein Bergan, Karsten Midtvedt

Oslo University Hospital - Rikshospitalet

Background: In renal transplant recipients, optimal tacrolimus concentrations are not definitely established. Based on the results of the Symphony study, we have applied low-target tacrolimus (trough concentrations 3–7 μg/l) in de novo standard risk renal transplant recipients since 2009. The objective of this study was to evaluate outcomes with this strategy in a clinical setting as compared to the trial data of the Symphony study.
Methods: A single-center study was conducted in standard risk renal transplant recipients, excluding immunized patients (DSA-positive, PRA >20% and ABO-incompatible) and HLA-identical transplants. Immunosuppression consisted of low-target tacrolimus, mycophenolate mofetil (1.5 g/day), low-dose prednisolone and basiliximab induction. One-year estimated renal function (Cockcroft-Gault), one-year biopsy-proven acute rejection rate and three-year graft- and patient survival were compared to the outcomes in the Symphony study.
Results: From January 1. 2009 to March 31. 2013, we included 406 standard risk renal transplant recipients. In total 68% of the 15 772 tacrolimus concentrations were within the therapeutic window as defined by the Symphony protocol. One year after transplantation, the mean ± SD GFR was 76.8 ± 28.3 ml/min (Symphony: 65.4 ± 27.0 ml/min, $p < 0.001$). Biopsy-proven acute rejections were seen in 14.5% of our patients (Symphony: 12.3%, $p = 0.35$). Kaplan-Meier estimates [95% confidence interval] of three-year graft- and patient survival were 96.6% [94.2–99.0%] (Symphony: 93%) and 95.0% [92.6–97.3%] (Symphony: 95%), respectively.
Conclusion: Low-target tacrolimus-based immunosuppression is as safe and effective in a clinical setting as reported in the original study trial.

O193 ALEMTUZUMAB INDUCTION ALLOWS BETTER REJECTION FREE GRAFT SURVIVAL IN COMPARISON TO BASILIXIMAB ALBEIT INCREASED POST TRANSPLANT VIRAL INFECTIONS

Vishwanath Siddagangaiah[1], Hemant Sharma[2], Nikhil Lal[1], Jason Rai[1], Matthew Howse[1], Daniel Ridgway[1], Ajay Sharma[1], Abdul Hammad[1], Sanjay Mehra[1]

[1]Royal Liverpool and Broadgreen University Hospitals; [2]Manchester Royal Infirmary

Background: 3C study concluded that in comparison to basiliximab, alemtuzumab induction reduces the risk of biopsy proven acute rejection (BPAR) in renal transplant recipients. In our experience at Liverpool, Alemtuzumab and Basiliximab induction safely provided steroid-free maintenance immunosuppressive regimen but the rejection and infection incidence were not established. We compared alemtuzumab and basiliximab induction followed by standard two drug maintenance immunosuppression to assess rejection & infection rate.
Methods: Data was collected retrospectively from patients transplanted between 1/08/2009 to 31/12/2013. 436 patients were analyzed; 235 received basiliximab, 198 received alemtuzumab & data was not available for 3 patients. Tacrolimus and mycophenolate mofetil were used as maintenance immunosuppression in both groups. Demographics, BPAR episodes, viral infections, & creatinine levels were analyzed using Medcalc 13.0 statistical software.
Results: Review of the data showed no significant differences for demographic details, graft & patient survival. Basiliximab group had increased incidence of BPAR, 52/235,22.1% as compared to Alemtuzumab 15/198, 7.5% (Yates correction <0.001, Fischers Exact test one tailed $p < 0.0001$). Median creatinine level at 6 weeks was 128 ± 21 μmol/l (Basiliximab) & 115 ± 16 μmol/l (Alemtuzumab). On the contrary, incidence of Viral infections was higher in the Alemtuzumab group vs Basiliximab [CMV (77/198 vs 57/235, Fischer Exact test one tailed $p < 0.0002$, Pearson Test $p < 0.0004$); BK (34/198 vs 17/235, Fischers Exact test one tailed $p < 0.0006$)].
Conclusion: Alemtuzumab induction significantly reduces the incidence of rejection but at the cost of increased viral infections. Our Study corroborates the 3C Trial findings. Further review of data over time will assess long term graft outcomes.

O194 DRUG DOSE, DOSE ADJUSTMENTS, AND RENAL FUNCTION AMONG DE NOVO LIVER TRANSPLANT RECIPIENTS RANDOMIZED TO ENVARSUS® TABLETS ONCE-DAILY VS. PROGRAF® CAPSULES TWICE-DAILY: RESULTS FROM A PHASE 2 STUDY

Derek Dubay[1], William Chapman[2], Lewis Teperman[3]

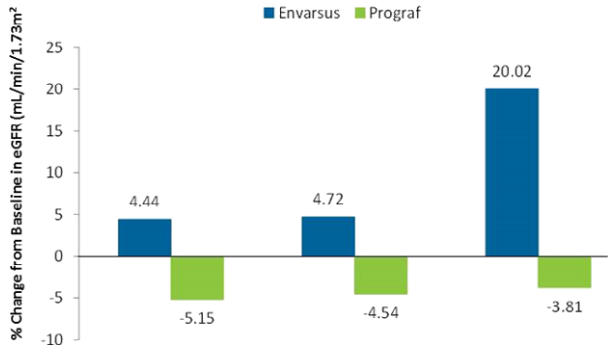
[1]University of Alabama at Birmingham, AL; [2]Washington University, St. Louis, MO; [3]New York University Medical Center, New York, NY

Envarsus® is a once-daily tacrolimus (tac) formulation that has shown increased bioavailability, lower peak, less peak-to-trough fluctuation, and similar efficacy and safety in stable liver and kidney transplantation and in de novo kidney transplantation vs. twice-daily tac (Prograf®). This study compared total daily dose (TDD), dose adjustments (DA), and renal function over 1 year between once-daily Envarsus and twice-daily Prograf in de novo liver transplants. Liver recipients were randomized to Envarsus ($n = 29$) 0.07–0.11 mg/kg once-daily (0.09–0.13 mg/kg for African-Americans) or Prograf ($n = 29$) at 0.10–0.15 mg/kg/day (divided twice-daily). Subsequent doses of both drugs were adjusted to maintain tac trough levels of 5–20 ng/ml through day 90 and 5–15 ng/ml thereafter. TDD for both drugs decreased overtime, and a dose reduction of ~20% was achieved with Envarsus; the mean TDD was lower for Envarsus vs. Prograf throughout the study. In the first 6 months, the mean number of DA was 9.4/patient for Envarsus and 10.8/patient for Prograf; in the first 14 days, the mean number of DA was Envarsus: 3.9/patient and Prograf: 4.8/patient. Improvement from baseline in renal function was slightly better throughout the study for Envarsus vs. Prograf, but the difference was not statistically significant. Estimated glomerular filtration rate (eGFR) increased in the Envarsus group vs. decreases for Prograf (Figure). Clinical efficacy (acute rejection, graft loss, death) was similar between the groups. Two patients died in each group; none were suspected to be related to study drug. Number of AEs, and SAEs were in line with expected, with no statistically significant difference between groups. Overall, the 3 most common AEs in both treatment groups were diarrhea (41%), nausea (38%), and headache (36%). These results add evidence that Envarsus may be an attractive alternative to Prograf in de novo liver transplantation, and that Envarsus can be given at ~20% lower dose than Prograf.

O195 EFFICACY AND SAFETY OF A COMBINATION SCHEDULE WITH ONCE-DAILY EVEROLIMUS AND ONCE-DAILY TACROLIMUS IN MAINTENANCE LIVER TRANSPLANTATION

Paolo De Simone, Paola Carrai, Arianna Precisi, Laura Coletti, Juri Ducci, Daniela Campani, Franco Filipponi
University of Pisa Medical School Hospital

Background: The pharmacokinetic profile of everolimus (EVR) allows for its once-daily (quaque die, QD) administration. We tested the efficacy and safety of a combination schedule of QD-EVR and reduced-exposure QD tacrolimus (QD-rTAC) over 12 months with a pilot, quasi-experimental study in adult, maintenance (≥6 months) liver transplant (LT) recipients.
Methods: Fifty-four LT recipients on TAC-based immunosuppression were enrolled and switched to 3 treatment arms on a 1:1:1 basis. Patients on QD-TAC were randomized to receive either QD-EVR (Group A; QD-EVR + rQD-TAC; *N* = 18) or twice-daily (bis in die, BID) EVR (Group B; BID-EVR + rQD-TAC; *N* = 18). Patients on BID-TAC received BID-EVR only (Group C; BID-EVR + rBID-TAC; *N* = 18). After EVR was in the target range (3–8 ng/ml), TAC exposure was minimized (3–5 ng/ml) until 12 months (M).
Results: The 12-M composite efficacy rate was similar across groups (94.5% (A); 100% (B); and 100% (C), respectively; p = ns). One case of mild biopsy-proven acute rejection (rejection activity index [RAI] = 6) was observed in Group A and C each, and there was only 1 death in Group C due to recurrent hepatocellular carcinoma. The most frequent adverse event was hyperlipidemia, with a 22.2%, 16.6%, and 16.6% incidence in Group A, B, and C, respectively (p = ns). Proteinuria ≥0.5 mg/g was observed in 1 case in each Group (0.5%; p = ns). The endpoint renal function improved by 4.5 ± 7.6, 5.1 ± 6.2, and 5.7 ± 6.8 ml/min/1.73 m^2 in Group A, B, and C, respectively (p = ns).
Conclusions: A full once-daily combination schedule of EVR and TAC allows for TAC minimization and shows comparable efficacy and safety vs. the twice-daily EVR regimen.



O196 EARLY INTRODUCTION OF EVEROLIMUS IN DE NOVO LIVER TRANSPLANTATION: FINAL RESULTS OF A MULTICENTER RANDOMIZED CLINICAL TRIAL (EPOCAL)

Umberto Cillo[1], Alessandro Vitale[1], Mauro Salizzoni[2], Michele Colledan[3], Giorgio Rossi[4], Umberto Baccarani[5], Luciano De Carlis[6], Alessandra Bertacco[1], Laura Saracino[1], Morales Rafael Ramirez[1], Salvatore Agnes[7]
[1]Liver Transplantation Unit Padua University Hosptital; [2]Azienda Ospedaliera-Universitaria S. Giovanni Battista di Torino Ospedale Molinette; [3]Ospedale Riuniti Bergamo; [4]Ospedale Maggiore Policlinico Milano; [5]Azienda Ospedaliera "S.M. Misericordia" Udine; [6]Ospedale Niguarda- Ca Granda Milano; [7]Policlinico Universitario Agostino Gemelli Roma

Introduction: Early introduction of Everolimus in patients undergoing liver transplantation (LT) remains controversial.
Methods: We performed a spontaneous, phase 2, multicenter (7 centers), randomized, open label, clinical trial. Patients were randomized in POD 7 in two possible arms using a 2 : 1 ratio, the study group (with Everolimus introduction in POD 8 and Tacrolimus reduction/weaning), and a control group (conventional immunosuppression). Primary endpoint: incidence of biopsy proven acute rejection (BPAR) and graft loss at 3 months after LT. Secondary endpoints: renal function, suspension of Tacrolimus, incidence of adverse events in a 24 months follow-up.
Results: In the intention-treat-analysis, 93 patients were enrolled in the study group and 47 in the control group. We did not find any significant difference between the two groups in patient-graft characteristics. The incidence of BPAR events at 3 months visit was 15% (12/80) in the study group and 5% (2/43) in the control group (p = 0.07). The primary endpoint for sample size calculation, defined as the incidence of BPAR <25% at 3 months after transplantation in the study group, has been reached. The BPAR incidence in the study group was significantly higher in patients with a too low exposure to Tacrolimus before achieving Everolimus target levels (36% vs. 10%, p < 0.01) while it was comparable in those with and without Tacrolimus weaning. Graft and patient survival were comparable in the two groups. The glomerular filtration rate (GRF) was similar at randomization, while it was significant better in the study group from 2° week onwards. The number of adverse events was comparable between the two groups with the exception of incisional hernias having a significantly higher incidence in the study group. Tacrolimus withdrawal was reached in 43% of study group patients.
Conclusion: Everolimus seems safe and effective when introduced early after LT.

009 ETHICS/LAW/PSYCHOSOCIAL/PUBLIC POLICY

O197 PRIORITISING RENAL TRANSPLANTATION BASED ON CLINICAL NEED: THE ROLE OF AN "URGENT" KIDNEY WAITING LIST

Emma Aitken[1], Vladyslav Shumeyko[2], Enric Murio[2], John Asher[2], David Kingsmore[2], Marc Clancy[2]
[1]Western Infirmary; [2]Western Infirmary, Glasgow

Background: In the UK, cadaveric kidneys are allocated for transplantation according to the NHS-BT Deceased Donor Organ Allocation Policy. This complex matching algorithm aims to provide equity of access by prioritising based on factors such as waiting time, HLA-match and age difference. However, unlike liver transplantation, it provides no opportunity to allocate on clinical need. There are a cohort of patients with end-stage vascular access (ESVA), who die from access loss on the waiting list.
Methodology: The current DCD organ sharing scheme permits local allocation of DCD kidneys. At our centre we have established an "expedited" list of patients with ESVA (bilateral central vein occlusion and survival, deemed by the MDT, to be 50 years old will first be allocated to these patients if a match exists. We describe our early (4 year) experience of such a policy.
Results: 22 patients with ESVA were identified. 18 were transplanted during the study period (9 via the "expedited" list, 6 via the national allocation policy, 3 live donors). The two patients with ESVA who were not transplanted both have a cRF of 100% and match score of 1. Half of those who were transplanted also had cRF >95%. Mean age and waiting time for patients getting "expedited' transplantation (n = 9) was comparable to the general transplant population (n = 420) (46 +/- 10 vs 48 +/- 13 years; p = 0.56 and 1305 +/- 925 days). 1-year patient and graft survival was 88.9%. 44.4% had DGF. Mean eGFR at 1 year was comparable to the general transplant cohort (62.0 +/- 13.4 vs 58.4 +/- 20.9 ml/min/1.73 m2; p = 0.71). In every case, the patient who would have been allocated the kidney according to the national algorithm, was transplanted within the subsequent year. Conclusions: Priority allocation of DCD kidneys to patients with failing vascular access has proven effective with acceptable outcomes and minimal negative impact on the global transplant population.

O198 PREDICTING MENTAL HEALTH AFTER LIVING KIDNEY DONATION: THE IMPORTANCE OF PSYCHOLOGICAL FACTORS

Lotte Timmerman, Mirjam Laging, Reinier Timman, Willij Zuidema, Denise Beck, Jan Ijzermans, Jan Van Busschbach, Willem Weimar, Emma Massey
Erasmus Medical Center

Background: Living donor kidney transplantation offers advantages to the patient, however, involves risks to the donor. In order to promote donor safety, this study investigated factors predictive of mental health after donation. Potential predictors were based on models of Lazarus (1999) and Ursin & Eriksen (2004) that describe predictors of mental health mediated by stress.
Methods: Living kidney donors (N = 151) participated in an interview before donation and completed validated questionnaires 2.5 months before, and 3 and 12 months after donation. Using multilevel regression models we examined whether psychological symptoms and wellbeing (BSI; PANAS; MHC-SF) were predicted by socio-demographic characteristics, appraisals, expectations (LDEQ), knowledge (R3K-T), social support (SSL), coping (COPE-Easy), and life events; and whether these relationships were mediated by stress (DASS).
Results: Donors without a partner showed a greater increase in negative affect over time. Younger age, lack of social support, expectations of negative health consequences, lower appraisals of manageability, and an avoidant coping style were related to more psychological symptoms over time. The latter three were mediated by stress. No religious affiliation, unemployment, history of psychological problems, less social support, expectations of negative health consequences, and lower appraisals of positivity were related to lower wellbeing.
Conclusion: This study identified a risk profile of negative psychological outcomes among living kidney donors. Professionals should examine this profile before donation and the need for extra psychological support in case of one or more risk factors. Support should be focused on the changeable risk factors or decreasing stress/psychological symptoms and increasing wellbeing.

O199 LIVING KIDNEY DONORS TRIGGERED BY SOCIAL MEDIA: DO THEY DONATE?

Willij Zuidema, Van De Jacqueline Wetering, Willem Weimar, Sohal Ismail, Dorthe Slaats, Sandra Middel, Joke Roodnat, Marcia Kho, Emma Massey, Michiel Betjes
Erasmus MC

Background: New categories of living kidney donors (LKD) are emerging such as specified unrelated donors triggered by stories in the (social) media. In addition, we recently launched a website with a possibility of registering interest in donation, resulting in an increase of requests for information about unspecified LKD. We evaluated how many of these potential living kidney donors actually do donate.
Methods: In 2013 and 2014, we prospectively collected data of all individuals who approached us with the intention to donate a kidney to a non-related recipient who solicited for a kidney via media e.g. Facebook (Facebook donors, FB) or by our website (website donors, WD). We distinguished four phases in our procedure. Information (by phone and booklets by post), medical and psychological screening, pre-operation and the kidney donation phase.
Results: Twenty potential FB donors contacted us for 5, sensitized specified patients. After the information 8 withdrew and 12 persons entered the screening. Seven FB donors could not donate to their intended recipient for immunological reasons and did not want to donate anonymously to another recipient. One donor decided to withdraw from the procedure. Two could not donate for medical reasons. One FB donor is still in screening for unspecified donation and we referred 1 to another center. Twenty-four WD indicated they were willing to donate a kidney anonymously and received information about it. Four withdrew from donation, 8 persons were referred to another center and 2 are considering donation. Ten WD started the screening, 1 withdrew and 5 donors are still in screening. Two are scheduled for donation and 2 already donated anonymously.
Conclusion: Ninety percent of the potential FB donors do not pass the information/screening phase, because of early withdrawal and immunological contraindications. In contrast, our preliminary data suggest that WD will contribute significantly to the number of unspecified donors.

O200 IS THERE A CORRELATION BETWEEN ACCEPTANCE OF THE CONCEPT OF BRAIN DEATH AND THE WILLINGNESS TO DONATE OR RECEIVE ORGANS IN MEDICAL PROFESSIONALS IN GERMANY?

Jutta Weiss[1], Thomas Bein[2], Josef Briegel[3], Kai-Uwe Eckardt[4], Götz Gerresheim[5], Dorothee Grammenos[5], Christoph Lang[4], Christopher Nieß[1], Florian Zeman[2], Thomas Breidenbach[1]
[1]Deutsche Stiftung Organtransplantation; [2]Universitätsklinikum Regensburg; [3]Ludwig-Maximilians-Universität München; [4]Universitätsklinikum Erlangen; [5]Klinikum Neumarkt

Background: It is known that a minority of people, including medical staff, do not accept the concept of brain death (cbd), which is the basis requirement of organ donation in Germany. This study examines the correlation between the acceptance of the cbd by professionals involved in organ donation with their own willingness to either donate organs or to accept a transplant in case of organ failure.
Methods: About 10000 questionnaires were distributed anonymously among medical staff involved in organ donation, in 50 Bavarian hospitals. All percentage figures refer to analyzable questionnaires.
Results: 2983 questionnaires were filled out. The majority of all respondents had a positive attitude towards organ donation (n = 2325, 81%); in total 71% (n = 2066) were willing to donate their organs after brain death (bd) and 57% (n = 1580) were willing to accept a transplant in the case of organ failure. The majority (n = 2367, 82%) of all respondents (physicians (90%, n = 736), nurses (79%, n = 1603)) accepted the cbd. In this group, 11% (n = 269) would not donate their organs in the case of bd. This contrasts with the 6% (n = 167) who disagree with the cbd, of whom 68% (n = 114) would not donate their organs. Of those accepting the cbd, 62% (n = 1470) would accept an organ in the case of organ failure, whereas 29% (n = 681) are uncertain and 9% (n = 208) would refuse. Among those who do not accept the cbd, 14% (n = 23) would accept an organ if needed, 55% (n = 92) would refuse.
Conclusion: Although transplantation is widely accepted within the society still a significant number of health professionals do not accept the cbd. Moreover only 2/3 of the medical staff accepting the cbd would accept an organ in the case they needed one. Further studies are necessary to evaluate the reasons for these results. We are convinced, that professional training is the key element to overcome the negative attitude.

O201 INFLUENCE OF MEDIA COVERAGE ON ATTITUDES OF MEDICAL PROFESSIONALS TOWARDS THE INSTITUTIONS RESPONSIBLE FOR ORGAN DONATION, ALLOCATION AND TRANSPLANTATION

Jutta Weiss[1], Thomas Bein[2], Josef Briegel[3], Kai-Uwe Eckardt[4], Götz Gerresheim[5], Dorothee Grammenos[5], Christoph Lang[4], Christopher Nieß[1], Florian Zeman[2], Thomas Breidenbach[1]

[1]Deutsche Stiftung Organtransplantation; [2]Universitätsklinikum Regensburg; [3]Ludwig-Maximilians-Universität München; [4]Universitätsklinikum Erlangen; [5]Klinikum Neumarkt

Background: The transplantation scandal in Germany had led to a severe loss of confidence. The press coverage on organ donation and transplantation since the beginning of the scandal was mainly negative. We analyzed whether this had a negative impact on the opinion of the professionals in regard to the three institutions responsible for organ donation (Deutsche Stiftung Organtransplantation = DSO), allocation (Eurotransplant = ET) and transplantation (transplant centers = TXC).
Methods: About 10 000 questionnaires were distributed anonymously among medical staff involved in the process of organ donation, in 50 Bavarian hospitals. Two groups were identified: Group1: Participants who didn't feel affected by press coverage and Group2: Participants who felt negatively affected. Within the groups, the attitude towards DSO, ET and TXC was analyzed.
Results: 2983 questionnaires were filled out. The majority of all respondents had a positive attitude towards organ donation (n = 23, 2581%). The number of usable questionnaires rating the institutions varied significantly (DSO: 1704, ET: 1297, TXC: 1466). The opinions of the respondents towards the different institutions in dependence of the influence by media coverage are summarized in the table:

Institution	Group 1 - *n* (%) pos	neg	Group 2 - *n* (%) pos	neg
DSO	1124 (93.6)	77 (6.4)	454 (90.3)	49 (9.7)
ET	871 (93.6)	60 (6.4)	315 (86.1)	51 (13.9)
TXC	859 (83.5)	170 (16.5)	224 (51.3)	213 (48.7)

Conclusion: Surprisingly the opinion of the professionals regarding the work of the DSO and ET seems not to be affected by the negative media coverage. The negative judgment towards the TXC by group2 is difficult to rate, because of the low sample size. Regaining trust, especially by medical professionals is important in order to overcome the low donation rates.

O202 MENTAL DISORDERS AMONG UNSPECIFIED LIVING KIDNEY DONORS

Sohal Ismail, W. Zuidema, Vd J. Wetering , M.T. Hilhorst, F.J.M.F. Dor, J.J.V. Busschbach, J.N.M. Ijzermans, E.K. Massey, M.G.H. Betjes, W Weimar

Erasmus University Medical Center

Background: In unspecified living kidney donation psychosocial screening is performed to determine whether donors are mentally stable enough to donate safely. The aim of this study is to investigate what proportion of these donors has been diagnosed with a post-donation mental disorder.
Methods: We retrospectively searched the medical records for individuals who were included as unspecified donors between May 2000 and January 2015 and registered which donors reported a diagnosis of a mental disorder during the yearly medical check-up. We also recorded whether donors attribute their acquired mental disorder to the donation.
Results: In total, 98 unspecified donors donated. Within this group 10% reported psychopathology within three years post-donation namely, posttraumatic stress disorder (n = 2), depression (n = 1), bipolar disorder with suicidal gestures (n = 2), unspecified psychiatric breakdown (n = 1), personality disorder not otherwise specified (n = 1) and depressive symptoms not meeting the full DSM-IV criteria (n = 3). Four donors (partly) attributed their decrease in mental health to the donation.
Discussion: Unspecified living kidney donors acquire mental disorders within three years post-donation. This percentage is comparable with the prevalence in the Dutch general population (9%). This finding could suggest that no additional care other than normal psychological care is warranted. Nevertheless, it is understandable that donors attribute a decrease in mental health at least in some degree to the 'life event' of the donation. Such attribution is not necessarily wrong, but justifies a duty of care. This emphasizes the need for a more detailed psychological follow-up of these donors in order to identify those at risk and to provide early psychological care. Using such follow-up data, studies can identify the association between the characteristics of the donation process and the donor and the change in mental status to inform future donors.

027 LUNG

O203 REPEAT LUNG TRANSPLANTATION OUTCOME; A SINGLE INSTITUTIONAL EXPERIENCE

Odeaa Al Jabbari, Walid K. Abu Saleh, Dewei Ren, Scott Scheinin, Thomas Kaleekal, Matthias Loebe, Brian Bruckner
Houston Methodist DeBakey Heart & Vascular Center

Background: Primary lung transplantation has demonstrated significant increases in volume across major centers in the world over the last several years. As more patients are transplanted there will be an increasing need for possible redo lung transplantation in the future. Herein, we report our large single center experience with redo lung transplantation.
Methods: A total of 785 lung transplants were performed at our institution between 2004 and 2014, with 60 patients undergoing redo lung transplant. Demographic, diagnoses, morbidity, and survival data were collected with primary endpoints from 30 days up to 3 years.
Result: The average age was 49 years (18–72), female (53%) vs 28 male (47%) with median LAS score at redo transplant of 48.2 (33.1–91.7). The main indication for retransplant was chronic graft rejection (BOS) (55 Patients, 92%). The 30, 90 day, 6 month, 1, 2, and 3 year. survival were 92%, 85%, 80%, 63%, 54% and 42% respectively. There is a significant increase in mortality in 1 year. The causes of death include infection/ septic shock (20%), stroke (10%), cardiac arrest (10%), acute rejection (25%), chronic rejection/ BOS (35%). after 1 year the major cause of death is chronic rejection.
Conclusions: In our experience with redo lung transplantation, 30 days mortality is similar to primary lung transplantation. Further investigation will be necessary to understand the reasons behind the significant increase in mortality in 1 year in this group of patients.

O204 ANTI-REFLUX SURGERY FOR GASTROESOPHAGEAL REFLUX DISEASE AFTER LUNG TRANSPLANTATION: BENEFIT FOR LUNG FUNCTION, ACUTE REJECTIONS AND RESPIRATORY INFECTIONS, A SINGLE CENTER EXPERIENCE

Abdul Monem Hamid[1], Antoine Roux[1], Olivier Boche[2], Jacques Leport[3], Marc Stern[1], Sandra De Miranda[1], Dominique Grenet[1], Benoit Douvry[1], Laurence Azuar-Beaumont[1], Lise Morer[1], Edouard Sage[4], Philippe Puyo[4], François Parquin[4], Clément Picard[1]
[1]Pneumology department, Lung Transplant Group, Foch Hospital; [2]Visceral Surgery department, Foch Hospital; [3]Gastroenterology department, Foch Hospital; [4]Thoracic Surgery department, Lung Transplant Group, Foch Hospital

Introduction: Gastroesophageal reflux disease (GERD) after lung transplantation (LT) is associated with chronic lung allograft dysfunction (CLAD). Anti reflux surgery (ARS) in LT need to be evaluated.
Objective: To describe GERD occurrence in LT recipients and evaluate the effect of ARS on lung allograft function.
Material and Methods: We retrospectively reviewed charts of 284 LT patients who had esophageal pH monitoring between 1991 and 2014. GERD was defined as a deMeester score >14.7. For patients who underwent ARS (fundoplication), the incidence of acute rejections (AR) and respiratory infections (RI) were compared before and after surgery. At 6 months post-ARS FEV1 were considered either improved (>110% from baseline), stabilized (91–109%) or deteriorated (<90%). Result are shown as median[IQR25–75] or mean ± SD.
Results: GERD was present in 199/284 patients (70%) who underwent testing at 4.5 months [3.1; 9.6] after LT. In the GERD group, the deMeester score was 44 ± 29. GERD was more frequent in the cystic fibrosis patients (81%, vs 55% $p < 0.001$) 59 patients (30%) underwent ARS 20 [13; 43] months after LT. Post-ARS, there was a decrease in AR from 1.04 ± 0.98 to 0.42 ± 0.99 episodes/year ($p = 0.001$) and RI from 0.75 ± 1.04 to 0.33 ± 0.6 episodes/year ($p = 0.007$). At 6 months, FEV1 improved in 37%, stabilized in 44%, and deteriorated in 17% of patients who had ARS. The number of patients with CLAD grading BOS ≥0p decreased significantly from 51 (86.4%, pre-ARS) to 28 (47.4%, post-ARS) ($n = 59$, $p < 0.0001$). The actual outcome of morbidity and 30-day mortality was (1.66% and 0% respectively),
Conclusion: Anti-reflux surgery is safe for well chosen patients results in a decrease of AR, IR, and an improvement in pulmonary function.

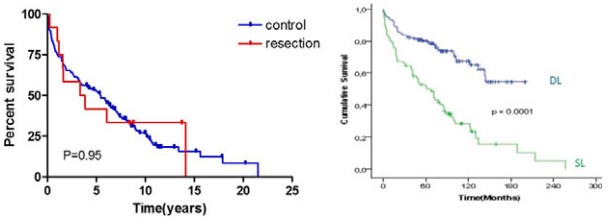
O205 RESECTION OF NATIVE LUNG FOR COMPLICATIONS AFTER SINGLE-LUNG TRANSPLANTATION (SLTX) FOR EMPHYSEMA (EMP) AND PULMONARY FIBROSIS (PF) CAN BE AVOIDED WITH DOUBLE-LUNG (DLTX) WITH SUPERIOR SURVIVAL

Dirk Van Raemdonck[1], Jan Nijs[1], David Ruttens[2], Stijn Verleden[2], Bart Vanaudenaerde[2], Willy Coosemans[1], Herbert Decaluwé[1], Philippe Nafteux[1], Paul De Leyn[1], Hans Van Veer[1], Marion Delcroix[1], Lieven Dupont[1], Robin Vos[1], Wim Wuyts[1], Arne Neyrinck[1], Geert M. Verleden[1]
[1]University Hospitals Leuven; [2]KU Leuven University

Compared to DLTx, recipients of SLTx have a higher risk to develop complications in native lung, such as cancer, opportunistic infection, hyperinflation or pneumothorax requiring parenchymal resection. We studied the incidence of native lung resections in all SL recipients with EMP and PF and compared survival with other SL recipients and also between SL versus DL recipients.

Between 07/1991-12/2014, 829 LTx (SL:158; DL:624; HL:47) were performed in 792 recipients. One CF patient with contralateral pneumonectomy prior to SL and re-LTx were excluded from the analysis. Since December 2011, SLTx was no longer performed at our institution for any indication.

Resection of the native lung was needed in 12/157 (7.6%) SL recipients [3F/9M; mean ± SE age 60 ± 2 years; 7L/5R] transplanted for PF (6) or EMP (6). The indication for resection was cancer (6) [NSCLC:5; SCLC:1], infection (4), hyperinflation (1), or pneumothorax (1). The extent of resection in the native lung was pneumonectomy (6), (VATS) lobectomy (2), wedge excision (2), LVRS (1), and bullectomy (1). Interval from LTx until resection was 1312 ± 381 days (1877 ± 655 days for cancer and 525 ± 231 days for infection). Ninety-day mortality after resection was 17% and 75%, respectively. Median and overall survival since LTx at 1, 3 and 5 years were 3.4 years, 91%, 58% and 42% in the resected group compared to 5.4 years, 75%, 61% and 53% in other SL recipients (NS) [Figure A]. In our cohort, survival after DLTx for EMP or PF was significantly better compared to SLTx; ($p < 0.001$) [Figure B].



Resection of native lung in SL recipients was necessary in 7.6% for better control of complications. Compared to lung cancer, native lung resection for an uncontrollable infectious problem was needed earlier with higher early mortality risk, but equal long-term outcome. DLTx for EMP or PF is now our preferred procedure to avoid native lung complications with superior long-term survival.

O206 DECREASE OF ALLERGIES AFTER LUNG TRANSPLANTATION IS ASSOCIATED WITH REDUCED BASOPHILS AND EOSINOPHILS

Mattheus Niedzwiecki[1], Yoshito Yamada[1], Ilhan Inci[1], Walter Weder[1], Wolfgang Jungraithmayr[2]
[1]University Hospital Zurich, Division of Thoracic Surgery; [2]University Hospital Zurich Division of Thoracic Surgery

Background: Allergies can develop as immunological responses upon antigen exposure similar to immune reactions after transplantation (Tx). Allergies can be transferred after bone marrow Tx, but their activity can change after Tx. The lung as a solid transplantable organ is particularly challenged by antigens from the air environment and thus renders patients more prone to allergies. We analyzed here the prevalence of allergies after lung Tx and monitored the course of leukocytes.
Methods: We systematically reviewed patients who underwent lung Tx between 1992 and 2014 ($n = 414$). Of these, we selected those who had a preexisting allergy before Tx. Allergies were defined as rhinitis symptoms due to pollen allergy. We analyzed the course of all leukocytes, lymphocytes, thrombocytes, neutrophils, basophils, and eosinophils in patients blood in which the status of allergy has changed.
Results: From a total of 414 lung Tx, 44 patients suffered from allergies before Tx (10.6%). In 20 of these patients (45.5%), allergies disappeared completely within one year after lung Tx and were persistently absent thereafter. In these patients, basophils decreased significantly (pre-Tx 51.0 ± 18 µl vs. 30 ± 11 µl post-Tx, $p < 0.012$) and also eosinophils decreased significantly (pre-Tx 210 ± 124 µl vs. 91 ± 38 µl post-Tx, $p < 0.0012$), while basophils and eosinophils of those patients whose allergies did not disappear did not decrease. Instead, they increased, even if not significantly.

Conclusions: Here, we showed that allergies disappear in almost half of cases after lung Tx. As a possible underlying cell responsible for the prevalence of allergies, we identified basophils and eosinophils as potential sources of histamine. The possible association between immunosuppressive drugs and the suppression of allergy-provoking cells and its mediators can give new insights into the development of allergies and its prevention.

O207 DSA CHARACTERISTICS ASSOCIATED WITH ANTIBODY MEDIATED REJECTION IN LUNG TRANSPLANTATION

Antoine Roux[1], Le Lan Ines Bendib[1], Sonia Holifanjaniaina[2], Kimberley Thomas[3], Abdul Monem Hamid[1], Clément Picard[1], Grenet Dominique[1], Sandra Demiranda[1], Benoit Douvry[1], Laurence Beaumont-Azuar[1], Edouard Sage[4], Morgan Le Guen[5], Jerome Devaquet[6], Elise Cuquemelle[7], Marc Stern[1], Magali Colombat[8], François Parquin[7], Caroline Suberbielle[9]

[1]Hopital Foch, Pneumology department; [2]Hopital Foch, Pathology Department; [3]Department of Pathology & Laboratory Medicine David Geffen School of Medicine UCLA, United States; [4]Hopital Foch, Thoracic Surgery Department; [5]Hopital Foch, Anesthesiology Department; [6]Hopital Foch, Intensive Care Unit; [7]Hopital Foch, Thoracic Intensive Care Unit; [8]Hopital Foch; [9]Hopital Saint-Louis, Service d'histocompatibilité

Background: DSA are frequently observed after LT and represent a pivotal role in AMR associated with clinical symptoms and histological abnormalities. In case of AMR,DSA characteristics are not yet fully defined
Methods/Materials: We retrospectively analyzed DSA characteristics and MFI values of 119 patients (pts) transplanted at our center (2010–2013). They were categorized into 3 groups: pts with DSA and AMR (AMR+), those with DSA without AMR (DSA + AMR-), and those with non-significant DSA (DSAns, one specificity, with an MFI = 500–1000 once). The global MFI (sum of DSA) was determined for the following categories: all DSA, class I or II, preformed, or de novo. The 'peak' time point was defined as the time of AMR, and the time of highest global MFI for AMR- patients.
Results: Only 2 AMR + pts exclusively had preformed DSA, whereas 1 AMR+ pt had solely class I DSA. Despite this rarety, distribution of class I or II specificities, as well as preformed or de novo did not differ between AMR+ and DSA + AMR- pts. All AMR + pts had DQ DSA (95%), except for one (no DQ mismatch). This frequency was significantly lower in DSA + AMR- (60%) and DSAns (46%) groups, $p = 0.0028$. Compared to DSA + AMR-, AMR+patients had significantly increased DSA specificities (mean $\pm$ SD; 3.4 ± 2.28 vs 1.8 ± 1.2, $p = 0.0015$), global peak MFI (med[IQR 25–75]; 11563[6867–16239] vs 2593[1485–5357], $p < 0.0001$), de novopeak MFI (8487[3847–12296] vs 1305 [0–2722], $p < 0.0001$),and class II peak MFI (10004[4399–13346] vs 1695[681–3365], $p < 0.0001$). Furthermore, ROC analysis revealed global peak MFI was the strongest indicator of AMR diagnosis (AUC = 0.87, 95 CI (0.8–0.94), $p < 0.0001$), and use of a double cutoff (MFI = 2000–15000) revealed 100% sensitivity, 100% negative predictive value, 96% specificity,and 91% positive predictive value.
Conclusion: DSA of AMR + pts have clearly different profiles compared to DSA of AMR- pts. The strong value of using global peak MFI for AMR diagnosis needs to be prospectively evaluated and validated.

O208 EARLY AGGRESSIVE TOTAL LYMPHOID IRRADIATION AFTER LUNG TRANSPLANTATION: A TOUGH BATTLE AGAINST CHRONIC LUNG ALLOGRAFT DYSFUNCTION

Simona Soresi[1], Anton Sabashnikov[2], Aron F. Popov[1], Andre R. Simon[1], John Smith[1], Anna Reed[1], Martin Carby[1]

[1]Royal Brompton; [2]University Hospital of Cologne

Introduction: The incidence of late graft failure in lung transplant (LTx) recipients remains higher than in other solid organ transplants. Total lymphoid irradiation (TLI) treatment is used as a second line treatment for chronic allograft dysfunction (CLAD). Previous studies assessing the efficacy and safety of this treatment included heterogeneous patient groups resulting in unclear conclusions.
Methods: Twentyseven patients who developed CLAD after LTx (surgery between 1992 and 2011) received aggressive second line treatment using TLI (from 03/2012 until 02/2014) All patients had already been on maximal pharmacological treatment with tacrolimus, MMF and steroids as well as azithromycin and pravastatin for at least 6 weeks. Patients were prospectively followed up for at least one year after TLI.
Results: The baseline immunosuppression regime was the same for all patients. One year after treatment, the mean FEV1 was no different from the baseline FEV1 (1.86 ± 0.59 vs. 1.73 ± 0.83 l, $p = 0.097$). The incidence of bronchiolitis obliterans syndrome (BOS) was 22% for BOS 1, 48% for BOS 2 and 30% for BOS 3. The median time from LTx to TLI treatment was 1123 (645;1750) days and the overall estimated cumulative survival after LTx in this demanding cohort was 81.2% at 3 years, 58.6% at 5 years and 46.8% at 10 years and later. The overall survival after TLI treatment commencement was 73.9% at 1 year and 58.6% at 2 years. Of 27 patients included 11 (41%) died during the post treatment follow-up and one patient underwent redo LTx. Out of this group, six (55%) patients were BOS 3 before TLI.
Conclusion: Our findings show that TLI is a safe and effective therapeutic option for CLAD, particularly effective when patients are referred at early stage. Larger prospective studies should be a goal of further research in order to confirm our preliminary results.

005 COMPOSITE TISSUES

O209 UTILIZING THE SKIN COMPONENT OF VASCULARISED COMPOSITE ALLOGRAFTS AS A PRE-REJECTION MARKER FOR INTESTINAL TRANSPLANTATION

Georgios Vrakas, Henk Giele, Rubens Macedo Arantes, Srikanth Reddy, Peter Friend, Anil Vaidya
Oxford University Hospitals

Introduction: Can the skin component of a synchronously transplanted vascularised composite allograft (VCA) be used as a pre-rejection marker for the intestinal transplant (IT)?
Methods: Recipients of a combined IT and VCA were studied and compared to those an earlier cohort who only received an IT.
Results: From October 2008 to January 2015, 32 patients had an IT. Fifteen patients had an IT without the VCA and 17 had an IT with a VCA. In the latter group, 15 had abdominal wall transplants (AWT) and 2 sentinel skin flaps (SSF). Induction immunosuppression was similar in both groups with Campath-1H (Genzyme, USA), 30 mg intravenously, 6 h after reperfusion and 24 h later. Maintenance was with Tacrolimus at a trough level of 8–12 ng/ml. At a mean follow-up of 40 months (range 5–65), 22 patients are alive and well. All VCA's were successful. There were 5 intestinal rejections in the IT alone group and 1 intestinal rejection in the IT + VCA group (lead time of 10 days between VCA and IT). There were 5 rejections in the VCA part of the IT+ VCA group. A further 5 patients in the IT group were falsely treated for biopsy proven rejection. This was later labelled as infection. False positive diagnosis of rejection was not observed in the IT + VCA group. Rejection of the intestine in the IT alone group resulted in a mean hospital stay of 45 days (range 15–63). Rejection of the VCA in the IT + VCA group resulted in a mean hospital stay of 4 days (range 3–5).
Discussion: VCA to complement IT provides a visual, dynamic canvas for remote immune monitoring of visceral grafts. The skin component of the VCA may act as an immunologic 'ghost target' that may help divert the cellular affect away from the IT. Conversely, intestinal graft dysfunction with a clear VCA is a dynamic visual canvas for the clinician. This helps to refute the diagnosis of rejection.

O210 BLOOD NKT CELLS MAY DIFFERENTIATE STABLE FROM REJECTING RECIPIENTS IN VCA

Emmanuel Morelon[1], Emilie Dugast[2], Palmina Petruzzo[1], Jean Kanitakis[1], Stefan Schneeberger[3], Annemarie Weissenbacher[3], Lionel Badet[1], Aram Gazarian[1], Chloe Paul[2], Annaick Pallier[2], Bernard Devauchelle[4], Sylvie Testelin[4], Olivier Thaunat[1], Olivier Thaunat[1], Sophie Brouard[2]
[1]Hospices Civils de Lyon; [2]INSERM U 643, ITUN; [3]Transplant and thoracic surgery, Innsbruck University; [4]Department of Maxillofacial surgery, CHU Amiens

Vascularized composite allotransplantation has emerged as a novel therapeutic option for treatment of limb amputation or face disfiguration. Profiling immune response of VCA patients (pts) may help for adapting immunosuppressive treatment and the prevention of chronic rejection.

The main objective of this study was to search for specific immune profile in stable or allo-reactive VCA pts.

Patients 13 pts (11 men and 2 women) were included: 10 of them received upper extremity allotransplantations (9 bilateral and 1 unilateral hand transplantations) and 3 face allotransplantations. The median HLA mismatches were 4 (1–6). The follow-up period ranged from 16 months to 14 years at the inclusion time. All recipients received an induction therapy with Thymoglobulin (n = 11) or alemtuzumab (n = 2); Initial maintenance treatment was based on steroids, tacrolimus and mycophenolate mofetil in all patients. B, T and NKT cell phenotype were assessed by flow cytometry in peripheral blood. At the inclusion time the patients did not show clinic signs of acute rejection and they underwent a cutaneous biopsy of the graft. Pts were divided in two groups according to the alloimmune response: group 1 included recipients without donor specific anti-HLA antibodies (DSA), low rate of acute rejection episodes, no signs of chronic rejection; group 2 included patients with DSA (seven patients developed DSA which were transient in two of them) or signs of graft vasculopathy or skin lesion suggestive of chronic rejection.

Naive, central and effector memory CD4+ and CD8+ T cells, induced and natural T regs, and B regs were not statistically different between the two groups. In contrast, the percentage of NKT among CD3+ T cells was significantly higher in group 2 prone to rejection (p = 0.008, Mann–Witney U-test).
In conclusion: These data suggest that a balance between NKT cells might play a role in VCA alloimmune response and that NKT cells phenotype might be considered for immunomonitoring.

O211 CHARACTERIZATION OF BONE MARROW DERIVED CD34+ EX VIVO CREATED HUMAN HEMATOPOIETIC CHIMERIC CELLS – A NOVEL TOLERANCE INDUCING STRATEGY IN TRANSPLANTATION

Tfaily Ewa Bryndza, Joanna Cwykiel, Kristin Dastych, Maria Siemionow
University of Illinois at Chicago

Background: Cell-based therapies represent a new approach for tolerance induction that could reduce the need life-long immunosuppression. The aim of this study was creation and preliminary characterization of the fused BM-derived CD34+ human hematopoietic chimeric cells (HHCC).
Methods: Fifteen ex vivo fusions were performed to create human BM-derived CD34+ HHCC. Briefly, CD34+ cells were isolated from two unrelated donors using MACS technology. Next, CD34+ cells from each donor were stained separately with PKH26 or PKH67 and fused using polyethylene glycol. Double (PKH26/PKH67) stained cells were sorted out and subjected to further assessments. Flow cytometry (FC), (CD34, CD133, CD117, CD90, CD4, CD19, CD14 and CD45RA markers, viability tests), confocal microscopy (CM), genotype HLA typing for class I and II antigens via PCR-rSSOP, STR and colony- forming unit (CFU) assay were used to characterize the properties of HHCC.
Results: FC and CM analysis confirmed CD34+ cell fusion and creation of HHCC. Using PCR-rSSOP we determined that HHCC share HLA class I and II antigens specific for both BM donors used for fusion. The presence of genetic material from both BM donors in HHCC was also confirmed by STR. After fusion ~99% of HHCC were viable and showed low level of apoptosis (2.7% and 1.2% of HHCC in early and late stages of apoptosis, respectively). Phenotype characterization showed expression of all assessed markers on the surface of HHCC. CFU assay showed that HHCC have clonogenic potential and can differentiate into all classes of myeloid and erythroid progenitor cells.

Conclusions: We successfully confirmed feasibility of ex vivo fusion of human BM-derived CD34+ hematopoietic cells leading to creation of HHCC. We characterized the viability, phenotype, genotype and clonogenic properties of HHCC. This unique concept of application of HHCC as a supportive therapy introduces new applications in transplant surgery for tolerance induction protocols in solid organ and

O212 SUBNORMOTHERMIC MACHINE PERFUSION WITH HEMOGLOBIN-BASED OXYGEN CARRIERS FOR TISSUE PRESERVATION IN VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Riccardo Schweizer[1], Sinan Oksuz[2], Chiaki Komatsu[3], Vijay S. Gorantla[3], Paulo Fontes[4]
[1]Department of Plastic and Hand Surgery, University of Zurich; [2]Gulhane Military Medical Academy, Department of Plastic Surgery, Istanbul; [3]Department of Plastic Surgery, UPMC, University of Pittsburgh; [4]Department of Surgery, Thomas E. Starzl Transplantation Institute, UPMC, University of Pittsburgh

Background: Vascularized composite allotransplantation such as hand/face transplantation is a clinical reality. Ischemia-reperfusion injuries (IRI) of vertical rectus abdominis muscle (VRAM) allografts were assessed in a preclinical large animal model comparing cold static preservation (CSP) with machine perfusion (MP) using a new cell-free hemoglobin-based oxygen carrier (HBOC) solution.
Methods: Pig VRAM allografts were procured and transplanted heterotopically (cervical) to recipients after 14 h cold ischemia time (CIT). Controls (n = 4) underwent CSP, the study group (n = 4) underwent MP/HBOC (21°C). The MP perfusate was assessed for arterial blood gases (pH, pO2, pCO2, BE, HCO3, lactate). Both groups had their allografts weighted before/after preservation. All recipients received triple-immunosuppression (Tacrolimus/MPA/Prednisone) for 7 day. Initial clinical and histopathological analysis was conducted. Subsequent studies included transcriptomics, proteomics and metabolomics.
Results: MP allografts were perfused at low pressures (55 mmHg), low flows (20–80 ml/min) and full oxygenation (FiO2 = 60% at 400 ml/min) over 14 h. The allografts perfused well and showed no signs of tissue edema or weight gain after MP. Lactate levels were kept under 4 after 14 h of MP. The pH was kept within physiologic range without the use of NaHCO3 infusions during MP. There were no signs of tissue damage over 14 h of MP in H&E and TUNEL stainings. There was a significantly lower amount of muscle fiber disruption and necrosis in the MP group compared to CSP flaps after transplantation. TUNEL staining showed a lower amount of apoptotic bodies in the MP group. Myoglobin blood levels were significantly higher at day 1 in the CSP group.
Conclusion: MP/HBOC provides effective oxygenation for VRAM allografts over an extended period (14 h) with no signs of endothelial cell damage/tissue edema. MP minimizes IRI when compared to CSP. Myoglobin release and histopathological damage were more pronounced after CSP.

O213 MONITORING OF ARTERIAL VESSELS IN UPPER EXTREMITY ALLOTRANSPLANTATION

Palmina Petruzzo[1], Luong Stephane[2], Danjou Fabrice[2], Gazarian Aram[2], Morelon Emmanuel[2], Badet Lionel[2]
[1]Department of Transplantation; [2]Hopital Edouard Herriot, Lyon

Background: Chronic rejection in vascularised composite allotransplantations has been described in animal models and in some clinical cases. GV has been the most common finding across cases of upper extremity transplantations, resulting in graft dysfunction and loss.
Methods: A prospective study has been performed to investigate intimal media thickness (IMT) of radial and ulnar arteries in the recipient and in the grafted extremities. Six bilateral hand allotransplantations, five men and one woman, performed between January 2000 and November 2012 were included in the present study as well as six controls matched for age, sex and tobacco abuse. All the subjects have undergone high frequency ultrasonography (Philips iU22 with probe L15-7io) at least once a year since September 2012.
Results: There was not a significant difference ($p = 0.075$; Mann-Whitney test) between the recipient and the grafted arteries: the mean IMT was 0.240 cm (0.225–0.261) in the recipient arteries and 0.289 cm (0.248–0.394) in the grafted arteries, although the values were higher in the grafted arteries particularly in the patient who developed a graft vasculopathy. Indeed, this patient showed, in all the points of the follow-up, higher values of IMT in all the grafted arteries compared to the other grafted patients and to the control subjects. The grafted patients (recipient arteries as well as grafted arteries) showed higher values of IMT compared to the control subjects, although this difference was significant only at level of the grafted ulnar arteries ($p = 0.030$; Wilcoxon signed ranks test).
Conclusion: On the basis of these data we have shown only a trend towards higher values of IMT in the arteries of the grafts compared to the recipient arteries while the patient who developed a graft vasculopathy showed significant higher values. The increased IMT could precede GV development and could be considered its early marker.

O214 POST TRANSPLANTATION HIGH-DOSE CYCLOPHOSPHAMIDE TREATMENT TO PROMOTE IMMUNE TOLERANCE AFTER VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Georg Furtmüller, Madeline Fryer, Byoungchol Oh, Sudipto Ganguly, Jeffrey Dodd-O, Giorgio Raimondi, Wp Andrew Lee, Leo Luznik, Gerald Brandacher
Johns Hopkins University School of Medicine

Background: Developing novel treatment concepts to minimize/avoid immunosuppression represents the prime task for vascularized composite allotransplantation (VCA) such as hand and face transplantation.
Methods: Murine skin, SOT (heart), and VCA (hind limb) transplants were performed across a full MHC mismatch barrier. Recipients were treated with a non-myeloablative dose of TBI and T-cell depletion and a single dose of post-transplant cyclophosphamide (PTCy). Donor BM and splenocytes (DBM) were injected at the time of transplantation. Post-transplant chimerism, V[beta]-TCR staining, MLR as well as secondary skin and SOT were performed.
Results: Untreated animals ($n = 5$) rejected skin grafts, SOT and VCA acutely within 14 ± 1 days, 9 ± 2 days, and 8 ± 1 days, respectively. The treatment regimen extended skin and SOT graft survival (32 ± 8; 65 ± 4, respectively) ($n = 5$). DBM augmentation lead to allograft survival of 150 days in skin and SOT. However, indefinite graft survival of >150 days was observed in all animals receiving the induction regimen and a VCA $\pm$ DBM. In groups receiving a VCA $\pm$ DBM, donor chimerism was detected at $22.51\% \pm 5.96\%$ and $30.17\% \pm 8.72\%$, respectively. Vβ-T cell receptor staining indicates a central tolerance mechanism. Long-term survivors showed donor-specific T cell unresponsiveness in-vitro (MLR) while demonstrated proliferation against 3rd party stimulators. In-vivo, tolerant animals accepted donor matched secondary skin, while 3rd party FVB/N skin was acutely rejected. Donor-matched SOT were accepted long-term.
Conclusion: Robust tolerance and immunosuppression-free long-term allograft survival can be induced with PTCy in a stringent fully MHC mismatched murine model of skin, heart, and vascularized composite allotransplantation.

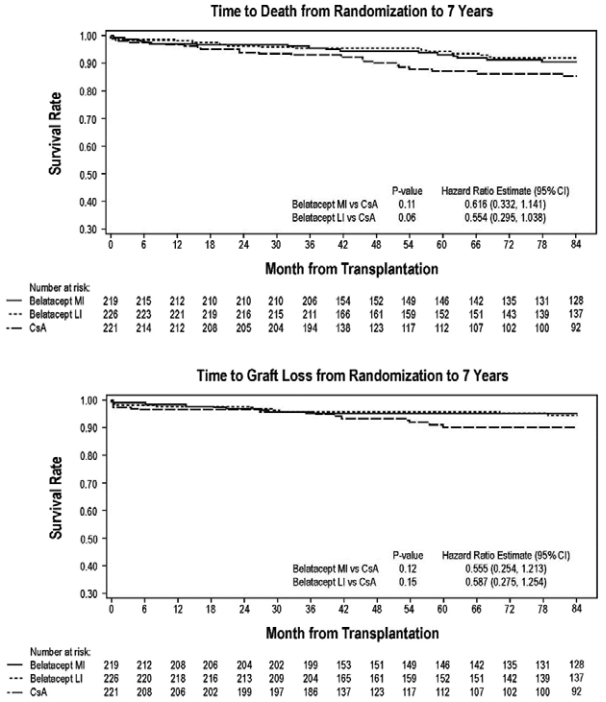
023 KIDNEY

O215* BELATACEPT PATIENTS HAD SUPERIOR GRAFT SURVIVAL COMPARED WITH CYCLOSPORINE PATIENTS: FINAL RESULTS FROM BENEFIT

Flavio Vincenti[1], Josep Grinyo[2], Lionel Rostaing[3], Kim Rice[4], Steven Steinberg[5], Marie-Christine Moal[6], Marty Polinsky[7], Herwig-Ulf Meier-Kriesche[7], Christian Larsen[8]

[1]University of California; [2]University Hospital Bellvitge; [3]University Hospital; [4]Baylor University Medical Center; [5]Sharp Memorial Hospital; [6]Hôpital de La Cavale Blanche; [7]Bristol-Myers Squibb; [8]Emory University

Background: Belatacept (bela)-treated patients (pts) had better graft survival at 7 years vs cyclosporine (CsA)-treated pts in BENEFIT. Here we report the individual contribution of death and graft loss to graft survival.
Methods: Kidney transplant recipients were randomized to more (MI) or less (LI) intensive bela or CsA regimens. All received basiliximab induction/MMF/CS. Outcomes were assessed for all randomized and transplanted pts at Yr 7. In a prospective analysis, time to death or death-censored graft loss was compared between treatment groups using a Cox regression analysis. Hazard ratio estimates and 95% confidence intervals were derived.
Results: A total of 666 pts were randomized and received kidney transplants in BENEFIT. For this analysis, there were 153/219 bela MI, 163/226 bela LI, 131/221 CsA evaluable pts. Over 7 years, a 43% risk reduction in death or graft loss was noted for pts receiving bela MI or LI vs CsA ($p = 0.02$, MI vs CsA; $p = 0.02$, LI vs CsA). A 38% reduction in the risk of death was noted for bela MI ($p = 0.11$) and 45% for bela LI ($p = 0.06$). For graft loss, a 45% risk reduction was noted for bela MI ($p = 0.12$) and 41% for bela LI ($p = 0.15$). Mean MDRD cGFR (mL/min/1.73 m^2; as observed) at Mo 84 was 74 for MI, 78 for LI, and 51 for CsA. Freedom from death, graft loss or cGFR <30 was seen in 85%, 86%, and 67% of MI, LI, and CsA pts, respectively. Acute rejection was observed in 24%, 18% and 10% of MI, LI, and CsA pts. SAEs rates were similar across treatment groups (71%, MI; 69%, LI; 76%, CsA). PTLD occurred in 3 MI (1 EBV+ [rate per 100 person-yrs, 0.09], 2 EBV- [1.50]), 2 LI (2 EBV+ [0.16]) and 2 CsA (1 EBV+ [0.10], 1 EBV- [0.61]) pts. All PTLD cases in bela-treated pts occurred before Month 24. Conclusions: At 7 years, bela conferred statistically superior graft survival compared with CsA that was attributable to the equal contributions of lower rates of graft loss and of patient death. The bela safety profile was consistent with that of previous reports.

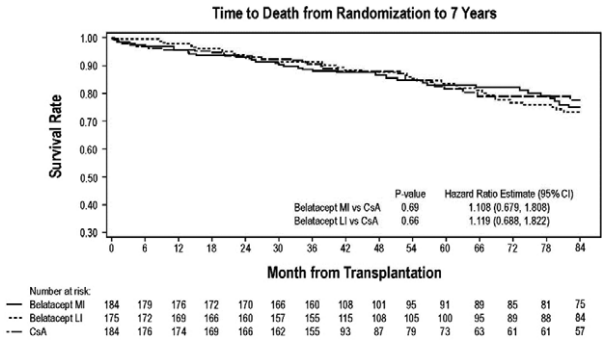


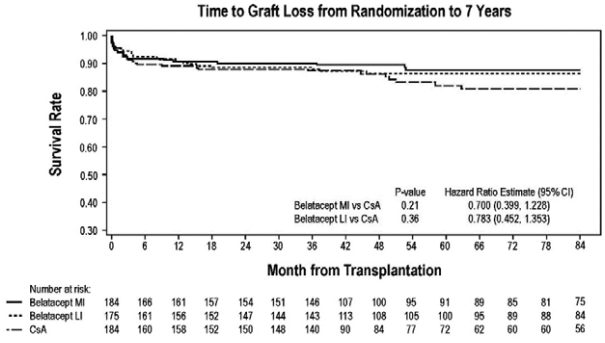
O216 LONG-TERM SURVIVAL OUTCOMES IN BELATACEPT-TREATED VS. CYCLOSPORINE-TREATED PATIENTS: FINAL RESULTS FROM BENEFIT-EXT

Antoine Durrbach[1], Pestana Jose Medina[2], Sander Florman[3], Rial Maria Del Carmen[4], Lionel Rostaing[5], Dirk Kuypers[6], Thomas Wekerle[7], Martin Polinsky[8], Herwig-Ulf Meier-Kriesche[8], Josep Grinyo[9]

[1]University Hospital of Bicêtr; [2]Hospital do Rim e Hipertensão; [3]Mount Sinai Medical Center; [4]Instituto de Nefrologia; [5]University Hospital; [6]University Hospitals Leuven; [7]Medical University of Vienna; [8]Bristol-Myers Squibb; [9]University Hospital Bellvitge

Background: At 7 years, recipients of extended criteria donor (ECD) kidneys who received belatacept (bela) had similar graft survival to cyclosporine (CsA)-treated pts. Here we report the individual contribution of death and graft loss to survival.
Methods: Recipients of ECD kidneys received more (MI) or less (LI) intensive bela or CsA. Assessments included all randomized and transplanted pts through 7 years. In this prospective analysis, time to death or death-censored graft loss was compared between treatment arms using a Cox regression model. Hazard ratio (HR) estimates were derived.
Results: In total, 543 pts were randomized and transplanted: 184 bela MI, 175 bela LI, and 184 CsA pts. HRs comparing time to death/graft loss were 0.915 for MI vs CsA ($p = 0.65$) and 0.927 for LI vs CsA ($p = 0.70$). There was an 11% increased risk of death ($p = 0.69$) and 30% risk reduction in graft loss ($p = 0.21$) for MI vs CsA. There was a 12% increased risk of death ($p = 0.66$) and trend toward numerically better graft survival (22% risk reduction; $p = 0.36$) with LI vs CsA. Mean MDRD cGFR (mL/min/1.73 m^2) as observed at Mo 84 was 57.6 MI, 59.1 LI and 44.6 CsA. Rates of freedom from death/graft loss or cGFR <30 were 53% MI, 55% LI and 36% CsA. Acute rejection occurred in 19% of pts in both bela groups and 16% of the CsA group. Serious AEs occurred in 87% MI, 89% LI and 84% CsA. Across MI, LI and CsA, the respective incidences per 100 person-yrs of serious infections (21.7, 15.8, 19.6), viral infections (21.0, 17.5, 19.1), fungal infections (9.8, 6.9, 11.0), and malignancies (3.7, 3.1, 3.4) were similar. Ten PTLD cases were observed: 2 MI ($n = 1$, EBV+ [incidence per 100 person-yrs, 0.11]; $n = 1$, EBV- [1.61]), 7 LI ($n = 2$, EBV+ [0.23]; $n = 5$, EBV- [6.10]) and 1 CsA (EBV+ [0.13]). Conclusions: At 7 years post-transplant, bela was associated with similar death/graft loss and improved renal function vs CsA. The bela safety profile was consistent with that previously reported.



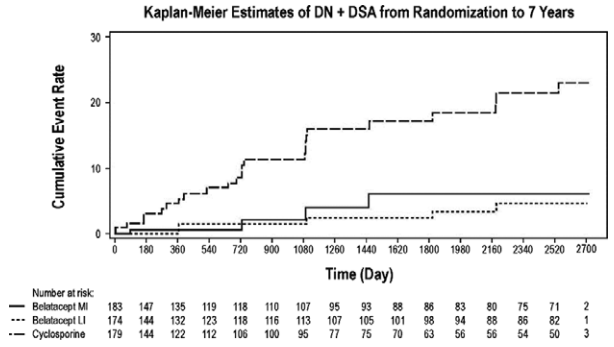


O217* EVALUATION OF DONOR-SPECIFIC ANTIBODIES THROUGH 7 YEARS WITH BELATACEPT: FINAL RESULTS FROM BENEFIT-EXT

Herwig-Ulf Meier-Kriesche[1], Robert Bray[2], Howard Gebel[2], Robert Townsend[1], Christian Larsen[2]

[1]Bristol-Myers Squibb; [2]Emory University

Purpose: Donor-specific antibody (DSA) is linked to an increased risk of antibody-mediated rejection and graft failure. De novo (DN) DSA specific to Class II HLA are associated with a worse prognosis than those specific to Class I. The 3-year intent-to-treat results from BENEFIT-EXT showed lower rates of DN DSA with belatacept. There was also a reduced rate of DN DSA with bela vs. CsA from baseline through Year 5 in the long-term extension (LTE) study. Here we report DN DSA rates from baseline through Year 7 in BENEFIT-EXT.
Methods: Recipients of extended criteria donor kidneys (UNOS extended-criteria deceased donor, anticipated cold ischemia time ≥24 h, donor with cardiac death) were randomized to bela MI or LI or CsA regimens. DN DSA rates were assessed for all randomized and treated patients from baseline through Year 7. The presence of DSA was established centrally by solid phase flow cytometry (FLowPRA™). Specificity (Class I and II) was assessed by LabScreen™ single antigen beads (One Lambda, Inc.). Kaplan-Meier estimates for the cumulative rate of development of DN DSA from randomization to study end were derived.
Results: In total, 543 patients were randomized and transplanted (n = 184, bela MI; n = 175, bela LI; n = 184, CsA). The cumulative event rates of DN DSA at Years 3, 5, and 7 for bela MI were 2.32, 6.21, and 6.21, respectively. The corresponding values for bela LI were 1.52, 2.39, and 4.48. The cumulative event rates at Years 3, 5, and 7 for CsA were 11.25, 17.07, and 21.30, respectively. Class I HLA specificity was seen in 5 MI-treated, 3 LI-treated, and 15 CsA-treated patients. Class II HLA specificity was seen in 2 MI-treated and 3 CsA-treated patients. Both Class I and II HLA formed in 4 patients in the CsA arm. Conclusions: Data from BENEFIT-EXT through Year 7 demonstrate a reduced incidence of DN DSA with bela (MI or LI) vs. CsA. Further study is required to determine if reduced incidence of DN DSA leads to better long-term outcomes.



O218* EFFICACY AND SAFETY OF THREE DIFFERENT TREATMENT REGIMENS IN DE NOVO RENAL TRANSPLANT PATIENTS: MONTH 48 FOLLOW-UP RESULTS OF THE HERAKLES TRIAL

M Guba[1], C Sommerer[1], V Kliem[1], O Witzke[1], J Jacobi[1], B Vogt[1], I Hauser[1], P Reinke[1], R Stahl[1], T Rath[1], M Porstner[2], D Baeumer[2], M Zeier[1], W Arns[1], F Lehner[1], K Budde[1]

[1]Herakles Study Group; [2]Novartis Pharma

Aim: To compare safety and efficacy of 3 different immunosuppressive (IS) regimens 4 years after renal transplantation (Tx).
Methods: 802 patients (pts) were included in this 1-year, prospective, open-label, randomised, controlled multi-centre study with observational follow-up (FU) until months (Mo) 60 post Tx. After induction therapy all pts received cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 Mo post Tx 499 pts were randomised 1:1:1 to either a) continue standard CsA (100–180 ng/ml) + EC-MPS (STD; n = 166) or convert b) to a calcineurin inhibitor (CNI)-free regimen with everolimus (EVR) (5–10 ng/ml) + EC-MPS (n = 171) or c) to a CNI-reduced regimen with EVR (3–8 ng/ml) + reduced CsA (50–75 ng/ml) (n = 162). All pts continued on steroids. At time of Mo48 FU interims-analysis data were available from 110 (73%) STD, 117 (79%) CNI-free and 111 (76%) CNI-low treated pts of the FU ITT population.
Results: From randomisation to Mo48 BPAR was reported in 19/151 (13%) STD, 24/149 (16%) CNI-free and in 23/147 (16%) CNI-low pts (ITT; p = ns). 5 deaths (3%) occurred in STD, 3 (2%) in CNI-free and 6 (4%) in the CNI-reduced group. 9 (6%) graft losses were observed in the STD, 6 (4%) in the CNI-free and 2 (1%) in the CNI-reduced group. Composite failure (BPAR, death, graft loss, loss to FU) occurred in 32, (21%) STD, 36 (24%) CNI-free, 39 (27%) CNI-reduced treated pts (Table). Premature discontinuation due to AEs occurred in 5 (3%) of STD, 5 (3%) of CNI-free and 1 (1%) of CNI-reduced pts (safety population) from Mo12 to 48. Renal function (cGFR, Nankivell, LOCF) was significantly improved by +6.8 ml/min/1.73 m^2 in favor of the CNI-free regimen at Mo48 (ITT; p = 0.02).
Conclusions: Mo48 results, from HERAKLES, show that IS regimen using EVR with reduced-dose or without CNI-exposure reflect an efficacious and safe therapeutic approach offering the opportunity for an individualized IS to minimize CNI-exposure.

Table: Safety and efficacy results

Safety Population, n (%) events during FU (Mo12–48)	**Standard (n=154)**	**CNI-free (n=151)**	**CNI-reduced (n=147)**
Infections	66 (43)	77 (51.0)	66 (45)
Severe infections	15 (10)	17 (11)	16 (11)
Infections leading to hospitalization	47 (31)	50 (33)	50 (34)
CMV	6 (4)	7 (5)	6 (4)
BKV	2 (1)	2 (1)	2 (1)
Hospitalizations due to (*primary reason*):			
Acute rejection	13 (8)	17 (11)	15 (10)
Cardiovascular event	7 (5)	7 (5)	5 (3)
Cerebrovascular event	2 (1)	0	2 (1)
GI event	8 (5)	5 (3)	9 (6)
Infection	43 (28)	44 (29)	44 (30)
Malignancy	3 (2)	9 (6)	4 (3)
Metabolic disorder	2 (1)	0	0
FU ITT Population, n (%) events from randomization to Mo48	**Standard (n=151)**	**CNI-free (n=149)**	**CNI-reduced (n=146)**
BPAR (rejection episode with final clinical diagnosis = acute rejection diagnosed by biopsy)	19 (13)	24 (16)	23 (16)
Banff grade IA	6 (4)	11 (7)	13 (9)
Banff grade IB	7 (5)	3 (2)	2 (1)
Banff grade IIA	2 (1)	1 (1)	2 (1)
Banff grade IIB	2 (1)	1 (1)	1 (1)
Banff grade III	0	1 (1)	0
unknown	2 (1)	7 (5)	5 (3)
Death	5 (3)	3 (2)	6 (4)
Graft loss	9 (6)	6 (4)	2 (1)

BPAR, biopsy-proven acute rejection; BKV, BK virus; CMV, cytomegalovirus; CNI, calcineurin inhibitor; FU, follow-up; GI, gastrointestinal; Mo, month; ITT, intent-to-treat.

O219 SUPERIOR RENAL FUNCTION IN AN EVEROLIMUS-BASED CYCLOSPORINE FREE REGIMEN VERSUS STANDARD CYCLOSPORINE/MYCOPHENOLATE AND REDUCED CYCLOSPORINE/EVEROLIMUS: FOLLOW-UP OF THE HERAKLES STUDY AT MONTH 48

T Rath[1], O Witzke[1], P Weithofer[1], M Guba[1], J Jacobi[1], B Vogt[1], I Hauser[1], R Stahl[1], P Reinke[1], D Baeumer[2], M Porstner[2], M Zeier[1], F Lehner[1], W Arns[1], K Budde[1], C Sommerer[1]

[1]Herakles Study Group; [2]Novartis Pharma

Aim: To follow up on renal function (GFR) at month (Mo) 48 after kidney transplantation (Tx) in patients (pts) on immunosuppressive regimen with different calcineurin inhibitor (CNI) exposures.
Methods: 802 pts were included in this prospective, open-label, randomised multi-centre study. After induction with basiliximab all pts received cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3Mo post Tx 499 pts were randomised 1:1:1 to either i) continue standard (STD) CsA (100–180 ng/ml) with EC-MPS (n = 166), ii) convert to a CNI-free regimen with everolimus (EVR; 5–10 ng/ml) + EC-MPS (n = 171) or iii) convert to CNI-reduced regimen CsA (50–75 ng/ml) with EVR (3–8 ng/ml) (n = 162).
Results: Here data from 48Mo observational follow-up are presented: GFR (Nankivell, ITT) was similar at randomisation 3Mo post Tx and had significantly improved at Mo12 by +5.6 ml/min (95% CI: [+2.9; +8.3]; p < 0.001) and remained significantly improved by +6.8 ml/min in favour of CNI-free regimen at Mo48 (p = 0.02) (Table). 54% of CNI-free, 36% of CNI-reduced and 44% of STD pts had an improvement in GFR at Mo48 (p = 0.09 CNI-free vs STD). All 3 groups had similar rejection rate since randomisation (13% STD, 16% CNI-free, and 16% CNI-low) and overall comparable safety profile. Median trough levels at Mo48 were: CsA 92 ng/ml in STD, 80 ng/ml in CNI-reduced pts and EVR 5.1 ng/ml in CNI-free, 5.0 ng/ml in CNI-reduced pts.
Conclusion: CNI-free as well as reduced CNI in combination with EVR regimens are both efficacious and safe regimens. CNI-reduced group had higher CsA levels than anticipated. The fact that CNI reduction was not fully accomplished might have prevented GFR differences compared to STD. However, CNI-free regimen was associated with better GFR maintained for 4 years post Tx. The results of this large trial confirm previous reports of improved GFR after CsA withdrawal with EVR in combination with EC-MPS.

Table: Renal function

eGFR (Nankivell) [ml/min/1.73m^2] ITT FU population to Mo48	**LS-Mean [95%CI]**	**Difference vs. STD**
Standard (n=142)	52.7 [47.5; 57.7]	-
CNI-free (n=138)	59.5 [54.3; 64.7]	6.8 [1.1; 12.6]
CNI-reduced (n=140)	51.0 [45.9; 56.1]	-1.7 [-7.4; 4.0]

(*) ANCOVA model with treatment, centre, donor type as factors and GFR value at covariate. Replacement of missing values: LOCF = last observation (>Randomisation) carried forward

CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; FU, follow-up; ITT, intent-to-treat; Mo, month; STD, standard.

O220 EXPLORATIVE ANALYSIS OF ZEUS AFTER 5 YEARS: HISTOLOGICAL ASSESSMENT FROM BIOPSY ANALYSES

U Eisenberger[1], O Witzke[1], I Hauser[1], F Lehner[1], C Sommerer[1], R Wuethrich[1], P Reinke[1], A Muehlfeld[1], K Heller[1], R Stahl[1], H Wolters[1], M Porstner[2], W Arns[1], K Budde[1]

[1]ZEUS Study Group; [2]Novartis Pharma

Background: Analysis of pathologists' assessments and histological data allow for deeper insight on patient outcome together with investigator's final clinical diagnoses. Here we present 5 year data from *de novo* transplant recipients after conversion to an everolimus (EVR)-based regimen and withdrawal of calcineurin inhibitor (CNI) therapy.
Methods: Analysis of histological and pathologists assessments from the prospective, open-label, controlled, multi-centre study ZEUS. 300 renal transplant (Tx) patients were randomized at month (Mo) 4.5 post Tx to either receive EVR plus enteric coated-mycophenolate sodium (EC-MPS) (n = 154) or cyclosporine (CsA) plus EC-MPS regimen (n = 146). After 12 Mo interventional core study observational follow-up (FU) on pts safety and efficacy was performed until Mo60 post Tx.
Results: Total number (nr) of biopsies performed and mean nr of biopsies per patient are overall similar in both groups until Mo60. Nr of pts with at least one rejection (as per final clinical diagnosis) was slightly higher in CNI group vs EVR group. Nr of pts with BPAR was higher in the EVR group especially due to mild, early acute rejections (mostly BANFF IA and IB). Nr of pts with histological evidence of chronic/sclerosing allograft nephropathy was similar in both groups, C4D staining positivity was found slightly higher in EVR group, however, pts with evidence of antibody mediated rejection was higher in CNI group as well as CNI induced toxicity (Table).

Conclusions: Data from histological assessments and reported final clinical outcomes show that an EVR-based regimen with early elimination of CNI therapy is as safe and efficacious as standard CNI therapy offering the opportunity to reduce CNI-induced toxicities on the allograft.

Table: Histological assessments

ITT Population	CNI	EVR
Number (%) of biopsies during total study period	282 (=100%)	306 (100%)
of these due to clinical event	177 (62.8)	176 (57.5)
of these due to follow-up biopsy	22 (7.8)	32 (10.5)
of these protocol biopsies	14 (5.0)	21 (6.9)
others (incl null-, blinded- and controll biopsies)	69 (24.4)	77 (25.1)
Number of biopsies per patient over total study period	1.93	1.99
Investigator initiated biopsies – Core Period	149	149
By protocol biopsies – Core Period	83	90
Investigator initiated biopsies – Follow-Up Period	42	48
By protocol biopsies – Follow-Up Period	8	19
	CNI (n=146)	**EVR (n=154)**
Final clinical diagnosis (multiple FDC per patient possible)		
Number (%) of patients with at least one rejection over total study period	91 (62.3)	88 (57.1)
Core Period: (most frequent diagnoses)		
Acute rejection diagnosed by biopsy	19 (13.0)	25 (16.2)
Borderline Lesion	20 (13.7)	17 (11.0)
CNI induced toxicity	20 (13.7)	17 (11.0)
Acute tubular necrosis	14 (9.6)	16 (10.4)
Borderline rejection	7 (4.8)	1 (0.7)
CAN	5 (3.4)	2 (1.3)
Follow-Up Period: (most frequent diagnoses)		
Acute rejection diagnosed by biopsy	6 (4.11)	9 (5.8)
Borderline Lesion	4 (2.8)	8 (5.2)
CNI induced toxicity	7 (4.8)	2 (1.3)
Infection	3 (2.1)	-
CAN	6 (4.11)	8 (5.2)
Pathologist's assessment of kidney allograft biopsy (excluding null-biopsies)		
N (%) of pts with histological evidence of acute/active rejection	22 (15.1)	32 (20.8)
N (%) of pts with histological evidence of chronic/sclerosing allograft nephropathy	15 (10.3)	15 (10.0)
N (%) of pts with positive C4D staining	10 (6.9)	17 (11.0)
N (%) of pts with evidence of antibody mediated rejection	6 (4.1)	3 (2.0)
N (%) of pts with presence of other lesions:		
CNI toxicity lesions	34 (23.3)	24 (15.6)
Acute tubular necrosis	22 (15.1)	18 (11.7)

CNI, calcineurin inhibitor; CAN, chronic allograft nephropathy; EVR, everolimus; ITT, intent-to-treat.

O221 MONTH 48 FOLLOW-UP RESULTS OF HERAKLES TRIAL ON THREE DIFFERENT TREATMENT REGIMENS AND SWITCHING OFF BEHAVIOUR IN DE NOVO RENAL TRANSPLANT PATIENTS

K Budde[1], C Sommerer[1], W Arns[1], O Witzke[1], M Guba[1], J Jacobi[1], B Vogt[1], P Reinke[1], I Hauser[1], R Stahl[1], T Rath[1], D Baeumer[2], M Porstner[2], F Lehner[1], V Kliem[1], M Zeier[1]

[1]Herakles Study Group; [2]Novartis Pharma

Aim: To compare switching off 3 different immunosuppressive (IS) regimens 4 years after renal transplantation (Tx).

Methods: 802 patients (pts) were included in this prospective, open-label, randomised, controlled multi-centre study with observational follow-up (FU) until Mo60 post Tx. After induction therapy with basiliximab all pts received cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 Mo post Tx 499 pts were randomised 1:1:1 to either i) continue standard CsA (100–180 ng/ml) + ECMPS (n = 166) (STD) or convert ii) to a calcineurin inhibitor (CNI)-free regimen with everolimus (EVR) (5–10 ng/ml) + EC-MPS (n = 171) or iii) to a CNI-reduced regimen with EVR (3–8 ng/ml) + reduced CsA (50–75 ng/ml) (n = 162). All pts continued on steroids.
Results: At 48 Mo post Tx 60 (36%) CNI-free, 43 (27%) CNI-reduced and 83 (51%) STD treated pts were still on their initial assigned treatment and available for Mo48 analysis. Among those pts who completed Mo48 FU visit non-switcher frequencies were 53% for CNI-free, 41% for CNI-reduced and 81% for STD treated pts. Drop-out frequency among FU ITT population from randomization to Mo48 was 17% for STD, 15% for CNI-free and 14% for CNI-reduced treated pts. Premature discontinuation due to AEs occurred in 5 (3%) of STD, 5 (3%) of CNI-free and 1 (1%) of CNI-reduced pts (safety population) from Mo12 to 48. Renal function (cGFR, Nankivell, LOCF) among those pts, who never switched off their assigned treatment GFR was significantly improved by +13.7 ml/min/1.73 m^2 in favour of the CNI-free regimen at Mo48 (ITT; $p < 0.001$).
Conclusions: Mo48 results from HERAKLES show that IS regimen using EVR with reduced-dose or without CNI-exposure reflect the opportunity for an individualised IS to minimize CNI-exposure. Drop-out rates over 4 years post Tx showed similar adherence rates between groups. Pts that never switched off the assigned CNI-free regimen reached a markedly improved GRF.

Subgroup/ Immunosuppressive medication at Mo 48		Treatment group name							
% related to ITT population entering FU		Standard		CNI-free		CNI-reduced		Total	
		N =151	100%	N=149	100%	N=146	100%	N= 446	100%
Drop Out/	*Unknown / drop out*	51	33.77	35	23.49	43	29.45	85	19.06
Switcher to	Tac	8	5.30	29	19.46	20	13.70	57	12.78
	CsA	2	1.32	22	14.77	32	21.92	56	12.56
	Everolimus	.	.	.	.	6	4.11	6	1.35
	Sirolimus	3	1.99	.	.	.	.	3	0.67
	MPA monotherapy	2	1.32	1	0.67	.	.	3	0.67
	CsA or Tac and azathioprine	1	0.66	.	.	2	1.37	3	0.67
	Everolimus and Tac	.	.	1	0.67	.	.	1	0.22
	CsA and Tac	.	.	1	0.67	.	.	1	0.22
	CsA and sirolimus	1	0.66	.	.	.	.	1	0.22
	Total	68	45.03	89	59.73	103	70.55	260	58.30
Non-Switcher	**Total**	83	54.97	60	40.27	43	29.45	186	41.70

eGFR (Nankivell) [ml/min/1.73m^2] Non-switcher pop. to Mo48	LS-Mean [95%CI]			Difference vs. STD [95%CI]			
Results from ANCOVA model (*) 95% CI (2-tail)	LS-Mean	LowerCL	UpperCL	LS-Mean	LowerCL	UpperCL	P-value
Standard (n=83)	61.51	56.86	66.17	-	-	-	
CNI-free (n=60)	75.19	69.92	80.46	+13.7	+19.1	+8.27	<.0001
CNI-reduced (n=43)	58.48	52.50	64.46	-3.03	+3.10	-9.16	0.3302

(*) ANCOVA model with treatment, center, donor type as factors and GFR value at V4/M3/BL2 as covariate
Replacement of missing values: LOCF = last observation (=Randomization) carried forward
ANCOVA, analysis of covariate; CI, confidence interval; CNI, calcineurin inhibitor; CsA, cyclosporine; eGFR, estimated glomerular filtration rate; FU, follow-up; ITT, intent-to-treat; LS, least squares; Mo, month; pop., population; STD, standard.

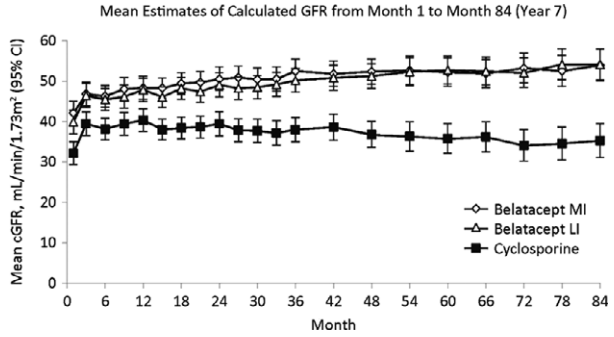
O222 SUPERIOR ESTIMATED GFR ASSOCIATED WITH BELATACEPT VS. CYCLOSPORINE TREATMENT: RESULTS FROM A MIXED EFFECTS MODELING ANALYSIS OF THE BENEFIT-EXT STUDY

Antoine Durrbach[1], Pestana Jose Medina[2], Rial Maria Del Carmen[3], Gerrit Grannas[4], Arthur Matas[5], Martin Polinsky[6], Herwig-Ulf Meier-Kriesche[6], Stephane Munier[6], Josep Grinyo[7]

[1]University Hospital of Bicêtre; [2]Hospital do Rim e Hipertensão; [3]Instituto de Nefrologia; [4]Medizinische Hochschule; [5]University of Minnesota; [6]Bristol-Myers Squibb; [7]University Hospital Bellvitge

Background: Belatacept (bela)-treated recipients of extended criteria donor (ECD) kidneys in BENEFIT-EXT had higher mean calculated GFR (cGFR) vs CsA pts at 3 years in the ITT population and at 5 years in the long-term extension population. Here we report cGFR derived from a longitudinal model analysis on the ITT population of BENEFIT-EXT through 7 years' follow-up. This model takes into account between-subject variability and intra-subject correlation of cGFR measurements across all time points. For missing data, it assumes Missingness At Random for per time point as-observed mean estimates.
Methods : Recipients of ECD kidneys received bela more (MI) or less (LI) intensive, or CsA regimens. Mean cGFR and 95% CIs were estimated from Months 1–84 for all randomized and transplanted pts using a repeated measures model with an unstructured covariance matrix. The difference in cGFR between treatments at each timepoint was also estimated. This model included treatment, time, and a time × treatment interaction (no further adjustment); time was regarded as a class variable (3-monthly intervals). A random slope longitudinal model was performed using time as a continuous variable and assuming linearity.
Results: Mean cGFR increased over 7 years for both bela treatment arms, but declined for CsA. The differences in mean cGFR between bela MI and CsA at Yrs 1, 3, 5, and 7 were 8.0, 14.5, 16.4, and 18.7 ml/min/1.73 m^2, respectively. The corresponding differences in mean cGFR between bela LI and CsA at Yrs 1, 3, 5, and 7 were 7.5, 12.2, 17.0, and 18.9 ml/min/1.73 m^2. These differences were statistically significantly in favor of each bela regimen

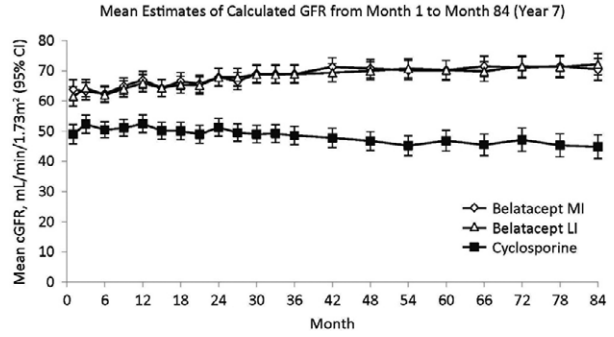
vs. CsA at all time points ($p < 0.0001$). Slope estimates from Yr 1–7: MI, 1.45 (95%CI: 0.94; 1.96); LI, 1.51 (1.02; 2.01); CsA: –0.01 (–0.55; 0.52).
Conclusions: In bela-treated patients, GFR continues to improve over time, with increasing divergence between treatment arms.



O223 BELATACEPT-TREATED PATIENTS HAD SUPERIOR ESTIMATED GFR VS. CYCLOSPORINE-TREATED PATIENTS: RESULTS FROM A MIXED EFFECTS MODELING ANALYSIS OF THE BENEFIT STUDY

Josep Grinyo[1], Lionel Rostaing[2], Barbara Bresnahan[3], Kim Rice[4], Flavio Vincenti[5], Stephane Munier[6], Martin Polinsky[6], Herwig-Ulf Meier-Kriesche[6], Rafael Reyes-Acevedo[7], Pestana Jose Medina[8]
[1]University Hospital Bellvitge; [2]University Hospital; [3]Medical College of Wisconsin; [4]Baylor University Medical Center; [5]University of California; [6]Bristol-Myers Squibb; [7]Hospital Miguel Hidalgo De Aguascalientes; [8]Hospital do Rim e Hipertensão

Background: Prior analyses of BENEFIT showed significantly higher mean calculated GFR (cGFR) with belatacept (bela) vs CsA at 3 years in the ITT population and at 5 years in the long-term extension cohort. Here we report cGFR derived from a longitudinal model analysis of BENEFIT on the ITT population for the 7-yr period. This model takes into account the between-subject variability and intra-subject correlation of cGFR measurements across all time points. For missing data, it assumes Missingness At Random for per time point as-observed mean estimates.
Methods: Recipients of living or standard criteria donor kidneys received bela more (MI) or less (LI) intensive, or CsA regimens. Mean cGFR and 95% CIs were estimated from Mos 1–84 for all randomized and transplanted pts using a repeated measures model with an unstructured covariance matrix. The difference in cGFR between treatment arms at each timepoint was also estimated. This model included treatment, time, and a time × treatment interaction, with no further adjustment; time was regarded as a class variable (3-monthly intervals). A random slope longitudinal model was performed using time as a continuous variable and assuming linearity.
Results: Mean cGFR increased slightly over 7 years for both bela treatment arms, but declined for CsA. The differences in mean cGFR between bela MI and CsA at Years 1, 3, 5, and 7 were 14.5, 20.3, 23.3, and 25.6 ml/min/1.73 m^2, respectively. The differences in mean cGFR between bela LI and CsA at Years 1, 3, 5, and 7 were 13.5, 20.4, 23.4, and 27.3 ml/min/1.73 m^2, respectively. The differences in cGFR were statistically significantly in favor of each bela regimen vs. CsA at all timepoints ($p < 0.0001$). Slope estimates from Yr 1–7: MI, 1.30 (95%CI: 0.83; 1.77); LI, 1.39 (95%CI: 0.93; 1.84); CsA, –1.04 (95%CI: –1.53; –0.54). Conclusions: The significant improvement in renal function seen with bela vs. CsA is sustained over 7 years, with increasing divergence between treatment arms over time.



O224 DEVELOPMENT OF PREDICTIVE SCORE OF POST-TRANSPLANT DIABETES INCLUDING CLINICAL AND GENETIC VARIABLES

Claudio Musetti[1], Marco Quaglia[1], Salvatore Terrazzino[2], Sarah Cargnin[2], Armando Genazzani[2], Tiziana Cena[3], Guido Merlotti[1], Piero Stratta[1]
[1]Universita' del Piemonte Orientale, Kidney Transplant Center; [2]Universita' del Piemonte Orientale, Pharmacogenetics Laboratory; [3]Universita' del Piemonte Orientale, Epidemiology Unit

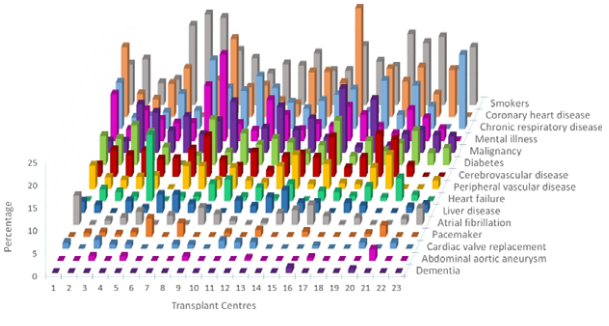
Background: New Onset Diabetes After Transplantation (NODAT) is associated with an increased morbidity and mortality following kidney transplantation (KTx): several polymorphism modulate its risk, but their clinical utility is still undefined. Aim of this study is to evaluate whether TCF7L2 SNP rs7903146 can predict the risk of NODAT in KTx recipients (KTR) when employed in an integrated model including clinical variables.
Methods: We investigated NODAT-free survival in 464 mainly Caucasian KTRs and analyzed its pre-transplant risk factors, including SNP rs7903146.
Results: Genetic analysis showed that 163 patients were CC (35.1%), 237 were CT (51.1%) and 64 were TT (13.8%): their 2-years incidence of NODAT was respectively 7.8%, 11.9% and 22.7%. At multivariate analysis, risk factors for NODAT were age (per year; HR = 1.027; 95%CI 1.003–1.051; p = 0.030), BMI at KTx >25 (HR = 3.03; 95%CI 1.78–5.14; p40, BMI >25, rs7903146 TT, previous transplants), patients with a score of 0, 1, 2 and 3 had respectively a 2-years NODAT risk of 2.0%, 5.1%, 18.8% and 40.0% ($p < 0.001$).
Conclusions: TCF7L2 SNP rs7903146 is strongly and independently associated with NODAT and is reliable in predicting the risk of NODAT prior to surgery when used together with few clinical variables. Genetic analysis of this polymorphism appears to be a promising tool to tailor immunosuppression in order to delay or prevent NODAT.

O225 COMORBIDITY IN DIALYSIS, WAITLISTED AND TRANSPLANT PATIENTS IN THE UNITED KINGDOM: FINDINGS FROM THE ATTOM STUDY

Diana Wu[1], Matthew Robb[2], Rommel Ravanan[3], Charles Tomson[4], John Forsythe[1], Clare Bradley[5], John Cairns[6], Christopher Dudley[3], Christopher Watson[7], Heather Draper[8], Wendy Metcalfe[1], Damian Fogarty[9], Paul Roderick[10], Andrew Bradley[7], Gabriel Oniscu[1]
[1]Transplant Unit, Royal Infirmary of Edinburgh; [2]NHS Blood and Transplant, Bristol; [3]Richard Bright Renal Unit, Southmead Hospital, Bristol; [4]Department of Renal Medicine, Freeman Hospital, Newcastle upon Tyne; [5]Health Psychology Research Unit, Royal Holloway, University of London; [6]Department of Health Services Research and Policy, London School of Hygiene and Tropical Medicine; [7]Department of Surgery, University of Cambridge and the NIHR Cambridge Biomedical Research Centre; [8]School of Health and Population Sciences, University of Birmingham; [9]UK Renal Registry, Bristol; [10]Primary Care and Population Sciences, Faculty of Medicine, University of Southampton

Background: Access to renal Transplantation and Transplant Outcome Measures (ATTOM) is a prospective study involving all 72 UK renal units, investigating the factors influencing access to and outcomes from transplantation in order to optimise equity of access, survival, quality of life and cost effectiveness.
Methods: 6842 patients aged 18–75 years were recruited to the study between 2011 and 2013, including 2621 incident dialysis (ID), 2262 incident transplant (IT) of whom 807 received living donor (LD) transplants and 1959 waitlisted matched control (MC) patients (matched for centre, age +/-5 years, time on waiting list, diabetes and kidney only/simultaneous kidney-pancreas transplant). Baseline data were collected at time of enrolment and analysed for descriptive comparisons using SAS® 9.4.

Figure 1. Variation in the comorbidity of transplant recipients between centres



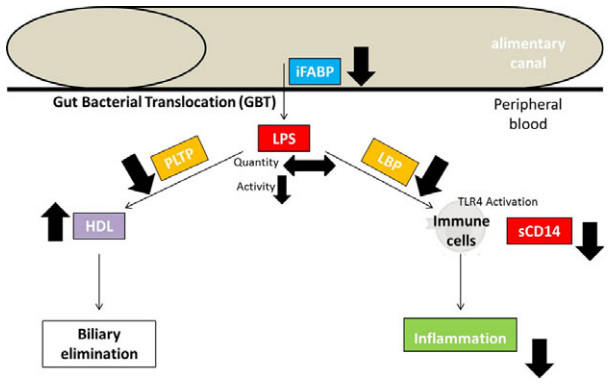
Results: There was a higher prevalence of comorbidity in ID patients compared with MC and IT patients, but no differences between MC and IT cohorts. There was significant geographical variation in the prevalence of comorbid diabetes, coronary heart disease (CHD), heart failure (HF), atrial fibrillation (AF), chronic respiratory disease, malignancy, mental illness and smoking in the ID population. There was significant centre variation in the age distribution and prevalence of diabetes, HF, AF, chronic respiratory disease and smoking amongst waitlisted as well as transplanted patients (Fig 1). LD transplant recipients were younger and fewer had CHD, HF, cerebrovascular disease and peripheral vascular disease compared with deceased donor (DD) recipients. Significant centre differences were noted in the prevalence of CHD, HF, malignancy and mental illness in patients receiving a DD transplant.
Conclusion: There are significant centre differences in the comorbidity profile of dialysis, waiting list and transplant patients in the UK. Further correlations with outcome and donor data are required to assess the impact of these variations on long-term outcome after transplantation.

O226 IMPACT OF RENAL TRANSPLANTATION ON GUT BACTERIAL TRANSLOCATION

Clémence Carron[1], Jamal Bamoulid[1], Jean-Paul Pais De Barros[1], Emilie Gaiffe[2], Philippe Saas[1], Laurent Lagrost[1], Didier Ducloux[1]
[1]Inserm; [2]FHU

Enhanced gut bacterial translocation (GBT) is thought to play a role in uremia-associated chronic inflammation. Both dysbiosis and increased gut permeability might explain GBT but its evolution after kidney transplantation (KT) is unknown. We aimed at describing GBT evolution and its impact on inflammatory status in renal transplant recipients (RTR). We prospectively included 100 RTR with available serum at time and one year post transplant. Intestinal integrity was evaluated through iFABP and GBT through bacteria products translocation (LPS, sCD14, LBP). Inflammatory biomarkers (IB), lipoproteins, and PLTP activity were simultaneously measured to determine LPS impact on inflammation and LPS binding to lipoproteins. GBT and IB were high at transplant time and significantly decreased 1 year after (sCD14 2.3 ± 0.6 vs. 1.2 ± 0.5 μg/ml, $p < 0.0001$; LBP 21.7 ± 6.6 vs. 17.7 ± 4.9 μg/ml, $p = 0.0004$; usPCR 25.3 ± 96.3 vs. 16.5 ± 29.6 μg/ml, $p = 0.04$, IL8 3.0 ± 4.6 vs. 1.7 ± 4.2 ng/ml, $p = 0.02$). iFABP decreased, but remained higher than in healthy controls (3.5 ± 2.1 vs. 1.9 ± 1.3 ng/ml, $p < 0.0001$). Probably due to persistent abnormal intestinal permeability, total LPS remained stable. Yet, LPS activity significantly decreased (6.8 ± 3.3 vs. 5.5 ± 1.8 EU/ml, $p = 0.03$). We concomitantly observed an increase in HDL and total cholesterol (0.42 ± 0.13 vs. 0.48 ± 0.15 g/l, $p = 0.0002$ and 1.63 ± 0.39 vs. 1.81 ± 0.50 g/l, $p = 0.001$) and a decrease in PLTP (11.0 ± 3.4 vs. 9.5 ± 2.6 pmol/h, $p < 0.0001$). LPS activity was correlated with cholesterol level ($r = 0.34$; $p = 0.001$). Altogether, this suggests that despite similar GBT after transplantation, consequences of translocation are decreased by higher LPS-binding fraction to HDL (figure 1). KT improves ESRD-related GBT. Increase in HDL after transplantation could bind circulating LPS and contribute to improvement of ESRD-associated inflammatory status. Clinical impact of GBT modifications remains to be addressed.

Figure1: Biomarkers of GBT and pathways of LPS binding: variations after KT



O227 DIABETES MELLITUS AND VITAMIN D DEFICIENCY FOLLOWING RENAL TRANSPLANTATION

Awena Le Fur[1], Marie Cécile Fournier[2], Florence Gillaizeau[2], Damien Masson[3], Magali Giral[1], Bertrand Cariou[4], Diego Cantarovich[1], Jacques Dantal[1]
[1]ITUN, Nantes University Hospital; [2]EA 4275 – SPHERE Biostatistics, Pharmacoepidemiology, and Human Sciences Research, Nantes; [3]Biochemestry Laboratory, Nantes; [4]Institut du Thorax, Nantes University Hospital

Post-transplant diabetes mellitus (PTDM) is a common and potential life threatening complication following kidney transplantation. Observational studies in the general population suggested an association between 25 hydroxyvitamin D [25 (OH)D] deficiency and type 2 diabetes. Such association remains however unknown in kidney transplant recipients.

A total of 444 patients were prospectively evaluated following primary kidney transplantation between January 2000 and December 2010. The 25 (OH)D level was measured by radioimmunoassay at the time of transplantation allowing to define 3 grades: deficiency (<10 ng/ml), insufficiency (≥10 and <30 ng/ml) and normal range (≥30 ng/ml).

At one year after transplantation, cumulative incidence of PTDM was 13.4%. Cox multivariate analysis indicated that 25 (OH)D deficiency (≤10 ng/ml) at the time of transplantation was an independent risk factor for PTDM within the first year after kidney transplantation (HR = 2.41). Other independent risk factors were tacrolimus (HR = 4.73 [1.86–12.04]), corticosteroids (HR = 1.99), increased BMI (HR = 1.72) for each increase of 5 kg/m^2) and age ≥55 years (HR = 2.21).

25 (OH)D deficiency (but not insufficiency) is a new independent risk factor for PTDM within the first year after kidney transplantation. Our study suggests that 25 (OH)D may be a marker of general health in kidney transplant recipients and could alert clinicians for PTDM risk.

O228 THE EFFECT OF HYPERCALCEMIA ON TUBULOINTERSTITIAL CALCIFICATION OF THE ALLOGRAFT

Aygul Celtik[1], Mumtaz Yilmaz[1], Sait Sen[2], Meltem Sezis-Demirci[1], Gulay Asci[1], Furkan Tamer[1], Banu Sarsik[2], Cuneyt Hoscoskun[3], Huseyin Toz[1], Ercan Ok[1]
[1]Ege University, School of Medicine, Department of Internal Medicine, Division of Nephrology; [2]Ege University, School of Medicine, Department of Pathology; [3]Ege University, School of Medicine, Department of General Surgery

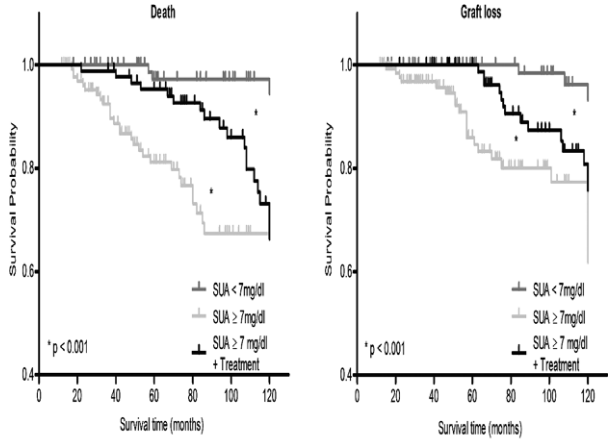
Objectives: Persistent hypercalcemia after kidney transplantation (KTx) may have adverse effects like osteoporosis, nephrocalcinosis and graft dysfunction. The aim is to evaluate patients with persistent hypercalcemia and assess the effect on tubulointerstitial calcification of the allograft.
Methods: 247 patients undergoing KTx between 2009 and 2011 were enrolled. Transient and persistent hypercalcemia was defined as hypercalcemia (corrected serum calcium >10.2 mg/dl) that persisted after KTx for 6 and 12 months, respectively. The severity of calcification in the zero-hour, 6th and 12th month protocol biopsies of patients with transient hypercalcemia ($n = 8$) and with persistent hypercalcemia ($n = 20$) were compared with a group of age, gender, dialysis duration, donor type, DGF and immunosuppressive regimen matched-control recipients ($n = 28$).
Results: Persistent hypercalcemia was detected in 8% of patients. Pretransplant PTH was only significant predictor of serum calcium level at 12 months. Longer dialysis duration was positive predictor of persistent hypercalcemia. Among patients with transient hypercalcemia, calcification scores in 6th month biopsies were increased when compared to that of zero-hour biopsies and it was higher than biopsies of matched-control group at 6th months and decreased to same scores with matched-control group in 12th month biopsies. The calcification scores were increased progressively from zero-hour to 12th months of KTx among recipients with persistent hypercalcemia. Multivariate analysis based on serum calcium and phosphate levels at 12 months and pretransplant PTH revealed that calcium was predictor of graft calcification at 12 months.
Conclusion: Persistently high level of serum calcium may cause tubulointerstitial calcification of the allograft especially if hypercalcemia persists beyond 6 months after KTx.

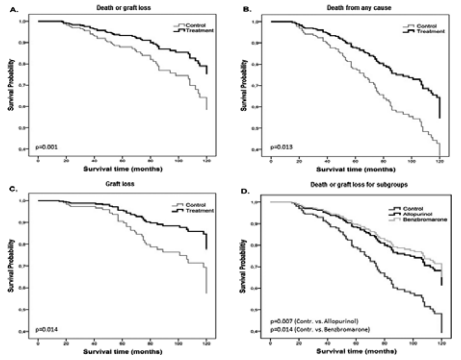
O229 TREATMENT OF ASYMPTOMATIC HYPERURICEMIA AFTER RENAL TRANSPLANTATION

Nikolaos Pagonas[1], Samad Kor[2], Felix Seibert[1], Nina Babel[2], Walter Zidek[2], Petra Reinke[2], Arnd Giese[1], Frederic Bauer[1], Timm Westhoff[1]
[1]University of Ruhr Bochum; [2]Charite-Universitätsmedizin Berlin

Introduction: Hyperuricemia is very common after renal transplantation. It is associated with an increased risk of cardiovascular events and graft loss. Todate, however, treatment is only recommended in symptomatic disease. No

data exist on the treatment of asymptomatic hyperuriceamia in renal transplant recipients.
Methods: 503 adult patients who underwent kidney transplantation at the CharitéUniversitätsmedizinBerlin between 1996 and 2011 were included in this retrospective study. Patients were followedupfor up to 120 months.
Results: At 12 months posttransplantation225 patients had a serum uric acid (SUA) >7 mg/dl: 52 patients were treated with allopurinol, 37 with benzbromarone and 136patients received no medication for hyperuricemia (control). At 12 months eGFR did not differ between groups ($p = 0.15$) but treated patients had a higher SUA ($p < 0.001$)compared to the control group. SUA lowering treatment was associated with a lower risk of allcausemortality (HR (95% CI) = 0.47 (0.26 0.85), $p = 0.013$) and graft loss (HR (95% CI) = 0.43 (0.22 0.84),$p = 0.014$) compared to control. At 120 months patients of the treatment group had lower SUA ($p = 0.001$) and higher eGFR ($p < 0.001$) compared tothe control group.
Conclusions: Treatment of asymptomatic hyperuricemia was associated with a substantial benefit in patient and graftsurvival.





O230 THE INTRARENAL RESISTIVE INDEX MEASURED AFTER TRANSPLANTATION IS DETERMINED BY THE UPSTREAM VASCULAR SYSTEM OF THE RECIPIENT

Line Heylen, Kathleen Claes, Ben Sprangers, Sam Heye, Liesbeth De Wever, Filip Claus, Raymond Oyen, Dirk Kuypers, Maarten Naesens
University Hospitals Leuven

Introduction: Recently, we demonstrated that the renal resistive index (RI), measured after renal transplantation, reflects recipient characteristics, like recipient age and central hemodynamics, and not so much allograft function nor histology. In this study, we provide further evidence.
Methods: Aortic calcifications were measured by X-ray in 262 recipients at the time of admission for renal transplantation. The severity of the calcific deposits at each lumbar vertebral segment was scored and the scores from both the anterior and posterior walls were summed. Patients were categorized according to the score as calcifications absent (score of 0) or present (score of 1 or higher). The RI was measured at baseline ($n = 195$) and at 3 ($n = 212$), 12 ($n = 177$) and 24 months ($n = 145$) after transplantation. Intima media tickness (IMT) ($n = 74$) and pulse wave velocity (PWV) ($n = 119$) were measured at the time of admission for transplant in a subgroup of recipients.
Results: There was a highly significant correlation between the severity of aortic calcifications of the recipient at transplantation and the RI measurements after transplantation (Spearman correlation coefficient = 0.32 at baseline; 0.46 at 3 Mo; 0.53 at 12 Mo; 0.47 at 24 Mo; $p < 0.0001$ at all time points). The RI was higher in the recipients with aortic calcifications compared to those who did not have aortic calcifications (at baseline: 0.75 ± 0.09 vs. 0.70 ± 0.09; at 3 Mo: 0.76 ± 0.07 vs. 0.70 ± 0.06; at 12 Mo: 0.75 ± 0.06 vs. 0.69 ± 0.06; at 24 Mo: 0.76 ± 0.07 vs. 0.69 ± 0.06; $p < 0.0001$ at all time points). IMT and PWV correlated with the RI as well (for IMT: Spearman correlation coefficient = 0.39 at baseline; 0.37 at 3 Mo; 0.49 at 12 Mo; 0.50 at 24 Mo; $p \leq 0.001$ at all time points; for PWV: Pearson correlation coefficient = 0.33 at baseline; 0.32 at 3 Mo; 0.29 at 12 Mo; 0.36 at 24 Mo; $p < 0.01$ at all time points).
Conclusions: The RI measured regularly after renal transplantation depends on characteristics of the upstream vasculature of the recipient.

O231 RENAL TRANSPLANTATION AT THE AGE OF 70: DOES IT IMPROVES OR FURTHER DETERIORATES CARDIOVASCULAR RISK OF THE RECIPIENT

Zelda Ncube[1], Qurat Hussain[1], Madiha Abbas[2], Abbas Ghazanfar[1]
[1]St Georges University Hospitals NHS Foundation Trust; [2]Croydon University Hospital NHS

Background: Renal Transplant (RTx) is associated with increase in life expectancy and improved quality of life. However its benefit at the age of 70 yrs and above is still under controversies. In this present study we have used QRISK cardiovascular disease risk algorithm (QRISK2 and QRISK lifetime) to estimate the cardiovascular disease (CVD) risk status of recipients above 70 years.
Material and Methods: In this retrospective analysis of RTx data of our unit between 2008 and 2012 we identified 37 recipients who received their RTx at or after 70 years of age [Cohort A]. We calculated their QRISK before RTx and then 1, 6 and 12 months after renal transplant and compared it with our patients who were <70 years of age [Cohort B]. We used SPSS 21 for statistical analysis. p value of <0.05 was considered significant.
Results: During that period of 5 years there were 489 RTx performed out of which 37 recipients (7.5%) were above 70 years. There was no significant difference in preoperative CVD risk between two recipients of two cohorts [$p = 0.6822$]. Likewise at 1, 6 and 12 months there was no significant difference of CVD risk between two cohorts [$p = 0.5624$, 0.4822, 0.5214 respectively]. In recipient's 70 years and above there was significant reduction in CVD risk noted at 6 and 12 months post RTx [$p = 0.0442$, 0.03682].

QRISK2 /QRISK-life time	Cohort A >70 ($n = 37$)	Cohort B <70 ($n = 452$)	Significance
Pre RTx (Mean)	18.5	16	0.6822
Post RTx 1mo (Mean)	19.5	16	0.3624
Post RTx 6mo (Mean)	12	14	0.5824
Post RTx 12mo (Mean)	9.5	11.5	0.5214

Conclusion: Age at 70 years is not associated with increased CVD risk following RTx. In contrary RTx significantly improves CVD risk at this age and hence improves long term morbidity and mortality.

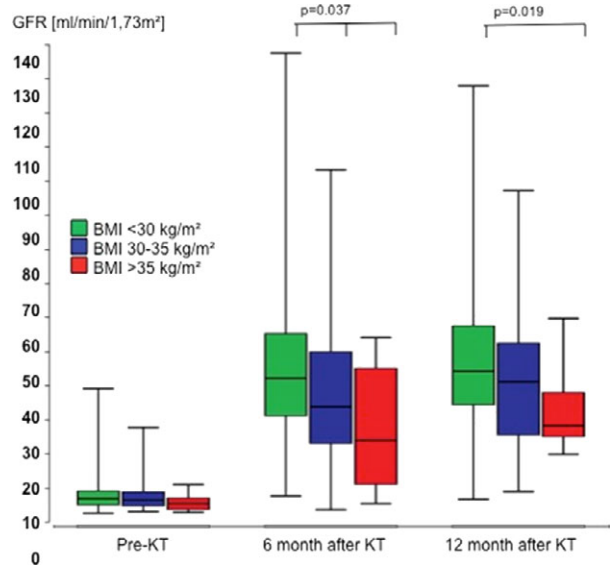
O232 INFLUENCE OF INCREASED RECIPIENT BODY MASS INDEX ON OUTCOME AFTER KIDNEY TRANSPLANTATION

Juliane Liese[1], Nils Bottner[1], Andreas Schnitzbauer[1], Guido Woeste[1], Ingeborg Hauser[2], Wolf O. Bechstein[1], Frank Ulrich[1]
[1]General and Visceral Surgery, Goethe University of Frankfurt; [2]Department of Nephrology, Goethe University of Frankfurt

The relationship between kidney transplant recipients body mass index (BMI) and outcomes after kidney transplantion (KT) is not fully understood and is discussed controversial. We studied the influence of BMI and clinically relevant outcomes among kidney transplant recipients.

Methods: In a retrospective single center study we included all patients who underwent kidney transplantation in our institution between 01/2007 and 12/2012. Beside demographic data and BMI, we analyzed the clinical course, rejection rates, delayed graft function, other adverse events as well as the new onset of diabetes mellitus and hypertonus after transplantation.
Results: During the study period we performed 386 KT (130 women, 256 men). Twenty-two patients were transplantated in the context of the ET Senior Kidney Transplant Program. We performed 23% living kidney donations. The median BMI was 25.9 kg/m^2. 17.4% of the recipients had a BMI >30 kg/m^2 and 3.9% a BMI >35 kg/m^2. BMI >30 kg/m^2 was significantly associated with primary non-function of the kidney ($p = 0.037$) and delayed graft function ($p = 0.018$). The creatinine clearance 12 month after KT was significantly lower in recipients with a BMI >30 kg/m^2. Multivariate analysis revealed recipient BMI, donor age, cold ischemic time and HLA mismatches as independent risk factors for a reduced creatinine clearance and delayed graft function.
Conclusions: Increased BMI at kidney transplantation is a predictor of adverse outcomes, including delayed graft function. These findings demon-

strate the importance of careful selection of patients and pre-transplant weight reduction, although the role of weight reduction for improving graft function is not clear yet and prospective studies ahave to follow.



025 LIVER

O233 TREATMENT WITH PROSTAGLANDIN E1 REDUCES RISK OF EARLY HCC RECURRENCE FOLLOWING LIVER TRANSPLANTATION

Kornberg Arno[1], Ulrike Witt[1], Jennifer Kornberg[2], Helmut Friess[1], Katharina Thrum[3]
[1]TU Munich, Department of Surgery; [2]LMU Munich, Department of Anesthesiology; [3]Helios Klinikum Berlin, Institut of Pathology

Background: There is evidence that ischemia reperfusion (I/R) injury promotes tumor outgrowth in liver transplant patients. Alprostadil, a prostaglandin E1 analog, was shown to attenuate I/R in liver surgery. The aim of this study was to analyze the tumor-specific impact of treating I/R with alprostadil in liver transplant patients with hepatocellular carcinoma (HCC).
Material/Methods: 106 liver transplant patients with HCC were retrospectively analyzed. Fifty-nine patients received systemic treatment with alprostadil (0.5 μg/kg/h) for up to 7 days following liver transplantation (LT), while 47 patients did not. The impact of PGE1-treatment on overall and recurrence-free survival was analyzed in uni- and multivariate analysis. Results

Three- and 5-year recurrence-free survival rates were significantly better in patients following alprostadil-therapy (87.9%; 85.7%) compared to those without PGE1-treatment (65.3%; 63.1%; $p = 0.003$). In multivariate logistic regression, absence of poor tumor differentiation ($p = 0.008$), absence of microvascular invasion ($p = 0.01$), AFP-level ≤400 IU/ml ($p = 0.034$) and treatment with alprostadil ($p = 0.042$) were independent and significant factors for preventing early HCC relapse. Treatment with PGE1 did not impact outcome in patients with Milan In tumors. In contrast, 3- and 5-year disease-free survival rates were significantly better in Milan Out patients following PGE1-therapy (84%; 78%) compared to those without alprostadil-treatment (31.3%; 25%; $p < 0.001$), respectively. In Milan Out patients, only absence of poor tumor differentiation (HR 6.5; $p = 0.001$) and treatment with PGE1 (HR 5.1; $p = 0.005$) were identified as independent predictors of recurrence-free survival.
Conclusion: Treating hepatic I/R injury with alprostadil reduces the risk of early HCC recurrence following LT. Particularly patients with HCC exceeding the Milan criteria seem to benefit from PGE1-treatment.

O234 TOTAL TUMOUR VOLUME AND ALPHA FETOPROTEIN FOR SELECTION OF TRANSPLANT CANDIDATES WITH HEPATOCELLULAR CARCINOMA: A PROSPECTIVE VALIDATION

Christian Toso[1], Glenda Meeberg[2], Roberto Hernandez-Alejandro[3], Jean-François Dufour[4], Paul Marotta[5], Pietro Majno[6], Norman Kneteman[2]
[1]Geneva University Hospitals; [2]University of Alberta; [3]London Health Sciences Centre; [4]University of Bern; [5]University of Western Ontario; [6]University of Geneva

Background: The selection of liver transplant candidates with hepatocellular carcinoma (HCC) is currently validated based on Milan criteria. The use of extended criteria has remained a matter of debate, mainly because of the absence of prospective validation.
Methods: The present prospective study recruited patients according to the previously proposed Total Tumor Volume (TTV ≤115 cm^3)/alpha fetoprotein (AFP ≤400 ng/ml) score. Patients with AFP >400 ng/ml were excluded, and as such the Milan group was modified to include only patients with AFP <400 ng/ml; these patients were compared to patients beyond Milan, but within TTV/AFP.
Results: From January 2007 to March 2013, 233 patients with HCC were listed for liver transplantation. Of them, 195 patients were within Milan, and 38 beyond Milan but within TTV/AFP. The average follow-up from listing was 33.9 ± 24.9 months. The risk of drop-out was higher for patients beyond Milan but within TTV/AFP (16/38, 42.1%), than for patients within Milan (49/195, 25.1%, $p = 0.033$). In parallel, intent-to-treat survival from listing was lower in the patients beyond Milan (53.8% vs. 71.6% at four years, $p < 0.001$). After a median waiting time of 8 months, 166 patients were transplanted, 134 patients within Milan criteria, and 32 beyond Milan but within TTV/AFP. They demonstrated acceptable and similar recurrence rates (4.5% vs. 9.4%, $p = 0.138$) and post-transplant survivals (78.7% vs. 74.6% at four years, $p = 0.932$).
Conclusion: Based on the present prospective study, HCC liver transplant candidate selection could be expanded to the TTV (≤115 cm^3)/AFP (≤400 ng/ml) criteria in centers with at least 8-month waiting time. An increased risk of drop-out on the waiting list can be expected but with equivalent and satisfactory post-transplant survival.

O236 CONDITIONAL COMPETING RISK OF TUMOR RECURRENCE AFTER LIVER TRANSPLANTATION OF PATIENTS WITH HEPATOCELLULAR CARCINOMA

Alessandro Vitale, Valeria Valli, Mohamed Moustafa, Elia Buroni, Francesco D'amico, Enrico Gringeri, Domenico Bassi, Daniele Neri, Giacomo Zanus, Patrizia Burra, Claudia Mescoli
University of Padua

Background: The risk of recurrence (RR) following liver transplantation (LT) of hepatocellular carcinoma (HCC) is typically reported censoring non-cancer related deaths and as actuarial rates from the date of surgery. The aim of this study is double: to assess the competing risk RR due to established HCC variables and to characterize conditional RR following LT of HCC patients.
Methods: A retrospective cohort study of 268 patients who underwent LT for HCC between January 1, 2000, and December 31, 2013, at one Italian Institution using liberal selection criteria (68% of patients beyond Milan criteria at explant pathology) was performed. A multivariate competing risk analysis of HCC recurrence of the study group was performed to identify indipendent predictors of RR among main pathological variables. Conditional 3-year RR (CRR3) estimates were finally calculated.
Results: Multivariate competing risk analysis identified microvascular invasion (hazard ratio = 3.66, 95% CI = 1.83–7.32, $p = 0.0002$) and alphafetoprotein >100 ng/ml (hazard ratio = 2.81, 95% CI = 1.41–5.63, $p = 0.0035$) as main independent RR predictors after LT. While actuarial RR increased over time from 11% at 3 years to 19% at 8 years, the CRR3 decreased over time among those patients who were recurrence-free. The CRR3 at 5 years - the probability to suffer HCC recurrence to postoperative year 8 after having already survived without recurrence to postoperative year 5 - was only 2% compared with 8-year RR of 19% ($p = 0.002$). Patients with the highest initial recurrence risk (presence of microvascular invasion or alphafetoprotein >100) demonstrated the greatest decrease in CRR as time elapsed.
Conclusions: Condtional RR estimates may provide critical quantitative information about the changing risk of recurrence over time among patients undergoing LT for HCC and therefore can be of significant value to patients and providers.

O237 POST-LIVER TRANSPLANTATION HEPATOCELLULAR CARCINOMA RECURRENCE WITH EVEROLIMUS TREATMENT: RESULTS FROM THE H2304 STUDY

L. Fischer[1], F. Saliba[1], P. De Simone[1], G. Dong[2], A. Speziale[3], P. Lopez[3], G. Junge[3], J. Fung[1]
[1]H2304 Study Group, Hamburg; [2]Novartis Pharmaceuticals Corporation, East Hanover, NJ; [3]Novartis Pharma AG, Basel

Background: Choice of immunosuppression is critical in hepatocellular carcinoma (HCC) recurrence in patients transplanted due to HCC. Several studies suggest a protective role of everolimus (EVR), an approved antineoplastic agent, on recurrence of HCC after liver transplantation (LTx). Here, we

Table 1: a) Baseline demographics, HCC characteristics and b) Outcomes

Parameters	EVR+rTAC N=67	TAC-WD N=69	TAC-C N=67
a) Baseline demographics and HCC characteristics			
Age (years), mean	57.6	58.1	58.5
Male, n (%)	53 (79.1)	50 (72.5)	58 (86.6)
Weight (kg), mean	77.4	77.4	73.9
HCV pos, n (%)	30 (44.8)	33 (47.8)	33 (49.3)
Prior treatment, n/N	33/67	41/69	27/67
Within Milan, n (%)	60 (89.6)	65 (87.0)	56 (83.6)
Nº lesions, mean (max.[a])	1.6 (5)	1.7 (10)	1.6 (5)
Largest lesion (cm), mean (max.[b])	2.7 (7.0)	2.3 (6.0)	2.7 (6.0)
TDD (cm), mean (max.[c])	3.9 (16.7)	3.1 (12.6)	3.9 (22.0)
AFP pos, n (%)	46 (68.7)	49 (71.0)	50 (74.6)
AFP (ng/mL), mean (max.[d])	100.6 (1382)	52.0 (372)	32.1 (261)
b) Outcomes			
Efficacy outcomes at M24, %			
BPAR	7.6	22.6	12.6
Graft loss	4.7	3.0	3.3
Death	6.3	6.4	4.9
Renal function (mL/min)			
ΔeGFR (RDN to M12)	1.7	2.9	-8.1
ΔeGFR (RDN to M24)	-4.9	0.4	-11.0
Cumulative HCC recurrence, %			
M12	0.7		1.5
M24	2.9		10.4
M36	3.7		11.9

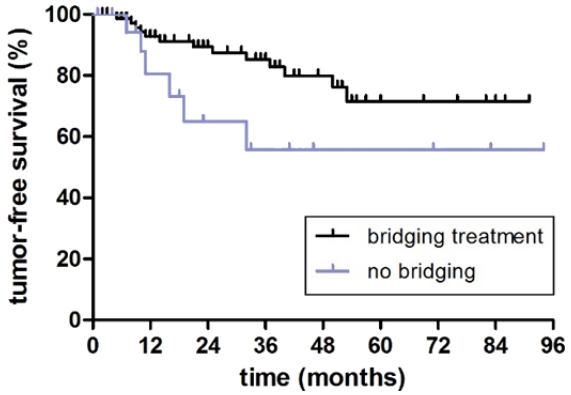
[a]max number of lesions defines whether in-/outside of Milan Criteria; [b]max diameter of largest tumor nodule defines whether in-/outside of Milan Criteria; [c]max total tumor diameter defines recurrences risk (e.g., up to 7 cm, or up to 11 cm are published cut-offs); [d]max AFP level defines recurrences risk (e.g., >500, >1000 ng/mL cut-offs)
AFP, alpha fetoprotein; BPAR, biopsy-proven acute rejection; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; M, month; RDN, randomisation; TTD, total tumor diameter

present HCC recurrence data from the H2304 (NCT00622869) study, up to 36 months (M) post-LTx in a subset of HCC patients.
Methods: Data were retrieved from the core study (24 month) and its extension (12 month) of *de novo* LTx recipients who received EVR (C0 3–8 ng/ml) plus reduced tacrolimus (rTAC, C0 3–5 ng/ml) or EVR (C0 6–10 ng/ml) with TAC withdrawal (TAC-WD) at M4 or standard TAC (TAC-C, C0 6–10 ng/ml). At the time of Tx, Milan criteria (prior/at Tx), number of tumor lesions, diameter of largest nodule, total tumor diameter (TDD), and alpha fetoprotein (AFP) levels were assessed. Data on patient outcomes and impact of EVR treatment and exposure were obtained at 12, 24, and 36M for patients entering into the extension phase.
Results: Across all groups, baseline demographics and HCC characteristics were comparable (Table 1a). Treatment outcome is presented in Table 1b. Incidences of BPAR (7.6%, 22.6% and 12.6%), graft loss (4.7%, 3.0% and 3.3%), and death (6.3%, 6.4% and 4.9%) were comparable for EVR + rTAC, TAC-WD and TAC-C, respectively. Change in renal function overtime was better with EVR treatments. Overall, HCC recurrence was low in the study (M12: 2, M24: 11, and M36: 13 patients). Incidence of HCC recurrence was lower in patients receiving EVR (EVR + rTAC and TAC-WD) vs TAC-C (2.9 vs 10.4%) at M24, and 3.7 vs 11.9% at M36.
Conclusion: HCC patients treated with EVR + rTAC had fewer BPARs and better renal function than TAC-C; and were similar to the overall population. The lower incidence of HCC recurrence in the EVR-treated patients needs further investigation. EVR + rTAC may offer an alternative immunosuppressive treatment in patients transplanted for HCC.

O238 LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA: EFFICACY OF BRIDGING TREATMENT

Sonia Radunz, Julia König, Tamás Benkö, Andreas Paul, Jürgen Treckmann
Department of General, Visceral and Transplantation Surgery, University Hospital Essen

Background: Liver transplantation has emerged as the standard of care for patients with cirrhosis and hepatocellular carcinoma (HCC) meeting Milan criteria and in select patients with HCC beyond Milan criteria. Loco-regional therapies are applied as bridging treatment in patients on transplant wait lists. The purpose of this study was to correlate bridging treatment with outcomes after liver transplantation for hepatocellular carcinoma and to evaluate associations between pathologic findings and patient outcomes.
Study Design: During an 8-year period, 121 patients with hepatocellular carcinoma underwent liver transplantation at our center. One hundred sixteen patients received deceased donor livers and five patients received live donor grafts. Demographics, type of bridging treatment, AFP, pathologic response, survival, and tumor recurrence were analyzed.
Results: Patients had a mean age 57 ± 8 years. HCC in 63 patients met the Milan criteria at diagnosis and at transplantation. Median lab MELD score was 10 [6–36]. Patients underwent one or more of the following bridging treatments: radiofrequency ablation (n = 22), transarterial chemoembolization (n = 60), radioembolization (n = 27), percutaneous ethanol injection (n = 3), sorafenib (n = 6). Twenty-one patients did not receive any bridging treatment. AFP before (18.2 [2.0–538184.0] IU/mL) and after (11.3 [1.5–48285.9]) bridging treatment did not differ significantly. Patients without any bridging treatment demonstrated statistically significantly reduced tumor-free survival (p 0.0367). Patients without necrosis on explant specimen and patients with AFP levels >200 IU/ml developed statistically significantly more frequentlyHCC (p 0.0180; p <0.0099; respectively).
Conclusions: Particularly in regions with long wait list times, e.g. EUROTRANSPLANT region, bridging treatment is effective in achieving tumor necrosis in potential liver transplantation candidates with HCC and may well improve survival.



O239 IMPACT OF LOCOREGIONAL THERAPIES ON SURVIVAL IN HCC PATIENTS LISTED FOR LIVER TRANSPLANTATION

Georg Györi[1], Moritz Felsenreich[1], Gerd Silberhumer[1], Thomas Soliman[1], Wolfgang Sieghart[2], Gabriela A. Berlakovich[1]
[1]Medical University of Vienna, Department of Transplantation; [2]Medical University of Vienna, Department of Hepatology

Background: Even in absence of prospective randomized trials, there is consensus, that patients with HCC listed for oLT should undergo locoregional treatment (LRT) while on the waiting list. Aim of this study was to assess and compare the effect of different LRTs on waiting list and post-transplant survival.
Methods: We performed a retrospective analysis of 150 consecutive patients listed for oLT between 2004 and 2011. Baseline data, modality of LRT (TACE, RFA, PEI) and tumor related outcomes according to mRECIST were analyzed in relation to intention to treat and overall survival after liver transplantation.
Results: Of 146 patients included in this study, 92 (63%) patients were transplanted, 30 (21%) patients were removed due to tumor progression, 13 (9%) patients died while on the waiting list and all others (7%) are still listed. 74 (51%) patients had viral hepatitis, 43 (29%) had alcoholic cirrhosis and 30 (20%) patients had other etiologies for cirrhosis. 81 patients (55%) received a TACE, 39 patients (27%) a RFA/PEI based regimen and 26 patients (18%) had no treatment. Transplant rates were equal in TACE and RFA based regimens. Number of lesions and size were not different between groups. Overall 1y, 3y and 5y intention to treat survival from listing was 80%, 59% and 50% respectively. Overall-survival from listing were comparable for downstaged and non-downstaged patients (p = 0.67).
Conclusion: Patients with HCC should undergo locoregional therapy for HCC during waiting time. We showed equal transplant rates for patients who underwent bridging and downstaging. Using mRECIST 27.2% pts. had complete response, 31.5% partial response, 28.3% stable disease and 13.0% progressive disease during LRT. Further, direct comparison of oLT rates between the different LRT strategies found no difference for dropout and post-oLT survival. Multimodality treatment might identify a cohort with poorer survival as this fact could be indicative for a more aggressive tumor biology.

O241 EFFECTIVE LOCO-REGIONAL TREATMENT BEFORE LIVER TRANSPLANTATION IS SIGNIFICANTLY RELATED WITH HIGHER INTENTION TO TREAT SURVIVAL

Giacomo Concone, Stefano Di Sandro, Andrea Lauterio, Alessandro Giacomoni, Vincenzo Buscemi, Fabio Ferla, Abdelwahab Ali Mahmoud, Riccardo De Carlis, Iacopo Mangoni, Luciano De Carlis
Ospedale Niguarda Ca' Granda

Background: To date the selection of the best candidates for Liver Transplantation (LT) due to Hepatocellular Carcinoma (HCC) has been mainly based on tumor morphological characteristics (nodule diameter and number), which have resulted to be independent risk-factors for short long-term survival and a high rate of tumor recurrence.
Methods: The study cohort included 400 patients transplanted because of HCC on liver cirrhosis since January 2000. Among these, 296 patients received previous locoregional treatment (LRT) for HCC. We defined effective procedure evaluating the absence of tumor residual at last CT/MRI before LT. One hundred sixty nine patients had effective procedure, whereas 98 not. Furthermore, patients were classified according to response to LRT before LT: progressive Group-A; complete Group-B; partial Group-C; stable Group-D.
Results: Effective procedures were associated with significantly better 5 year post-LT survival: 92% vs 76% (p-value: 0.003). The 3- and 5-year overall survival rates were 65.5% and 48.9% for group-A vs 84.8% and 74.6% for Group-BCD (p = 0.01). The 3- and 5-year disease-free survival rates were 74% and 74% for Group-A and 95.7% and 93% for groups BCD (p = 0.007). HCC progression was the only independent risk factor according to Cox-regression p = 0.014 - OR 4.4 (1.35–14.3).
Conclusion: Following aggressive HCC treatment before LT, imaging progression while on the waiting list was a strong predictor of high HCC recurrence rate also in patients who met the Milan criteria. Lack of imaging progression can contribute towards the selection of good transplant candidates for HCC together with the Milan criteria. Effective procedure protect patients to develop tumor progression before LT and recurrence after LT.

O242 SOLUBLE CYTOKINE PROFILES AND COMPLICATIONS AFTER LIVER TRANSPLANTATION

Denis Efimov, Aliaksei Shcherba, Ala Karytka, Sergey Korotkov, Alexander Dzyadzko, Olga Lebedz, Oleg Rummo

RSP center for organ and tissue transplantation

Background: It has been shown that several cytokines can be considered as a surrogate markers of adverse outcomes after liver transplantation (LT) [O. Millan et al. Clin Immunol, 2010; Zhou TB et al. Transpl Immunol, 2011] The aim of the study was to evaluate the association between different cytokines levels and post liver transplant complications.
Materials/Methods: A prospective case-controlled study was conducted. 52 consecutive adult full-size DBD LT were enrolled. Study period: January 2013 – January 2014. Interleukin (IL)-2, 6, 8, 17, 23, TNF-a, MIP-1a, VEGF were measured in 3 blood samples by multiplex immunoassay (Luminex 200): in hepatic veins 1 h after reperfusion, in peripheral blood 24 and 72 h after reperfusion consequently. Liver biopsy was performed at the back-table for CD68 staining. Investigated outcomes: early allograft dysfunction (EAD), biopsy-proven acute rejection (BPAR), biliary and infectious complications and graft survival were assessed according to standard criteria.
Results: Surprisingly none of measured cytokines were associated with EAD and BPAR. The correlation between donor ICU stay and IL-6 in the 1st sample ($r = 0.4$, $p = 0.03$), donor liver CD68 expression and IL-23 in the 1st sample ($r = 0.62$, $p = 0.03$), donor liver ballooning degeneration and IL-2 in the 1st sample ($r = 0.77$, $p = 0.02$), hospital stay and IL-6 in the 3rd sample ($r = 0.61$, $p = 0.001$), hospital stay and VEGF in the 3rd sample ($r = -0.71$, $p = 0.0004$) were revealed. There were statistically significant difference in IL-6 and VEGF in the 3rd sample in groups with and without biliary complications (34 (28;116) vs. 13 (3;16), $p = 0.018$ and 50 (40;51) vs. 102 (84;259), $p = 0.002$; pg/ml). The significant difference in IL-6 in the 3rd sample in cases with and without wound infection (219 (85;2182) vs. 15 (3;30), $p = 0.006$; pg/ml) was revealed.
Conclusion: Our study supports the concept of liver damage in donor as well as the role of angiogenesis in biliary complications and regeneration.

O243 REDUCED RENAL FUNCTION INCREASES RISK OF CARDIOVASCULAR EVENTS IN LIVER TRANSPLANT RECIPIENTS AT 2-YEARS POST-LIVER TRANSPLANT

C. Escrig[1], G. Bader[1], G. Dong[2], P. Lopez[1], P. Bernhardt[1], G. Hustache[1]

[1]Novartis Pharma AG; [2]Novartis Pharmaceuticals Corporation

Purpose: Liver transplant recipients (LTxR) are at high risk of cardiovascular disease (CVD). Impaired glomerular filtration rate (GFR) is one of the factors involved in increasing the risk of CVD. In the H2304 study, use of everolimus (EVR) with reduced tacrolimus (rTAC) provided adequate immunosuppression and better renal function at 12 and 24 months (M) vs standard TAC (TAC-C). In this post-hoc analysis, we have evaluated if renal function at LTx is associated with an increased risk of CVD events after 24M follow up.
Methods: H2304 is a multicentre, open-label study, in which 719 *de novo* LTxR were randomised (1:1:1), on day 30, to receive EVR + rTAC ($n = 245$) or EVR with TAC withdrawal (TAC-WD, $n = 231$) at M4 or TAC-C ($n = 243$). Adverse events (AEs) related to cardiovascular events (SMQ, major adverse cardiac events [MACE]) were used to determine the cardiovascular risk of this population.
Results: At M24, overall incidence of cardiovascular events was 4.47%. (2.18%, 4.08% and 7.02% for TAC-WD, EVR + rTAC and TAC-C arms, respectively). At M24, occurrence of MACE was significantly associated with eGFR at baseline ($p = 0.0001$). Using spline function, an eGFR <38 ml/min was associated with a higher risk of MACE [HR (95%CI): 1.05 (1.00–1.11), ($p = 0.046$)]. Overall rate of MACE at M24 was 8.57% and 4.26% when eGFR <38 ml/min and eGFR ≥38 ml/min, respectively.
Conclusions: This data suggests that LTx recipients are at risk of developing CVD as early as 2-years and that impaired renal function at transplantation further increases their risk. Immunosuppressive regimens aiming to provide improvements in renal function in LTx recipients might help to reduce CV risk and improve long-term outcomes.

O244 5-YEAR FOLLOW-UP OF THE PROTECT RANDOMISED LIVER TRANSPLANTATION STUDY SHOWED SUPERIOR RENAL FUNCTION WITH EVEROLIMUS AND EARLY CALCINEURIN INHIBITOR WITHDRAWAL

Martina Sterneck, Gernot Kaiser, Nils Heyne, Nicolas Richter, Falk Rauchfuss, Andreas Pascher, Peter Schemmer, Lutz Fischer, Christian-Georg Klein, Silvio Nadalin, Frank Lehner, Utz Settmacher, Daniel Gotthardt, Martin Loss, Peter Wimmer, Hans Schlitt

PROTECT study group

Purpose: At the end of the 12-month (M) PROTECT core study (NCT00378014), *de novo* liver transplant recipients (LTxR) who switched from a calcineurin inhibitor (CNI) based immunosuppression to a CNI free everolimus (EVR) based regimen showed numerically better renal function. The renal function benefit with EVR was maintained up to 3 years post LTx. Here we present the 5-year follow-up data from the PROTECT study.
Methods: PROTECT was an open-label, parallel-group, randomised-controlled study in which LTxR received basiliximab and CNI-based immunosuppression with/without corticosteroids. Between Week 4 and 8, patients were randomised 1:1 to receive EVR or continue CNI. In the EVR group, CNI was completely withdrawn after 8 weeks. Patients who completed the core study were asked to enter the extension study and continue their randomised treatment. Key endpoints included change in renal function measured by estimated glomerular filtration rate (eGFR) by Cockcroft-Gault (CG), efficacy failure (composite of biopsy-proven acute rejection [BPAR], graft loss, death, or loss to follow-up), and incidence of adverse events (AEs) and serious AEs (SAEs).
Results: A total of 81 patients entered the extension study (41, EVR group; 40, CNI group). At M 59 post randomisation, the adjusted mean eGFR was significantly higher in the EVR group compared to the CNI group, with a benefit of 12.4 ml/min using CG [95%CI: 1.2; 23.6; $p = 0.0301$]. During the extension period, 3 deaths occurred (EVR, 1; CNI, 2). There were no cases of graft loss. Two BPAR occurred in the EVR group. SAEs occurred in 26 (63.4%) and 28 (70.0%) of the patients in the EVR and CNI group, respectively. Commonly reported AEs were incisional hernia, nasopharyngitis, peripheral edema, diarrhea, and back pain.
Conclusion: In comparison to CNI based immunosuppressive treatment EVR-based CNI free immunosuppression resulted in better renal function and comparable patient and graft outcomes after 5 years follow-up.

Table: Renal endpoints at Month 59 post randomisation (all extension study population)

	EVR (n=41)	CNI (n=40)	Difference [95% CI]	P-value
Randomisation/Baseline				
eGFR, Cockcroft-Gault (mL/min)	81.8 (23.6)	81.2 (24.1)	-	0.909*
eGFR, MDRD4 (mL/min/1.73 m²)	79.0 (23.5)	77.8 (24.4)	-	0.814*
eGFR, Nankivell (mL/min/1.73 m²)	92.0 (20.5)	89.2 (20.1)	-	0.535*
Month 59 post randomisation				
eGFR, Cockcroft-Gault (mL/min)				
Unadjusted	86.8 (30.2)	74.5 (25.5)	-	0.052*
Adjusted	87.5 [78.0; 97.0]	75.1 [64.6; 85.5]	12.4 [1.2; 23.6]	0.0301#
Adjusted change from randomisation	6.0 [-3.5; 15.5]	-6.4 [-16.9; 4.1]	-	-
eGFR, MDRD4 (mL/min/1.73 m²)				
Unadjusted	77.0 (26.0)	65.3 (21.1)	-	0.029*
Adjusted	79.1 [70.1; 87.2]	67.6 [58.6; 76.6]	11.4 [1.8; 21.1]	0.0214#
Adjusted change from randomisation	0.7 [-7.5; 8.9]	-10.8 [-19.8; -1.8]	-	-
eGFR, Nankivell (mL/min/1.73 m²)				
Unadjusted	92.5 (18.7)	80.3 (20.7)	-	0.007*
Adjusted	94.3 [87.0; 101.6]	83.1 [75.1; 91.0]	11.2 [2.7; 19.7]	0.0107#
Adjusted change from randomisation	3.7 [-3.6; 10.9]	-7.5 [-15.5; 0.4]	-	-

*t-test (between group comparison).
#ANCOVA.
Unadjusted values are shown as mean (SD). Adjusted values shown as least square mean values and 95% CI are obtained from ANCOVA analysis with treatment and centers as factors and eGFR at randomisation as covariate.
ANCOVA, analysis of covariance; CI, confidence interval; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; MDRD4, modification of diet in renal disease (4-variable).

O245 SHOULD WE USE DCD GRAFTS IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS?

Irene Scalera, Thamara Perera, Hynek Mergental, Garret Roll, Darius Mirza, John Isaac, James Ferguson, Paolo Muiesan

Queen Elizabeth Hospital

Patients with primary sclerosing cholangitis (PSC) awaiting liver transplantation (LT) may benefit from liver grafts from donation after circulatory death (DCD). PSC LT candidates are generally younger and fitter, compared with non-PSC recipients, to withstand the reperfusion syndrome typical of DCD grafts. A worse graft survival has been reported using DCD grafts for LT of PSC patients (PSCdcd) compared to non-PSC recipients (nonPSCdcd). The aim of this study was to analyse PSC recurrence, vascular and biliary complications after LT for PSC using grafts from DCD donors. All consecutive LT performed for PSC between 2006 and June 2014 were included in the study. PSCdcd were compared to nonPSCdcd and PSC recipients of DBD grafts (PSCdbd). Donor and recipient characteristics were collected from a prospectively held LT database. Outcomes analysed included vascular/biliary complications, disease recurrence and patient/graft survival. Twenty PSCdcd were included and compared with 205 nonPSCdcd and 69 PSCdbd. Donor and recipient characteristics were similar in all three groups, a part for lower Donor Risk Index and longer CIT in DBD grafts. Patient survival was comparable in the 3 groups at 1–3–5 years: in PSCdcd it was 95%, 95% and 79%, in nonPSCdcd 89%, 82% and 73% and in PSCdbd 91%, 85% and 85%. Similarly graft survival at 1–3–5 years was 80%, 80% and 67% in PSCdcd, 87%, 80% and 71% in nonPSCdcd and 91%, 83% and 79% in PSCdbd. Among the DCD groups there was a higher rate of hepatic artery thrombosis in PSCdcd (15%vs.6%; $p = 0.03$) and no difference in biliary complications. Among PSC groups, DCD recipients showed a higher incidence of vascular and biliary complications including a greater incidence of ischaemic cholangiopathy compared to PSCdbd. PSC recurrence rates were similar in both PSC groups. Despite the higher rate of vascular and biliary complications, DCD grafts do not seem

to adversely impact mid- and long-term LT outcomes in selected PSC recipients.

O246 ACUTE CELLULAR REJECTION AS RELEVANT RISK FACTOR IN THE DEVELOPMENT OF BILIARY STRICTURES AFTER LIVER TRANSPLANTATION

Federico Mocchegiani[1], Paolo Vincenzi[1], Daniele Nicolini[1], Roberto Montalti[1], Martina Coletta[1], Andrea Risaliti[2], Marco Vivarelli[1]
[1]Universita Politecnica Delle Marche; [2]Universita di Udine

Abstrac:
Background: The purpose of this study was to investigate risk factors associated with Biliary Strictures after Liver Transplantation with particular reference to immunological features as acute and chronic rejection, crossmatch positivity, ABO incompatibility and primary biliary cirrhosis.
Material and Methods: The study included 249 patients, in 339 adult liver transplantation performed from August 2005 and December 2014, with a radiological or surgically proven biliary stricture. We studied about 29 potential risk factors for biliary strictures analyzing donor, preoperative and postoperative recipient variables.
Results: A biliary stricture was diagnosed in 61 patients (24.5%), the anastomotic type and the non anastomotic type complicated the transplantation in 41 (16.5%) and 12 (4.8%) cases respectively. Eight patients (3.2%) presented both forms. Univariate analysis using logistic regression showed that primary biliary cirrhosis ($p = 0.009$), the donor age >46 years old ($p = 0.008$), donor risk index >1.91 ($p = 0.039$), the occurrence of acute cellular rejection ($p = 0.019$) and of biliary leak ($p = 0.002$) were all significantly associated with the development of biliary stricture. At the multivariate analysis, the Primary Biliary Cirrhosis ($p = 0.016$), the donor age >46 years ($p = 0.008$), the acute cellular rejection ($p = 0.026$) and the biliary leak ($p = 0.004$) appeared to be the only variables independently associated with the development of a biliary stricture.
Discussion: In addition to the consolidated risk factors as cholestatic disease, biliary leak and donor age, immunological factors as acute cellular rejection emerged as being important variables associated to the development of biliary strictures after liver transplantation. Other immunological features as chronic rejection, crossmatch positivity and ABO incompatibility didn't find relevance in our sieries.

O247 EFFICACY OF SPLENIC ARTERY EMBOLIZATION (SAE) FOR LATE ONSET REFRACTORY ASCITES DUE TO PORTAL HYPERPERFUSION (PHP) AFTER LIVER TRANSPLANTATION

Riccardo Pravisani[1], Ilaria Rispoli[1], Gian Luigi Adani[1], Dario Lorenzin[1], Vittorio Cherchi[1], Stefania De Martino[1], Stefano Scarsini[1], Alessandro Vit[2], Massimo Sponza[2], Pierluigi Toniutto[3], Andrea Risaliti[1], Umberto Baccarani[1]
[1]Liver Transplant Unit, Department of Medical and Biological Sciences; [2]Division of Interventional Radiology; [3]Department of Internal Medicine

Portal hyperperfusion (PHP) is an hemodynamic condition which may constitute the pathogenic mechanism for refractory ascites and hepatic hydrothorax after liver transplantation (LT). The diagnosis is established by exclusion of other causes of increased sinusoidal pressure/resistance such as cellular rejection or toxicity and outflow obstruction. The pathophysiology underlying PHP is not completely understood but seems to be related to both a direct damaging effect of elevated portal venous pressure and hepatic arterial hypoperfusion with graft ischemia caused by the hepatic artery buffer response (HABR). Therefore even increased hepatic artery resistive index or elevated liver enzymes are reported to be possible clinical feature of PHP in addiction to refractory ascites and hydrotorax. Splenic artery embolization (SAE) has been already demonstrated to be a safe and effective technique to treat the PHP and herein our clinical experience is presented. 23 transplanted patients with a diagnosis of late onset refractory ascites and persistent hydrothorax underwent SAE 110 $\pm$ 61 (range 22–240) days after LT. Preliminary graft biopsy was negative for cellular rejection and cavogram with hepatic venous pressure measurement excluded outflow obstruction (mean trans-anastomosis gradient 0 $\pm$ 2.94 mmHg). The SAE was performed with proximal occlusion of the splenic artery and no cases of splenic abscess, infection or bleeding occurred. Post procedure diameter of the spleen showed a slightly significant reduction (15.25 $\pm$ 1.91 vs 14.15 $\pm$ 2.3 cm, $p = 0.047$) while PV velocity and wedge hepatic venous pressure (WHVP) were significantly reduced by SAE (PV velocity: 57.31 $\pm$ 24.34 vs 39.65 $\pm$ 19.14 cm/second, $p = 0.01$; WHVP: 22.54 $\pm$ 6.93 vs 16.78 $\pm$ 3.27, $p = 0.03$). Clinically, the 33% decrease in PV flow and 26% in WHVP were associated with the resolution of symptoms in all the cases, demonstrating that SAE is a safe and effective technique to treat refractory ascites or persistent hydrothorax due to PHP.

O248 ITALIAN OBSERVATIONAL STUDY ON RENAL FUNCTION (SURF): RENAL FUNCTION DECLINE IS HIGHER IN THE FIRST YEAR AFTER LIVER TRANSPLANTATION

Paolo De Simone[1], Mauro Salizzoni[2], Matteo Cescon[3], Luciano De Carlis[4], Giovan Giuseppe Di Costanzo[5], Alfonso Avolio[6], Alfredo Di Leo[7], Donato Donati[8], Rossella Brusa[9], Stefano Fagiuoli[10]
[1]University of Pisa Medical School Hospital; [2]San Giovanni Battista Hospital; [3]Sant'Orsola University Hospital Bologna; [4]Niguarda Hospital Milan; [5]Cardarelli Hospital Naples; [6]Catholic University Rome; [7]Bari University Hospital; [8]Varese Hospital; [9]Novartis Italy; [10]Papa Giovanni XXIII Hospital Bergamo

SURF was a multicenter, observational study aiming at: 1) exploring the prevalence of chronic kidney disease (CKD) (defined as eGFR <60 ml/min/1.73 m^2) in adult liver transplant (LT) recipients from 6 to 60 months (M) (cross-sectional phase, T0); 2) assessing the velocity (=slope) of renal function (RF) deterioration from LT to enrollment; and 3) prospectively reassessing the RF slope 12 M after inclusion and introduction of recommendations for CKD (longitudinal phase, T12). The SURF recommendations stratified patients in 6 alert categories for renal dysfunction (from very low to very high) based on eGFR (>90; 89–60; 59–30; <30 ml/min/1.73 m^2), proteinuria (0.5 g/day), and slope of eGFR deterioration (−4 ml/min/year).

A total of 1002 patients were enrolled, and 738 (73.6%) were followed up for 12 M. Prevalence of CKD was 15.56% at transplant ($N = 874$), 25.25% at study inclusion ($N = 1002$), and 27.91% after 12M from inclusion ($N = 738$). The mean change in eGFR (standard deviation [SD]) from LT to T0 was -18.1 ± 35.7 ($p < 0.0001$) mL/min/1.73 m^2 and -1.6 ± 16.0 ($p = 0.0061$) from T0 to T12. The median change in eGFR slope (25thp;75thp) was −13.8 (−36.4;3.5) mL/min/1.73 m^2/year in the first year (Y) after LT ($N = 665$); −0.7 (−10.6;7.7) in the second Y ($N = 561$); −0.3 (−9.1;8.9) in the third Y ($N = 366$); −0.3 (−6.7;6.6) in the fourth Y ($N = 253$); −0.2 (−6.5;8.7), in the fifth Y ($N = 129$ patients); −0.3 (−8.2;6.8) in the sixth Y ($N = 34$). Patients with eGFR >90 ml/min/1.73 m^2 were 53% at LT, 29% 6 M after LT and a similar proportion from 1 to 6 Y from LT, when it was 25%.

RF deterioration was detected in LT population, and the decline was higher during the first year from LT. A stabilization was observed afterwards. RF protection with introduction of kidney sparing immunosuppressive strategies is highly needed, focusing on the early period after LT.

O249 REGENERATIVE CAPACITY OF BILIARY EPITHELIUM AND IMMUNE RESPONSE TO BACTERIAL INFILTRATION DETERMIN BILIARY COMPLICATIONS AFTER LIVER TRANSPLANTATION

Henrik H.G. Junger, Stefan M. Brunner, Hans J. Schlitt, Stefan Fichtner-Feigl
University Medical Center Regensburg

Background: This study aimed to determine the onset of bile duct (BD) damage and elucidate the molecular mechanism underlying biliary complications after liver transplantation (LTx).
Methods: BD injury after cold storage was quantified by the BD damage score (BDDS) and correlated with patient outcome. Bacterial infiltration was determined by Fluorescence in situ Hybridisation (FISH) for bacterial antigens. Further, BD samples were analysed by immunohistochemistry for Aggrecan, Cytokeratin, CDH18 and Ki-67, whole genome microarray and gene set enrichment analysis.
Results: Patients with BD damage after cold storage had a significantly increased risk of biliary complications and graft loss ($p < 0.0001$; $p = 0004$). In damaged BDs with biliary complications reduced mRNA levels of cell-adhesion, adherens-junction and focal-adhesion-molecules (FDR q-value 0.049; 0.003; 0.049) were detected compared to damaged BDs without biliary complications reflecting the integrity and regenerative capacity of the BD epithelium. Immunohistochemistry showed reduced expression of Aggrecan, Cytokeratin, CDH-18 and Ki-67 in these groups. FISH analysis demonstrated equal distribution of bacterial infiltration of BDs, however, mRNA analysis detected enrichment of gene programs characterizing immune response to bacterial infection and FC-gamma mediated phagocytosis (FDR q-value 8.4*10–4; 0.046) in BDs without biliary complication.
Conclusions: Histological BD damage detected after cold storage is a prognosticator for biliary complications and graft loss after LTx. Damaged BDs show loss of adhesion molecule integrity. Following BD damage during cold storage, functional regenerative capacity of the biliary epithelium and enhanced immune response to bacterial infiltration are able to rescue BDs and prevent biliary complications after LTx.

O250

POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDER IN ADULT PATIENTS AFTER LIVER TRANSPLANTATION

Ahad Eshraghian, Mohammad Hadi Imanieh, Saman Nikeghbalian, Alireza Shamsaeefar, Kourosh Kazemi, Bita Geramizadeh, Heshmatollah Salahi, Seyed Ali Malek-Hosseini

Organ Transplant Center, Shiraz University of Medical Sciences

Background: Post-transplant lymphoproliferative disorder (PTLD) represents a group of abnormal lymphoid proliferations that may occur after both solid organ and hematopoietic transplantation. Here in, we aimed to investigate survivals of patients with PTLD and associated risks after adult liver transplantation.
Methods and Materials: A cross -sectional study was conducted among adult patients (>18 years) who underwent liver transplantation at Shiraz transplant center, Shiraz, Iran between February 2008 and February 2014. Clinical and laboratory information of patients were collected using a questionnaire containing data regarding age, sex, time of liver transplantation time of PTLD, survival of patients, immunosupprersive regimen, rejection episodes and type of allograft.
Results: There were 1095 adult liver transplants during this time period that PTLD was diagnosed in 12 patients. The incidence of PTLD was 1.09% in our adult liver transplant patients. The underlying liver disease in these patients were: hepatitis B induced liver cirrhosis in 3 patients, Cryptogenic liver cirrhosis in 3 patients, primary sclerosing cholangititis in 2 patients, autoimmune hepatitis in 1 patient, Wilson disease in 1 patient, hepatitis C induced liver cirrhosis in 1 patient and Budd-Chiari syndrome in 1 patient. Nine patients were male and 3 patients were female. The overall post-PTLD survival at 3 months was 82.5% (±SE 11%), at 1 year was 73.3% (±SE 13%) and at 5 years was 58.7% (±SE 16.9%). Four patients developed PTLD in the first 12 months after surgery and 8 patients after this time period. There was no statistically significant difference between tacrolimus level, steroid and tacrolimus dose, age, gender, rejection episodes and Epstein- Barr virus (EBV) infection and PTLD onset in our patients.
Conclusion: PTLD may occur as a post liver transplant complication in adult patients. Long-term survival is still possible with rapid and aggressive treatment.

019 ISCHEMIA/REPERFUSION INJURY/PRESERVATION

O251 THE EFFECT OF ISCHEMIA DURING 12 HOUR EX VIVO LUNG PERFUSION: ANALYSIS OF LUNG PHYSIOLOGY AND METABOLIC ACTIVITY

Simon Becker[1], Jasmin Steinmeyer[1], Murat Avsar[2], Jawad Salman[2], Axel Haverich[2], Gregor Warnecke[2], Matthias Ochs[1], Anke Schnapper[1]
[1]Institute of Functional and Applied Anatomy - Hannover Medical School; [2]Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School

Clinical ex vivo lung perfusion (EVLP) is currently performed using a cellular or acellular perfusate. Before EVLP, potential donor lungs are subjected either to short cold static preservation (CSP) when using the portable Organ Care System™ or a longer CSP period when using a stationary EVLP circuit. This study aimed to compare the impact of CSP duration on lung physiology and metabolism during prolonged EVLP.

Lungs of 15 female pigs (body weight 57.7 ± 9.1 kg) were retrieved following a standard procedure. Whereas 5 lungs underwent short CSP of 65 ± 8 min (sCSP-group), 10 lungs were subjected to an extended CSP time of 24 h (eCSP-group). Subsequently, EVLP was performed for 12 h using a perfusion flow of 70 ml/kg/min in a closed atrium circuit. Lungs from the sCSP-group and 5 lungs from the eCSP-group were perfused on the basis of an acellular and the remaining 5 lungs from the eCSP-group on the basis of a cellular perfusate composition. Hourly, organs were monitored on aerodynamic and functional parameters. From glucose, lactate and potassium concentrations in the perfusate, consumption/production rates and fluxes were calculated, respectively.

Airway pressures, lung compliance and oxygenation indices were within a physiological range and no significant differences were observed between the 3 groups during 12 h of EVLP. Glucose consumption and lactate production were 2-fold higher during the first hour of perfusion in the eCSP-groups and remained significantly higher up to 4 h of EVLP compared to the sCSP-group. An efflux of potassium from the perfusate fluid was only observed during the first hour of perfusion in the eCSP-groups.

In summary, groups only differed with respect to higher metabolic activity during the first few hours of EVLP after an extended CSP time. We hypothesize that the additional glucose utilization was partly due to active potassium transport into the lung in order to recover ionic homeostasis of lung cells after extended CSP.

	eCSP Group		sCSP Group
	acellular EVLP	cellular EVLP	
Glucose utilization, 1st hour [mmol]	4.07±0.37	3.93±0.40	2.14±0.66
Mean glucose utilization, 2nd-12th hour [mmol/h]	1.19±0.36	1.09±0.36	1.11±0.53
Lactate production, 1st hour [mmol]	4.18±0.27	4.18±0.58	2.11±0.40
Mean lactacte production, 2nd-12th hour [mmol/h]	1.75±0.62	1.453±0.60	1.61±0.60
K^+ efflux/influx from perfusate, 1st hour [mmol]	-1.48±0.32	-1.88±0.34	0.74±0.10
K^+ efflux/influx from perfusate, 2nd-12th hour [mmol/h]	0.24±0.17	0.38±0.16	0.34±0.20

O252 NORMOTHERMIC REGIONAL PERFUSION RESUSCITATES DCD LIVERS BY MITOCHONDRIAL PROTECTION

Debabrata Roy[1], Russell Jamieson[2], Karl Morten[3], Alireza Morovat[4], Constan Coussios[5], Peter Friend[2]
[1]John Radcliffe Hospital NHS Trust; [2]Nuffield department of surgical sciences; [3]Nuffield department of obstetrics and Gynaecology; [4]Nuffield department of Biochemistry; [5]Department of Biomedical Engineering

Objective: Donation after circulatory death (DCD) donors are increasingly important in alleviating the donor organ shortage. The provision of oxygen after the inevitable hypoxic period may be important in the recovery of cellular energetics. We have studied the effects of in-situ oxygenated normothermic regional perfusion (NRP) on cellular energy metabolism and mitochondrial function.
Methods: Porcine livers were subjected to 60 min warm ischaemia followed by 1 h of normothermic regional perfusion (Group NP). This was followed by a limited period of cold storage (bench work), followed by 23 h of normothermic perfusion (as a surrogate for transplantation). The control group (Group C) did not receive NRP, but was otherwise treated in the same way. Mitochondria were isolated from sequential liver biopsies and analysed for ATP content and mitochondrial function. Haemodynamic parameters were recorded during normothermic perfusion and the perfusate was analysed for markers of hepatocellular injury.
Results: Cellular ATP levels declined sharply during 60 min of warm ischaemia in both groups. In Group C, mitochondrial function and ATP levels continued to decline during both warm ischaemia and the subsequent cold preservation on the bench. NRP improved mitochondrial function and ATP levels significantly in Group NP livers. This effect was maintained during normothermic perfusion and was associated with greater functional recovery of the group NP livers, with superior bile production, acid-base homeostasis and reduced hepatocellular injury.
Conclusions: These data suggest that mitochondria sustain progressive damage during sequential warm and cold ischaemia followed by reperfusion. Normothermic regional perfusion immediately following warm ischaemia confers mitochondrial resilience to ischaemia-reperfusion injury and may have therapeutic benefits in DCD liver transplantation.

O253 CONTROLLED OXYGENATED REWARMING OR NORMOTHERMIC MACHINE PERFUSION FOR ENDISCHEMIC LIVER RECONDITIONING BEFORE TRANSPLANTATION?

Dieter Paul Hoyer[1], Bastian Luer[2], Patrik Efferz[2], Andreas Paul[1], Thomas Minor[2]
[1]University Hospital Essen; [2]University Hospital Bonn

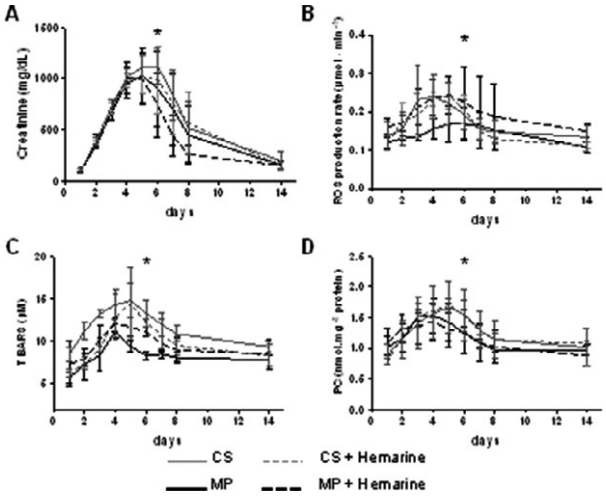
Background: The beneficial effect of different non-hypothermic liver preservation modalities has been shown in various models. This study compares controlled oxygenated rewarming (COR) to Normothermic Machine Perfusion (NMP) to resuscitate liver grafts after preceding cold storage.
Methods: Pig livers were preserved overnight for 18 h by simple cold storage at 4°C. Three hours prior to reperfusion grafts were put on a machine perfusion device (LiverAssist®)with portal (3 mmHg, continuous flow) and arterial (30 mmHg, pulsatile flow) perfusion for reconditioning by either COR or NMP (n = 6, resp.). COR was carried out for 3 h with the new Custodiol-N solution (shown slightly superior to KPS1 in pilot experiments), slowly increasing temperature from 8 to 20°C during the first 90 min. NMP was carried out with diluted autologous blood (Hb = 6 g/dl) at 37°C for 3 h. In both cases, perfusate was oxygenated to pO2 >500 mmHg. All livers were rinsed with 500 ml of cold saline solution and kept at room temperature for 20 min to simulate time of engrafting. Then, liver viability was tested for 180 min during isolated sanguineous reperfusion in vitro.
Results: Activity of the mitochondrial inducer caspase 9, as well as cleaved keratin 19 as marker for actual apoptosis were significantly lower after COR. COR also resulted in significantly lower enzyme leakage and higher bile production ($p < 0.05$) during reperfusion.
Conclusion: The adapted gentle rewarming seems to mitigate mitochondrial induction of apoptosis after cold storage and resulted in superior graft recovery compared to reconditioning by normothermic machine perfusion.

O254 A NEW O2 CARRIER APPLIED TO A DCD PORCINE MODEL FOR KIDNEY PRESERVATION

Massimo Abelli[1], Elena Ticozzelli[1], Alessandra Vezzoli[2], Simona Mrakic-Sposta[3], Carolina Bianco[4], Ilaria Benzoni[4], Federica Meloni[5], Di Tor Vajana Jacopo Ferrario[4], Benedetta Maiga[4], Valérie Polard[6], Emanuela Cova[7], Andrea Bottazzi[8], Eloisa Arbustini[9], Maristella Gussoni[10]

[1]General Surgery Department, IRCCS Policlinico San Matteo Foundation, Pavia; [2]Institute of Bioimaging and Molecular Physiology, National Council of Research (CNR), Segrate (MI); [3]Institute of Bioimaging and Molecular Physiology, National Council of Research (CNR), Segrate (MI); [4]General Surgery Department, University of Pavia; [5]Respiratory Diseases IRCCS Policlinico San Matteo Foundation,Pavia; [6]Hemarina S.A., Morlaix; [7]Respiratory Diseases Department,IRCCS Policlinico San Matteo Foundation, Pavia; [8]Intensive Care Department, Fondazione IRCCS Policlinico San Matteo, Pavia; [9]Center for Inherited Cardiovascular Diseases, IRCCS Policlinico San Matteo Foundation, Pavia; [10]Department of Pathophysiology and Transplantation–Physiology Section, University of Milan, Milan

Background: Kidneys from all donor types respond to Ischemia–Reperfusion (I/R) by pathophysiological processes involving hypoxia and/or re-oxygenation and, by the others, oxidative stress (OxS). The increased production of Reactive Oxygen Species (ROS) leads to oxidation of lipids and proteins and eventually to cellular dysfunction and death.
Methods/Materials: This study aims to examine the effects of hypothermic pulsatile perfusion in RM3 (MP) on I/R injury, compared with static storage (CS) solutions, adopting a porcine kidney model. Kidney was subjected to 70 min of warm ischemia prior to 8 h of MP or CS preservation with or without addition of Hemarine-M101-an O2 carrier with high-O2 affinity- to the perfusion solution. Plasma samples were collected during 14 days after transplantation by assessing: creatinine, biomarkers of OxS (i.e protein carbonyls (PC), thiobarbituric acid (TBARS)) by immune-enzymatic methods and Reactive Oxygen Species (ROS) production by Electron Paramagnetic Resonance [Mrakic-Sposta S. et al. Oxidative Medicine and Cellular Longevity 2014:306179]
Results: The results are shown in the panel. Values are expressed as mean ± SD. Significant differences ($p < 0.05$) resulted between CS and MP with and without Hemarine in Creatinine (A), ROS production (B) TBARS (C) and PC (D). Values in MP were significantly lower compared to CS. In MP with Hemarine, Creatinine and PC increased at day 6 ($p = 0.01$) accordingly, followed by a faster recovery of kidney function.
Conclusions: In all CS groups a severe level of renal dysfunction was observed, reaching higher levels and longer recovery times of plasmatic creatinine, oxidative damage biomarker concentrations and ROS production rate. MP, particularly MP-He, appeared more suitable to substain the I/R with better function recovery and lower oxidative stress activation.

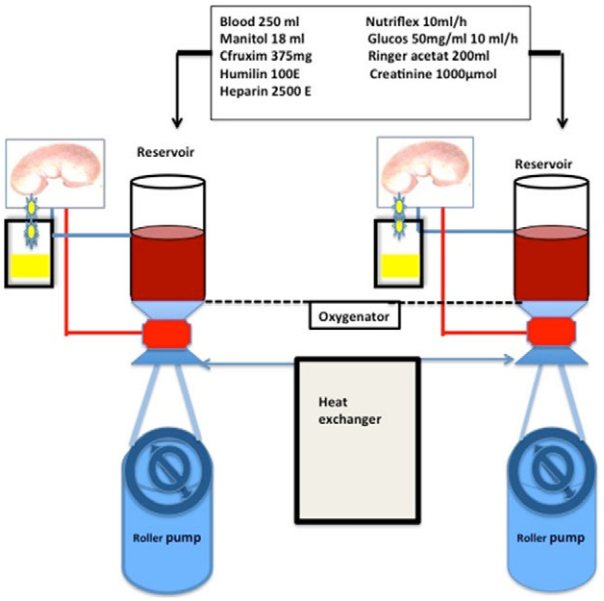


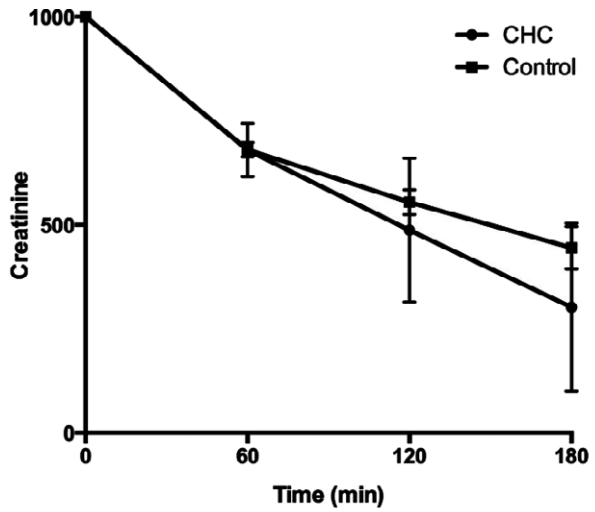
O255 PERFUSION OF PORCINE KIDNEYS WITH MACROMOLECULAR HEPARIN AMELIORATES EARLY ISCHEMIA REPERFUSION INJURY

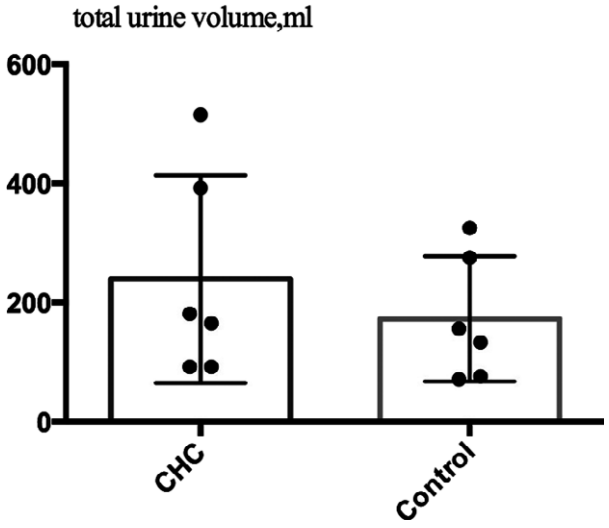
Amir Sedigh[1], Sofia Nordling[2], Rolf Larsson[2], Erik Larsson[2], Bo Norlin[3], Norbert Lübenow[3], Fredrik Lennmyr[3], Gunnar Tufveson[4], Peetra Magnusson[2], Thomas Lorant[1]

[1]Department of Surgical Sciences, Section of Transplantation Surgery, Uppsala University, Uppsala, Sweden; [2]Department of Immunology, Genetics and Pathology, Section of Clinical Immunology, Uppsala University, Uppsala, Sweden; [3]Department of Surgical Sciences, Section of Anaesthesiology and Intensive Care, Uppsala University, Uppsala, Sweden; [4]Department of Surgical Sciences, Section of Transplantation Surgery, Upp-sala University, Uppsala, Sweden.

Background: Ischemia-reperfusion injury (IRI) is the most significant antigen-independent nonspecific insult, inevitably linked to organ transplantation. Endothelial glycocalyx is degraded during the earliest events in I/R. Previously we have been able to demonstrate the feasibility of coating the surface of the renal artery with a heparin conjugate (CHC; Corline Systems AB, Uppsala, Sweden) during hypothermic machine perfusion (HMP), in Lifeport® kidney transporters (Organ Recovery Systems, Chicago, IL). The purpose of this study was to assess protective effects of coating the renal vessel walls, against IRI and early kidney function.
Methods: In pigs ($n = 6$) brain death was achieved by raising the intracranial pressure (ICP) through stepwise increasing the volume of an epidurally placed balloon to the point of exceeding the mean arterial pressure (MAP) creating a negative cerebral perfusion pressure (CPP). Both kidneys, ($n = 6$) in each group, were preserved for 18 h by HMP. In total 50 mg CHC was added to one of the HMP system (experimental group). Blood, urine and histological samples were collected during the subsequent 3 h ex vivo normothermic perfusion with an oxygenated autologous blood using cardiopulmonary bypass machine.
Results: Serum creatinine was reduced faster in the experimental group ($p = 0.023$). The total urine volume was larger in the experimental group 239 ± 71 ml compared to the control group 172 ± 42 ml, ($p = 0.031$). Histologically tubular changes were less frequent in the experimental group ($p = 0.045$). No difference was seen between the groups regarding tromboelastography.
Conclusions: Perfusion of porcine kidneys with CHC during HMP ameliorates early IRI and improves organ function close after reperfusion. No increased risk of bleed was seen with this treatment. This is a protective intervention strategy that potentially may improve the outcome of kidney transplantation in a clinical setting.







O256 PARAMETERS OF MACHINE PERFUSION EVALUATING QUALITY OF KIDNEYS FROM DCD/ECD DONORS

Guodong Chen, Min Han, Jiang Qiu, Changxi Wang, Xiaoshun He, Lizhong Chen

The First Affiliated Hospital of Sun Yat-Sen University

Background: Donors from donation after cardiac death (DCD) and expanded criteria donors (ECD) have poorer outcomes compared to standard deceased donors (SCD). It is important to evaluate quality of kidney from DCD/ECD before transplantation. In this study, machine perfusion was used to preserve the DCD/ECD kidneys with the aim to investigating whether the parameters of machine perfusion could predict the quality of kidneys from DCD/ECD donors.
Methods: 36 pairs of kidneys from DCD/ECD donors were harvested in our hospital from July 2011 to Aug 2014. All kidneys were preserved with machine perfusion (Life Port), and parameters of machine perfusion were collected. All kidneys were biopsied before transplantation. The kidneys were discarded if histology results showed glomerulosclerosis or interstitial fibrosis were more than 20%. The primary endpoints were delayed graft function (DGF) and graft loss. Postoperative complications and 1-year serum creatinine levels were also recorded.
Results: Seven pairs of kidneys (19.4%) were discarded before transplantation. During machine perfusion, 1-h resistant index (RI) were significantly higher and 1-h flow rate were significantly lower in discarded kidneys compared to the kidneys that were transplanted (p0.4) and 36 cases in low RI group (RI $\leq$0.4). DGF rate were significantly higher in the high RI group (72.7% vs. 27.8%). 1-year serum creatinine levels were also significantly higher in the high RI group ($p < 0.05$). Acute rejection rate and 1-year graft survival were comparable between the two groups.
Conclusion: Parameters of machine perfusion are good tools for evaluating quality of kidneys from DCD/ECD donors, and predicting the DGF and 1-year graft function after transplantation.

O257 HYPOTHERMIC PULSATILE PRESERVATION OF KIDNEYS FROM UNCONTROLLED DECEASED DONORS AFTER CARDIAC ARREST

Palmina Petruzzo[1], Xavier Matillon[2], Ricardo Codas[2], Fabrice Danjou[2], Celine Dagot[2], Emmanuel Morelon[2], Lionel Badet[2]

[1]Department of Transplantation; [2]Department of Transplantation

Background: Kidneys from uncontrolled donors after cardiac arrest (uDCD) suffer from a period of warm ischemia between cardiac arrest and cold flushing. The present study was performed to evaluate the effectiveness of perfusion machine in uDCD and the parameters influencing intrarenal vascular resistance (IR).
Materials: Forty-four kidneys from uncontrolled donors after circulatory death (uDCD) were included in this study. The potential donors (Maastricht category I and II) underwent cardiopulmonary resuscitation by assisted ventilation and chest compression. After the arrival at the hospital and the no-touch period of 5 min, the organs were preserved with in situ cold perfusion (IGL-1 solution) using an aortic double-balloon triple-lumen catheter, "Gillot sonde" or a normothermic subdiaphragmatic extracorporeal membrane oxygenation. All the harvested kidneys were machine perfused using hypothermic (1–4°C) pulsatile perfusion (RM3, Waters Medical System). Kidneys with IR >0.5 mmHg/ml/min after 6 h of perfusion were discarded.
Results: There was one PNF, while 37 recipients (84.1%) experienced DGF. Four graft losses were reported: one kidney was removed for venous thrombosis 6 days after the transplantation; the other ones were lost for chronic rejection. A linear regression model showed that IR values at the end of perfusion were associated with MDRD variations at 3 and 6 months after transplantation ($p = 0.049$ and $p = 0.01$, respectively). IR at the beginning of perfusion was influenced by the in situ cold perfusion procedure ($p = 0.001$), donor smoking ($p = 0.002$), warm ischemia time ($p = 0.021$); while at the end of the perfusion IR was influenced by the in situ cold perfusion procedure ($p = 0.008$), donor sex ($p = 0.001$) and donor serum creatinine values ($p = 0.002$).
Conclusion: Donor characteristics and warm ischemia time influenced IR, which was associated with renal function.

O258 END-ISCHEMIC HYPOTHERMIC IN-HOUSE MACHINE PERFUSION IMPROVES 1-YEAR GRAFT SURVIVAL IN ECD KIDNEYS

Anja Gallinat[1], Vera Amrillaeva[1], Dieter Hoyer[1], Peri Kocabayoglu[1], Jürgen Treckmann[1], Marieke Van Meel[2], Undine Samuel[2], Thomas Minor[3], Andreas Paul[1]

[1]Universityhospital Essen; [2]Eurotransplant; [3]Universithospital Bonn

Background: Preservation by continuous hypothermic machine perfusion (HMP) decreases DGF rates and improves graft survival in ECD-DBD kidneys (Treckmann J. et al. Transpl int. 2011;24:548–54). The aim of this pilot study was to provide clinical data comparing end-ischemic in-house HMP (e-HMP) with conventional cold storage (CS).
Patients and methods: Between 11/2011 and 8/2014, 52 ECD kidney donor pairs (mean age: 66 (50–81) years) were included in this prospective single center study. From each donor pair, one kidney, initially stored on ice has been reconditioned by e-HMP upon arrival at our center, whereas the contralateral kidney remained on ice until being transplanted at another ET-center. Two pairs have been excluded due to preservation unrelated early arterial /venous thrombosis. Data from the remaining 50 pairs were analyzed looking at the incidence of DGF and 1-year graft and patient survival. Data were compared by parametric and non-parametric tests, graft and patient survival by Kaplan-Meier analysis.
Results: All 50 e-HMP kidneys were transplanted, while 6 out of 50 (12%) CS contralateral kidneys were discarded due to medical reasons. Mean recipient age was 65.5 (28–82) years for e-HMP and 66.5 (24–79) years for CS preserved kidneys. Waiting time, number of zero HLA mismatches and re-transplant rate were similar in both groups. CS kidneys had a shorter cold ischemia time (CS vs. e-HMP: 697 vs. 832 min, $p = 0.003$). We recorded a DGF-rate of 12% in the e-HMP arm vs. 20.4% in the CS arm ($p = 0.24$). 1-year graft survival was significantly improved by e-HMP compared to the CS group (98% vs. 86%, $p = 0.02$). Patient survival (92% vs. 88%) was not statistically different.
Conclusion: We observed that e-HMP leads to a reduction of DGF from 20.4% to 12% as well as to a significant improvement in one year graft survival. The use of e-HMP did not result in kidneys being discarded.

O259

THE EFFECT OF MACHINE PERFUSION ON ENDOTHELIAL APOPTOSIS: A NOVEL HUMAN TISSUE MODEL

Yazin Marie[1], Nuor Jamil[2], Helen Caldwell[3], Rebecca Telfer[4], Souad Messahel[5], Nicholas Inston[1]

[1]University Hospitals Birmingham NHS Trust; [2]Durham and Darlington NHS foundation Trust, Newcastle Upon Tyne; [3]Breakthrough Research Unit, Western General Hospital, Edinburgh; [4]College of Medical and Dental Sciences-University of Birmingham, Birmingham; [5]Human Biomaterials Resource Centre- University of Birmingham, Birmingham

Background: Hypothermic Machine Perfusion (HMP) is used for organ preservation (OP) yet the mechanisms of action are still unclear. The effect of flow cessation during OP on vascular endothelium prior to transplantation is largely unknown. The aim of this study was to assess the effects of HMP versus static cold storage (SCS) on endothelial apoptosis in a human tissue model.
Methods: Fresh human gonadal veins from cadaveric (cGV) and living kidney donors (lGV) were bisected with half formal fixed at time zero (T0), the other half SCS stored for 24 hours (T24NF)inUniversity of Wisconsin (UW) solution and analysed using immunohistochemistry (IHC) for Caspase-3 and transmission electron microscopy (TEM). A second phase study using trisected cGVs where the third part was attached to a novel flow model using a standard HMP device with pulsatile flow of UW solution for 24 h (T24F). Caspase-3, cell counting and qualitative assessment of vessel morphology was performed by two independent blinded pathologists. Local ethics board approval was obtained.
Results: 15 donor veins were assessed. Five lGV, and five cGV in phase 1, and five cGVs in phase 2. Baseline Caspase-3 expression was higher in cGV than lGV (5.65 +/- 3.7 vs 3.2 +/- 1.8). SCS resulted in a significant rise in Caspase-3 in T24NF samples. TEM findings confirmed morphological changes consistent with apoptosis. T24F showed a reduction of Caspase-3 expression over baseline expression (mean = 1.07, SD = 0.39; p = 0.0001), T24NF showed a significant increase in Caspase-3 (1.4–4 folds) (mean = 2.81, SD = 1.37; p = 0.0001).
Conclusions: Pre-transplant endothelial apoptosis is higher in cGVs at T0. SCS increases apoptosis that is avoided by HMP. The effects of EC injury leading to apoptosis have been shown to activate pro-fibrotic pathways and the physical benefits of flow may be underestimated. This simple model is proposed to allow further study in both SCS and HMP to detetermine the effect of flow in organ perfusion.

015 INFECTIONS

O260 HIGH INCIDENCE OF HERPES ZOSTER AFTER KIDNEY, LIVER, HEART AND LUNG TRANSPLANTATION

Nicole Van Besouw, Stefan Roest, Dominique Bovée, Herold Metselaar, Jan Van Weezel, Annemiek Van Der Eijk, Rogier Hoek, Willem Weimar, Olivier Manintveld, Marcia Kho
Erasmus MC

Background: Primary varicella zoster virus (VZV) infection causes varicella and lifelong latent infection in neural ganglia from which it may reactivate leading to herpes zoster (HZ). Immunocompromised transplant recipients are at risk to develop HZ and severe clinical complications. Therefore, we investigated the incidence of HZ after the first organ transplant and analysed the severity of HZ.
Methods: The records of patients after the first kidney (KTx: $n = 434$), first liver (LTx: $n = 277$), heart (HTx: $n = 222$) and lung (LuTx, $n = 120$) transplantation were analysed for VZV-PCR DNA and clinical signs of HZ.
Results: VZV infection was clinically diagnosed and confirmed by PCR in 40 KTx, 16 LTx, 38 HTx and 16 LuTx recipients. 5 patients who were VZV IgG negative pre-transplantation, developed primary VZV infection 2.8 and 4.7 years post-KTx, 4.7 and 7.8 years post-HTx, and 1.2 years post-LuTx.

38 patients developed HZ post-KTx; 8/38 had complicated HZ: 2 had disseminated HZ (>3 dermatomes) of whom one died of the complications, 2 had involvement of head and trunk, 2 only of the head, and 2 had cranial nerve involvement. 16 patients developed HZ post-LTx: 2/16 had disseminated HZ. 36 patients developed HZ post-HTx; 7/36 had complicated HZ: 2 had systemic dissemination and 5 had cranial nerve involvement. 15 patients developed HZ post-LuTx: 2/15 had systemic dissemination, of whom one died six days later.

The overall incidence rate of HZ post-KTx (14.0 cases/1000 PY), LTx (24.8 cases/1000 PY), HTx (30.8 cases/1000 PY) and LuTx (37.1 cases/1000 PY) was significantly higher than an age-matched healthy population (7–8 cases/1000 PY).
Conclusion: HZ is a frequent complication after kidney, liver, heart and lung transplantation. Boosting the VZV immune response by prophylactic VZV vaccination pre-transplantation may limit the incidence and severity of HZ post-transplantation.

O261 VACCINATION TO PREVENT THE HIGH HERPES ZOSTER INCIDENCE AFTER RENAL TRANSPLANTATION

Marcia Kho, Willem Weimar, Dominique Bovée, Marieken Boer - Verschragen, Joke Roodnat, Jacqueline Van De Wetering, Annemiek Van Der Eijk, Nicole Van Besouw
Erasmus MC

Introduction: Herpes zoster (HZ) is the clinical manifestation of varicella zoster virus (VZV) reactivation and occurs more frequently in people with a suppressed immune system. We studied the incidence of HZ in a cohort of renal transplant recipients. Moreover we assessed the efficacy of vaccination to increase VZV IgG titres in kidney transplant candidates to comparable levels as in healthy persons.
Methods: In a cohort of 522 renal transplant recipients, transplanted between 2003 and 2008, incidence and complications of HZ were analysed up to July 31, 2013. In a prospective study, patients ≥50 years awaiting renal transplantation ($n = 26$) were vaccinated with Zostavax®. Gender and age-matched kidney transplant donors ($n = 27$) were included as controls. VZV-specific IgG titres were determined before, 1 and 3 months after vaccination.
Results: HZ prevalence was 21.3%. HZ incidence was 12.5 cases/1000 person years (PY) under immunosuppressive therapy (IS) in patients <50 years, and 22.7 cases/1000 PY in patients ≥50 years. HZ incidence in the general population is significantly lower (7–8 cases/1000 PY). Complications (bacterial infections, systemic dissemination, death) only occurred in HZ cases under IS. After vaccination, VZV-IgG titres significantly increased at 1 and 3 months compared to before vaccination in both patients (1 mo: $p < 0.0001$, 3 mo: $p = 0.0001$) and donors (1 mo: $p < 0.0001$, 3 mo: $p < 0.0001$). The increment in VZV-IgG titers from pre-vaccination to 1 and 3 months post vaccination was comparable between patients and donors. One patient had a mild HZ episode at 11 months post-transplantation (16 months post-vaccination).
Discussion: HZ incidence post renal transplantation is high. Remarkably, in contrast to hepatitis B vaccination, VZV vaccination equally increased virus specific IgG titres in patients with renal failure compared to healthy individuals. ESRD patients can be effectively vaccinated to prevent herpes zoster.

O262 IMPACT OF ANTIVIRAL PROPHYLAXIS ON THE INCIDENCE OF αLPHA-HERPESVIRIDAE INFECTIONS IN SOLID ORGAN TRANSPLANT (SOT) RECIPIENTS: A NATIONWIDE COHORT STUDY

Cecilia Martin-Gandul[1], Delphine Zbinden[1], Susanne Stampf[2], Christian Van Delden[3], Hans Hirsch[2], Paola Gasche-Soccal[3], Maja Weisser[2], Nicolas Müller[4], Christoph Berger[4], Alexia Cusini[5], Christian Garzoni[5], Katia Boggian[6], Manuel Pascual[1], Pascal Meylan[1], Oriol Manuel, Stcs The[1]
[1]University of Lausanne; [2]University of Basel; [3]University of Geneva; [4]University of Zurich; [5]University of Bern; [6]University of St. Gallen

Background: The use of prophylaxis against CMV may potentially reduce the incidence of herpes simplex (HSV) and varicella zoster virus (VZV) infections in SOT recipients. We determined the incidence and risk factors for the development of HSV/VZV infections and assessed the impact of antiviral prophylaxis on the incidence of these infections.
Methods: Patients included in a nationwide cohort of SOT recipients (Swiss Transplant Cohort Study) from 5/08 to 12/13 were analyzed. Antiviral preventive strategies (prophylaxis vs. preemptive approach for CMV) were established according to each center protocol. Risk factors for the development of HSV/VZV infection were assessed by logistic regression.
Results: 2368 patients (56% kidney, 20% liver, 10% lung, 7.3% heart, others 6.8%) were analyzed. 75/95% were seropositive for HSV/VZV at transplant. 198, 78, and 16 patients developed HSV, VZV, and both infections. 19% of HSV infections were non-mucocutaneous. 93/98% of HSV/VZV infections received antiviral therapy. Incidence at 1 year post transplant of HSV/VZV infections were 6.9/2.4% in kidney, 8.1/1.5% in liver, 1.3/0% in lung, 11/5.8% in heart. Incidence of HSV/VZV infection was 4.5/13% in patients with and without antiviral prophylaxis. By CMV serostatus, in D-/R- the incidence at 1 year was 3.7/11% in patients with and without antiviral prophylaxis. In D +/ R- or R+, the incidence of HSV/VZV infection was 4.1% in patients receiving anti-CMV prophylaxis and 14% in patients followed by the preemptive approach. By Cox regression, variables associated with HSV/VZV infections were lung transplant (HR 0.22, $p < 0.001$, compared to heart transplant), antiviral prophylaxis (HR 0.39, $p < 0.001$) and female gender (HR 1.35, $p = 0.02$).
Conclusion: HSV/VZV infections were prevalent in our population, with a significant rate of non-mucocutaneous involvement. AntiCMV and antiherpes prophylaxis had a significant impact in reducing the incidence of HSV/VZV infection after transplant. CMV D-/R- and patients followed by the preemptive approach should receive specific antiherpes prophylaxis.

O263 SIMULTANEOUS PANCREAS/KIDNEY TRANSPLANTATION INCREASES THE RISK OF LATE-ONSET BKV-ASSOCIATED NEPHROPATHY

Thomas Schachtner, Marina Zaks, Andreas Kahl, Petra Reinke
Charite Campus Virchow Clinic - Department of Nephrology and Internal Intensive Care

Opportunistic infections have increased in simultaneous pancreas/kidney transplant recipients (SPKRs) with BKV-associated nephropathy (BKVN) being the most important infectious cause of kidney allograft loss. However, a comparison of BKVN in kidney transplant recipients (KTRs) and SPKRs is lacking.

Here, we studied all KTRs and SPKRs at our single transplant center between 2003 and 2012. 24 of 1098 KTRs (2.2%) and 11 of 106 SPKTs (10.4%) were diagnosed with biopsy-proven BKVN with allograft loss due to BKVN in 3 KTRs (12.5%) and 1 SPKTs (9.1%). A control group of 598 KTRs and 95 SPKTs without BKVN was used for comparison.

SPKRs showed an increased incidence of BKVN compared to KTRs ($p < 0.05$). Most KTRs showed early-onset BKVN, whereas SPKRs were more likely to develop late-onset BKVN ($p < 0.05$). Interestingly, lymphocyte-depleting induction, CMV-reactivation, acute rejection episodes, and poor HLA-matching increased the risk of early-onset BKVN in KTRs ($p < 0.05$), while no risk factors could be identified for SPKRs developing BKVN. No differences were observed for patient and allograft survival between KTRs and SPKRs with BKVN ($p > 0.05$). However, SPKRs showed higher peak BKV-loads and were more likely not to recover to baseline creatinine after BKVN ($p < 0.05$).

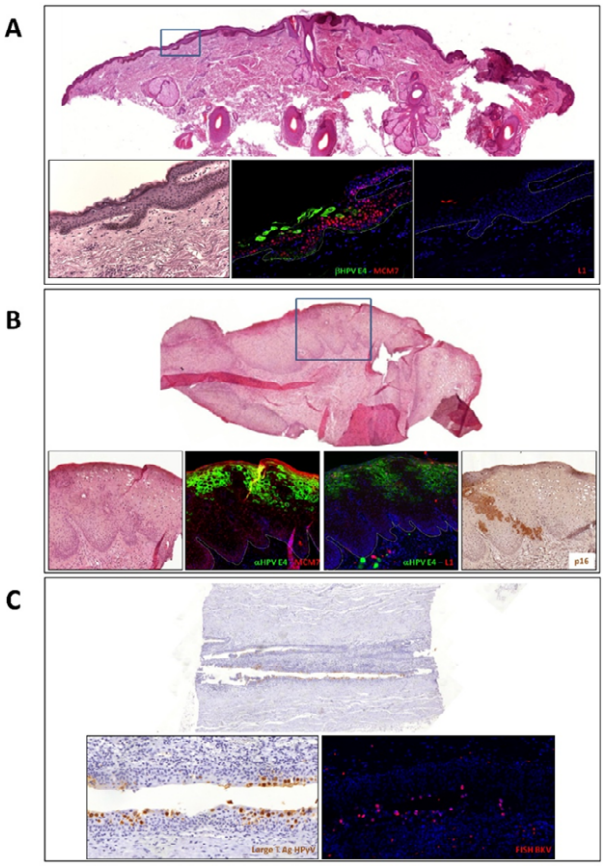
Although SPKRs show more risk factors for early-onset BKVN as lymphocyte-depleting induction only, higher rates of acute rejection, and poorer HLA-matching compared to KTRs, no increased incidence of early-onset BKVN has been observed. In contrast, SPKRs are at highly increased risk of late-onset BKVN, which may result from a chronic inflammatory state and chronic high-dose immunosuppression after simultaneous kidney/pancreas transplantation.

O264 SMALL DNA TUMOUR VIRUS INFECTIONS IN TUMOURS FROM A COHORT OF KIDNEY TRANSPLANT RECIPIENTS

Claudio Musetti[1], Cinzia Borgogna[2], Tiziana Cena[3], Federica Calati[2], Olivero Carlotta[2], Renzo Boldorini[4], John Doorbar[5], Marisa Gariglio[2], Piero Stratta[1]
[1]Kidney Transplant Center, Universita' del Piemonte Orientale; [2]Virology Unit, Universita' del Piemonte Orientale; [3]Epidemiology Unit, Universita' del Piemonte Orientale; [4]Pathology Unit, Universita' del Piemonte Orientale; [5]Department of Pathology, University of Cambridge

Introduction: Virus-associated malignancies are an ominous complication of kidney transplantation. Among these, small DNA tumor viruses, including papillomaviruses (HPV) and polyomaviruses (HPyV), are regarded as causing agents.
Methods: Immunostaining for HPV and HPyV proteins (E4, L1, and Large T antigen) was performed in 96 skin, 12 anogenital and 2 kidney tumors (RCC) available from our cohort of 982 KTR enrolled since 1998. As both RCC developed after a BKV nephritis, we've also performed PCR analysis and genotyping in urine and blood samples.
Results: Out of the 982 KTR included in this study (mean age 51.2 ± 12.4 years, 63% males), 60 pts (67% males) had developed a skin cancer (123 lesions) and 55 a non-skin tumor: 10 pts (20% males) had 20 áHPV-related anogenital lesions, and 13 pts a RCC (93% males). Among known risk factors, only azathioprine use was associated with skin (25% vs. 6.2%) and anogenital tumor development (12.5% vs. 0.8%). In addition, RCC were more frequent (3/16; 18.7%) among patients with a BKV nephritis (RR = 18.0, $p < 0.001$). Out of the 10 patients with anogenital lesion, 3 (30%) displayed positivity for αHPV proteins by immunohistochemistry. Out of 26 patients with skin lesion, 5 (19.2%) were positive for βHPV protein expression. Both RCC patients were BKV type IV positive in blood and urine. LargeT antigen expression was well evident in the tumor-free kidney and ureter.
Discussion: Our data demonstrate that active infection by ubiquitous viruses, usually present in low or inactive state in the normal setting, can be detected in tumor from different sites in the immunocompromised host and it is not restricted to áHPV in the genital sites. The áHPV and BKV activation found in the proximity of transformed tissues points to their possible involvement in the carcinogenetic process.

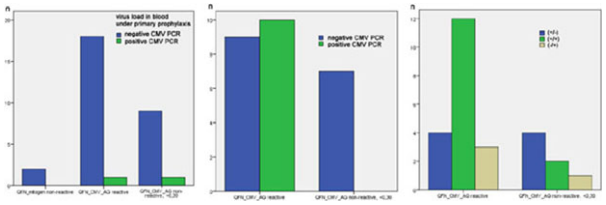
Figure: virus expression patterns. (a) Basal cell carcinoma: Distribution of βHPV E4, βHPV L1 and cellular MCM7. (b) Vaginal carcinoma in situ: Distribution of αHPV E4, L1, and cellular MCM7 and p16. (c) Kidney tumor: Distribution of HPyV Large T antigen and BKV DNA.



O265* REACTIVATION OF CYTOMEGALY VIRUS OCCURS RAPIDLY AFTER THE END OF PRIMARY ORAL ANTIVIRAL PROPHYLAXIS IN KIDNEY TRANSPLANTATION IN SPITE OF REACTIVE QUANTIFERON((R))-CMV RESPONSE

Inge Derad, Philip Muck, Jan Rupp, Martin Nitschke, Birgit Obermann
University of Luebeck

Although specific antiviral prophylaxis of cytomegaly virus (CMV) infection has improved the transplant outcome, about half of these patients will develop CMV infection. CMV infection still bears an increased risk of acute and chronic organ failure, rejection and graft loss. QuantiFERON-CMV allows the assessment of the individual cellular immunity against CMV by detecting the production of IFN-gamma following *in vitro* stimulation with CMV antigens. Preliminary studies have shown a correlation between a lack of detectable cell-mediated immunity measured by the QuantiFERON-CMV assay and a higher incidence of CMV infection in D+/R−. In a prospective study 32 kidney transplant patients (D+/R−, D+/R+, D−/R+) were consecutively examined before finishing primary CMV prophylaxis and 8 ± 4 weeks after end of primary prophylaxis. QuantiFERON-CMV assessed CMV specific cellular immune response. CMV PCR (IU/ml) was measured in whole blood and evaluated in relation to QuantiFERON-CMV. Specific patient data including immunological risk were recorded.32 patients (16 female, mean 53.1 ± 15.5 years) were transplanted between February 2012 and November 2013. Patients had triple or quadriple immunosuppressive therapy, with lymphocyte depleting therapy in 18 patients (56.3%). Influences of the lymphocyte depleting therapy led to a higher amount of CMV reactivation. Four patients (12.5%) had BK blood viremia. Results of the QuantiFERON-CMV in relation to CMV reactivation are shown in Fig. 1a and b and Fig. 2 displays the influence of CMV D/R status. In the present cohort with most patients being D+/R+, those patients ($n = 10$) with CMV reactivation all had cellular immunity against CMV as assessed by QuantiFERON((R))-CMV, no patient was non-reactive, whereas 7 out of 16 patients without viral reactivation were non-reactive. This is in contradiction to a previous study, finding non-reactive QuantiFERON-CMV to be a risk in D+/R− patients to develop CMV viremia.



O266 EVEROLIMUS REDUCES CYTOMEGALOVIRUS INFECTION IN RENAL TRANSPLANT RECIPIENTS RECEIVING PREEMPTIVE CMV PROPHYLAXIS

Gionata Spagnoletti, Maria Paola Salerno, Francesca Apponi, Valentina Bianchi, Evaldo Favi, Albero Pedroso Josè, Jacopo Romagnoli, Franco Citterio
Department of Surgery, Renal Transplantation Unit, Università Cattolica S. Cuore

Introduction: Aim of this study was to evaluate the efficacy and safety of preemptive prophylaxis of CMV infection in kidney transplant recipients (KTx) receiving polyclonal antibodies induction and randomized to an immunosuppressive regimen with/without everolimus.
Methods: One-hundred-eighty-two KTx were randomized to maintenance immunosuppressive therapy based on calcineurin inhibitors (CNIs) in combination with MMF (103 pts, MMF) or everolimus (76 pts, EVE). All KTx received induction with thymoglobulin (total median dose 200 mg). CMV-PCR was periodically monitored during the first year and positive patients were treated with ganciclovir.
Results: At 6 months follow-up 82/182 pts (45%) become CMV-PCR positive without any other symptomatology, 9/182 pts (4.9%) had CMV syndrome. Between 6 and 28 months (median follow-up) other 7/182 pts (3.8%) become CMV-PCR positive without any other symptomatology, 5/182 pts (2.7%) had CMV syndrome and 1/182 pts (0.5%) had mild CMV pneumonitis. Median time for CMV-PCR positivity finding was 30 days after transplantation. Patients receiving everolimus showed significantly lower CMV-PCR positivity (CMV-PCR+: EVE versus MMF 31% vs. 69%, $p < 0.001$). CMV syndrome was not significantly different in patients receiving everolimus or MMF (13% vs. 15%, p = ns).
Conclusions: Our data suggest that in KTx receiving induction therapy with thymoglobulin, everolimus significantly reduce CMV infection within a preemptive prophylaxis strategy of CMV disease.

O267 CONTRIBUTION OF POPULATION PHARMACOKINETICS TO DOSE OPTIMIZATION OF GANCICLOVIR/ VALGANCICLOVIR IN SOLID ORGAN TRANSPLANT PATIENTS

Ariadna Padullés[1], Helena Colom[2], Oriol Bestard[3], Josep Maria Cruzado[3], Eduardo Melilli[3], Núria Sabé[4], Raul Rigo[5], Jordi Niubó[6], Joan Torras[3], Laura Lladó[7], Nicolas Manito[8], Anna Caldés[3], Josep Maria Grinyó[3], Núria Lloberas[3]
[1]Pharmacy Department Hospital de Bellvitge; [2]Biopharmaceutics and Pharmacokinetics Unit, Pharmacy and Pharmaceutical Technology Department, School of Pharmacy, University of; [3]Nephrology Department, Hospital Bellvitge; [4]Infectious Diseases Department, Hospital Bellvitge; [5]Biochemistry Department, Hospital Bellvitge; [6]Microbiology Department, Hospital Bellvitge; [7]Digestive Surgery Department, Hospital Bellvitge; [8]Cardiology Department, Hospital Bellvitge

Background: Anti-cytomegalovirus treatment of solid organ transplant (SOT) patients with ganciclovir (GCV) or valganciclovir (VGCV) following the manufacturer's dosing recommendations may result in either over or under-exposure to the drug. However, Bayesian prediction based on a population pharmacokinetics model has been suggested to optimize GCV/VGCV dosing achieving target area under the curve (AUC) values (between 40–50 μg h/ml).
Methods: We conducted a two arm, randomized, open-label, clinical superiority trial (superiority margin = 40%) in adult SOT patients receiving GCV/VGCV either as prophylaxis or treatment of CMV infection. Group A: GCV/VGCV doses according to the manufacturer's recommendations. Group B: GCV/VGC doses based on target AUC using a Bayesian prediction model. Drug exposure achieved was evaluated.
Results: Fifty-three consecutive SOT patients were recruited in the study. 88.4% (23/26) of patients with CGV/VGCV adjusted according to the Bayesian prediction model (group B) reached therapeutic target AUC values, whereas only 18.7% (5/27) of patients in Group A did so, achieving the desired 40% superiority margin of the study design ($p < 0.001$, 95% CI for the difference: 54–86). The required time to reach target AUC values was significantly longer in Group A as compared to Group B (55.9 ± 8.2 vs. 15.8 ± 2.3 days respectively, $p < 0.001$). A numerically shorter time to viral clearance was observed in Group B as compared to Group A (12.5 vs. 17.6 days, $p = 0.125$, respectively). The incidence of CMV relapse (Group A: 66.67% [8/12]; Group B: 9.01% [1/11]) and late CMV disease (Group A: 36.7% [4/11]; Group B: 7.7% [1/13]) were both higher in Group A than in Group B. No relevant differences in drug-related toxicity were observed.
Conclusions: GCV/VCGV dose adjustment based on a population pharmacokinetics Bayesian prediction model optimizes GCV/VGCV exposure and shortens the time to achieve a therapeutic AUC target in SOT patients.

O268 ONE-SHOT VERSUS MULTIDOSE PERIOPERATIVE ANTIBIOTIC PROPHYLAXIS AFTER KIDNEY TRANSPLANTATION: A RANDOMIZED, CONTROLLED, CLINICAL TRIAL

Giuseppe Orlando[1], Tommaso Maria Manzia[2], Roberto Sorge[3], Giuseppe Ilaria[2], Roberta Angelico[2], Daniele Sforza[2], Luca Toti[2], Andrea Peloso[4], Timil Patel[5], Ravi Katari[5], Joao Paulo Zambon[5], Andrea Maida[6], Maria Paola Salerno[7], Katia Clemente[8], Pier Paolo Di Cocco[8], Luca De Linda[8], Laura Tariciotti[2], Famulari Antonio[9], Franco Citterio[7], Giuseppe Tisone[2], Francesco Pisani[8], Jacopo Romagnoli[6]
[1]WFSOM; [2]Department of Experimental Medicine and Surgery, Tor Vergata University, Rome; [3]Section of Transplantation and the Biometrics Laboratory, Tor Vergata University, Rome; [4]Department of General Surgery, IRCCS Policlinico San Matteo, University of Pavia, Pavia; [5]Wake Forest School of Medicine, Winston Salem, NC, USA; [6]Renal Transplantation Unit, Catholic University, Rome; [7]Department of Surgery, Transplantation Service, Catholic University of Sacred Heart, Rome; [8]Section of Transplantation, Department of Surgery, University of L'Aquila, L'Aquila; [9]Transplant Unit, Department of Surgery, University of L'Aquila, San Salvatore Hospital, L'Aquila

Background: Perioperative antibiotic prophylaxis may prevent surgical site infection (SSI) after renal transplant even if an optimal therapy has not been achieved yet. Most common regimes include the irrigation of the wound or the use of long-term, multidose antibiotical regimes.
Methods: Patient were enrolled in a prospective, randomized, multicenter, controlled trial in order to compare a single dose of antibiotic VS a multidose regimen of systemic antibiotic prophylaxis. Inclusion criteria were adult recipient of a deceased or living donor kidney allograft with no history of infection in the last 18 months of dialytic therapy. Exclusion criteria were age 30/m^2, hemoglobin levels <8 g/dl and white blood cell count <3000 cell/ml at the moment of RT. Primary and secondary endpoints of this study were to estimate the incidence of SSI and to assess other infection in the first postoperative month respectively.
Results: Two hundred five patients were enrolled and randomized for receiving a single (Group A [$N = 103$]) or a multidose (Group B [$N = 102$]) antibiotic therapy. In both groups the incidence of SSI and urinary tract infection were analogous. Three cases of superficial SSI (2 in group A [2%] vs. 1 in group B [1%]) occurred. No differences were recorded for dehiscence of surgical wound (5 in group A [5%] vs. 2 in group B [2%]). Secondary endpoint showed similar trend for SSI and urinary tract infection in both groups.
Conclusion: This study is the first randomized, controlled, multicenter trial comparing two different preoperative prophylaxis regimes in a relative homogeneous patient population undergoing renal transplantation. Antibiotic resistance is one of the modern healthcare system. For this reason we believe that to equality of results the single dose regimen is preferred in a non-diabetic, non-morbidly obese, adult renal transplant recipients.

023 KIDNEY

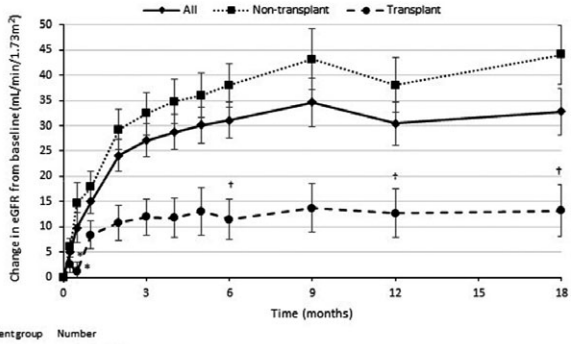
O269 EFFICACY AND SAFETY OF ECULIZUMAB IN ATYPICAL HAEMOLYTIC URAEMIC SYNDROME (AHUS) PATIENTS WITH OR WITHOUT RENAL TRANSPLANT

Christophe Legendre[1], Josep Campistol[2], Thorsten Feldkamp[3], Giuseppe Remuzzi[4], Laurent Weekers[5], Neil Sheerin[6]

[1]Universite Paris Descartes and Hôpital Necker; [2]Hospital Clinic, University of Barcelona; [3]University Hospital Schleswig-Holstein, Kiel; [4]Mario Negri Institute, Bergamo; [5]University of Liège Hospital; [6]Newcastle University

Background: Patients (pts) with aHUS often progress to end-stage renal disease and are transplanted. Eculizumab has been reported to inhibit thrombotic microangiopathy irrespective of renal transplant status (Keating G, Drugs, 2013;73:2053). Here, we evaluate the outcome of pts with native and transplanted kidneys included in four prospective studies of eculizumab.
Methods: Patient data were pooled from four prospective phase 2 trials of eculizumab with long-term extensions. Median (range) eculizumab treatment time was 71 (0–186) weeks. Efficacy and safety outcomes were analysed *ad-hoc* for all pts ($N = 100$). Here we compare the results for non-transplant (native kidney) pts ($n = 74$) and transplant pts ($n = 26$).
Results: The mean (standard error [SE]) estimated glomerular filtration rate (eGFR) at start of treatment was 24.2 (2.5) and 25 (3.3) ml/min/1.73 m^2 for native kidney pts and transplant pts, respectively. There was a significant increase in mean eGFR from baseline in both groups over time (figure). The mean (SE) eGFR at 18 months was 65.7 (5.3) and 41.5 (6) ml/min/1.73m^2 for native kidney pts and transplant pts, respectively. At baseline, 52 (70%) of native kidney pts and 15 (58%) of transplant pts had a platelet count $<150 \times 10^9$/l, respectively. The mean (SE) increase in platelet count ($\times 10^9$/l) at 18 months in these subgroups was 135.6 (15.4) for native kidney pts and 83.1 (29.8) for transplant pts. Eculizumab was well tolerated: most adverse events were mild or moderate. Two pts had a meningococcal infection, one native kidney pt (serogroup B) and one transplant pt (serogroup unknown). Both pts had been vaccinated and were not on prophylactic antibiotics at the time of infection. Infection resolved with antibiotic treatment and the native kidney pt continued to receive eculizumab.
Conclusion: Eculizumab was well tolerated by aHUS pts with native and transplant kidneys. In pts with aHUS, eculizumab improved renal function and platelet count in pts irrespective of renal transplant status. A larger improvement was seen in eGFR and platelet values in pts with native kidneys. This could be due to different disease characteristics in transplanted kidneys, a reduced capacity for functional recovery or later diagnosis of disease. Optimal disease management should reduce the need for transplantation in aHUS patients. These data suggest that early recognition and treatment of aHUS is important to minimise irreversible organ damage.

Figure. Mean change in eGFR from baseline for all pts and those with or without a renal transplant



Patient group	Number							
All	37/97/45/86/95	96	95	94	96	80	70	55
Non-transplant	22/71/29/60/69	71	70	70	71	57	49	35
Transplant	15/26/16/26/26	25	25	24	25	23	21	20

P<0.01 for all time points compared to baseline except **P*>0.05 and †*P*<0.02

O270 EVOLUTION OF RENAL FUNCTION IN RENAL ALLOGRAFT RECIPIENTS UNDER VARIOUS EVEROLIMUS BASED IMMUNOSUPPRESSIVE REGIMENS

Chrysanthi Skalioti[1], Smaragdi Marinaki[1], Maria Darema[1], Sofia Lionaki[1], Nikos Antonakopoulos[2], George Zavos[3], John Boletis[1]

[1]Nephrology Department & Renal Transplantation Unit, Laiko General Hospital; [2]Novartis Hellas; [3]Renal Transplantation Unit, Laiko General Hospital

Background: Long-term allograft survival still remains a major challenge in kidney transplantation.
Aim: To estimate the evolution of renal function in patients receiving different immunosuppressive regimens based on everolimus (EVR).
Methods: Ninety nine renal allograft recipients were included in a 12-month open label, non-interventional, prospective, single-center study. Patients were divided in two groups, *de novo* and late conversion to EVR.
Results: Group A included 40 patients, who received de novo calcineurin inhibitor (CNI) plus EVR. Median time post-transplantation was 33.06 months (IQR18.25–42.85). Mean eGFR the first month after transplantation (MDRD) was 54.89 ± 19.08 ml/min, while mean proteinuria was 0.54 ± 0.38 g/24 h. At the end of follow up, mean eGFR and mean proteinuria significantly improved (65.49 ± 20.79 ml/ min; p: 0.011 and 0.157 ± 0.089 g/24 h; p: 0.002 respectively). Group B consisted of 59 patients; 49 of them had initially received mycophenolic acid (MPA) plus CNI while 10 had been on Azathioprine plus CNI. Median time of initial immunosuppression was 37 months (IQR 14.75–112.5). Initial immunosuppression was switched to: MPA plus EVR in 49 patients, CNI plus EVR in 4 and EVR with steroids in 6. The main indications for conversion were malignancies and biopsy proven CAI. Mean eGFR 1 month post-transplantation and at the time of conversion were 50.79 ± 17.83 ml/min and 57.39 ± 19.17 ml/min respectively (p: 0.014). After conversion to EVR, mean eGFR increased significantly (66 ± 24.89 ml/min; p: 0.006). Mean proteinuria was 0.509 ± 0.530 g/24 h the first post-transplant month and it remained stable at 0.415 ± 0.431 g/24 h until study completion. Two acute rejection episodes occurred after conversion to EVR. At the end of follow up, patient and death-censored graft survival were 97% and 100% respectively.
Conclusions: In kidney transplant recipients, EVR either *de novo*, or after conversion with or without CNI is a safe and effective treatment that preserves renal function.

O271 M-TOR INHIBITORS-INDUCED PNEUMONITIS IN RENAL TRANSPLANTED PATIENTS: A SINGLE CENTER EXPERIENCE

Alberto Mella, Maria Messina, Antonio Lavacca, Ana Manzione, Massimo Gai, Stefania Bussolino, Maria Cristina Di Vico, Fabiola Pagani, Giuseppe Paolo Segoloni, Luigi Biancone

Nephrology Dialysis and Transplantation, Department of Medical Sciences, Città della Salute e della Scienza Hospital, Turin

Introduction and Aims: The mTOR inhibitors drugs (mTORi) can cause non-infectious pneumonitis (mTORi P) with an interstitial (NSIP) or organizing damage (OP). The overall experience about mTORi P in renal transplanted patients (RTx) is limited.
Methods: We performed a retrospective analysis among the mTORi-treated RTx in our Center between 01/01/1997–12/31/2011: 434/1052 (continous or intermittent protocols).
Results: Prevalence of mTORi P was 3.9% (17/434). mTORi P population characteristics: 3/17 everolimus-treated and 14/17 sirolimus-treated; 7/17 patients mTORi treated ab-initio; ratio M/F 8/1; median age 58 years (min-max 35–70); risk factors (smoke, a pre-existant pneumopathy, CMV infection) in 5/17; "classic" mTORi P symptoms (fever+cough+dyspnoea) in 6/17. m-TORi P-related characteristics: median time between symptoms and radiological demonstration 716 days (min-max 66–3176); median mTORi serum levels at 6, 3, 1 months before mTORi P and at diagnosis 7, 8, 7.6 and 7.3 ng/ml (p = NS); mTORi levels higher than targeted in 2/17. Diagnosis: CT scan in 16/17 patients (10 OP, 6 NSIP, one case radiologically negative). 17/17 with altered pulmonary functional tests (PFTs), 5/11 lympocytic alveolitis on bronchoalveolar lavage (BAL). Treatment: Withdrawal of mTORi drug in 17/17, associated with steroid therapy in 3/17. Outcomes: 17/17 alive; symptoms resolution in 9/17 at ≤3 months, 7/17 at ≤6 months, 1/17 at ≤12 months; renal function unnchanged 1 year after mTORi withdrawal.
Conclusions: In the AA' experience mTORi P is not such a rare adverse event as often reported. An early diagnosis, a multidisciplinary approach and a well-defined diagnostic pathway (CT scan, PFTs and BAL) allow to a positive outcome. The AA'suggest: (i) to consider mTORi P also without classic symptoms or sovranormal mTORi serum levels (ii) to withdraw mTORi after diagnosis of mTORi P, if not contraindicated (iii) to associate corticosteroids in case of severe pulmonary impairment.

O272 DONOR AND RECIPIENT FACTORS THAT INFLUENCE EFFICACY OUTCOMES: 12 AND 24-MONTH MULTIVARIATE ANALYSES FROM KIDNEY TRANSPLANT RECIPIENTS RECEIVING EVEROLIMUS WITH REDUCED CALCINEURIN INHIBITOR

C. Escrig[1], G. Colussi[2], R. Roland[3], M. Vergara[4], Z. Wang[4], P. Bernhardt[1]
[1]Novartis Pharma AG; [2]Niguarda Cà Granda Hospital; [3]University Hospital L. Pasteur; [4]Novartis Pharmaceuticals Corporation

Purpose: Donor and recipient characteristics like age, gender, race and delayed graft function (DGF) may affect efficacy outcomes post-transplant (Tx) independent of immunosuppression. Here, we present data from a multivariate analysis of such factors and efficacy outcomes in *de novo* kidney Tx recipients (KTxR).
Methods: In A2309 (NCT00251004), a 24-month (M), multicentre, open-label trial, 833 KTxR were randomised (1:1:1) to everolimus (EVR; C0 3–8 or 6–12 ng/ml) with reduced cyclosporine (rCsA) or to mycophenolic acid (MPA) with standard (s) CsA; all with steroids. Primary endpoint (composite efficacy failure: treated biopsy-proven acute rejection, graft loss, death or loss to follow-up) and possible effect of donor and recipient variables (by Cox proportional hazard modelling) were assessed at M12 and M24.
Results: Of 716 (86.0%) patients completing the study, composite efficacy failure occurred in 197 patients (23.6%) at M12 and 242 (29.1%) at M24. At M12, males (HR 1.50; 95% CI 1.08, 2.10; p = 0.017), African-Americans (HR 1.68; 95% CI 1.08, 2.60; p = 0.021), patients with HLA mismatch ≥3 (HR 1.42; 95% CI 1.00, 2.02; p = 0.049) and patients with DGF (HR 2.75; 95% CI 1.82, 4.16; p < 0.0001), along with increasing donor age (HR 1.01; 95% CI 1.00, 1.03; p = 0.022), were at a significantly higher risk of composite efficacy failure. At M24, increasing recipient age was associated with significantly lower risk, whereas males, African-Americans, patients with DGF, and increasing donor age remained associated with significantly higher risk of composite efficacy failure (Table 1). Treatment with EVR + rCsA versus MPA + sCsA showed similar composite efficacy failure rate.
Conclusions: Males, African-Americans and increasing donor age were associated with significantly higher composite efficacy failure rate; patients with DGF showed the highest risk both at M12 and M24. In this analysis, treatment regimen was not associated with outcome, confirming equal efficacy of EVR + rCsA versus MPA + sCsA.

Table 1: Cox proportional hazard regression analysis for composite efficacy endpoint[a] at M24 (ITT population)

Variables	HR	95% CI	p-value
EVR 3–8 ng/mL vs MPA	1.20	0.87, 1.64	0.27
EVR 6–12 ng/mL vs MPA	0.98	0.71, 1.37	0.93
Recipient male vs female	1.35	1.00, 1.82	**0.05**
Increasing recipient age in years	0.99	0.98, 1.00	**0.03**
Recipient Black vs non-Black	1.62	1.09, 2.42	**0.02**
Diabetes (yes vs no)	1.12	0.80, 1.56	0.51
DGF (yes vs no)	2.60	1.78, 3.82	**<0.01**
HLA mismatch ≥3 vs <3	1.35	0.98, 1.85	0.06
Increasing PRA in percentage[b]	1.02	0.99, 1.04	0.23
Donor male vs female	0.86	0.66, 1.12	0.26
Increasing donor age in years	1.01	1.00, 1.02	**0.01**
Donor Black versus non-Black	0.97	0.58, 1.63	0.91
Donor living versus deceased	1.13	0.69, 1.83	0.63
Increasing cold ischaemia time in hours	1.01	0.98, 1.04	0.54

[a]Treated biopsy-proven acute rejection, graft loss, death or loss to follow-up.
[b]Most recent evaluation.
CI, confidence interval; DGF, delayed graft function; EVR, everolimus; HLA, human leukocyte antigen; HR, hazard ratio; ITT, intent-to-treat; M, month; MPA, mycophenolic acid; PRA, panel reactive antibody.

O273 CORRELATION BETWEEN TACROLIMUS VARIABILITY AND THE PRESENCE OF DNDSA

Vannesa Teng, Marc Clancy, Kathryn Stevens, Julie Glen, Ann-Margaret Little
University of Glasgow

Tacrolimus is the optimal choice of calcineurin inhibitor used to prevent immunological rejection and *de novo* donor specific antibodies (dnDSA) development. It exhibits intrapatient variability (IPV), a measure of magnitude and frequency of blood concentration deviations from the ideal target range. A high IPV is associated with adverse renal outcomes but a causal relationship has not been confirmed. This relationship may be causal through suboptimal immunosuppression leading to dnDSA development. Thus, we aim to determine if tacrolimus variability correlates with dnDSA presence. This study includes 305 consecutive adult renal transplant recipients in the West of Scotland between January 2007 and December 2011. Prospective data was collected from Manzen tissue-typing and renal unit database. Serum samples were screened for Class I and II HLA IgG antibodies, using LABScreen PRA and Single Antigen Bead test. dnDSA is defined as specificity corresponding to current HLA mismatch and was absent pre-transplant, with an MFI ≥ 500 (cutoff). Out of 305 patients, 26 patients (8.6%) developed dnDSA with a mean follow-up of 4.0 ± 1.3 years post transplant. Based on the population median of 16.0% for tacrolimus IPV, 157 patients (51.5%) were assigned to high variability (HV) group and 148 patients (48.5%) were assigned to low variability (LV) group. HV group were more likely to have previous late acute rejections (13.5% vs. 5.4%, p = 0.016), dnDSA development (12.2% vs. 7%, p = 0.02) and had poorer allograft survival rate (HR = 6.43, p = 0.005). After adjusting for late acute rejections, a high tacrolimus IPV was independently associated with dnDSA development (OR = 1.04, p = 0.032). In conclusion, adverse graft outcome is associated with both high tacrolimus IPV and dnDSA presence, between which a significant correlation has been found. There is a potential that increased surveillance and identifying therapies to reduce IPV can improve long-term graft outcomes and thus, should be undertaken.

O274 IVIG AND HIGH DOSE STEROIDS EFFECTIVELY SLOW RENAL FUNCTION LOSS IN TRANSPLANT GLOMERULOPATHY

Kasia Sablik, Caspar Looman, Marian Clahsen-Van Groningen, Madelon Van Agteren, Michiel Betjes
Erasmus MC

Background: Transplant glomerulopathy (TG) is a major cause of kidney graft loss in the long term with no established effective therapy yet. At our transplantation centre, patients with TG within the context of chronic allograft rejection are treated with intravenous immunoglobulins (IVIG) and pulse methylprednisolone (MP). In this study we analysed the efficacy of this treatment.
Methods: From January 2007 until January 2015, 36 patients with biopsy proven TG were treated with IVIG/MP. All patients underwent a biopsy because of progressive decrease in renal function (eGFR) or *de novo* proteinuria at least 1 year post transplantation. The median time after transplantation was 6 years (range 1–19 years). After TG was diagnosed, patients were administered three doses of 1 g intravenous methylprednisolone combined with a single dose of IVIG (1 g/kg body weight). The efficacy of the treatment was analysed by comparing the slope of eGFR loss 12 months prior to treatment to the course of the eGFR in the 12 months after treatment by linear multilevel analysis. Clinical characteristics were analysed for association with outcome.
Results: Treatment with IVIG/MP resulted in a significant change in eGFR gradient from −0.8 ml/min/1.73 m^2/month pre-treatment to −0.4 ml/min/1.73 m^2/month post treatment (p < 0.001). This translated into an average reduction of eGFR loss of 4.6 ml/min/1.73 m^2 in the 1st year after treatment. Out of the 36 patients, 5 were considered complete non-responders. Renal function at start of treatment or the presence of proteinuria (>1 g/l) were not associated with response to IVIG/MP. Remarkably, late diagnosis and treatment of TG (>6 years after transplantation) responded significantly better to therapy (p < 0.001). Additionally the treatment effect on proteinuria was examined by including all patients with proteinuria (n = 24), defined as 0.5 g/l, at time of biopsy. Prior to IVIG/MP, the proteinuria increased with an average of 53 mg/l/month with a significant change in slope thereafter (p = 0.0069) leading to a decrease of 13 mg/l/month.
Conclusion: IVIG/MP treatment for TG significantly slows the progression of eGFR loss and reduces proteinuria.

O275 THERAPEUTIC DRUG MONITORING OF MYCOPHENOLIC ACID DURING COMBINED TACROLIMUS THERAPY

Zou Yuangao, Shi Yunyin, Bai Yangjuan, Su Zhenzhen, Li Yi, Wang Lanlan
West China Hospital of Sichuan University, China

Background: Mycophenolic acid (MPA) as a selective inhibitor of lymphocyte proliferation, its levels have demonstrated a good correlation with the risks for acute rejection and side effectsits in renal transplanted patients. Therapeutic drug monitoring (TDM) of MPA is recommended to include full area under the concentration–time curve (AUC). But full AUC estimates are not practical for routine monitoring, so limited sampling strategy have been suggested.
Methods: Plasma MPA was measured by EMIT in samples taken before the morning dose of mycophenolate mofetil, 0.5, 2 and 4 h post-dose, The truncated AUC0–4 was calculated (AUC = 14.81 + 0.8*C0.5 + 1.56*C2 + 4.8*C4), completing 4248 MPA concentration-time points from 1105 adult kidney transplant recipients (770male, 335female, age 35 ± 10 years). Besides MPA, patients received tacrolimus.
Results: AUC of MPA in adult kidney transplant recipients receiving a fixed dose of mycophenolate mofetil 750 mg twice daily were recommended therapeutic range (35–75 μg h/ml). In ours study group the mean AUC0–4 level was 62.57 ± 20.20 μg h/ml. The AUC0–4 level was above the therapeutic range in 23.4% of patients. The mean values for C0 was 3.61 ± 2.34 mg/l, but C0 showed a low correlation with AUC0–4 h (r^2 = 0.311).
Conclusions: MPA TDM-based MMF dosage adjustment enabled us to administer MMF more confidently, and it will be a useful tool to cope with the wide pharmacokinetic variability of MMF after kidney transplantation.

O276 CARDIOVASCULAR RISK PROFILE AFTER CONVERSION FROM TACROLIMUS TO EVEROLIMUS IN RENAL TRANSPLANT PATIENTS ON MAINTENANCE

Julio Pascual[1], José M. Morales[2], Ana Sánchez-Fructuoso[3], Daniel Serón[4], Joan M. Díaz[5], Federico Oppenheimer[6], Manuel Rengel[7], Domingo Hernández[8], Josep M. Cruzado[9], On Behalf of Evita Study Group[10]
[1]Servicio Nefrología. H. del Mar. Barcelona; [2]Fund. Investigación H. 12 Octubre. Madrid; [3]Servicio Nefrología. H. Clínico San Carlos. Madrid; [4]Servicio Nefrología. H. Vall Hebron. Barcelona; [5]Servicio Nefrología. Fundació Puigvert. Barcelona; [6]Unidad Trasplante Renal. H. Clínic i Provincial de Barcelona. Barcelona; [7]Servicio Urología. H. Gregorio Marañón. Madrid; [8]Servicio Nefrología. H. Carlos Haya. Málaga; [9]Servicio Nefrología. H. Bellvitge. IDIBELL. Barcelona; [10]xxxx

Background: Our aim was to compare the cardiovascular (CV) risk profile of everolimus (EVL) in a regimen with mycophenolic acid (MPA) versus tacrolimus (TAC) + MPA in maintenance renal transplant (RT) recipients.
Methods: 24-month, open-label, parallel-group, multicenter, randomized, phase II study in patients 18–70 years, first/second RT, more than 6 months and <3 years post-transplant, on TAC+MPA. Patients with serum creatinine ≥2 mg/l; glomerular filtration rate (GFR) ≤ 40 ml/min; proteinuria ≥ 500 mg/day, severe rejection and PRA ≥ 20%, were excluded.
Results: A total of 71 patients were randomized and 60 patients were included in the ITT population (28 EVL and 32 TAC). Demographic and clinical baseline characteristics were well balanced between groups [mean (SD) age 48.3 (12.5) years, 58.3% male, BMI 26.5 (4.3) kg/m^2, 90.0% first transplant, 83.3% PRA0%, mean time since transplantation 1.5 (0.8) years, and proteinuria 0.188 (0.119) g/day]. LVH and cardiac biomarkers showed a reduction at month-24 in EVL and TAC groups, which showed statistical differences between treatments in the Procollagen type I N-terminal propeptide (PINP, ANCOVA analysis, p = 0.004, Table 1). Pulse wave velocity (PWV) and systolic/diastolic ambulatory blood pressure (ABP24 h) were well controlled at baseline and during the study in both groups. Creatinine clearance and GFR showed a significant improvement in EVL group versus TAC (ANCOVA p = 0.030 and p = 0.014, respectively) and was maintained at month-12 and month-24 (Table 1). No cell/humoral rejection/graft loss in EVL or TAC was observed. The number of adverse events (AE) was: 132 EVL vs. 74 TAC (8 and 5 were serious AEs, respectively).
Conclusion: CV profile improves in both treatment groups showing a decrease in LVH and in cardiac biomarkers at month-24. This fact is reinforced by the normal PWV and ABP values observed throughout the study. Renal function improved in EVL group at month-6 and was maintained during the study.

Table 1. CV profile and renal function. ITT population.

	TAC group (*N* = 32)	EVL group (*N* = 28)
Left ventricular hypertrophy, *n* (%)		
Baseline	19 (59.4)	16 (57.1)
Month-12	20 (62.5)	17 (60.7)
Month-24	12 (37.5)	12 (42.9)
Pulse wave velocity (m/s), mean (SD)		
Baseline	7.2 (1.9)	7.3 (1.6)
Month-6	7.0 (1.6)	7.4 (1.7)
Month-24	7.6 (1.7)	7.1 (1.7)
N-terminal pro-brain natriuretic peptide (pg/ml), median (Q1–Q3)		
Baseline	144.8 (56.3–341.9)	110.6 (55.7–256.0)
Month-6	115.5 (117.2–480.7)	123.4 (114.9–386.9)
Month-24	105.7 (42.0–188.2)	79.6 (36.0–215.2)
Procollagen type I N-terminal propeptide[1](μg/l), median (Q1–Q3)		
Baseline	54.2 (41.4–109.0)	63.2 (34.7–88.9)
Month-6	57.1 (40.3–69.6)	40.2 (36.4–57.7)
Month-24	50.4 (43.7–69.5)	43.9 (26.9–81.0)
Glomerular filtration rate[2] (ml/min/1.73 m^2), mean (SD)		
Baseline	59.9 (13.4)	58.7 (15.0)
Month-6	56.0 (11.2)	62.8 (18.1)
Month-12	57.8 (11.4)	61.2 (19.2)
Month-24	57.7 (10.5)	60.8 (17.4)

[1]Ancova analysis (p = 0.0044), [2](p = 0.0143).

O277 EARLY CONVERSION TO EVEROLIMUS IN *DE NOVO* RENAL TRANSPLANT RECIPIENTS ACHIEVES BETTER RENAL FUNCTION PRESERVATION: 12-MONTH RESULTS FROM THE ELEVATE STUDY

Johan W De Fijter[1], Hallvard Holdaas[2], Antonio Speziale[3], Guido Junge[3], Zailong Wang[4], Josep M. Cruzado[5], Markus Van Der Giet[6]
[1]Leiden University Medical Centre, Leiden; [2]Oslo University Hospital, Oslo; [3]Novartis, Basel; [4]Novartis Corporation, East Hanover, NJ; [5]Bellvitge University Hospital, Barcelona; [6]Charite - Universitätsmedizin Berlin, Berlin

Background: Long-term exposure to calcineurin inhibitors (CNIs) contributes to unfavorable long-term outcomes, including inferior renal function and premature graft loss with vascular lesions, glomerulosclerosis and interstitial fibrosis (IF/TA). The ELEVATE study (NCT01114529) evaluated whether early CNI to everolimus (EVR) conversion in renal transplant recipients (RTRs) provides better preserved renal function without compromising efficacy compared with standard CNI. Here, we present the 12-month (M) results.
Methods: ELEVATE is a 24-months, multicentre, open-label trial, in which *de novo* RTRs were randomised (RND) (EVR, *N* = 360; CNI = 357) at 10–14 weeks post-transplant to convert from CNI to EVR (C0 6–10 ng/ml) or continue standard CNI (C0, tacrolimus: 5–10 ng/ml, cyclosporine: 100–250 ng/ml); all received enteric-coated mycophenolate sodium + steroids. The primary end point was change in estimated glomerular filtration rate (eGFR; 4v-MDRD) from RND to M12. Main secondary end points were composite efficacy failure of treated biopsy-proven acute rejection (Banff ≥ IB), graft loss, or death and safety.
Results: Mean eGFR was significantly higher at all time-points after RND in EVR versus CNI group. At M12, mean eGFR in EVR versus CNI group was 64.4 vs. 60.4 ml/min/1.73 m^2 (p = 0.031) and 66.7 vs. 61.1 ml/min/1.73 m^2 (p = 0.002) for intent-to-treat and on-treatment analysis, respectively. In two patients in the EVR group and one in the CNI group proteinuria (≥3 g/day) was reported. At M12, the Kaplan-Meier incidence of composite efficacy failure was comparable between the groups. Overall, 12.5% of RTRs on EVR returned to CNI while 1.4% on CNI switched CNI medication. The incidence of adverse events (AEs) and serious AEs was comparable (Table). Currently, the M24 results are awaited.
Conclusion: Early conversion to EVR therapy at 10–14 weeks post-transplant versus continued CNI leads to better preserved renal function with comparable overall efficacy and safety.

Table: Incidence of key efficacy and safety outcomes at Month 12

	EVR group (N = 344)	CNI group (N = 356)	p-value
Efficacy outcomes, n (%)			
Composite efficacy failure*	19 (5.9)	12 (3.9)	0.263
- Treated BPAR ≥IB	15 (4.4)	7 (2.0)	0.084
- Graft loss	2 (0.6)	3 (0.8)	1.000
- Death	5 (1.5)	4 (1.1)	0.749
BPAR	31 (9.0)	17 (4.8)	0.035
Treated BPAR	27 (7.8)	17 (4.8)	0.119
AR	42 (12.2)	21 (5.9)	0.003
Treated AR	35 (10.2)	19 (5.3)	0.017
Safety outcomes, n (%)	**(N = 335)**	**(N = 359)**	
Any AE	295 (88.1)	297 (82.7)	-
Any serious AE	141 (42.1)	143 (39.8)	-

*Kaplan-Meier incidence (%) reported for composite efficacy failure (treated BPAR Banff ≥IB, graft loss, or death).

AE, adverse event; AR, acute rejection; BPAR, biopsy-proven acute rejection; CNI, calcineurin inhibitor; EVR, everolimus.

O278 SURGICAL INFORMED CONSENT PROCEDURES IN LIVE DONOR NEPHRECTOMY IN THE NETHERLANDS

Kirsten Kortram, Jan Ijzermans, Frank Dor
Erasmus MC

Background: There are many uncertainties when it comes to informed consent in general, let alone in living kidney donors. A recent systematic review demonstrated that there is no consensus on how the informed consent procedure in live donor nephrectomy should be arranged and practices vary.
Materials and Methods: A web-based survey was created and sent to all kidney transplant surgeons in the Netherlands (*n* = 50). Questions were divided into four subgroups: personal experience, hospital logistics, contents of informed consent and actual informed consent procedure. Surgeons were asked how often they mentioned 23 items regarding short- and long-term risks.
Results: Response rate was 96% (*N* = 48), of which 30 were involved in living donor education. Respondents were transplant (53%), vascular (30%), abdominal surgeons (10%), and urologists (7%) from all eight kidney transplant centers. Informed consent procedures vary between centers, ranging from assumed to signed consent. Some respondents from the same center report different procedures. Bleeding was the only complication that every surgeon mentioned. Risk of death was always mentioned by 16 surgeons (53%),

sometimes by 11 (37%), but three surgeons (10%) never disclosed this disastrous complication. Those who did disclose the risk of death reported different mortality rates, ranging from 0.003% to 0.1%. Mentioning frequencies for all other complications varied. Short-term complications were more frequently disclosed than long-term complications.
Conclusion: Important complications are not always mentioned during the surgical informed consent process for live donor nephrectomy. Informed consent procedures vary, and surgeons from the same center sometimes report different practices. To ensure donor safety, and to optimally prepare living kidney donors for the procedure, a standardized informed consent procedure for live donor nephrectomy is highly desirable. A nationwide inventory project has been initiated to achieve this.

O279 ROBOT-ASSISTED LIVE DONOR NEPHRECTOMY IN A HIGH VOLUME CENTRE: THE WAY TO GO?

Shiromani Janki[1], Karel W.J. Klop[1], Michiel G.H. Betjes[2], Turkan Terkivatan[1], T.C. Khe Tran[1], Jan N.M. Ijzermans[1]
[1]Division of HPB and Transplant Surgery, Department of Surgery, Erasmus MC, University Medical Center, Rotterdam; [2]Division of Nephrology and Transplantation, Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam

Background: It is of utmost importance to minimize the risks of the donation procedure and maximize donor safety by evaluating and implementing minimal invasive surgical techniques. In this study we evaluated the clinical relevance and learning curve of robot-assisted donor nephrectomy (RADN) in a high volume centre.
Methods: Between January 2012 and May 2014 40 robot-assisted donor nephrectomies were performed by two da Vinci® certified transplant surgeons. Donors were eligible provided a left sided nephrectomy was indicated and approval for donation was given by a multidisciplinary team of transplant surgeons, nephrologists and anesthesiologists. Data on operative time, blood loss, complications, quality of life, kidney function, and graft survival were prospectively collected. All left-sided laparoscopic donor nephrectomies (LDN) performed in the aforementioned period by our two da Vinci® certified transplant surgeons were selected as control group.
Results: There were significant differences between the robot and laparoscopy group in preoperative BMI (median 23.8 [17.9–38.0] vs. 26.1 [18.5–37.0] $p = 0.001$), WIT (median 3.5 min [1–9] vs. 3 min [1–6], $p < 0.001$), operative time (median 191.0 min [114–282] vs. 165.0 min [85–241], p 0.032), blood loss (median 100 ml [10–1100] vs. 200 ml [20–800], $p = 0.027$), and quality of life dimension score "bodily pain" at 3 months follow-up (estimate 90.0 [16.4] vs. 98.5 [6.3], $p = 0.035$), respectively. Costs of RADN are approximately €2000 more per procedure compared to LDN. More low grade post-operative complications were observed in the laparoscopy group. No difference was found between the robot and laparoscopic technique on donor kidney function at 3 months post-donation.
Conclusion: Robot-assisted live donor nephrectomy for renal transplantation has a relatively short learning curve, and results in a favourable outcome for donor as well as recipient, comparable to a laparoscopic approach. Due to high costs we prefer the laparoscopic technique in a tertiary high volume centre.

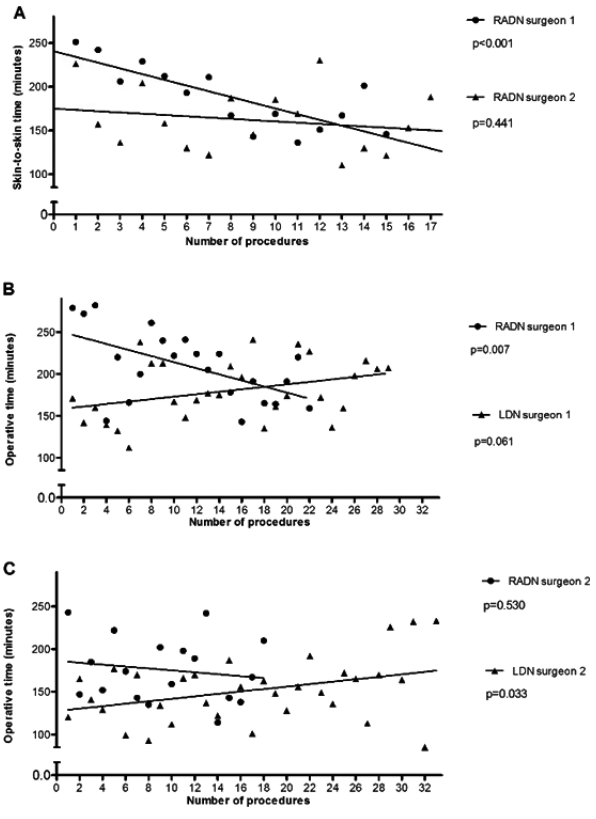


Figure 1. The skin-to-skin time in minutes of RADN for surgeon 1 and 2 (a), and the operative time in minutes of RADN and LDN for surgeon 1 (b) and surgeon 2 (c). The p-values represent the improvement of the slope during the performed procedures.

O280 THE PREVAILING PREFERENCE FOR LEFT NEPHRECTOMY IN LIVING DONOR TRANSPLANTATION DOES NOT ADVERSELY AFFECT DONOR OR RECIPIENT OUTCOMES

Scot Robertson[1], Lucy Garrard[1], Niaz Ahmad[2], Shahid Farid[2], Magdy Attia[2], Richard Baker[3], Richard Feltbower[4]
[1]University of Leeds; [2]Department of Transplantation, St James University Hospital, Leeds; [3]Department of Renal Medicine, St James University Hospital, Leeds; [4]Department of Epidemiology, University of Leeds

Background: Reservations continue to exist in the procurement of the right donor kidney for the purposes of live transplantation. We present the largest national study to date comparing donor and recipient outcomes following right or left laparoscopic donor nephrectomy.
Methods: A total of 5393 patients undergoing laparoscopic donor nephrectomy (LDN) and their transplant recipients between January 2003 and December 2012 with a mean follow up 3.8 years were included in the study from 24 centres across UK. Donor outcomes for analysis: hospital stay, intra/post-operative complications, use of anti-hypertensive drugs, 1-year serum creatinine, eGFR, and creatinine clearance. Recipient outcomes for analysis: Delayed graft function (DGF), primary non function (PNF), 1-year serum creatinine, eGFR and graft survival.
Results: Of the 5393 donors, 4568 (84.7%) were left-LDN and 825 (15.3%) right-LDN. No significant difference was observed in all donors outcomes between right and left LDN, except in incidence of post-operative wound infection (3.7% vs. 1.2%, $p < 0.001$). In transplant recipients there was no difference in DGF (5.7% vs. 4.3%), PNF (1.8% vs. 1%), 1 year serum creatinine, eGFR and graft survival in recipients receiving kidneys from right or left LDN respectively. Recipient patient and graft survival analysis produced no significant difference ($p = 0.547$, $p = 0.126$ respectively) between left and right kidney recipients.
Conclusion: We present the largest study to date comparing donor and transplant outcomes following right or left LDN and have shown no significant differences in terms of safety and function. Prevailing bias for left kidney nephrectomy does not disadvantage the donor or recipient in living kidney donor transplantation.

O281 POTENTIAL KIDNEY DONORS WITH ASYMPTOMATIC MICROSCOPIC HEMATURIA: HISTOPATHOLOGICAL FINDINGS AND OUTCOMES

Ehab Hammad, Hassan Aleid, Tariq Ali, Ihab Ibrahim, Hadeel Almanea
King Faisal Specialist Hospital & Research Center

Background: Microscopic hematuria is not uncommon in potential kidney donors. After excluding urological causes, most of these donors require kidney biopsy to exclude underlying renal causes of hematuria such as glomerulonephritis. We reviewed biopsy findings of donors with microscopic hematuria and studied short-term outcomes of recipients who received kidneys from donors with hematuria but with no pathology.
Methods: We included kidney donors who had microscopic hematuria which was not due to urological causes. All of them underwent native kidney biopsies which were examined by light microscopy, immunofluorescence and electron microscopy.
Results: Out of 750 donors, 23 with microscopic hematuria underwent kidney biopsy. Mean age was 32.7 ± 6.28. Seventeen donors had 1+ hematuria, 6 donors had 2+ or more in urine dipstick. All of them had red cell on urine microscopy. Six (26%) biopsies showed histopathological abnormalities of which, 4 were thin basement membrane disease (17.3%) and 2 were IgA nephropathy (8.7%). These 6 patients were excluded from donation. The remaining 17 patients (74%) had no significant pathology on the renal biopsies. Their mean age was 34.4 ± 7.9. Nine (39.1%) of these donors have donated their kidneys and 6 (26%) donors are in the process of donating. The other 2 (8.7%) donors were excluded due to other medical/recipient related issues. The recipients of kidneys from donors with microscopic hematuria with no pathology have a mean SBP of 121 ± 9.5, mean DBP 74 ± 8.5 and mean serum

creatinine of (69 μmol/l ± 21.3) at 3 month follow up. Their last urine analysis showed absence of hematuria in 6 of them, 3 showed +ve dipstick and microscopic hematuria; their original disease was cystic kidney disease, chronic GN and diabetes.
Conclusions: Our study showed that 26% of our donors had abnormal pathology on renal biopsy. Isolated hematuria requires extensive work up including renal biopsy to identify donors who may have underlying glomerulonephritis.

O282 DONOR AND RECIPIENT OUTCOMES WITH LAPAROSCOPIC DONOR NEPHRECTOMY IN DONORS WITH COMPLEX RENAL VASCULAR ANATOMY

Ahmed Fahmy, Ahmed Dakkak, Moaz Mandoura, Wael Habhab, Najla Zabani, Naef Alsulaimani, Ahmed Al Hashemy
King Faisal Specialist Hospital and Research Center

Introduction: Laparoscopic donor nephrectomy (LDN) is considered the gold standard for donor nephrectomy. Up to 30% of the general population has multiple renal vessels, or complex anatomy making it highly likely to encounter such anatomy in the donor population. We report our experience with LDN in the setting of complex vascular anatomy comparing recipient outcomes in the standard and complex anatomy groups.
Methods: Between February 2011 and February 2015 we performed 186 LDNs. 184 were left sided (98.9%) and two were right sided. Standard anatomy was found in 133 donors, while mulitpe renal arteries and anomalous venous anatomy were encountered in 53 (28.5%). Complex vascular anatomy consisted of multiple arteries in 41 cases (22%), with two arteries in 38 and three arteries in 3 donors. Complex venous anatomy was found in 20 donors (10%) with muliplte veins in 7, retroaortic vein in 11, circumaortic vein in 1 and left sided cava in 1. A single anomaly was found in 49 donors while 4 cases (2%) had multiple anomalies. Mean operative time was 204 min in the standard anatomy group and 233 min in the complex anatomy group. Mean warm ischemia time in the standard and complex group was 3.21 and 3.86 min respectively. One donor in the standard anatomy group required reexploration for bowel obstruction. Recipients mean serum creatinine for the standard anatomy group at 1 week, 1 month and 3 months post operatively were 109, 96,and 92 μmol/l respectively, while for the complex anatomy group were 107, 96, 94 μmol/l respectively. There was no statistically significant difference between the two groups. Delayed function occurred in one patient (1.8%) in the complex anatomy group with serum creatinine at end of 3 months down to 78 μmol/l.
Conclusion: LDN in patients with complex vascular anatomy is safe in experienced centers. The warm ischemia time remains within acceptable range with no added risk to the donor and without negative impact on the outocome of transplantation.

O283 HAND-ASSISTED RETROPERITONEOSCOPIC DONOR NEPHRECTOMY OFFERS MORE LIBERAL USE OF RIGHT KIDNEYS: AN EXPERIENCE OF 455 CASES

Baris Akin, Soykan Barlas, Murat Dayangac, Ayse Sinangil, Vedat Celik, Tevfik Ecder
Istanbul Bilim University Florence Nightingale Hospital

Hand-assisted retroperitoneoscopic (HARP) donor nephrectomy offers a safer alternative to laparoscopic approach, combining the advantages of manual control while avoiding intraabdominal organ injuries and adhesions. In February 2009, after 3 years of experience with hand-assisted laparoscopic nephrectomy (HALN), we implemented HARP technique for all donor nephrectomies. We reviewed the records of 455 consecutive living donor kidney transplantation procedures performed between February 2009 and December 2014. HARP technique requires two trocars and a hand port placed through paramedian (n = 434) or Pfannenstiel (n = 21) incision. Only the first right HARP case was performed with extra trocar for liver retraction. Three donors died during follow-up period. The rate of right donor nephrectomy at HALN technique (4 out of 103, 3.8%) increased to 18.9% with adoption of HARN technique. The mean age was 44.3 and BMI was 27.4. The female ratio was 57.5%. 73 donors had multiple renal arteries (14 right, 59 left). None of the patients had blood transfusion, conversion to open surgery, readmission or reoperation. The mean dissection time was 101.3 min. The demographics and mean dissection time were statistically similar for both sides. 84 patients had peritoneal opening during procedure. Wound infection (n = 6) and incisional hernia (n = 11) were other complications. One patient had renal artery thrombosis secondary to intimal flap at the recipient artery. One patient had delayed graft function. All other transplanted kidneys had immediate function. There were two recipients with ureter stenosis. HARP approach offers safe and effective results and avoids the intraperitoneal complications of HALN technique. In our experience, switch from HALN to HARP technique significantly increased our rate of right donor nephrectomies. A more liberal use of right kidneys can help to increase the living donor pool and increase the long term donor safety by saving the better kidney to the donor.

O284 SHIFTING PARADIGMS IN LIVE KIDNEY DONATION: ATTITUDES OF TRANSPLANT PROFESSIONALS

Jeff Lafranca, Emerentia Spoon, Jacqueline Van De Wetering, Jan Ijzermans, Frank Dor
Erasmus MC, University Medical Center, Rotterdam

Background: The transplant community increasingly accepts extended criteria (EC) live kidney donors, because of organ shortage, although long-term follow-up data of these donors and their respective recipients is still lacking. Great differences are present in acceptance of these donors, and guidelines do not offer clarity. The aim of this survey was to reveal these differences and to get an insight in both center policies as well as personal beliefs of transplant professionals.
Methods: An online survey was sent to 1128 ESOT members. The questionnaire consisted of an objective part asking for center policies regarding acceptance of EC live kidney donors, and a subjective part for transplant surgeons, regarding their personal beliefs in this matter. Questions about several EC, pre-operative imaging, multidisciplinary team discussions and operative techniques were included. Comparisons were made between transplant centers of three regions in Europe (Northwest, Mediterranean and East) and between other countries worldwide.
Results: 331 questionnaires were completed by professionals from 55 countries (30 in Europe). 55% were transplant surgeons, 35.3% were nephrologists. Significant differences exist between regions in acceptance of donors with EC. Median refusal rate for potential live donors is approximately 15%. Furthermore, differences are seen regarding pre-operative work-up, specialists who perform screening and preoperative imaging. Almost a quarter of transplant professionals sometimes deviate from their center policy, resulting in more or less comparable personal beliefs regarding EC.
Discussion: By performing this survey amongst a large group of transplant professionals, we gained insight in both center policies as well as personal opinions of acceptance of EC donors. Variety is seen, proving the need for a standardized approach in selection. Although short-term outcome of these donors seems to be good, long-term follow-up is warranted to ensure donor safety.

O285 VASCULAR MULTIPLICITY SHOULD NOT BE A CONTRA-INDICATION FOR LIVE KIDNEY DONATION AND TRANSPLANTATION

Jeff Lafranca, Mark Van Bruggen, Hendrikus Kimenai, Khe Tran, Turkan Terkivatan, Michiel Betjes, Jan Ijzermans, Frank Dor
Erasmus MC, University Medical Center, Rotterdam

Background: Whether vascular multiplicity in live kidney donors should be considered as relative contraindication and 'extended donor criterion' is still under debate, as around 10% of the European centers only accept donors with singular anatomy.
Methods: From 2006 to 2013, data from all live kidney donors (n = 951) was collected, and retrospectively reviewed. Vascular anatomy as imaged by MRA, CTA was compared with intraoperative findings. Furthermore, the influence of vascular multiplicity on outcome of donors and respective recipients was studied.
Results: In 237 donors, vascular multiplicity was present, 58.4% of them had bilateral multiplicity. CTA had higher accuracy levels compared to MRA regarding renal vascular anatomy assessment in this cohort. Regarding outcome of live donors with vascular multiplicity, warm ischemia time (WIT) and skin-to-skin time were significantly longer if arterial multiplicity (AM) was present (5.1 vs. 4.0 min and 202 vs. 178 min). Skin-to-skin time was significantly longer and complication rate (Clavien-Dindo grade I) was higher in donors with venous multiplicity (VM) (203 vs. 180 min and 17.2% vs. 8.4%). Analysis of renal transplant outcome in recipients showed a significantly increased WIT (30 vs. 26.7 min), higher rate of DGF (15.7% vs. 5.7%) and lower rate of BPAR (6.9% vs. 13.9%) in patients receiving a donor kidney with AM compared to donor kidneys with singular anatomy. Most importantly, recipients had no impaired graft- and patient survival.
Conclusion: CT-scan proves to be superior to MRA regarding correct pre-operative anatomical imaging of a live kidney donor. Although significant differences were found in WIT and skin-to-skin time, we conclude that vascular multiplicity should not be considered as a contra-indication to donation, since it has little impact on clinical outcome in the donor. Furthermore, renal transplant recipients receiving a donor kidney with multiple arteries or veins have excellent outcome.

O286*

CONTEMPORARY SURGICAL OUTCOMES FROM 900 HAND ASSISTED LAPAROSCOPIC DONOR NEPHRECTOMIES

Zubir Ahmed, Nizam Mamode
Guy's Hospital

Introduction: Despite over 5000 hand assisted laparoscopic donor nephrectomies (HALDN) having been performed in the United Kingdom robust outcome data is still lacking to inform patient choice and to identify areas for quality improvement. We performed a detailed analysis of all patients undergoing HALDN in the UK's largest centre.
Method: Data was collected separately by one surgeon and major outcome measures separately validated by another senior surgeon. Patient demographics, intraoperative details and postoperative complications (short and longterm) were recorded.
Results: 900 patients underwent HALDN between 2003 and 2013. 51.8% were female with a mean age of 44.6 (SD11.7) years. 85% were left sided cases. 14 cases were performed in 2003 and 136 in 2013. This increase mirrored national trends. Mean postoperative hospital stay was 3.93 (SD 1.44) days and was unchanged over the decade. Multiple arteries were encountered in 241/900 cases. 5/900 underwent open conversion with none in the last 200 cases. 20% of patient suffered a surgical complication, 18% were of an infectious aetiology. 3.3% suffered a major complication (clavien 3/4) with 3% requiring reoperation. A binary logistic model investigating factors predictive of surgically significant complications revealed operating surgeon experience (OR1.1 1.03–1.18) and departmental experience (measured in quartiles of caseload OR 1.2–1.67) to be the only implicated factors. Incisional herniation (IH) was revealed as a significant medium term complication (cohort incidence 5.8% median time to onset 8 months) with a supraumbilical incision independently predictive of IH (OR 3 1.33–10.8). There were no patient deaths or need for dialysis in this period.
Conclusions: Although safe, HALDN confers a significant amount of short and medium term postoperative morbidity. Factors associated with this are modifiable and should form part of of future interventional trials in living donation.

025 LIVER

O287 AN SINGLE-CENTER EXPERIENCE OF ABO INCOMPATIBLE LIVING DONOR LIVER TRANSPLANTATION WITH NEW SIMPLIFIED INTRAVENOUS IMMUNOGLOBULIN PROTOCOL: A PROPENSITY SCORE MATCHING ANALYSIS

Dong Kim Joo, Lak Choi Dong, Ryeon Choi Hye
Catholic University of Daegu College of Medicine

Background: Since various innovative strategies including local infusion therapy and rituximab have been introduced, outcomes of ABO incompatible (ABO-I) living donor liver transplantation (LDLT) have remarkably improved. However, the consensus of the ABO-I LDLT protocol remains undetermined. Herein, we describe 25 cases of ABO-I LDLT with our simplified protocol and compare the outcomes with those in ABO compatible LDLT.
Methods: We analyzed the outcomes via retrospective review of 182 adult LDLT cases including 25 ABO-I LDLT cases from January 2011 to December 2014. A propensity sore model was used to compare two groups. The desensitization protocol comprised plasma exchange, rituximab, intravenous immunoglobulin without local infusion therapy and splenectomy. The preoperative anti-ABO antibody titer was achieved $\leq$32 by performing plasma exchange.
Results: The median age of recipients was 51 years (range, 35–66) and the median MELD score was 15 (range, 7–37). The initial range of isoagglutinin IgM and IgG titers were 1:1–1:256 and 1:4–1:2048, respectively. The comparisons between two groups showed no significant difference in patient demographics and perioperative variables except postoperative hospital stay ($p = 0.02$). Although significant rebound elevation in isoagglutinin titer during postoperative periods was observed in three cases, Neither C4d staining in the graft nor clinical signs of antibody mediated rejection was not apparent in these cases. Neither diffuse intrahepatic biliary stricture nor biliary anastomotic stricture were encountered at all ABO-I LDLT patients with the mean follow-up of 22.6 $\pm$ 17.2 months. The incidence of graft vascular thrombosis was similar in both groups and significant differences in overall and graft survival were not observed between the two groups.
Conclusion: ABO-I LDLT can be performed safely under this new simplified protocol and may be proposed when ABO compatible donors are not available.

O288 OUTCOME OF LIVING DONOR LIVER TRANSPLANTATION USING PARTIAL LIVER ALLOGRAFTS WITH DUAL ARTERIAL SUPPLY

Won Lee Kyo, Hyuk David Kwon Choon, Woo Cho Chan, Nuri Lee, Kyu Oh Dong, Gon Na Byung, Yong Choi Jin, Wontae Cho, Seunghwan Lee, Man Kim Jong, Sung Choi Kyu, Berm Park Jae, Joo Kim Sung, Jae-Won Cho, Suk-Koo Lee
Samsung Medical Center

Background: When multiple donor hepatic arteries (HA) are present in living donor liver transplantation (LDLT), whether all HAs require reconstruction remains debatable.
Methods: From May 1996 to June 2014, 1098 cases of LDLT were performed at Samsung Medical Center (Seoul, Korea). We excluded ABO incompatible cases and retransplantation cases from the analysis. Data of 1098 cases were retrospectively reviewed. Our center's criteria for HA anastomosis in case of multiple HAs is to check for pulsatile back-flow from the smaller HA during the donor procedure and also during the recipient procedure. A priority is set on anastomosis of both HAs, unless good pulsatile back-flow is evident during both the donor and the recipient procedures.
Results: Out of the 1098 cases, 74 cases (74/1098, 6.7%) were done using liver allografts with 2 HAs. 30 were right lobe (RL) grafts, 9 were left lobe (LL) grafts and 35 were left lateral section (LLS) grafts. Among the 30 RL grafts, we anastomosed both HAs in 19 cases and one HA in 11 cases. The two groups did not show differences in donor and recipient age, GRWR, cold ischemia time, macro- and microscopic steatosis of the graft, type of bile duct anastomosis and number of bile duct anastomoses. Postoperative results showed similar levels of maximum AST and ALT during the 1st post-transplant week. Rate of biliary complications were not different between the two groups. One case of HA thrombosis occurred (both HAs anastomosis group). Among the 44 LL and LLS grafts, we anastomosed both HAs in 29 cases and one HA in 15 cases. The two groups did not show differences in donor and recipient clinical characteristics. Postoperative outcome were not different between the two groups. One case of HA stenosis occurred (one HAs anastomosis group).
Conclusion: Using our criteria for HA anastomosis, we were able to achieve similar outcomes among LDLT cases using allografts with two HAs.

O289 ADVANCES OF DONOR OPERATION IN LIVING DONOR LIVER TRANSPLANTATION FROM OVER 1000 CASES AT A SINGLE INSTITUTION

Kyung-Suk Suh, Suk-Won Suh, Kwang-Woong Lee, Nam-Joon Yi, Won Lee Hae, Youngrok Choi, Hyeyoung Kim, Jaehong Jeong
Surgery, Seoul National University College of Medicine, Seoul

Background: Lots of surgical advances in donor operation in living donor liver transplantation (LDLT) were achieved and, recently, it has been standardized with excellent outcomes. In the present study, we address our experience over 1000 cases of donor operation in LDLT.
Materials and Methods: Between January 1999 and February 2014, three chronological periods were investigated: the initial period (1999–2004, $n = 239$); the period wherein the right liver with middle hepatic vein reconstruction was primarily used (2005–2010, $n = 422$); and the period wherein the right liver was exclusively used with a standardized protocol including a preoperative diet program in donors who had fatty liver, replacement of systemic heparinization, central catheterization, intraoperative cholangiography and preoperative liver biopsy with localized heparin washout, peripheral catheterization, preoperative magnetic resonance cholangiography and spectroscopy, the exact mid-plane parenchymal dissection technique, and incremental application of minimally invasive incisions (2011–2014, $n = 339$).
Results: The proportion of patients aged, more than 50 years (2.5% vs. 4.7% vs. 8.8%) increased, whereas those of patients with a remnant liver volume, <30% (3.3% vs. 12.8% vs. 6.1%) and macrosteatosis, more than 10% (7.9% vs. 11.1% vs. 3.5%) decreased in recent period. A minimal incision (0 vs. 13.0% vs. 36.9%) was increased, and operative time (292.7 min vs. 290.0 min vs. 276.3 min) and hospital stay (12.4 days vs. 11.2 days vs. 9.7 days) were reduced. Overall morbidity (26.4% vs. 13.3% vs. 7.7%) including major, grade III (1.7% vs. 1.9% vs. 0.3%) and biliary (7.9% vs. 5.0% vs. 0.3%) complications were markedly reduced. No intraoperative transfusion was required. No cases of irreversible disability or mortality were noted.

Complication	1999–2004 ($n = 239$)	2005–2010 ($n = 422$)	2011–2014 ($n = 339$)
Grade I			
Wound problem	9 (3.8%)	12 (2.8%)	4 (1.2%)
Pleural effusion	18 (7.5%)	2 (0.5%)	6 (1.8%)
Transient bile leakage	9 (3.8%)	14 (3.3%)	1 (0.3%)
Transient bleeding on drain	3 (1.3%)	10 (2.4%)	0
Paralytic ileus	7 (2.9%)	4 (0.9%)	7 (2.1%)
Grade II			
Bile leakage	7 (2.9%)	5 (1.2%)	0
Pneumonia	0	2 (0.5%)	2 (0.6%)
Psychologic	2 (0.8%)	1 (0.2%)	1 (0.3%)
Hypersensitivity to drug	1 (0.4%)	1 (0.2%)	0
Intraabdominal bleeding	2 (0.8%)	1 (0.2%)	2 (0.6%)
Pulmonary artery embolism	0	0	1 (0.3%)
Intractable ascites	5 (2.1%)	2 (0.5%)	0
Cholangitis	0	0	1 (0.3%)
Grade IIIa			
Biliary stricture	0	1 (0.2%)	1 (0.3%)
Biliary leakage	3 (1.3%)	1 (0.2%)	0
Pneumothorax	1 (0.4%)	0	0
Grade IIIb			
Intraabdominal bleeding	0	6 (1.4%)	0
Rotation of the liver	0	0	1 (0.3%)
	67 cases among 63 donors	62 cases among 56 donors	27 cases among 26 donors

Conclusion: Advances in donor operation for LDLT with excellent outcomes were achieved in recent periods.

O290 LONG TERM OUTCOMES OF LIVING DONOR LIVER TRANSPLANTATION FOR PRIMARY BILIARY CIRRHOSIS – JAPANESE MULTICENTER STUDY

Hiroto Egawa[1], Satoshi Teramukai[2], Shotaro Sakisaka[3], Koji Umeshita[4], Shinji Uemoto[5]
[1]Tokyo Women's Medical University; [2]Kyoto Prefectural University; [3]Fukuoka University; [4]Osaka University; [5]Kyoto University

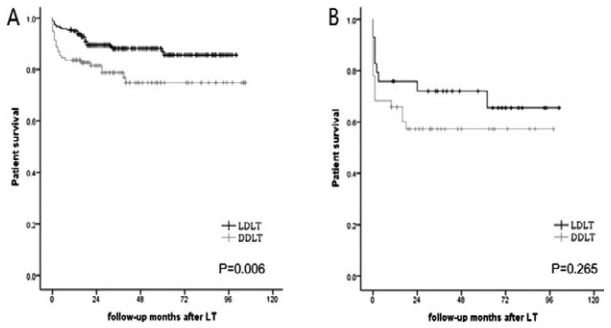
We collected data of 451 patients undergoing primary liver transplantation for primary biliary cirrhosis (PBC) in 28 centers until end of 2010 according to Registry by Japanese Liver Transplant Society.
Patients: Donors were blood relatives in 331, relatives in law in 6, spouses in 105, domino in 1, and deceased donors in 6. Recipient age ranged from 28 to 70 years and donor age ranged from 18 to 66 years. MELD score ranged from 2 to 57, and updated Mayo score ranged from 0 to 96.7. AMA ranged from 0 to 2560, and IgM ranged from 25 to 2024. Number of HLA A-B-DR mismatch was

0 in 20, 1 in 32, 2 in 89, 3 in 123, 4 in 49, 5 in 39, 6 in 19 patients. Graft type was left in 232, right in 216, and whole in 3 patients. Graft recipient weight ratio (GRWR) ranged from 0.45 to 3.25.
Results: Overall patient survival was 76.2%, 80%, and 52.5% in 5, 10, 15 years after LT, respectively. The most frequent cause was infections, malignancies, and hepatic failure, in each period of within 1 year, 1 year by 10 years, later than 10 years after transplantation. Multivariate analysis showed that recipients 61 years old or older, GRWR smaller than 0.8, HLA-mismatch 4 - 6, and husband donors were significant risks for patient survival. PBC recurrence occurred in 67 patients and treatment including steroid was effective histologically and or biochemically. Multivariate Landmark analysis at 1 year including pre-and pot-operative factors showed IgM 554 or greater, and initial immunosuppression with CyA were significant risks for PBC recurrence. There was no significant impact of PBC recurrence on patient survival. Subgroup analysis suggested that immunosuppression regime of tacrolimus followed by cyclosporine might minimize PBC recurrence. In conclusions, risks for patient survival and PBC recurrence were different and the recurrence had no significant impact on patient survival. Awareness for *de novo* malignancies is important to improve long term outcomes.

O291 OUTCOMES OF LIVING AND DECEASED DONOR LIVER TRANSPLANT RECIPIENTS ACCORDING TO THE MELD SCORE

Juhan Lee, Geun Lee Jae, Hwan Song Seung, Jin Joo Dong, Hoon Han Dae, Ki Ju Man, Sub Choi Jin, Kim Soon Il, Soo Kim Myoung
Yonsei University College of Medicine

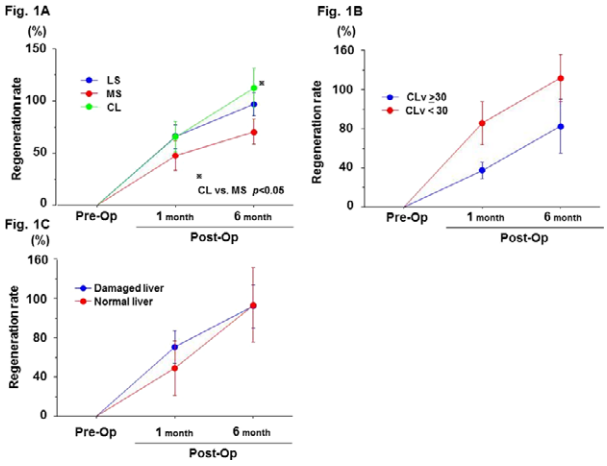
Background: Living donor liver transplantation (LDLT) has developed as an alternative to decease donor liver transplantation (DDLT) to overcome the critical shortage of deceased organ donations. However, the evidence supporting a LDLT for high model for end stage liver disease (MELD) score recipient is weak. We compared the outcomes of LDLT and DDLT according to MELD scores.
Methods: The study included 425 adult patients who underwent liver transplantation between 2006 and 2013 at Severance Hospital (268 LDLT, 157 DDLT). Patients with re-transplantation and fulminant liver failure were excluded from the study. Recipients were categorized according to their MELD score into low (MELD score $\leq$ 25) and high (MELD score $>$ 25) MELD group.
Results: Recipient characteristics were similar between LDLT and DDLT, with the exception of higher MELD score in DDLT group (19.85 vs. 13.52, $p < 0.001$). The DDLT donors were significantly older than LDLT donors (42.55 vs. 31.48, $p < 0.001$). Hepatocellular carcinomas were present in 57.4% of the recipients (60.8% in LDLT vs. 51.6% in DDLT, $p = 0.063$). The median follow-up was 32 months (range, 0 to 105 months). LDLT demonstrated significantly better patient survival than DDLT in low MELD group (86.9% vs. 74.8% at 5 years, $p = 0.006$). Survival after LDLT was not inferior to DDLT in high MELD group (72.1% vs. 57.3% at 5 years, $p = 0.265$).
Conclusion: LDLT provided similar survival to DDLT in high MELD score recipients. Thus, when deceased donor organs are scare, a high MELD score should not be a contraindication to LDLT.

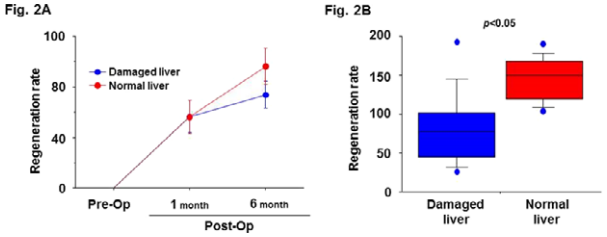


O292 THE LIVER REGENERATION OF LEFT LOBE AFTER RIGHT LIVER RESECTION: THE COMPARISON AMONG CAUDATE LOBE, LATERAL AND MEDIAN SEGMENT

Yu Saito, Hiroki Teraoku, Masato Yoshikawa, Shinichiro Yamada, Tetsuya Ikemoto, Yuji Morine, Satoru Imura, Mitsuo Shimada
Department of Surgery Tokushima University

Background: We previously reported that that reconstruction of short hepatic vein (SHV) might be taken into consideration in case of over 30 ml of preoperative caudate lobe volume (CLv) (ESOT 2011). In this study, as the surrogate model of extended left liver graft with complete SHV reconstruction, we compared the liver regeneration rate of remnant left lobe in each segment after right liver resection.
Methods: Twenty-six patients with right liver resection in our department were enrolled in this study. The patients were divide into two groups, damaged liver group with primary liver tumors ($n = 19$) and normal liver group with metastatic or benign liver tumors ($n = 7$). The CT volumetry of each segment (CL, lateral segment [LS] and median segment [MS]) was performed using Synapse Vincent® before the operation, one from 2 month and 6 months after the operation.
Results: In all patients, the regeneration rate of the CL significantly better than LS or MS at 6 months after the operation (CL; 113 $\pm$ 86%, LS; 97 $\pm$ 50%, MS; 71 $\pm$ 56%, CL vs. MS $p = 0.04$) (Fig. 1A) and, the regeneration rate of the preoperative CLv 30 ml at both 1 and 6 months after operation (CL 30 ml; 37 $\pm$ 26%, at 1 month after operation, $p = 0.07$) (Fig. 1B). There was no significant difference in the regeneration pattern of CL between damaged and normal liver group (Fig. 1C). The liver regeneration rate in left lobe in the patients with damaged liver group was significantly worse than that of the patients with normal liver group at 6 months after the operation. Especially, the regeneration pattern of LS in damaged liver group was significantly worse than that of normal liver group.
Conclusion: Our results suggested that the regeneration of CL might be better than LS or MS especially in case of over 30 ml of preoperative CLv in the remnant liver after right liver resection.





O293 POST-OPERATIVE THROMBOCYTOPENIA MAY DETERMINE THE FATE OF ADULT-TO-ADULT LIVING DONOR PARTIAL LIVER TRANSPLANTATION: PROPOSAL OF THROMBOTIC MICROANGIOPATHY (LTX-TMA) SCORE

Hirokazu Tanaka[1], Koichiro Hata[1], Hirofumi Hirao[1], Yusuke Okamura[1], Toyonari Kubota[1], Osamu Inamoto[1], Shintaro Yagi[1], Kohei Ogawa[1], Yasuhiro Fujimoto[1], Akira Mori[1], Hideaki Okajima[1], Toshimi Kaido[1], Rene Tolba[2], Shinji Uemoto[1]
[1]Division of Hepato-Biliary-Pancreatic Surgery and Transplantation, Department of Surgery, Kyoto University Hospital; [2]Institute for Laboratory Animal Science, RWTH Aachen University

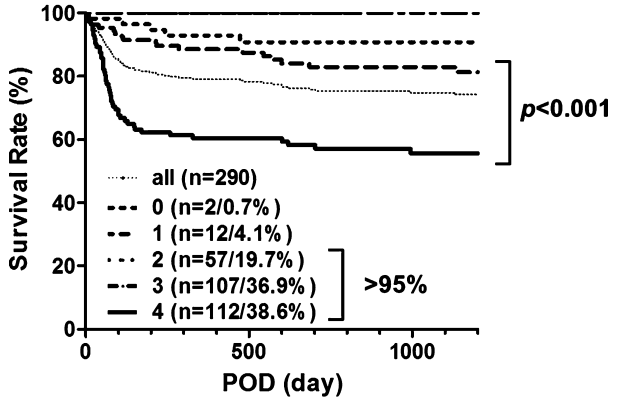
Background: It has recently been recognized that post-operative thrombocytopenia may predict the outcome of liver transplantation (LTx), however, the underlying pathophysiologies have not been fully elucidated. Here we propose the concept of "LTx-associated thrombotic microangiopathy (LTx-TMA)", as the crucial pathology in post-LTx thrombocytopenia.
Methods: We retrospectively analyzed the clinical characteristics/data in the consecutive 290 cases of primary adult partial LTx in our single center between April 2006 and March 2013, with special interest to the diagnostic criteria of

transplant-associated TMA (TA-TMA), as follows: (i) thrombocytopenia (PLT < 50 000 mm^3), (ii) hemolytic anemia (Hb500 IU/l), and (iv) appearance of schistocytes.
Results: Among various clinical parameters, only post-LTx platelet count was significantly associated with 6-months mortality. Moreover, patients' survival was clearly deteriorated according to the degree of thrombocytopenia. Regarding the TA-TMA criteria, thrombocytopenia occurred in 253 out of 290 cases (87.2%), hemolytic anemia was observed in 271 (93.4%), LDH elevation in 166 (57.2%), and schistocytes appeared in 205 (70.7%).

	(+)	(−)
PLT < 5.0 × $10^4/mm^3$	253 (87.2%)	37 (12.8%)
Hb < 8.0 g/dl	271 (93.4%)	19 (6.6%)
LDH > 500 IU/l	166 (57.2%)	124 (42.8%)
Schistocyte (+)	205 (70.7%)	85 (29.3%)

Of note, 95.2% (276 cases) exhibited at least 2 characteristics of TA-TMA, and surprisingly, 112 cases (38.6%) met all 4 characteristics by 60 post-operative day (POD). Furthermore, 6-months mortality in 14 cases those met only 0 or 1 criteria was 0%, while that in the 112 recipients met with all 4 criteria increased up to 25%. As summarized in the figure, the more factors were fulfilled, the more deteriorated the patients' survival was.

TMALS score may reflect the prognosis after LTx



Conclusion: Although multifactorial pathologies may involve the post-LTx thrombocytopenia, LTx-TMA plays a pivotal role in such critical pathology. Thus, LTx-TMA seems not only to be an important predictor of prognosis, but to be a novel therapeutic target for improving high mortality and morbidity after LTx.

O294 IMPACT OF DONOR AGE ON THE OUTCOME OF ADULT LIVING DONOR PARTIAL LIVER TRANSPLANTATION: SINGLE-CENTER EXPERIENCE IN 316 PATIENTS

Toyonari Kubota[1], Koichiro Hata[1], Takashi Sozu[2], Hirokazu Tanaka[1], Hirofumi Hirao[1], Yusuke Okamura[1], Osamu Inamoto[1], Kohei Ogawa[1], Yasuhiro Fujimoto[1], Akira Mori[1], Hideaki Okajima[1], Toshimi Kaido[1], Shinji Uemoto[1]
[1]Department of Surgery, Hepato-Biliary-Pancreatic and Transplantation, Kyoto University of Medicine; [2]Department of Biostatistics, Kyoto University Graduate School of Medicine

Background: It is well-known that the donor age is an important prognostic factor in deceased donor liver transplantation (DDLT). However, little is known about the influence of donor age on the outcome in living donor liver transplantation (LDLT).
Methods: We retrospectively analyzed the consecutive 316 cases of primary adult LDLT (recipient age > 18 years-old) in our single center between April 2006 and September 2013. The 316 recipients were divided into 5 groups according to the donor age: 20's, n = 60 (19%); 30's, n = 72 (23%); 40's, n = 57 (18%); 50's, n = 95 (30%); 60's, n = 32 (10%), respectively. The recipients' survival and various clinical factors were investigated and compared among the 5 groups.
Results: The overall recipient survival proportion in Group-20's was significantly higher than in the other groups, as summarized in Fig. 1 (p = 0.008, <0.001, <0.001,, and 0.005, vs. 30's, 40's, 50's, and 60's, respectively), while no significant difference was observed as compared with recipient age. Regarding the background etiologies of cirrhosis, Group-20's demonstrated significantly better recipients' survival proportion than in others both in HCV-related (p = 0.084, 0.006, 0.006, and 0.024 vs. 30's, 40's, 50's, and 60's, respectively), and in the other etiologies (p = 0.02, 0.006, 0.038, and 0.05, respectively). Univariate analysis revealed that donor age (p = 0.002) and the graft type (p = 0.006) were significant risk factors for 6-months mortality of adult LDLT recipients, whereas in multivariate analysis, only donor age was identified as an independent risk factor (p = 0.013) for poor recipients' survival, as detailed in Table-1. The odds ratio of donor age was 1.04/year, indicating 5.84-times higher risk in recipients' perioperative mortality with 65 years-old donors, compared with 20 years-old.
Conclusions: Donor age is an independent, strong prognostic factor in adult-to-adult LDLT, rather than various clinical factors of recipients.

Fig.1 *Overall Survival of Adult LDLT Recipients According to donor age*

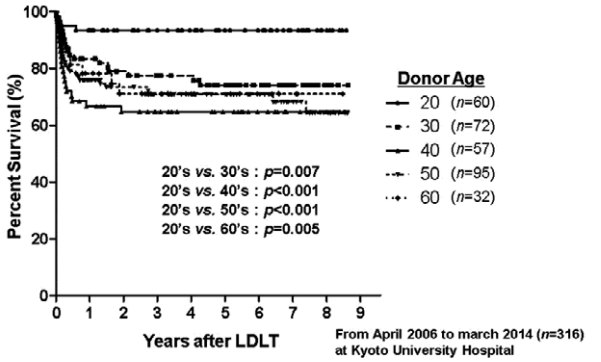


Table.1 *Prognostic Factors of Perioperative Recipints' Survival after LDLT (Multivariate Analysis)*

	Odds ratio	95% CI	P value
Donor age	1.04	1.01〜1.08	0.013
Graft type (Right or Left lobe)	0.47		0.084
ABO compatibility (identical/ compatible/ incompatible)			0.282
GRWR (%)	1.43	0.18〜11.4	0.732
MELD score	1.02	0.97〜1.07	0.493
Final PVP	1.09	0.97〜1.23	0.149

GRWR: Graft Recpient Weight Ratio
MELD: Model for End-stage Liver Disease
PVP: Portal Venous Pressure

O295 THE MELD-ADJUSTED GRAFT-TO-RECIPIENT WEIGHT RATIO THRESHOLD FOR LIVING DONOR LIVER TRANSPLANTATION

Arzu Oezcelik, Murat Dayangac, Yildiray Yuzer, Yaman Tokat
General and Transplantation Surgery Istanbul Science University

Background: The graft-to-recipient weight ratio (GRWR) is an important selection criteria for living donor liver transplantation (LDLT). The generally accepted threshold for adults is known to be 0.8. However, in patients with low MELD-score we reduced this threshold. The aim of this study was to evaluate the results of these patients with GRWR < 0.8.
Patients and Methods: Between 2005 and 2013, 446 patients underwent LDLT for end-stage liver disease. All recipients who had a GRWR <0.8 were identified. The clinical characteristics, MELD-score, preoperative, intraoperative and postoperative data, re-transplantations, graft and patient survival were retrospectively analyzed. The results were compared to patients with GRWR ≥ 0.8.
Results: There were 43 patients (10%), who underwent right lobe LDLT with GRWR < 0.8. Out of these patients, seven (2%) had a GRWR of 0.6. The mean MELD-score was 14. There were no intraoperative complications. Postoperative complications, such as biliary leakage or stricture, were seen in 10 patients (23%). Of all 43 patients three died perioperatively within 1 month and one patient underwent re-liver transplantation due to graft failure. The mean hospital stay was 18 days. The 1-year survival rate was 93% (Figure). The overall survival was 38 month. None of the patients had a MELD-score above

20. The comparison of the results with the patients who had a GRWR ≥0.8 has shown no significant difference but MELD-score and BMI, which were both significantly higher.
Conclusion: Based on the results of our study, we conclude that the GRWR can be reduced safely even to 0.6 in patients with low MELD-score. More criteria are needed in order to individualize the GRWR threshold.

	GRWR < 0.8 (n = 43)	GRWR ≥0.8 (n = 403)	p-Value
Age (mean)	51 years	50 years	0.9
BMI (mean)	29	26	0.0001*
MELD-score (mean)	14	19	0.01*
Hospital stay (mean)	18 days	20 days	0.4
Postop. complications	10 (23%)	128 (31%)	0.2
Periop. mortality	3 (7%)	40 (9%)	0.8
Re-Transplantation	1 (2%)	11 (2%)	1.0
One-year-survival	93%	91%	0.5

O296 A CONSECUTIVE SERIES OF 100 CONTROLLED DCD-LIVER TRANSPLANTATIONS

Olivier Detry[1], Arnaud Deroover[1], Hieu Ledinh[1], Nicolas Meurisse[1], Marie-France Hans[1], Jean Joris[2], Séverine Lauwick[2], Abdour Kaba[2], Michel Meurisse[1], Pierre Honore[1]
[1]Department of Abdominal Surgery & Transplantation, University of Liege, CHU Liege; [2]Department of Anaesthesiology, University of Liege, CHU Liege

Introduction: Donation after circulatory death (DCD) have been proposed to partially overcome the organ donor shortage. DCD-LT remains controversial, with reported increased risk of graft loss and retransplantation. The authors retrospectively reviewed a single centre experience with controlled DCD-LT in a 12-year period.
Patients and Methods: 100 DCD-LT were consecutively performed between 2003 and 2014. All donation and procurement procedures were performed as controlled DCD in operative rooms. Data are presented as median (ranges). Median donor age was 57 years (16–83). Median DRI was 2.16 (1.4–3.4). Most grafts were flushed with HTK solution. Allocation was centre-based. Median recipient MELD score at LT was 15 (7–40). Mean follow-up was 35 months. No patient was lost to follow-up.
Results: Median total DCD warm ischemia was 19 min (10–39). Median cold ischemia was 235 min (113–576). Median peak AST was 1132 U/l (282–21 928). Median peak bilirubin was 28 mg/dL. Patient survivals were 90.7%, 75.5% and 70.7% at 1.3 and 5 years, respectively. Graft survivals were 88.7%, 72.1% and 67.1% at 1.3 and 5 years, respectively. Biliary complications included mainly anastomotic strictures and extrahepatic main bile duct ischemic obstruction, that were managed either by endoscopy or hepatico-jejunostomy. No PNF or graft loss due to ischemic cholangiopathy was observed in this series.
Discussion: In this series, DCD LT appears to provide results similar to classical LT. Short cold ischemia and recipient selection with low MELD score may be the keys to good results in DCD LT, in terms of graft survival and avoidance of ischemic cholangiopathy. If symptomatic ischemic cholangiopathy is diagnosed, adequate management with endoscopy and surgical hepaticojejunostomy may avoid graft loss and retransplantation.

O297 UTILITY OF INTERPOSITION DACRON GRAFTS FOR RECONSTRUCTION OF ANTERIOR SECTOR DRAINAGE VEINS IN RIGHT LOBE LIVING DONOR LIVER TRANSPLANTATION

Murat Dayangac, Sezgi Barlas, Ali Kagan Gokakin, Sema Baghaki, Barkin Malkoc, Altan Alim, Yalcin Erdogan, Arzu Oezcelik, Yildiray Yuzer, Yaman Tokat
Istanbul Bilim University Florence Nightingale Hospital

Reconstruction of anterior sector (AS) drainage veins using interposition homologous or prosthetic grafts has been an established technique in right lobe (RL) living donor liver transplantation (LDLT). Material of choice used for this type of reconstruction have been cryopreserved homologous grafts, because of lower patency rates reported for prosthetic grafts. However, with relative shortage of cryopreserved grafts, prosthetic grafts have the advantage of their unlimited availability. This study investigates short-term patency rate of polyester (Dacron®) grafts used as venous conduit for AS drainage of RL grafts.

Between January 2014 and December 2014, 51 of 80 (63%) patients who underwent LDLT in our institution received a RL graft with AS venous reconstruction including isolated segment 5 (n = 5), isolated segment 8 (n = 6), or combined segment 5 and 8 (n = 40) drainage. A separate accessory inferior right hepatic vein reconstruction was also performed in 16 (31%) patients. All reconstructions were performed using Dacron grafts.

Dacron graft patency was investigated in 75% (n = 38) of the patients using either Doppler ultrasound (n = 25) or computed tomography (n = 29). Dacron graft was patent in 32 of 38 patients (84.2%) in a median time of 37 (10.0–97.5) days after LDLT. In 6 patients with AS venous outflow obstruction, no significant clinical consequence was observed. There was 1 perioperative mortality due to sepsis and 1 graft loss due to initial poor function, which needed retransplantation. In a median follow-up of 7 (5–10) months, 49/51 (96%) patients were alive. Dacron grafts have high short-term patency rates comparable to those of cryopreserved homologous grafts; thus, they offer an excellent source of interposition material for reconstruction of AS drainage veins in RL LDLT.

O298 OUTCOMES FOLLOWING LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA USING DONOR AFTER CIRCULATORY ARREST VERSUS DECEASED BRAIN DEAD DONOR GRAFTS

Markus Selzner[1], Trevor Reichman[2], John Seal[2], Rachel Evers-Meltzer[2], Ian Carmody[2], Anand Ghanekar[1], Ian Mcgilvray[1], Mark Cattral[1], Paul Greig[1], Humberto Bohorquez[2], Ari Cohen[2], David Bruce[2], George Loss[2], Gonzalo Sapisochin[1]
[1]University of Toronto; [2]Ochsner Medical Center

Background: Transplantation of liver grafts from donors after circulatory death (DCD) is now an accepted practice with equivalent outcomes when compared with standard deceased brain dead (DBD) donors. The outcomes of patients transplanted in the setting of hepatocellular cancer (HCC) with DCD versus DBD still remain controversial due to questionable poorer outcomes with DCD donors. However, prior studies have focused only on overall survival and ignored the impact of recurrence on survival.
Methods: A multicenter review of a combined HCC database (Ochsner Medical Center and Toronto General Hospital) was performed from 1/2008 to 12/2013.
Results: 385 patients (41 DCD and 344 DBD) were identified and included in the analysis. There were 49 recurrences (14%) in the DBD group versus 6 recurrences (14%) in the DCD group (p = 0.946). The recurrence-free survival was equivalent for the DCD versus DBD groups (p = 0.819). Similarly, overall 1/3 year survival was 94%/85% and 92%/83% for the DBD versus the DCD groups, respectively. In multivariate regression analysis, lymphovascular invasion, tumor number (>5), and tumor size (>5 cm) were shown to be significant predictors of tumor recurrence not donor type.
Conclusion: DCD liver transplants when performed in experienced centers yield equivalent oncologic outcomes for patients transplanted with HCC.

O299 BILIARY RECONSTRUCTION IN LIVER TRANSPLANT PATIENTS WITH PRIMARY SCLEROSIS CHOLANGITIS

Saman Nikeghbalian[1], Alireza Shamsaeefar[1], Mohammad Shafiee[1], Nasrin Motazedian[2], Seyed Ali Malekhosseini[2]
[1]Shiraz Organ Transplant Center; [2]Shiraz HIV/AIDS Research Center

Objectives: Traditionally Roux-en-Y hepaticojujenostomywas the method of choice for biliary reconstruction in primary sclerosingchollangitis (PSC) in patients undergoing orthotopic liver transplantation. In this study, we compared the result of duct to duct anastomosis versus Roux-en-Y hepaticojujenostomy as biliary reconstruction in patients with primary sclerosingchollangitis who underwent liver transplant in Shiraz organ transplant center.
Methodsand Materials: There were 69 patients with primary sclerosingchollangitis who underwent liver transplant. Mean follow up period was 36.5 months (18–55 months). We performed duct to duct reconstruction in those patients who had grossly normal bile duct during hepatectomy. In 29 cases duct to duct reconstruction was done and Roux-en-Y hepaticojujenostomy reconstruction in 40 cases. Data collecting form contained biliary complications (leak, stricture, and cancer in the remnant bile duct), documented episodes of rejection, and morbidity.
Results: In duct to duct group, two patients presented with anastomotic site stricture and one patient developed chollangiocarcinoma in distal bile duct which underwent pancreaticoduodenectomy (3/29). In Roux-en-Y group, five patients developed anastomotic stricture in the follow up (5/40). This difference was not significant (p value = 0.999). Also documented episodes of rejection were similar between two groups (Chi square test, p value = 0.66) and there was no significant difference.
Discussion: We concluded that duct to duct reconstruction is safe and maybe the choice method for biliary reconstruction in some patients with PSC. In addition, due to innovations in ERCP, management of strictures in duct to duct group was more easy and feasible in comparison to revision of Roux-en-Y hepaticojujenostomy

O300 ISCHAEMIC CHOLANGIOPATHY IN DCD LIVER TRANSPLANTATION: RADIOLOGICAL FEATURES

Irene Scalera, Anna Murray, Simon Olliff, Brinder Mahon, John Isaac, Hynek Mergental, Darius Mirza, Thamara Perera, Garret Roll, Paolo Muiesan
Queen Elizabeth Hospital

Ischaemic cholangiopathy (IC) is one the main causes of morbidity and graft loss after liver transplantation from donation after circulatory death (DCD). The diagnosis is based on magnetic resonance cholangio-pancreatography (MRCP) features. An existing classification based on donor after brain death grafts was tested by 2 independent expert liver radiologists, blinded to each other's reports, on our DCD liver recipients diagnosed with IC. The scores produced were highly inconsistent. We then looked for MRCP imaging patterns of IC in DCD grafts, which are consistent and easily recognizable, aiming to correlate these to clinical outcomes. All the IC cases were identified from a prospectively collected DCD liver transplant database. MRCP images were reviewed independently by two expert liver radiologists. MRCP features were identified and the clinical outcomes correlated. Of 254 DCD liver transplants, 22 (8.7%) cases of IC were diagnosed. Mean time from grafting to development of IC was 5.3 $\pm$ 6.4 months. Two recipients with IC died, one patient was retransplanted, 6 were managed with endoscopic procedures and 13 treated conservatively. Three main MRCP features of IC were identified: biliary strictures (BS), leaky ducts (LD) and filling defects (FD). Two patients had FD alone and one LD and FD combined. BS were identified in 19/22 patients. Of these, 6 had associated FD. Three cases showed all 3 radiological characteristics and were associated with the worst outcomes: one patient was retransplanted and two patients listed for regrafting. Imaging-related characterization of IC may be relevant to predict prognosis and for a prompt therapeutic management. This preliminary observational study of MRCP features of IC shows that coexisting BD, FD and LD are associated with worst prognosis and need for retransplantation. Further studies are required to analyze the correlation between the MRCP appearance and clinical outcomes of DCD recipients affected by IC.

O301 THE LENGTH OF PRE-ANASTOMOTIC COMMON HEPATIC DUCT IS A RISK FACTOR OF BILIARY COMPLICATIONS AFTER LIVER TRANSPLANTATION?

Roberto Montalti[1], Federico Mocchegiani[1], Daniele Nicolini[1], Andrea Vecchi[1], Paolo Vincenzi[1], Andrea Giovagnoni[2], Marco Vivarelli[1]
[1]Chirurgia dei Trapianti - Ospedali Riuniti – Ancona; [2]Radiology - Ospedali Riuniti - Ancona

Introduction: The biliary anastomosis is still considered the Achilles' heel of liver transplantation (LT) because the incidence of biliary stricture is approximately 30%. It is common opinion that the length of the pre-anastomotic common hepatic duct should be as short as possible, in order to preserve the biliary blood supply, although this data was never analyzed. The purpose of present study was to verify if the length of the pre-anastomotic common hepatic duct influence the rate of biliary strictures or leakage in patients undergoing LT.
Materials and methods: From 8-2005 to 4-2014 we performed 323 liver transplants in 307 patients. The inclusion criteria were: first LT, duct-to-duct anastomosis with T-tube drainage, minimum follow-up of 3 months, the possibility of measuring of the distance of the extrahepatic bile duct at the cholangiography in POD 7. In 202 patients the distance between the first order biliary bifurcation and the biliary anastomosis was measured.
Results: After a mean follow-up of 43 $\pm$ 28 months, 46 (22.8%) patients developed a biliary stricture, of which 38 (18.8%) were anastomotic strictures. 15 (7.4%) patients developed a biliary leakage. The average length of the pre-anastomotic common hepatic duct of the graft was 34.1 $\pm$ 9.8 mm in patients who didn't develop a biliary stenosis vs. 36.3 $\pm$ 9.9 mm in patients who experienced a biliary stricture ($p = 0.19$). The average length was 34.2 $\pm$ 9.7 mm vs. 36.3 $\pm$ 10.5 mm in patients who didn't develop an anastomotic stricture vs. patients who developed an anastomotic stricture, respectively ($p = 0.23$). The average length of the pre-anastomotic bile duct was 34.3 $\pm$ 9.5 mm vs. 38.9 $\pm$ 13 mm, in patients who didn't develop a biliary leakage and who developed a biliary leakage, respectively ($p = 0.078$).
Discussion: Contrary to popular belief, the length of the pre-anastomotic common hepatic duct is not a risk factor statistically associated to the developement of biliary compliclications after LT.

O302 SOMATOSTATIN INFUSION ALLOWS REVERSIBLE GRAFT FLOWS AND PRESSURE GRADIENT MODULATION IN CLINICAL LIVER TRANSPLANTATION: RESULTS OF A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL

Mauricio Sainz Barriga[1], Jurgen Van Limmen[2], Bjorn Heyse[2], Baerdemaeker Luc De[2], Isabel Colle[3], Roberto Ivan Troisi[4]
[1]HPB & Liver Transplantation Department, Tours University Hospital & Medical School; [2]Anesthesiology Department, Ghent University Hospital & Medical School; [3]Gastroenterology Department, Ghent University Hospital & Medical School; [4]General & HPB Surgery Department, Ghetn University Hospital & Medical School

Background: Despite liver transplantation (LT) previous portal hypertension and hyperdynamic status do not immediately ameliorate, exposing the graft to the risk of hemodynamic (HD) damage, mainly when the graft cannot accommodate to HD stress. To evaluate the safety and the pharmacological properties of somatostatin (SST) as hepatic HD modulator a prospective randomized double blind, placebo-controlled study was designed.
Methods: Patients with intraoperative hepatic vein pressure gradient (HVPG) $\geq$10 mmHg, were allocated in a 1:2 ratio to either receive placebo (P) or SST (S). All included patients received a 5 cc bolus injection. Group S received STT and group P normal saline. Afterwards, group S received a continuous infusion of SST during 5 days starting at the anhepatic phase, while P group received the same amount of standard intravenous fluids. A 20% reduction of portal vein flow (PVF) or HVPG during bolus infusion was defined as responder to treatment.
Results: 21 patients were included in group S and 12 in group P. No early graft failure or patient death was observed. At a median follow up of 24 months (IQR 14–42), all patients but 5 (2 in group P and 3 in group S) were in good health with normally functioning grafts. All patients in group S were responders to STT treatment. HD comparison of group S with group P revealed: PVF decrease ($p = 0.02$), HVPG decrease ($p = 0.016$), and a trend for higher hepatic artery flow (HAF) ($p = 0.058$). No vascular thrombosis was observed. No significant differences in AST and INR peaks as well as in proteomic expression between both groups were observed.
Conclusions: SST has proven to be safe and useful to consistently reduce PVF and HVPG allowing accommodation of the graft to the HD stress early after LT. The trend for HAF increase could be particularly interesting in partial graft transplantation where its value can be decreased.

O303 THE CZECH EXPERIENCE WITH SPLIT LIVER TRANSPLANTATION INCLUDING FULL LEFT/FULL RIGHT: 2 YEARS OF THE PROGRAM, 15 SPLITS AND 30 TRANSPLANTS

Jiri Fronek, Libor Janousek, Matej Kocik, Milos Kucera, Martin Oliverius, Eva Kieslichova, Eva Pokorna, Pavel Trunecka
Institute for Clinical and Experimental Medicine (IKEM), Prague

Background: Split liver transplantation can help with organ shortage and serve some of the small adults and pediatric recipients. The systematic split liver program has been introduced in Czech Republic in Jan 2013.
Methods: Retrospective analysis of 15 split liver procedures. Initial experience in 1998 included two classical splits and three transplants, two pediatric recipients died from graft dysfunction early after transplant, one adult lived for 6 years. One classical split was done in 2009, both recipients are alive. Split liver program has been set up at our unit in 2013, this means mandatory split if donor meats predetermined criteria.
Results: There were 15 split liver procedures performed since 2013, of those 12 classical for child and adult, 3 full left/full right for 2 adults. All procedures we performed *in vivo*. Mean deceased donor age was 29.5 years (SD 17.5), weight of 86.5 kg (SD 21.5). All recipients except one child are alive and well, all the 29/30 recipients developed immediate graft function. Some 5 patients had biliary leak, treated with stent placement in 1 case, four cases treated with re-operation. In one case we did full split for two fulminant liver failure patients, husband and wife who poisoned themselves by mistake with mushroom amanita phalloides. One of the recipients was AB0i on top of that. Since 2013 the average waiting time for pediatric recipients has changed from 271 days down to 35 days. There was no death of the pediatric recipient on the waiting list since the introduction of the program.
Conclusions: Split liver transplantation has been introduced and helped to decrease the waiting time for pediatric transplant, it may help to avoid deaths on the waiting list as well. Full left/full right split liver for two adults is feasible, in case of two fulminant liver failure patients it served both recipients successfully, in two other cases we have successfully treated four stable small adults.

O304

REAL-TIME MEASUREMENTS OF TISSUE OXYGEN MICROTENSION AS A MARKER OF BILE DUCT VIABILITY IN LIVER TRANSPLANTATION

Elena Navarro-Rodriguez, Ruben Ciria-Bru, Javier Medina-Fernandez, Belen Gallardo-Herrera Ana, Marina Sanchez-Frias, Dolores Ayllon-Teran Maria, Sebastian Rufian-Peña, Pedro Lopez-Cillero, Javier Briceño-Delgado
HU Reina Sofia

Aims: To evaluate bile duct viability by assessing its microvascular quality using an innovative real-time oxygen tension device by testing different areas in both donor and recipient's side. The findings were correlated subsequently with immunohistochemical and histopathological results.
Methods: Observational prospective cohort study with 18 patients included from November 2013 to September 2014. Tissue oxygen microtension measurements were made using Oxylite® device in different areas of recipient and donor's bile duct intraoperatively after biliary anastomosis was made.
Results: Mean oxygen microtension value in the graft bile duct at anastomosis level was 106 (92.5–118) mmHg, being 125 (108.5–134.5) mmHg 1.5 cm proximal to the hilar plate. Mean micro-oxygenation value in the bile duct recipient was 117.5 (100.5–150) mmHg, whilst a value of 138 (119–183) mmHg was observed 1.5 cms distal to the anastomosis. Tissue oxygen microtension was statistically higher in distal areas to section border of the biliary anastomosis, with an overall pO2 increase distal to the anastomosis of 17.94 mmHg ($p < 0.001$) and 21.61 mmHg ($p < 0.001$) in the graft and recipient, respectively. Biliary anastomosis was performed above the cystic duct insertion in the donor bile duct in 10 patients, with significant higher values of pO_2 microtension ($p = 0.017$). Histological injury grade 2–3 in biliary mural stroma and grade 1–3 in peribiliary vascular plexus of graft's bile duct graft were associated with lower tissue oxygen pressure, as well as injury grade 2 in biliary epithelium and grade 1–3 in peribiliary vascular plexus of recipient's bile duct were associated with lower micro-oxygenation ($p < 0.05$).
Conclusion: Our results domonstratrates that terminal border of donor and recipient bile duct are low-vascularized areas. Tissue microoxygenation improves significantly in areas close to the hilar plate and to the duodenum in the donor and recipient's sides, respectively.

007 DONATION/RETRIEVAL

O305 LIVING DONATION HIGH QUALITY PRACTICES: LIDOBS NETWORK RECOMMENDATIONS

Ana Menjivar[1], Marti Manyalich[2], Christina Papachristou[3], David Paredes[2], Ignacio Revuelta[2], Constantino Fondevila[2], Fritz Diekmann[2], Jordi Colmenero[2], Xavier Torres[2], Entela Kondo[2], Entela Kondo[4], Entela Kondo[4], Entela Kondo[4]

[1]Fundacio Clinic of Barcelona; [2]Hospital Clínic of Barcelona; [3]Charité Hospital; [4]LIDOBS Network

Introduction: LIDOBS is a scientific network and shall function as a platform where international professionals, actively working in Living Organ Donation (LOD), can exchange knowledge, engage in discussions, and set priorities following the values- Safety, quality and transparency. Recommendations on best practice for LOD were developed in the context of LIDOBS International Conference on Living Donation- High Quality Practices, Barcelona, 2014. This Conference has received funding from the European Union.
Objective: To achieve and formulate consensus and recommendation for LOD practices in order to assure high quality practices. To set up a community of experts in LOD Programs that will continue to expand and increase the knowledge. To disseminate the results of EU projects on LOD.
Methodology: Structured six Working groups

Tool used- LIDOBS Consensus Canvas: to measure the Impact and Feasibility of each recommendation.

Stages: Preconference: Starting point was previous discussions and references documents to moving forward to achieve recommendations on strategies and actions to be taken. Conference: Proposed recommendations were presented to the Conference attendees and underwent a televoter process. Postconference: a consultation process was developed to define the final conclusions.
Results: Conference attendance: more than 100 participants, 55 Institutions from 31 countries, 4 Continents.

Outcomes: Consensus on 22 recommendations for High quality practices. Network Expansion: 28 professionals from 20 Institutions from 13 countries signed the Consortium agreement. Active Online platform for networking.
Conclusions: The establishment of international consensus in terms of ethical and legal aspects, protection practices, medical-psychosocial follow-up's and registration issues will protect Living donors' health and safety. The safety of the living Donors, the quality and transparency of LOD programmes are and will remain the main aims of LIDOBS.

O306 COMPREHENSIVE COMPUTERIZED LIVING LIVER DONOR EVALUATION TO INCREASE CANDIDATES CONSIDERED FOR DONATION

Hoylan Fernandez[1], Amar Gupta[1], Peter Kim[1], Tiffany Anthony[1], Nicolas Onaca[1], Richard Ruiz[1], Greg Mckenna[1], Robert Goldstein[1], Marlon Levy[1], Goran Klintmalm[1], Claus Niemann[2], Giuliano Testa[1]

[1]Baylor University Medical Center; [2]Medsleuth

Public interest in living liver donation (LLD) has increased over the years. Donation is greatly dependent on recipient efforts to identify and recruit donor candidates. We implemented (April 2014) a web-based software platform (BREEZE TRANSPLANT™, MedSleuth, Inc.) to increase access to patients, expedite patient work-up and improve efficiency. Here we report our initial experience with the software.

The software was designed to triage patients not meeting basic eligibility criteria for LLD (e.g., BMI, age, Hep C) and elicit a comprehensive transplant-specific medical history for those LLD candidates meeting basic eligibility criteria. To elicit this medical history the software employs branch chain logic and machine learning to generate a customized questionnaire for each LLD candidate. All completed questionnaires were immediately available for healthcare providers. We performed a service line performance analysis pre and post software implementation.

134 LLD candidates were evaluated in 2013; 152 from April-December 2014 (annualized 190; $p < 0.05$ increase vs. 2013); and ~300 projected for 2015. 2015 LLD transplant volume is projected to increase 40–60% vs. 12 in 2014. LLD data availability (e.g., screen out reason, medications, medical/ surgical history, health related behavior, demographics, donor-recipient relationship) significantly improved post software implementation. The software ruled out 26.3% of LLD candidates (most frequently for BMI, no insurance) and elicited comprehensive medical histories for the remaining candidates (mean age = 40 ± 11 years, 65.2% female, 34.8% male, median time to complete = 16.3 min).

Patient-centered web-based software can be effectively administered to facilitate LLD candidacy evaluation. The introduction of an automated intake system increased the total number of LLD candidates and projected donors. Automated evaluation expedites initial screening, with a reduction in staff time devoted to non-elgible donors.

O307 PREDICTORS OF LOW EGFR AFTER LIVING KIDNEY DONATION IN A SOUTH-EAST ASIAN POPULATION FROM SINGAPORE

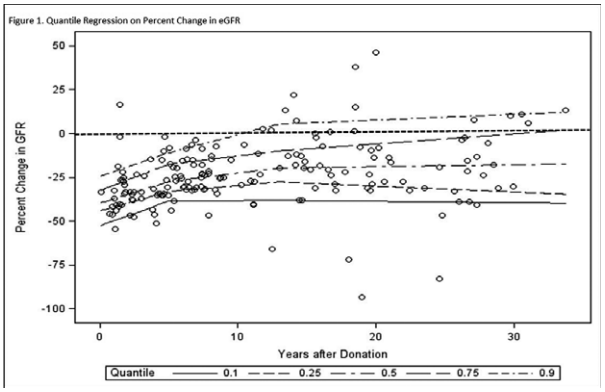
Yu Tan Ru[1], Carson Allen John[2], Yi Shern Kee Terence[1], Weng Kit Lye[2], Tazeen Hasan Jafar[1]

[1]Singapore General Hospital; [2]Duke-NUS Graduate Medical School

We analysed data on a retrospective cohort of kidney donors to determine the pattern of and factors associated with change in kidney function after kidney donation and determine a suitable cut-point of pre-donation eGFR which predict risk of low eGFR after kidney donation.

Quantile regression analysis of percent change in CKD-EPI eGFR from pre- to post-nephrectomy level was performed and comparisons of demographic and clinical characteristics of the entire cohort were done according to period after donation: short-term (>6 months to <5 years post-donation), mid-term (5 to <10 years post-donation), long-term (≥10 years post-donation). Univariate and multivariate logistic regression and ROC analyses were performed to investigate and evaluate predictors of low eGFR of <60 ml/min post donation.

174 donors with mean age of 40.7 years were recruited. They were predominantly female (63.8%) and of the Chinese race (73%). Median (range) follow-up was 7.8 (0.1–34) years. 30 donors (17%) developed low eGFR <60 ml/min. 43.1% ($n = 75$) of donors recovered 75% or more of pre-nephrectomy eGFR after 5 years post donation. 9.8% ($n = 17$) exhibited 100% recovery of pre-nephrectomy eGFR after an average of 19.2 years. After controlling for potential confounders, high pre-nephrectomy eGFR and more years after donation were protective for post-donation eGFR <60 mL/min. Risk of eGFR <60 mL/min was reduced by 6% for each unit increase in pre-donation eGFR (OR:0.94, 95% CI:0.91–0.97, $p = 0.0002$) and by 7% for each additional year since donation ((OR: 0.93, 95% CI: 0.87–0.997, $p = 0.040$). ROC analysis was done using pre-donation eGFR as a predictor of low eGFR<60 ml/min. AUC (95% CI) was 0.748, 95% CI 0.656 – 0.840) resulting in an eGFR cut-off value of 100 ml/min (sensitivity 0.80, specificity 0.61, PPV 0.29, NPV 0.94).



Overall kidney function is well preserved following kidney donation in Southeast Asian donors. Low levels of pre-nephrectomy eGFR predict sub-optimal kidney function post donation.

O308 A POST-NEPHRECTOMY INFLAMMATORY SYNDROME: A COMMON COMPLICATION POST LAPAROSCOPIC LIVE DONOR NEPHRECTOMY?

Erin Mcilveen[1], Marc Clancy[2]
[1]NHS GGC; [2]Western Infirmary, NHS GGC

Background: Living kidney donation involves exposing a healthy individual to risks of major surgery. Laparoscopic techniques reduce pain and provide better cosmesis, however, little is known about the abdominal inflammatory response post laparoscopic nephrectomy.
Methods: Data was collated for all laparoscopic hand-assisted live donor nephrectomies performed 2009–2014. Readmissions, post-operative imaging and raised inflammatory markers (WBC/CRP) were identified.
Results: 150 live donors were performed June 2009–December 2014 with a conversion rate of 2%, 113 (75.3%) left and 37 (24.7%) right. Average stay was 4.6 days. 56 (37.33%) patients re-presented on average 18.21 days post-op and stayed an average of 3 days. 52 (92.86%) patients presented with abdominal pain and/or pyrexia. 30 (53.57%) had a raised CRP >40 on admission with a normal WCC. Post operative imaging was performed in 50 (33.33%), US abdomen in 30 (47.62), CT Abdomen 23 (36.51%) and CTPA 9 (14.28%). Commonest finding on CT was of non-specific, post-operative change. This occurred in 45 (90%) and often included the presence of soft tissue or fat stranding. 37 (66.07%) of those readmitted were discharged with a diagnosis of post-operative change or non-specific abdominal pain. Of those readmitted with no diagnosis, 15 (36.59%) were given antibiotics. None had positive microbiology.
Conclusion: A third of patients post hand-assisted live donor nephrectomy were readmitted 2–3 weeks post operatively. The reason for this is unknown although it is postulated that residual perinephric fat left behind after laparoscopic donation, may undergo fat necrosis causing an inflammatory response. Although there were no long-term sequelae, removal of the perinephric fat at nephrectomy may prevent such readmissions.

O309 LONG TERM QUALITY OF LIFE AFTER LIVING RELATED LIVER DONATION – A SINGLE CENTER EXPERIENCE

Safak Gül-Klein[1], Katharina Grüttner[1], Robert Sucher[1], Anja-Kathrin Mühlisch[1], Sascha Ketzel[1], Christina Papachristou[2], Andreas Pascher[1], Daniel Seehofer[1], Gero Puhl[1], Johann Pratschke[1], Volker Schmitz[1]
[1]Department of General, Visceral and Transplantation Surgery - Charité - Universitätsmedizin Berlin; [2]Medical Clinic for Internal Medicine and Psychosomatics - Charité - Universitätsmedizin Berlin

Background: Increased donor organ shortage is inevitably paralleled by a compensatory rise in living liver donation and transplantation. Despite growing demand of living donors, only few long-term postoperative health related quality of life (HRQOL) studies exist. We therefore aimed to investigate the long-term quality of life after living related liver donation.
Material and Methods: Between 1999 and 2013, HRQOL was assessed at different time-points after donation by means of short form-36 (SF-36) analysis that comprises a physical (PCS) and mental component summary (MCS). Data were subsequently compared to a representative cross section ($n = 2892$) of the German population.
Results: Of the 105 donors enrolled in this study 41 (39%) had SF-36 data. Mean age at evaluation was 43.3 years (range: 19–70 years), 59% were female, and 60% were married. Donor survival was 100%, however one (0.95%) donor had to be transplanted 6 years after donation due to development of secondary biliary cirrhosis. 6.7% ($n = 7$) of donors required reoperation due to bleeding (1.4%, $n = 2$), partial venous thrombosis (0%), bilioma (7%, $n = 10$ postoperative course, 0.7%, $n = 1$ follow-up) and wound dehiscence (5%, $n = 7$). In the long term MSC and PSC scores of liver living donors were equal or higher when compared to the German population. However 8.5% ($n = 4$) would not repeat a living donation again.
Discussion: Liver living donation does not negatively affect HRQOL. Long-term results of living related liver donors demonstrate equal to superior HRQOL scores when compared to the German population.

O310 DONOR SAFETY FOLLOWING LIVING DONOR LIVER TRANSPLANTATION – ROMANIAN EXPERIENCE

Bogdan Dorobantu, Vlad Brasoveanu, Emil Matei, Irinel Popescu
Fundeni Clinical Institute

Background: Transplant community has to overcome the primary issue of the growing gap between the number of patients awaiting liver transplantation and available organs. Therefore living donor liver transplantation has become an option and major concerns when performing ALDLT include ensuring the donor's safety and having an adequately sized liver graft for the recipient. Advances in surgical techniques, however, have helped improve safety, minimize risks and allow LDLT to be widely offered in a continuous scarcity of liver grafts.
Objective: We evaluated the safety of donors after partial liver graft donation for A-A LDLT and A-C LDLT performed in our center.
Methods: From October 2000 to November 2014, 114 patients underwent LDLT using right lobe, left lobe or left lateral liver grafts in our center (including 2 dual grafts transplantation). Forty-seven donors were men and sixty-seven were women (range, 19–55 years; median age, 34 years). In A-A LDLT the right hemi liver grafts (72) were usually obtained by transecting the liver on the right side of the middle hepatic vein (MHV) with sectorial or inferior veins reconstruction, but also sometimes with MHV, as for the left hemi liver grafts (3) usually with S1. In the A-C LDLT 4 right and 8 left hemi liver grafts were used for teenagers and 27 left lateral sections for smaller children.
Results: These donor residual liver volumes was >30.5%. We did not experience any donor mortality. Only 6 donors developed Clavien grade 3 complications (5.2%) consisting in 3 bile leaks, 2 pleural effusions and 1 hemoperitoneum. All donors were fully recovered and returned to their previous occupations.
Conclusions: LDLT using a right hemi liver graft has become a standard option. Live liver donation was a safe procedure in our center encouraging it as a valid alternative in selected cases.

O311 ALTRUISTIC KIDNEY DONATION IN NORTHERN IRELAND: WHO, WHAT, WHEN, WHERE AND WHY

Judi Graham[1], Aisling Courtney[2]
[1]Belfast Trust; [2]Belfast Trust, Belfast City Hospital

Background: Non-directed altruistic (unspecified) kidney donors (NDAD) are an increasing proportion of living donors. Since January 2012 UK donors have had the choice to enter a pooled donation scheme or to donate directly to the national transplant waiting list. This study focuses on the outcomes for all NDAD in Northern Ireland from 2011–14.
Methods: From a retrospective review of a prospectively kept database, donor demographics, serum creatinine pre and post donation, kidney allocation and peri-operative complications were evaluated, in addition to motivating reasons for donation.
Results: There were 20 NDAD, 12 (60%) were female, mean age was 55 years (range 27–71 years.). 12 (60%) were in full time employment. 6 (30%) donors chose to enter the pooled donation scheme and 4 (20%) actually donated this way. The remainder donated directly to the deceased donor waiting list. Initially, there were no local matches but subsequently 3 (15%) kidneys were matched locally after the original recipient withdrew at short notice. 5 (25%) of donors had a peri-operative complication: 2 developed a pneumothorax (1 requiring a chest drain), 1 symptomatic hyponatraemia (Na+ 117 mmol/l), 1 post-operative ileus, 1 hypertension. The mean creatinine rose from 71 μmol/l to 103 μmol/l after donation. Underlying motivation included the desire to help another person in 10 (50%), strong faith was a contributing factor in 10 (50%), 11 (65%) had a family member or friend with chronic illness or renal problems, 18 (90%) were involved with other altruistic acts such as blood donation and charity work, and 9 (45%) were in caring professions. A smaller cohort identified donation with the need to rectify feelings of guilt and injustice.
Discussion: NDAD make a valuable contribution to kidney donation but the full potential is not realized at present. Entry into the pooled scheme should be encouraged. For the majority of donors giving a kidney is a natural extension of established altruistic behaviour.

O312 A SINGLE CENTRE'S FIVER YEAR EXPERIENCE WITH ALTRUISTIC KIDNEY DONATION

Helena Crawshaw, Katherine Thomas, Sanjay Sinha, James Gilbet, Anand Muthusamy
John Radcliffe Hospital, Oxford

Introduction: Since 2008 there have been a growing number of non-directed (altruistic) kidney donors in the UK, with 259 to date. This study focuses on 26 non-directed donors and 16 recipients of kidneys from non-directed donors at our centre over 5 years.
Method: A retrospective review of a prospectively kept database was done; donor demographics, pre and post donation renal function was noted using serum creatinine levels (sCr); and reasons underlying altruistic donation were assessed. The outcome in recipients receiving an altruistic kidney was also analysed.
Results: 15/26 (58%) of donors were female. Ages ranged from 22 to 81 and the average age was 55 years. 19/26 (73%) underwent left-sided nephrectomy. All grafts had primary function with 1 kidney removed on day 1, due to renal vein thrombosis. After donation, at a median 13 month follow up (range 6–26 months) mean sCr rose from 70 to 103 (47%). In recent donors at a median 1 month follow up (range 1–3 months) mean sCr increased from 60 to 95 (58%). There is no evidence of correlation between age and increase in creatinine levels although there is correlation between increasing age and higher final creatinine level.

Four themes motivating donation are emphasised: the desire to help, personal exposure to renal disease/transplants, media coverage and regular blood donation.

All 16 recipients had primary function post-transplant and are dialysis independent. The mean sCr measured at a median 11.5 months (range 6–24 months) decreased from 696 to 122 (82%). For more recent transplants at a median 1 month follow up (range 1–3 months) the mean sCr decreased from 697 to 101 (86%).

Discussion: Altruistic kidney donation greatly benefits transplant recipients. Donors retain satisfactory renal function and age does not appear to be a risk factor for donors. To maximise the benefits, non-directed donors should be encouraged to participate in the living donor sharing scheme.

O313 ORGAN DONATION AFTER EUTHANASIA ON SPECIFIC PATIENTS' REQUEST IN BELGIUM

Dirk Ysebaert[1], Olivier Detry[2], Guy Verfaillie[3], Dimitir Mikhalski[4], Dirk Van Raemdonck[5]

[1]Antwerp University Hospital; [2]University of Liege; [3]UZ Brussels; [4]Erasme University Hospital; [5]Leuven University Hospital

Euthanasia is since 2002 legalized in Belgium for adults under strict conditions. The patient must be in a medically futile condition, of constant and unbearable physical or mental suffering that cannot be alleviated, resulting from a serious and incurable disorder caused by illness. This implies that also non-terminal not-cancer patients can request for euthanasia for instance in case of debilitating neurological disorder.

From 2005 till 2015 more than 25 patients, suffering from diverse neuropsychiatric diseases, got their request for euthanasia granted, and subsequently asked spontaneously for the possibility of organ donation. The involved physicians, the transplant teams and the Institutional Ethics Committees, had the well-discussed opinion that this strong request for organ donation after euthanasia could not be denied. A clear separation between the euthanasia request, the euthanasia procedure and the organ procurement procedure was judged necessary. After extensive preparation, finally, in Belgium, 17 patients got their wish for organ donation after euthanasia fulfilled, in several academic or non-academic hospitals and in different regions. Several requests and preparations were started for other patients but ultimately did not lead to organ donation due to patients' personal choices or logistically reasons. The euthanasia procedure was carried out by three physicians involved in the euthanasia granting. After clinical diagnosis of cardiac death, the procurement team came in and performed the organ procurement similar as in a DCD type III procedure. Almost always, liver, two kidneys and sometimes lungs and pancreatic islets were successfully recovered and transplanted, after allocation by Eurotransplant.

The possibility of organ donation after their euthanasia provides a very much improved self-image of these patients, and adds something really positive to the unfortunate end-of-life of these patients.

031 PEDIATRIC TRANSPLANTATION

O314 RENAL TRANSPLANTATION IN SMALL PAEDIATRIC RECIPIENTS – A COMPARATIVE STUDY OF 350 CASES

Pankaj Chandak[1], Jelena Stojanovic[2], Rajesh Sivaprakasam[3], Nizam Mamode[4], Francis Calder[4], Jonathan Olsburgh[4], Chris Callaghan[4], Martin Drage[4], John D Taylor[4], Geoff Koffman[4], Judy Taylor[5], Stephen D. Marks[2], Nicos Kessaris[4]

[1]Guy's and St Thomas' and Great Ormond Street Hospitals NHS Trust; [2]Great Ormond Street Hospital; [3]Royal London Hospital, London, UK; [4]Guy's and St Thomas' Hospitals; [5]Evelina Childrens Hospital, London

Background: Renal transplantation (RTx) is the gold standard treatment modality for children with end-stage kidney disease providing imporved quality of life, growth and patient survival compared to dialysis. However, there are increased challenges in pediatric renal transplant recipients (pRTR) under 20 kg including surgical, metabolic, immunological and peioperative aspects. This study aims to determine if there is a difference in patient and graft survival in those paediatric recipients weighing <20 kg at the time of transplant compared to those >20 kg.
Methods: Data was retrieved from a prospectively collected database (apart from the weight at time of transplant and the last eGFR, which was collected retrospectively) from two large Pediatric Transplant Units in UK. Cases with incomplete data were excluded.
Results: A total of 350 children underwent kidney transplantation between 2005 and 2014. Group 1 included 90 cases (57M, 33F) of pRTR with a weight <20 kg (Median age 3, IQR 2.25) and Group 2 had 260 pRTR (146M, 114F) with a weight ≥20 kg (Median age 13, IQR 5, $p < 0.001$). 83 cases from Group 1 have a functioning graft at last follow up (5 failed, 2 died) and 230 in Group 2 (29 failed, 1 died). In Group 2 there were 5 en-bloc kidneys, one of which thrombosed intra-operatively. The median donor age (years) was 38 (IQR13) and 41 (IQR12) for Group 1 (66M, 24F) and 2 (142M, 117F) respectively ($p < 0.001$). In Group1 there were 63 live donors, 25 DBD and 2 DCD donors. In Group 2 there were 150 live donors, 104 DBD and 6 DCD donors. 1/90 in Group 1 and 25/260 in Group 2 underwent their 2nd or 3rd transplant. The last median eGFR was 59 (IQR26) and 49 (IQR23) in Group 1 and 2 respectively ($p < 0.001$). Both groups had equal median follow up of 3 years (IQR 4).
Conclusions: Despite the obvious differences between the two groups, we conclude that the overall patient and graft survival is comparable between children < 20 and those >20 kg at the time of transplantation in this large pediatric cohort.

O315 EFFECTIVENESS OF THE FINNISH MODEL OF ADOLESCENT TRANSITION TO ADULT CARE IN KIDNEY TRANSPLANT RECIPIENTS

Fernanda Ortiz[1], Patrik Finne[1], Ulla Sandholm[2], Kai Rönnholm[2]

[1]Helsinki University Hospital; [2]Helsinki University Hospital, Hosp of Children and Adolsecents

Background: Transition of kidney transplanted adolescents (KT) to adult care is problematic. We analyzed the effectiveness of the transition model (TM) developed in Finland in 2005.
Material and methods: 239 Finnish pediatric patients received a KT between 1986 and 2013, of whom 132 were transferred to adult care. In 2005 a TM was implemented in patients from the Helsinki area. We compared graft survival, graft function deterioration and associated risk factors before and after year 2006.
Results: 76% of the 132 patients had their first and 19% their second KT functioning at transition. Mean transition age was 18.4 years and mean follow-up 7.9 years. After transition 5% died after returning to dialysis and 9% with a functioning KT. We subdivided the population into three groups: graft loss, creeping creatinine (50% increase from transition) and stable function 5 years post-transition. Age at transplantation had a significant impact on the GS (age 15–18 years, RR 1.5; $p = 0.04$). TM implemented in Helsinki had not effected GS nationally in the different time periods ($p = 0.04$). However, the TM effectively decreased the risk of achieving a composite end-point of graft lost, death and creaping creatine in the Helsinki area.
Conclusions: Graft lost within 5 years after transferring to adult care occurred in 20% of the Finnish KT adolescents. The implementation of a TM successfully reduced the risk of graft loss, death and creatinine increase.

O316 HEPATIC LIVER TRANSPLANTATION IN PEDIATRIC PATIENTS UNDER 10 KG: COMPARATIVE STUDY

Gloria Gonzalea[1], Mario Uribe[2], Andrea Alba[1], Bessie Hunter[1], Luis Paqui[2], Carlos Acuña[3]

[1]Hospital Dr Luis Calvo Mackenna/Clinica Las Condes; [2]Hospital Dr Luis Calvo Mackenna/Del Salvador/Clinica Las Condes; [3]Hospital Dr Luis Calvo Mackenna

Introduction: Liver Transplant (LT) in patients under 10 kg is a surgical challenge. More technical complications have been reported. The aim of this study is to compare our results of LT in patients weighting under 10 kg with transplanted pediatric patients with a weight over 10 kg.
Method: Our data base was analyzed and all patients transplanted at our center between 1996 and February 2015 were included. Results in the group under und over 10 kg, were compared, concerning hepatic artery thrombosis (HAT), portal vein thrombosis (PVT) and biliary complications (BC). Statistical analysis was performed using Chi square test and Odds ratio. Kaplan Meier curves and Log Rank were used for graft and patient actuarial survival. Patients under 10 kg were divided in 2 groups; (i) patients transplanted between 1996 and 2005 and (ii) patients transplanted between 2006 and 2015.
Results: 207 LT were performed in 171 patients, 19.8% (41 LT in 39 patients) weighted 10Kg): PVT 17.1%/3.6% (OR 5.49 (1.73 a 17.3 $p = 0.003$); HAT 7.3%/10.8% ($p = 0.86$); BC 14.9%/22% ($p = 0.21$). Actuarial patient and graft survival at 1 y 5 years were: 89.2% and 83.7% in 10Kg ($p = 0.92$ y $p = 0.15$). Analysis by period of transplantation of patients <10 kg: Group 1 (20 LT in /17 patients- Group 2: 21 LT in 20 patients. No statistical differences concerning TP ($p = 0.62$); TA ($p = 0.27$); CB ($p = 0.15$) were found between these two periods. Graft survival at 1 and 5 years: 65% and 60% in group 1 and 85.7% and 80.9% in group 2 ($p < 0.05$). Patient survival 88.8% and 77.7% in group 1 vs. 85.7% and 80.95% in group 2 ($p = 0.59$).
Conclusions: PVT presented more frequently in the group <10 kg. Our series includes patients with a weight range between 3.7 and 10 kg with similar results in other parameters studied. No significant differences in complications rate were found between the 2 periods of the study, but graft survival improves in the second period, probably because of a better management of the complications. LT can

O318 HEPATIC ARTERIAL THROMBOSIS: ROLE OF TYPES OF VASCULAR RECONSTRUCTION IN PEDIATRIC LIVER TRANSPLANTATION

Mario Uribe[1], Gloria Gonzalez[2], Luis Paqui[2], Jorge Godoy[2], Bessie Hunter[2], Andrea Alba[2]

[1]Hospital Dr Luis Calvo Mackenna/Del Salvador/Clinica Las Condes; [2]Hospital Dr Luis Calvo Mackenna

Background: Hepatic Arterial thrombosis (HAT) presents in up to 20% of pediatric liver transplantation (PLT). The type of anastomosis could be related with this outcome. The aim of this study was to classified different types of anastomosis and compares the association with the rate of arterial thrombosis to propose a risk classification.
Methods: Demographic and surgical data of patients transplanted in our center between 1996 and February 2015 were analyzed. A classification based on arterial anatomy, number of anastomosis and vascular prosthesis was made: Type I: end-to-end hepatic anastomosis; type II: direct end-to-side aortic anastomosis; type III: iliac by-pass to the aorta; type IV: end-to-end anastomosis in living donor graft (LDG); type V: multiple vascular anastomoses; type VI: use of vascular prosthesis. Rate of HAT was determined for each type of anastomosis and Odds Ratio (two-tailed Fisher exact test) was analyzed.
Results: 207 PLT were performed in 171 patients, 90 males, median age 5.5 years (6 months to 17 years). Indications were: Biliary atresia 80 (38.6%), acute liver failure 45 (21.7%), retransplantation 36 (17.3%), other 46 (22.4%). LDG 65 (31.4%) Type of anastomosis; Type I 80 (38.6%), type II 18 (8.7%), type III 18 (8.7%), type IV 46 (22.2%), type V 45 (21.7%) Type VI 0. There were 21 HAT (10.1%), 13 (61.9%) were successfully revascularized, 8 required retransplantation. Rate of HAT was 5 for type I (6.3%) $p = 0.37$, 5 for type II (27.8%) OR 3.4 (1.1 A 10.5), $p = 0.03$, 5 for type III (27.8%) OR 3.4 (1.1 A 10.5) $p = 0.03$, 4 for type IV (8.7%) $p = 0.76$, 2 for type V (4.4%) $p = 0.24$.
Conclusions: Rate of HAT is similar compared with the reported in literature. Any type of aortic by pass is related with increased risk of HAT. Multiple anastomoses are not related with higher risk. This classification could help to compare, in a more accurate way, complications related to technical aspects of arterial anastomoses, among series.

O320*

CLASS II (ANTI-DQ/ANTI-DR) DONOR SPECIFIC ANTIBODY (DSA): PREVALENCE AND ASSOCIATION WITH HISTOPATHOLOGY IN LONG-TERM PEDIATRIC LIVER TRANSPLANT (TX) RECIPIENTS WITH NORMAL LIVER TESTS: IWITH TRIAL

Sandy Feng[1], John Bucuvalas[2], Sai Kanaparthi[3], Bryna Burrell[3], Demetris A. Jake[4], Katharine Spain[5], Iwith Investigators[6]

[1]UCSF; [2]Cincinnati Children's Hospital; [3]Immune Tolerance Network; [4]University of Pittsburgh; [5]Rho, Inc.; [6]NIAID/NIDDK/ITN

Background: We aim to describe the prevalence of Class II DSA (α-DQ/α-DR) and association with histopathology among stable pediatric liver tx recipients with normal liver tests enrolled in iWITH (NCT00320606), a 12 center prospective trial of immunosuppression withdrawal.
Methods: Subjects (n = 157) had screening liver biopsy, Class II antibody (Ab) screening (FlowPRA Screening™) and characterization (LabScreen® Single Antigen™ assay). Assignment of donor specificity was based on historical (deceased) or current (living) donor HLA typing. Regression models examined associations between demographics, DSA, and histopathology cluster assignment.
Results: 157 biopsies from 79M/78F recipients of 47 living/110 deceased (74 whole/36 partial) grafts obtained 8.9 ± 3.5 years after tx segregated into 3 distinct clusters (Fig. 1): (i) +interface activity (IA)/ ± fibrosis; (ii) IA/+fibrosis; (iii) IA/—fibrosis. 67 (43%) subjects had no Class II DSA and 56 (36%) had Class II DSA; DSA presence/absence could not be determined for 34 (22%) subjects (Tables 1A-C). 123 subjects with known DSA status (67—/56+) make up the DSA cohort. In the entire cohort (n = 157), deceased vs. living donor was associated with Cluster 1 vs. 3 assignment: OR 4.12; 95% CI 1.32–12.88; there were no living donor recipients in Cluster 1 (Fig. 2). In the DSA cohort (n = 123), α-DQ DSA with maximum MFI >20 000 was strongly associated with Cluster 1 vs. 3 assignment (Table 2): OR 10.8; 95% CI 2.76–42.5. Ten of 14 (71%) subjects with α-DQ DSA of maximum MFI >20 000 were in Cluster 1 (Fig. 3). For Cluster 2 vs. 3 assignment, neither DSA presence nor type were risk factors; only age at biopsy was associated with fibrosis: OR 1.15/month increment; 95% CI 1.02–1.28.
Conclusions: Stable long-term pediatric liver allografts can harbor significant subclinical pathology. The association of deceased donor and high MFI α-DQ DSA with IA suggests immunologic injury while that of age at biopsy with fibrosis suggests non-immunologic injury.

FIG 1: Histopathological Clusters

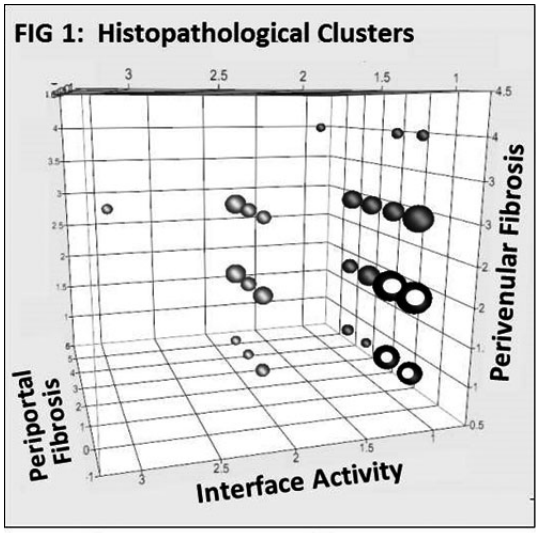


Fig 2: Distribution of living / deceased donor recipients in Cluster 1 vs. 3

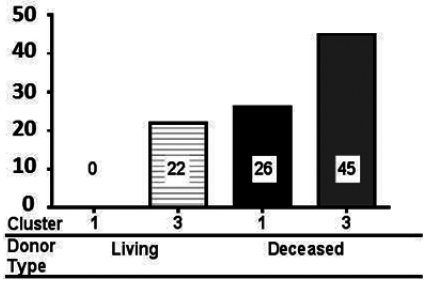


Fig 3: Distribution of subjects in Cluster 1 vs. 3 according to DSA type and MFI

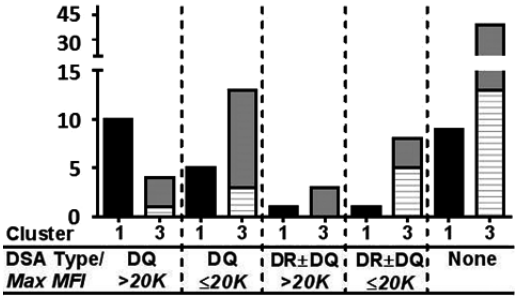


TABLE 1A: Prevalence of Class II DSA n=157

AlloAb status	Donor specificity	N
Negative		62
Positive	No	5
	Yes	56
	Unknown	27
Unknown		7

TABLE 1B: Prevalence of Class II DSA among living / deceased donor recipients: DSA cohort; n=123

Class II DSA	Deceased	Living
Negative	51	16
Positive	46	10
αDQ	34	5
αDR ± αDQ	12	5

TABLE 1C: Prevalence of αDQ alone vs α DR ± α DQ DSA: DSA cohort; n=123

Cluster	1	2	3
No DSA	9	19	39
αDQ alone	15	7	19
αDR ± αDQ	2	4	11

Table 2: Risk of Assignment to Cluster 1 vs 3 by DSA Type and Maximum MFI

DSA Type / Max MFI		OR	95% CI	P value
α-DQ / >20,000	REF: No DSA	10.8	2.76-42.5	0.008
α-DQ / ≤20,000		1.67	0.47-5.88	0.497
α-DR ± α-DQ / >20,000		1.44	0.13-15.6	0.812
α-DR ± α-DQ / ≤20,000		0.54	0.060-4.90	0.535

O321 PREFORMED AND *DE NOVO* DSA DO NOT PREDICT ACUTE CELLULAR REJECTION IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS

Dominique Schluckebier[1], Laetitia-Marie Petit[1], Dominique Belli[1], Jean Villard[2], Barbara Wildhaber[3], Sylvie Ferrari-Lacraz[2], Valérie Mclin[1]
[1]Swiss Center for Liver Disease in Children, Geneva University Hospitals; [2]Department of Immunology, Geneva University Hospitals; [3]Division of Pediatric Surgery, Geneva University Hospitals

Background: The impact of donor-specific alloantibodies (DSA) is a well-known risk factor on short and long-term outcomes in kidney and heart transplantation. Their role in liver transplantation (LT) remains unclear. Recent studies in adult recipients suggest that the presence of preformed or *de novo* DSA might have an unfavorable influence on transplant survival.
Aim: To determine whether the presence of donor-specific anti-HLA antibodies (DSA) predict acute cellular rejection in pediatric liver transplant recipients.
Method: Retrospective single-center study of children aged 0–16 years having undergone liver transplantation between January 1, 2005 and December 31, 2013. A systematic collection of biological samples, clinical, pathology and laboratory information was performed for each patient at 1, 3 and 5 years following transplantation and at the time of clinically indicated liver biopsies. Biopsies were analyzed using the BANFF criteria. HLA typing and anti-HLA antibodies were performed in all patients before LT and at least once after LT, except in patients who died within 1 month of transplantation. DSA measurement and specificity was performed using the LABScreen® Mixed or the LABscreen® Single Antigen (one Lambda) method (table).
Results:
Discussion: In this small, single center retrospective study, DSA do not seem to be associated with an increased incidence of acute cellular rejection or with the severity of the rejection. Further, pre-formed or *de novo* DSA were not associated with graft demise.

O322 POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDER IN PEDIATRIC PATIENTS AFTER LIVER TRANSPLANTATION: SURVIVAL AND RISK FACTORS

Ahad Eshraghian, Mohammad Hadi Imanieh, Seyed Mohsen Dehghani, Saman Nikeghbalian, Bita Geramizadeh, Ali Bahador, Seyed Ali Malek-Hosseini
Organ Transplant Center, Shiraz University of Medical Sciences

Background: Post-transplant lymphoproliferative disorder (PTLD) represents a group of abnormal lymphoid proliferations that may occur after both solid organ and hematopoietic transplantation. They include a clinical and pathological spectrum ranging from benign lymphoproliferation to an aggressive fatal and widespread lymphoma. Here in, we aimed to investigate survivals of patients with PTLD and associated risks after pediatric liver transplantation.
Methods and Materials: A cross sectional survey was conducted among pediatric patients (<18 years) who underwent liver transplantation at Shiraz transplant center, Shiraz, Iran between February 2005 and February 2015. Clinical and laboratory information of patients were collected using a questionnaire containing data regarding age, sex, time of liver transplantation time of PTLD, survival of patients, immunosupprersive regimen, rejection episodes and type of allograft.
Results: There were 640 pediatric liver transplants during this time period. A total of 39 pediatric patients developed PTLD during their follow up. The incidence of PTLD after liver transplantation was 6.09% in our study population. The overall post-PTLD survival at 6 months was 74.4% (±7%), at 1 year was 68.9% (±7.5%) and at 5 years was 49.5% (±15.4%). Mortality was significantly higher in patients with multi-organ involvement ($p = 0.001$). EBV positivity was observed in 92.85% in alive patients versus 44.44% in non-survived patients ($p = 0.017$). Age, sex, type of allograft, rejection episode, tacrolimus and steroid dose, and serum tacrolimus level were not associated with post-PTLD survival ($p > 0.05$). EBV positive patients had higher survival compared to EBV-negative PTLD patients (60.58 ± 7.62 vs. 5.58 ± 2.72 months) ($p = 0.002$).
Conclusion: PTLD is a rather prevalent post liver transplant complication in pediatric patients. EBV- negativity and multi organ involvement were associated with mortality. However, EBV-positive patients had much more better survival than EBV-negative patients with better response to therapy.

Rejection (Banff)	Preformed anti-HLA (%)	Preformed DSA (%)	*De novo* DSA (%)	No DSA (%)	Graft survival (%)	Total patients (%)
Light	1 (4)	1 (8)	1 (4)	0	2 (100)	2 (3)
Moderate	3 (11)	3 (23)	7 (28)	6 (23)	15 (94)	16 (25)
Severe	1 (4)	0	2 (8)	3 (12)	4 (80)	5 (8)
Total rejection	5 (17)	4 (31)	10 (40)	9 (35)	21 (91)	23 (36)
No rejection	23 (82)	9 (69)	15 (60)	17 (65)	36 (88)	41 (64)
Total	28	13	25	26	57	64

023 KIDNEY

O323 EARLY BEDSIDE REMOVAL VERSUS DELAYED CYSTOSCOPIC REMOVAL OF URETERIC STENTS FOLLOWING LIVE DONOR RENAL TRANSPLANTATION: A RANDOMIZED PROSPECTIVE STUDY

Nalaka Gunawansa[1], Mandika Wijeyaratne[1], Rezni Cassim[1], Chathu Sahabandu[2]

[1]National Hospital Sri Lanka; [2]National Institute of Nephrology, Dialysis and Transplantation

Introduction: Stenting of the allograft ureter across the neo-ureterocystostomy is accepted as standard practice in renal transplantation. This has shown to minimize Major Ureteric Complications (MUC) and the stent is usually removed 4–6 weeks post-transplant by flexible cystoscopy. However, this allows for the increased risk of post-transplant Urinary Tract Infections (UTI) and also requires a second invasive procedure along with the related logistic and cost implications.
Methods: A prospective randomized study was done, comparing delayed (day 28) cystoscopic stent removal (group-1) with early (day-06) bedside removal (group-2) in Live Donor Renal Transplants (LDRT). In group-2 the ureteric stent was tied to the tip of the urinary catheter intra-operatively and was removed along with the catheter at the bedside. Both groups were prospectively followed up for MUC and UTI.
Results: There were 382 (Group-1, 179; Group-2, 203) consecutive LDRT between January 2009 and August 2013. The mean follow up was 16 (12–36) months. There were two instances of ureteric anastomotic stenoses detected on routine follow up (9 and 14 weeks post-transplant). Both these were in Group 1 and required re-stenting for a longer period. No surgical intervention was required. There were 42 (11%) culture proven UTI during follow up, 19 in Group-1 and 23 in Group-2, $p = 0.5$
Conclusion: Tying of the ureteric stent to the urinary catheter intra-operatively and early bed-side removal is a safe alternative to the conventional practice. We have not observed any significant difference with regards to the incidence of post-transplant MUC and UTI. In turn, it allows greater convenience to the patient and cost-effectiveness to the institution, especially in centralized transplant services. Hence, We recommend this as the standard method for stenting the allograft ureter during renal transplantation.

O324 BARIATRIC SURGERY IN RENAL TRANSPLANT CANDIDATES AND RECIPIENTS: A SINGLE CENTER EXPERIENCE

Inbal Golomb, Eytan Mor, Eviatar Nesher, Michael Gurevitch, Janos Winkler, Andrei Keidar

Rabin Medical Center

Background: Obesity (BMI > 35) is a relative contraindication for transplantation due to increased risk of mortality, complications and graft loss. Bariatric surgeries are the most effective method of weight loss and remission of obesity related comorbidities. Data regarding the effect of bariatric surgeries on renal transplant patients is scarce.
Patients and Methods: Data were collected from all bariatric surgeries performed on renal transplant candidates and recipients patients between 2011 and 2015 at our center and included demographics, renal-function and comorbidities. Nineteen patients with a functioning transplanted kidney and five transplant candidates underwent bariatric surgery. One listed patient underwent a successful renal transplantation. Mean follow-up was 18 months (2–47, 15 patients over 12 months). The mean age was 54 and 46 years in transplant recipients and candidates, respectively.
Results: All patients lost weight but using the criteria of loss of >50% of excess weight the procedure was successful in 86%. Subjects' mean preoperative BMI was 42 and 40 kg/m^2 versus a postoperative BMI of 29 and 27 kg/m^2 in transplant recipients and candidates, respectively. Mean hospital stay was 4 days. The majority of comorbidities improved or resolved. In transplant recipients renal function showed a significant improvement as manifested by a decrease of urinary protein (1086 mg/day to 679), and creatinine (1.34 mg/% to 1.15). No graft rejection was encountered. Two patients developed major complications.
Conclusions: Bariatric surgery provided effective weight loss and induced remission of obesity-related comorbidities in renal transplant candidates and recipients. No adverse effects on graft function and immunosuppression were seen. These results have important implications in the clinical setting when offering kidney transplantation to morbidly obese candidates.

O325 UMBILICAL VEIN CATHETER VERSUS DOUBLE J STENT FOR URETERIC ANASTOMOSIS IN RENAL TRANSPLANTATION: A SINGLE CENTRE, OPEN LABEL, RANDOMIZED TRIAL

Vishnu Swaroop, Arshiya Pereira, Stella Smith, Omar Masood, Ravi Pararajasingam, Titus Augustine, Hany Riad, Sanjay Mehra, Afshin Tavakoli

Manchester Royal Infirmary

Introduction: Double J (DJ) stents are used routinely in renal transplantation to prevent ureteric complications. A recent Cochrane review of literature showed that routine prophylactic stenting reduces the risk of major ureteric complications.
Aim: To compare the use of Umbilical Vein Catheter (UVC) to DJ stents in renal transplants. Primary end point was ureteric complication rate. Secondary end points were UTIs, re-operation or radiological interventions, and cost effectiveness.
Methods: 300 patients were randomized using a sealed envelope technique.151 to DJ stents and 149 to UVC. There was a significant conversion rate from UVC to DJ stent, intra-operatively (30%). Eventually 187 DJ stents and 98 UVCs were included in final analysis. Intention to treat (ITT) and per protocol (PP) analysis were done. Fishers' test was used for two-sided p values. Absolute risk with 95% confidence interval (CI) and number need to treat (NNT) were calculated.
Results: Patient demographics were similar in both groups. Both ITT and PP analyses showed no significant increase in ureteric complications with UVC ($p = 0.1194$ and 0.1286, respectively; AR 3.5%; 95%CI = −1.22 to 8.21; NNH = 29). ITT showed a significant increase in UTIs in DJ stent group ($p < 0.0001$) with AR 21.6%, 95% CI = 11.5–31.7; NNT = 5. But the PP analysis failed to show any significant difference between the 2 groups ($p = 0.5937$; AR 3.56; 95% CI = −7.71 to 14.84; NNT = 29). There was a significant cost difference between the 2 groups with DJ stents costing £848 per patient (cost of stent itself and day surgery procedure of flexible cystoscopic removal) and UVCs costing just £0.80. This resulted in a savings of over £83 000 in this study.
Conclusions: This prospective randomized trial showed that UVCs are comparable to DJ stents in terms of ureteric complications. However, ITT analysis showed a higher risk of UTIs with DJ stents. But there was a significant conversion rate. UVCs need to be evaluated in future trials to tap the potential cost benefits.

O326 ROBOTIC KIDNEY TRANSPLANTATION: AN UPDATE

Raquel Garcia Roca, Carlos Martinez Parra, Sandra Garcia Aroz, Ivo Tzvetanov, Hoonbae Jeon, Enrico Benedetti, Jose Oberholzer

UIC

Introduction: Candidates for kidney transplantation (KT) with BMI>35 kg/m^2 are frequently denied access to transplant. When listed, they wait an average of 2 years longer than those with a normal BMI. The surgical site infection rate (SSI) nears 20–30% in morbidly obese recipients, which has been associated with significantly worse graft survival and trend to inferior patient survival. The robotic surgical approach focuses on minimizing and preventing SSI in order to provide equal opportunity transplantation for obese candidates with similar outcomes
Methods: Since June 2009, all suitable KT candidates with a BMI ≥30 kg/m^2 were considered for the robotic approach, in particular those with an abdominal panus. This is a retrospective chart review, analyzing intra-operative and post-operative data as well as the short-term outcomes
Results: From 143 robotics KT cases, most recipients had living donor (84.6%) with a mean follow up of 23.8 ± 15.9 months. The intra-operative data is shown in Table 1. The mean age was 47 ± 11 years with a mean BMI of 41.7 ± 7 kg/m^2 (range 29–59.5). The SSI rate was 2% ($n = 3$) and the rate of other wound complication was 6.9% (8 seromas and 2 dehiscences). Conversion to open technique was due to poor exposure ($n = 3$) or for vascular reasons ($n = 5$). Out of 6 urinary complications, 4 were repaired successfully with robotic assistance and two were treated conservatively. The mean calculated GFR at 6 month was 57 ± 19 ml/min/1.73 m^2 with a 8.6% delayed graft function. The 1-year patient and graft survival rates were 97.8% and 95.6% respectively (includes 2 due to non-compliance).
Conclusion: The minimal invasive approach to KT in obese patients significantly reduces the risk of SSI with acceptable surgical complication rates. Robotic-assisted KT offers safe access to transplantation for obese patients otherwise denied access the opportunity.

CIT (hrs)	4.3±5.2
WIT (min)	44.5±10.6
Length of surgery (hours)	5.1±1.3
Blood loss (cc)	140.9±135.9
Hospital days	7.8±7.1

O327 POST-TRANSPLANTATION ENCAPSULATING PERITONEAL SCLEROSIS IN THE NETHERLANDS: DECREASING INCIDENCE BUT STILL HIGH MORTALITY

Michiela Betjes[1], Hans Kalberg[1], Mario Korte[2]
[1]Erasmus Medical Center; [2]Albert Schweitzer Ziekenhuis

Background: Encapsulating peritoneal sclerosis (EPS) is a rare complication of peritoneal dialysis and is defined by progressive fibrotic thickening of the peritoneal membrane leading to small bowel obstruction. EPS after kidney transplantation (post-KT EPS) occurs in 1–3% of all transplanted PD patients and carries a significant mortality. The Dutch EPS registry was started in 2009 for prospective data collection of EPS patients in the Netherlands.
Methods: New cases were identified by contacting all Dutch nephrologists twice yearly. Medical records of suspected EPS cases were then acquired and reviewed by a steering committee. The diagnosis of EPS was made according to international accepted criteria.
Results: From January 2009 to January 2014, 65 cases have been reviewed of which 43 with a confirmed diagnosis of EPS. Twenty-three cases (53.5%) were identified as post-KT EPS. Follow-up of 13 suspected early cases showed 5 progressing into full EPS. The average age was 54.1 ± 14.8 years with a median duration of PD treatment of 60 months. The majority of cases of post-KT EPS was diagnosed within 12 months after transplantation. A striking finding was the sharp decrease in EPS incidence in the last 2 years from an initial 1.0% in 2009 to 0.2% in 2013. This observation could not be explained by a similar change in number of PD patients or kidney transplantations. Compared to a historical cohort, significantly more EPS patients were treated with steroids (42%) or tamoxifen (56%). In addition more EPS patients underwent surgical treatment (33%) consisting of peritonectomy and enterolysis. However, mortality did not change and remained high at 40% in the first year after diagnosis. Discussion: The incidence of post-KT EPS has significantly decreased in the Netherlands but the mortality remains high. Increased use of biocompatible dialysis fluids and early withdrawal of patients at risk for EPS may have contributed to the decrease incidence.

O328 KIDNEY TRANSPLANTATION IN THE UK

Lisa Bradbury[1], Rachel Johnson[1], Christopher Watson[2]
[1]Organ Donation and Transplantation Directorate, NHS Blood and Transplant, Fox Den Road, Bristol; [2]University Department of Surgery, Addenbrooke's Hospital, Cambridge and the NIHR Cambridge Biomedical Research Centre

This review reports key figures about kidney transplantation in the United Kingdom (UK). It presents information about donor, transplant list, transplant activity and survival after kidney only transplant for all 24 centres performing kidney transplantation.

Data were obtained from the UK Transplant Registry on kidney transplant activity between April 2004 and March 2014. Survival estimates are reported at 5-years post-transplant for the period April 2005 to March 2009. Results are described separately according to the type of donor (deceased [DD] and living [LD]). Patient survival from listing is reported at 10 year post registration for a DD adult kidney only transplant between 2002 and 2013. The centre specific results for survival estimates are adjusted for differences in risk factors between the centres.

On 31 March 2014, there were 5590 adult patients on the UK active kidney transplant list a 7% decrease in the number of patients a year earlier. The length of time a patient waits for a kidney transplant varies across the UK. The median waiting time for adult DD kidney only transplant is 1082 days and varies between centres ranging from 572 days to 1768 days. There were 2930 adult kidney only transplants performed in the UK in 2013/14 an increase of 9% compared to 2012/13. Of these, 1101 were from DBD donors, 779 were from DCD donors and 1050 were from LD. The national rate of graft survival 5 years after first adult DD kidney only transplant is 86%. These rates vary between centres, ranging from 81% to 92% (risk-adjusted [RA]). The national rate of graft survival 5 years after first adult LD kidney only transplant is 91% ranging from 84% to 97% (RA) between centres. The national rate of 10 year patient survival from listing for DD kidney only transplants in adult patients is 75% ranging from 69% to 84% (RA) between centres.

The report presents comprehensive information relating to kidney transplant activity in the UK

O329 COMPLEMENT- AND NON-COMPLEMENT-BINDING *DE NOVO* DONOR SPECIFIC ANTI-HLA ANTIBODIES AND KIDNEY ALLOGRAFT SURVIVAL

Florent Guerville[1], Gwendaline Guidicelli[2], Sébastien Lepreux[3], Chris Wiebe[4], Olivier Thaunat[5], Valérie Dubois[6], Jonathan Visentin[2], Thomas Bachelet[1], Emmanuel Morelon[5], Peter Nickerson[4], Pierre Merville[1], Jean-Luc Taupin[2], Lionel Couzi[1]
[1]Service de Néphrologie-Transplantation-Dialyse, CHU Bordeaux; [2]Service d'Immunologie, CHU Bordeaux; [3]Service d'Anatomopathologie, CHU Bordeaux; [4]Department of Medicine and Immunology, University of Manitoba; [5]Service de Transplantation, Néphrologie et Immunologie Clinique, Hospices Civils de Lyon; [6]Laboratoire d'histocompatibilité, Etablissement Français du Sang, Lyon

The aims of our study were to describe the incidence and risk factors for the appearance of C1q-binding *de novo* donor-specific anti-HLA antibodies (DSA) and their long-term impact. Using Luminex® Single Antigen Flow Bead assays, 346 pre-transplant non-sensitized kidney recipients were screened at 2 and 5 years post-transplantation for *de novo* DSA, which was followed when positive by a C1q Luminex® assay. Twelve (3.5%) and 8 (2.5%) patients had C1q-binding *de novo* DSA at 2 and 5 years, respectively. A *de novo* DSA mean fluorescence intensity higher than 6237 and 10 000 at 2 and 5 years, respectively, predicted C1q binding. HLA mismatches and ciclosporine A were independently associated with an increased risk of C1q-binding *de novo* DSA occurrence. When *de novo* DSA were analyzed at 2 years, the 5-year death-censored graft survival was similar between patients with C1q-non-binding *de novo* DSA and those without *de novo* DSA, but was lower for patients with C1q-binding *de novo* DSA ($p = 0.003$). When *de novo* DSA were analyzed at 2 and 5 years, the 10-year death-censored graft survival was lower both for patients with C1q-non-binding *de novo* DSA if detected at 2 and 5 years and with C1q-binding *de novo* DSA, than for patients without *de novo* DSA ($p = 0.0004$ and $p = 0.002$, respectively). Those results were partially confirmed in 2 validation cohorts. In conclusion, C1q-binding *de novo* DSA are associated with graft loss occurring quickly after their appearance. However, the long term persistence of C1q-non-binding *de novo* DSA could also lead to lower graft survival.

O330* ECULIZUMAB IN PREVENTION OF ACUTE ANTIBODY-MEDIATED REJECTION IN SENSITIZED DECEASED-DONOR KIDNEY TRANSPLANT RECIPIENTS: 1-YEAR OUTCOMES

Denis Glotz[1], Graeme Russ[2], Lionel Rostaing[3], Christophe Legendre[4], Steven Chadban[5], Josep Maria Grinyo[6], Nizam Mamode[7], Gunnar Tufveson[8], Lionel Couzi[9], Paolo Rigotti[10], Yvon Lebranchu[11], Silvio Sandrini[12], William Marks[13]
[1]Hospital Saint-Louis; [2]Royal Adelaide Hospital; [3]CHU Rangueil; [4]Hospital Necker; [5]Royal Prince Alfred Hospital; [6]Hospital Universitari de Bellvitge; [7]Guy's Hospital; [8]Uppsala University Hospital; [9]Hôpital Pellegrin; [10]Azienda Ospedaliera di Padova; [11]Hôpital Bretonneau - CHU de Tours; [12]Spedali Civili di Brescia; [13]Alexion Pharmaceuticals, Inc.

Complement activation by preformed DSA is a major mechanism of acute antibody-mediated rejection (aAMR) in sensitized, deceased-donor, kidney transplant recipients (SKTR). In a previous interim study report, eculizumab (Ec), a C5 inhibitor, appeared effective for prevention of aAMR in SKTR compared to historical controls. We report 1-year data from this ongoing, open-label, single-arm trial.
Methods: SKTR defined as: current DSA >3000MFI as detected by SAB; or B-cell or T-cell flow cytometric crossmatch ≥300 and ≤500 mean channel shift; or historical positive complement-dependent cytotoxicity crossmatch to donor HLA. All recipients received Ec 1200 mg postoperative day (POD) 0 prior to reperfusion, 900 mg on POD 1, 7, 14, and 28, and 1200 mg at weeks 5, 7, 9. Rabbit ATGa was used for induction and corticosteroids, tacrolimus, and mycophenolate for maintenance immunosuppression. Posttransplant (PT) plasmapheresis was not allowed. The primary composite endpoint was clinically significant, biopsy(bx)-proven aAMR grade II/ III (Banff 2007), (based on centrally read bx), death, graft loss, or loss to follow-up at 9 weeks PT. Graft and patient survival were estimated by Kaplan–Meier.
Preliminary Results: 80 candidates were transplanted (48 F, 32 M); median age 52 year (range, 24–70). 9/80 SKTR met the 9 week composite endpoint based on local bxs (11.3% [95% CI 5.3%, 20.3%]). 5 of the 9 SKTR had aAMR (6.3%) compared to 30% expected for historical controls. Graft survival at 6 and 12mo was 93.7% and 87.1%, respectively; patient survival at 6 and 12 months was 97.4%. Mean creatinine levels (mg/dl) at baseline, 1 and 12 month PT were, 7.44 (±2.52), $n = 78$; 1.86 (±1.07), $n = 74$; and 1.80 (±1.11), $n = 45$, respectively. No new safety signals were identified.
Conclusions: Ec appeared to be effective in reducing the incidence of aAMR in SKTR. Patient and graft survival and kidney function at 1 year were similar to those expected for nonsensitized KTR. Ec was well tolerated. Updated data to be presented.

O331 IMMUNOGLOBULIN G DONOR-SPECIFIC ANTI-HLA ANTIBODY SUBCLASSES AND KIDNEY ALLOGRAFT ANTIBODY-MEDIATED INJURY

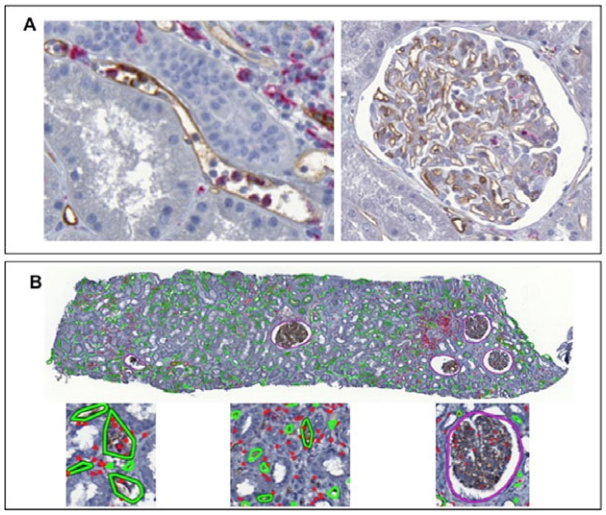
Carmen Lefaucheur[1], Denis Viglietti[1], Christophe Legendre[2], Denis Glotz[1], Alexandre Loupy[2], Adriana Zeevi[3]
[1]Saint-Louis Hospital, Paris; [2]Necker Hospital; [3]UPMC Pittsburgh

Experimental and clinical data suggest that antibodies have different pathogenicities according to their IgG subclasses. We investigated the association between IgG subclasses of circulating anti-HLA antibodies and antibody-mediated kidney allograft injury. Among 635 consecutive kidney transplantations performed between 2008 and 2010, we enrolled patients with DSA detected in the first year post-transplant. We assessed the DSA-positive patients for DSA characteristics – specificity, HLA class specificity, MFI level, C1q-binding, and IgG subclasses – together with the graft injury phenotype at the time of sera evaluation. A total of 125 DSA-positive patients were included: 51 (40.8%) with acute antibody-mediated rejection (aABMR), 36 (28.8%) with sub-clinical ABMR (sABMR) and 38 (30.4%) without ABMR. The MFI of the immunodominant DSA (iDSA) was 6724 ± 464, with 41.6% showing C1q positivity. The distribution of iDSA IgG1-4 subclasses among the population was 75.2%, 44.0%, 28.0% and 26.4%, respectively. An unsupervised principal component analysis integrating iDSA IgG subclasses revealed that aABMR was mainly driven by IgG3 iDSA, whereas sABMR was driven by IgG4 iDSA. IgG3 iDSA was associated with a shorter time to rejection ($p < 0.001$), increased microcirculation injury ($p = 0.002$) and C4d capillary deposition ($p < 0.001$). IgG4 iDSA was associated with later allograft injury with increased allograft glomerulopathy and interstitial fibrosis/tubular atrophy lesions ($p < 0.001$ for all comparisons). When the iDSA HLA class specificity, MFI level, C1q-binding and IgG subclasses were integrated in a survival Cox model, IgG3 iDSA and C1q-binding iDSA were strongly and independently associated with allograft failure: hazard ratio = 4.8 ($p = 0.003$) and 3.6 ($p = 0.03$), respectively. The IgG DSA subclasses identify distinct phenotypes of kidney allograft antibody-mediated injury. IgG3 iDSA is a strong determinant of long-term allograft failure beyond conventional DSA assessment features.

O332 COMPUTER-ASSISTED INFLAMMATION ANALYSIS (CIA) OF KIDNEY-GRAFT BIOPSY: THE POLYGRAPH OF ANTIBODY-MEDIATED REJECTION

Antoine Sicard[1], Vannary Meas-Yedid[2], Maud Rabeyrin[3], Stephanie Ducreux[4], Alice Koenig[1], Jean-Yves Scoazec[3], Frederique Dijoud[5], Lionel Badet[6], Valerie Dubois[4], Emmanuel Morelon[1], Jean-Christophe Olivo-Marin[2], Olivier Thaunat[1]
[1]Hospices Civils de Lyon, Hôpital Edouard Herriot, Service de Transplantation, Néphrologie et Immunologie Clinique, Lyon; [2]Quantitative Image Analysis Unit, Institut Pasteur; [3]Service d'anatomopathologie Hôpital Edouard Herriot Lyon; [4]Etablissement Français du Sang, Lyon; [5]Service d'anatomopathologie, Groupement Hospitalier Lyon EST; [6]Service d'urologie et transplantation rénale, Lyon

Introduction: The diagnosis of antibody-mediated rejection (AMR) requires the presence of (i) donor specific antibodies (DSA), and (ii) microvascular inflammatory lesions on kidney graft biopsy. Histological assessment relies on semiquantitative lesion scoring according to Banff classification, which faces limitations in terms of diagnostic accuracy and risk prediction. We have developed a new method of computerized image analysis allowing fine characterization of the quality and intensity of graft inflammation.
Method: Renal biopsies from 57 kidney recipients who fulfilled the criteria for AMR were analyzed. Double immunohistological stainings were performed with anti-CD31 (capillaries) and respectively anti-CD68 (macrophages), CD3 (T lymphocytes), CD66b (granulocytes), or CD20 (B lymphocytes) (Fig. 1a). Computer-assisted Inflammation Analysis (CIA) was used to quantify the number of cell of each type in interstitium, glomeruli and peri-tubular capillaries (Fig. 1b). The ability of DSA to bind C3d was assessed using flow bead assays.
Results: 34 patients had C3d + DSA and 23 had C3d-DSA. Although allograft survival was lower in the C3d+ goup ($p < 0.001$), Banff scores for antibody-mediated lesions (g+ptc) were similar in the 2 groups. In contrast, CIA revealed differences between groups. Patients with C3d+DSA had a higher number of monocytes/10^3 pixels in both peritubular capillaries (0.11 vs. 0.04, $p = 0.002$) and glomeruli (0.03 vs. 0.01, $p = 0.018$). Interestingly, the monocyte and neutrophil infiltrations were also more intense in the interstitium of the C3d+ group (0.13 vs. 0.04, $p = 0.004$ and 0.004 vs. 0.0005, $p = 0.02$).
Conclusion: CIA is a novel reproducible approach allowing topographical quantification of graft inflammation. Using CIA, we observed that histopathological features of complement-binding DSA are different from that of non-complement binding DSA. Analyses are currently performed to determine whether CIA is useful to stratify the risk of graft loss in AMR.



O333 MARKERS OF ENDOTHELIAL TO MESENCHYMAL TRANSITION: EVIDENCE FOR ANTIBODY-ENDOTHELIUM INTERACTION DURING ANTIBODY MEDIATED REJECTION IN KIDNEY RECIPIENTS

Alexandre Hertig[1], Yi-Chun Dubois-Xu[2], Julie Peltier[3], Isabelle Brochériou[4], Arjang Djamali[5], Caroline Suberbielle[6], Eric Rondeau[7]
[1]APHP Hopital Tenon Urgences Nephrologiques et Transplantation Rénale; [2]Inserm; [3]APHP; [4]APHP, Hopital Tenon, Pathology; [5]Wisconsin University; [6]APHP, Hopital Saint Louis; [7]APHP, Hopital Tenon

Background: Antibody-mediated rejection (ABMR) is a leading cause of allograft loss. The efficacy of treatment depends on accurate diagnosis at an early stage. Sensitive and reliable markers of antibody-endothelium interaction during ABMR are not currently available for routine use.
Methods: Using immunohistochemistry, we retrospectively studied the diagnostic value of three markers of endothelial-to-mesenchymal transition (EndMT) (fascin1, vimentin, and hsp47) for ABMR in 53 renal transplant biopsies, including 20 with ABMR, 24 with cell-mediated rejection, and 9 normal grafts. We validated our results in a second independent set of 74 unselected biopsies.
Results: Endothelial cells of the peritubular capillaries in grafts with ABMR expressed strongly fascin, vimentin, and hsp47, whereas those from normal renal grafts did not. The level of expression of these EndMT markers was significantly associated with current ABMR criteria including capillaritis, glomerulitis, peritubular capillary C4d deposition, and donor-specific antibodies. These markers allowed us to identify C4d-negative ABMR and to predict late occurrence of disease. EndMT markers were more specific than capillaritis for the diagnosis and prognosis of ABMR and predicted late (up to 4 years after biopsy) renal graft dysfunction and proteinuria. In the second independent set of 74 renal graft biopsies, the EndMT markers for the diagnosis of ABMR had a sensitivity of 100% and a specificity of 85%. Fascin expression in peri-tublar capillaries was also induced in a rat model of ABMR.
Conclusion: EndMT markers are a sensitive and reliable diagnostic tool to detect endothelial activation during ABMR, and predict late loss of allograft function.

O334 EARLY ACUTE HUMORAL REJECTION IN THE ABSENCE OF ANTI-HLA ANTIBODIES: CLINICO-PATHOLOGICAL DESCRIPTION FROM A FRENCH NATION-WIDE STUDY

Marianne Delville[1], Philippe Gatault[2], Magali Giral[3], Olivier Thaunat[4], Nadia Arzouk[5], Marc Hazzan[6], Nassim Kamar[7], Pierre Merville[8], Cyril Garrouste[9], Sophie Caillard[10], Alexandre Hertig[11], Christophe Mariat[12], Joseph Rivalan[13], Dominique Bertand[14], Marc Ladriere[15], Vincent Vuiblet[16], Christophe Legendre[17], Dany Anglicheau[17]
[1]Hopitla Necker APHP; [2]CHU de Tours; [3]CHU de Nantes; [4]CHU de Lyon; [5]Hopital La pitié Salpétrière; [6]CHU de Lille; [7]CHU de Toulouse; [8]CHU de Bordeaus; [9]CHU de Clermont-Ferrand; [10]CHU de Strasbourg; [11]CHU de Tenon; [12]CHU de St Etienne; [13]CHU de Rennes; [14]CHU de Rouen; [15]CHU de Nancy; [16]CHU de Reims; [17]Hopital Necker

Antibody mediated rejection (ABMR) is associated with poor transplant outcome. Pathogenic alloantibodies are usually directed against HLA antigens. However evidence of ABMR in the absence of anti-HLA antibodies strongly suggests the implication of non-HLA antibodies. Despite the severity of non-HLA ABMR, the available data remain elusive. We implemented a retrospective nation-wide study. Inclusion criteria were: first or retransplantion, deceased or

living-donor; acute allograft dysfunction during the first 3 months; allograft biopsy showing a total score of g+ptc≥3 according to the Banff classification; absence anti-HLA DSAs using Luminex®.47 patients were included. Recipient age was 46 ± 2 years. Male/female ratio was 3/1. Mean time of dialysis was 3.7 ± 0.6 years. Donor age was 51 ± 2 years. Cold ischemia time was 15.8 ± 1.5 h. All patients but one received induction therapy (85% basiliximab, 13% ATG), CNI (72% tacrolimus, 28% cyclosporine), MPA and steroids. Rejection was diagnosed at day 15 ± 4 in the absence of graft function recovery in 47% of patients. The remaining 25 patients defined a poor serum creatinine nadir of 228 ± 28 μmol/l at 24 ± 9 days. Acute graft dysfunction (318 ± 42 μmol/l) led to the diagnosis of acute rejection at day 25 ± 6. No anti-HLA DSA were observed. Mean g+ptc score was 3.9 [3–6]. In addition, intimal arteritis (v ≥ 1) and interstitial inflammation (i ≥ 1) were observed in 48% and 72% of cases, respectively. Additional lesions included red blood cell extravasation and thrombotic microangiopathy in 21% and 23% of cases, respectively. Therapeutic strategies included steroids pulses (89%), ATG (23%), rituximab (30%), IVIG (45%) and plasmapheresis (60%). Primary non function was observed in 2 cases and one patient was back to dialysis at 2 years. At last follow up (3 ± 1 years), serum creatinine was 182 ± 15 μmol/l. Non-HLA ABMR is a severe condition associated with graft lost. Additional mechanistic analyses are upcoming.

013 IMMUNOBIOLOGY/BASIC SCIENCE

O335* PRECLINICAL EFFICACY OF ANTI-IL7 RECEPTOR MONOCLONAL ANTIBODIES IN NON HUMAN PRIMATE

Nicolas Poirier[1], Lyssia Belarif[1], Hoa Le-Mai[2], Caroline Mary[1], Jeremy Hervouet[2], David Minault[2], Virginie Thepenier[1], Stéphanie Le Bas-Bernardet[2], Elisabeth Nguyen[2], Jean-Paul Soulillou[2], Sophie Brouard[2], Bernard Vanhove[1], Gilles Blancho[2]
[1]Effimune; [2]INSERM UMR 1064

Interleukin 7 is a limiting and non-redundant cytokine sustaining T lymphocyte proliferation, survival and homeostasis. Almost all T lymphocytes express the IL-7 receptor (IL-7R), with a particular exception for natural regulatory T-cells (Treg), constituting a rare opportunity to selectively target effectors (Teff) while sparing Treg. We recently reported that IL-7R blockade promotes long-term allograft survival in mice and favors the Treg/Teff ratio. Here we evaluated novel anti-human IL-7R monoclonal antibodies (mAbs) at the preclinical level.

Anti-IL-7R mAbs dose-dependently inhibited IL-7-induced phosphorylation of STAT5 (IC50 = 0.04 μg/ml) as well as proliferation of both CD4+ and CD8+ human T cells (IC50 = 0.2 μg/ml). IgG1 and IgG4 anti-IL-7R mAbs were evaluated *in vivo* in a tuberculin-induced delayed-type hypersensitivity (DTH) model in baboons previously sensitized with BCG vaccine. Pharmacokinetics analysis after a single IV administration at 10 mg/kg ($n = 3$ per group) showed an elimination half-life of up to 1 week. No serum cytokine was induced by the antibody. Both IgG1 and IgG4 anti-IL-7R mAbs strongly inhibited erythema (80–90%) and skin infiltration by T cells and macrophages after intradermal tuberculin challenge performed few hours after mAbs injection. Blood and lymph nodes T lymphocytes numeration as well as IFNg-secreting cells frequency measured by ELISPOT remained stable over-time even with IgG1 mAbs suggesting no depletion *in vivo*. Interestingly, while animals treated with excipient ($n = 3$) had reproducible DTH responses 1, 2, 3 and 4 months later, DTH responses remained weak in all anti-IL7R mAbs treated animals over this period of time, even though the drug had been for long eliminated.

In conclusion, this PK/PD and safety profile assessment were compatible with a clinical development. The persistent effect seen in this antigen-specific memory response model confirms the potential to control alloimmune responses on the long term.

O336* SELECTIVE CD28 BLOCKADE BLUNTS MEMORY T LYMPHOCYTES REACTIVATION IN NON HUMAN PRIMATES

Nicolas Poirier[1], Melanie Chevalier[2], Caroline Mary[1], Jeremy Hervouet[2], David Minault[2], Paul Baker[3], Simon Ville[2], Stéphanie Le Bas-Bernardet[2], Nahzli Dilek[2], Lyssia Belarif[1], Elisabeth Cassagnau[4], Linda Scobie[3], Bernard Vanhove[1], Gilles Blancho[4]
[1]Effimune; [2]INSERM UMR 1064; [3]Glasgow Caledonian University; [4]CHU Nantes

Selective CD28 blockade, i.e. blockade of CD28 but not CTLA-4 and PDL-1, suppresses T cells activation while enhancing regulatory T cells and tolerance in immunologically naïve animals. While CD28 dominantly controls priming of naïve T-cell and hence memory cell generation, the role of CD28 on established memory T cells remains controversial. We compared the efficacy of FR104, a novel monovalent humanized CD28 antagonist, with the CD80/86 antagonist Belatacept to prevent memory T cell reactivation.

The proliferation of purified human naïve and memory T cells stimulated with alloantigens (allo-Ags) or HLA–restricted peptides from CMV, EBV, Flu and tetanus toxin was measured. FR104 was as effective on memory as it is on naïve T cells to prevent proliferation induced by either allo-Ags or viral peptides. Belatacept inhibited similarly naïve and memory T cell proliferation stimulated with allo-Ags but not when stimulated with viral peptides. We then investigated antigen-specific memory T-cell reactivation *in vivo* in a delayed-type hypersensitivity (DTH) model in baboons sensitized with BCG vaccine. Administration of FR104 dose-dependently ($n = 3$ per group) prevented erythema development and inflammatory skin infiltrates. Unexpectedly, while all animals treated with excipient ($n = 3$) had reproducible monthly DTH responses over 6 months, DTH responses in animals treated with 10 mg/kg FR104 stayed minimal over that period of time, even after drug elimination. No modification of memory T lymphocytes subsets and numbers and no reactivation of chronic latent viruses occurred. In contrast, administration of Belatacept at 10 mg/kg ($n = 3$) was not able to prevent DTH response suggesting that CTLA-4 and/or PD-L1 coinhibitory signals tightly control memory T cell reactivation.

These findings indicate that memory T cell responses are controlled by both CD28 and CTLA-4/PDL1 cosignals and suggests that FR104 might lead to higher therapeutic indexes in transplantation compared to Belatacept.

O337 SELECTIVE BLOCKADE OF THE CD28/B7/CTLA4 PATHWAY WITH MONOVALENT ANTI-CD28 ANTIBODIES VERSUS TARGETING OF B7 WITH BELATACEPT, IN NON-HUMAN PRIMATE KIDNEY ALLOGRAFT

Simon Ville[1], Nicolas Poirier[2], Bernard Vanhove[2], Gilles Blancho[3]
[1]INSERM; [2]Effimune SAS; [3]INSERM UMR1064

CTLA4-Ig is a biologics which targets B7 and prevents its interaction with CD28 and to some extent to CTLA-4. In spite of its effectiveness in inhibiting alloimmune responses, clinical experience in kidney transplantation revealed a high incidence of "belatacept-resistant graft rejection" allocated to heterologous immunity implicating memory cells. Inhibiting the CTLA-4 pathway is the main drawback of B7-specific blocking strategy. Owing to different requirement in costimulatory and co-inhibitory signals to control naïve and memory T cells, selectively targeting CD28 instead of B7 could have advantage. We performed assessment of FR104, a selective CD28 pegylated-Fab' antagonist versus CTLA4-Ig (LEA26Y) in kidney allograft in the baboon. Biologics were used *de novo* together with an initial 1 month treatment with low dose of tacrolimus that was weaned between months 1 and 2, then recipients where under monotherapy with biologics. In CTLA4-Ig group ($n = 4$), all recipients developed severe acute cellular rejection before, during or just after tacrolimus weaning and bolus of steroids were inefficient. In FR104 group- ($n = 5$), only two animals developed an acute rejection episode just after tacrolimus weaning, that could be reversed by steroids. Two recipients were accidentally lost and three still had a stable kidney function at 1 year. A transcriptional analysis of month 1 biopsies did not reveal significant differences, with the remarkable exception of Il-21 which was lower in FR104-treated animals. As the main source of Il-21 is CD4 T follicular helper (Tfh), we assessed *in vitro* proliferation of stimulated Tfh (CXCR5+ICOS+) using human tonsils and found that inhibition was more effective with FR104 than with CTLA4-Ig. This was also true for Tfh memory responses to KLH in mice. These results suggest that the selective blockade of CD28 was more efficient than CTLA4. The mechanism of action could be dependent of Il21, a cytokine know to be antitolerogenic.

O338 PROMOTION OF IMMUNOREGULATION BY SELECTIVE BLOCKADE OF HUMAN CD28 COSTIMULATORY SIGNALING

Fadi Issa[1], Masaaki Zaitsu[1], Joanna Hester[1], Kate Milward[1], Bernard Vanhove[2], Kathryn Wood[1]
[1]University of Oxford; [2]INSERM

Background: Targeting the CD28-CD80/86 co-stimulation pathway with CTLA4-Ig is a promising alternative to current immunosuppression. However, clinical trial data have shown an increase in rates of acute rejection with CTLA-4 Ig treatment in comparison to conventional immunosuppression. This may be related to interference with physiological co-inhibitory CTLA-4 and PD-1 signaling, and blockade of CD28 may circumvent this obstacle. In this study we investigate the hypotheses that a non-activating PEGylated monovalent anti-CD28 antibody (FR104) would suppress alloimmune responses *in vivo* and facilitate adoptive cellular therapy with regulatory T cells (Treg).
Methods: Using a humanised mouse model, we treated mice that had received human skin transplants with FR104 (5 mg/kg), CTLA4-Ig (10 mg/kg) or PEG only control intravenously. All mice were started on the treatment regimen three weeks post-adoptive transfer of allogeneic human peripheral blood mononuclear cells (PBMCs) to ensure adequate levels of human leukocyte chimerism. In a separate set of assays, mice were also treated with *ex vivo*-expanded Treg in order to assess whether Treg activity would be impaired.
Results: FR104 significantly prolonged skin allograft survival compared with CTLA4-Ig (median survival time 56 vs. 31 [$p = 0.002$]). The number of graft infiltrating CD4+ and CD8+ cells was significantly reduced by FR104 treatment as compared with CTLA4-Ig treatment. Interestingly however, the number of infiltrating human FOXP3+ cells was preserved in mice treated with FR104 but reduced with CTLA4-Ig treatment. Finally, in mice treated with FR104, the activity of adoptive Treg therapy was preserved and even synergised with FR104, facilitating long-term human skin transplant survival. In contrast, Treg cell therapy failed in mice treated with CTLA4-Ig, with all grafts being rejected. This highlights the importance of CTLA4 for Treg suppression and provides a new avenue for clinical costimulatory blockade.

O339* A NOVEL, BLOCKING ANTI-CD40 MONOCLONAL ANTIBODY PROLONGS NON-HUMAN PRIMATE RENAL ALLOGRAFT SURVIVAL IN THE ABSENCE OF B-CELL DEPLETION OR THROMBOEMBOLIC EVENTS

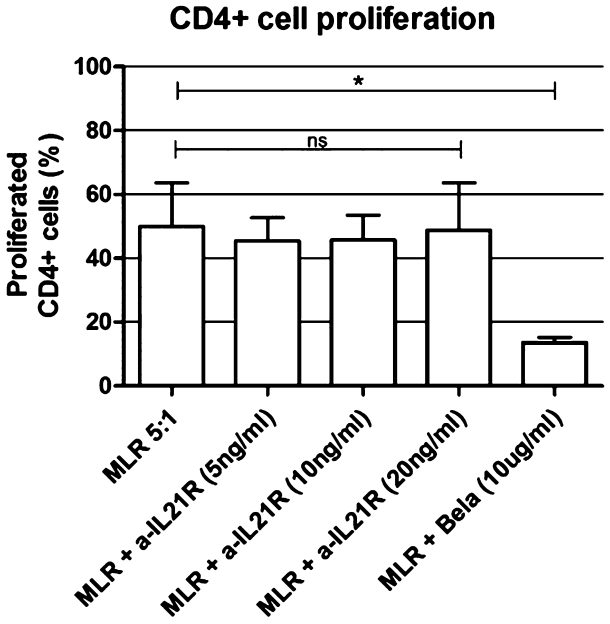
Francisco Cordoba[1], Grazyna Wieczorek[1], Maxime Audet[2], Martin A. Schneider[1], Pascal Espie[1], Lukas Roth[1], Christoph Heusser[1], Christian Bruns[1], Dhavalkumar Patel[1], Boerje Haraldsson[1], James S. Rush[1]
[1]Novartis Institutes for Biomedical Research, Basel; [2]Hôpital de Hautepierre, Strasbourg

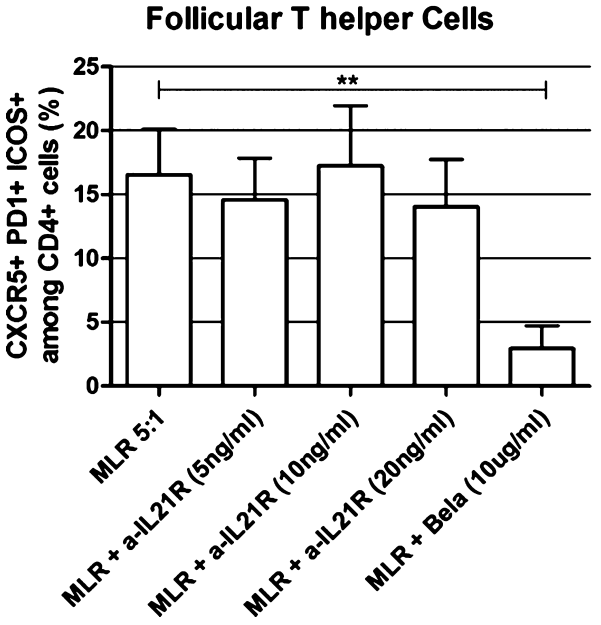
Purpose: CD40-CD154 pathway blockade significantly prolongs renal allograft survival in non-human primates. However, antibodies (Abs) targeting CD154 were associated with an increased incidence of thromboembolic complications precluding clinical development. Here, we assessed relative contribution of B-cell depletion to the efficacy of anti-CD40 blockade.
Methods: We developed a novel, human Fc-silent anti-CD40 monoclonal Ab (CFZ533) that will not cause Ab-dependent cellular cytotoxicity or complement-dependent cytotoxicity and tested it alongside an Fc-competent, B-cell depleting version of the same antibody in MHC-mismatched cynomolgus monkey renal allograft transplantation.
Results: Allograft survival was prolonged in animals treated with the Fc-competent anti-CD40 Ab (52, 22, 24 days) vs. untreated monkeys (survival ~7 days; $n = 9$). Well-functioning allografts survived up to 100 days in CFZ533-treated animals (100, 100, 100, 98, 78 days) at which point the experiment was terminated and graft morphology examined by histology. In contrast to good graft morphology in the CFZ533-dosed group, acute cellular rejection (ACR) was observed in animals treated with Fc-competent anti-CD40. While both Abs completely disrupted splenic germinal centers in transplanted animals (indicating a full tissue pharmacodynamic effect), peripheral blood B-cell depletion was only observed with the Fc-competent Ab. CFZ533 was well-tolerated and there was no evidence of thromboembolic events. Additionally, in most animals CFZ533 largely suppressed a gene signature associated with ACR.
Conclusions: The data indicate that CD40 pathway blockade in the absence of B-cell depletion maintained very high allograft quality and function up to 100 days post-transplantation. Thus, use of the Fc-silent anti-CD40 Ab CFZ533 appears to be an attractive approach for preventing solid organ transplant rejection and treating autoimmune diseases involving T-cell-dependent humoral immune mechanisms.

O340 NOVEL INSIGHTS IN B CELL MEDIATED ALLO-IMMUNE RESPONSES BY TARGETING HUMAN GERMINAL CENTER FOLLICULAR T-B CELL INTERACTIONS

Yannick Rakké[1], Kitty De Leur[1], Gretchen De Graav[1], Frank Dor[2], Carla Baan[1], Marcella Franquesa[1]
[1]Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; [2]Division of HPB & Transplant Surgery, Department of Surgery, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Background: Antibody-mediated rejection (ABMR) has been increasingly recognized as an important cause of allograft dysfunction. We hypothesize that T-B cell interactions are crucial in ABMR. Germinal center CD4+CXCR5+PD1+ICOS+Bcl6+ T follicular helper (Tfh)-cells activate B cells, and play a pivotal role in the generation of efficient antibody responses through costimulatory molecules and cytokines. Here, we investigated whether blockade of the CD28−CD80/86 costimulatory pathway and the interleukin (IL)-21 signaling pathway hampers B cell differentiation into antibody-producing plasmablasts.
Methods: Sorted T and B cells from human donor-derived spleens were co-cultured in a mixed lymphocyte reaction, mimicking lymphoid organ germinal center reactions in the absence and presence of an IL-21 receptor antagonist (anti-IL21R) (5, 10, 20 ng/ml), and belatacept targeting the CD28-CD80/86 (10 μg/ml). Proliferation and differentiation of T and B cell subsets were analyzed after 7 days using flow cytometry.
Results: CD4 T cell proliferation was not inhibited by anti-IL21R, while belatacept had a significant effect (3.7 fold reduction, $p = 0.01$; Fig. 1). Furthermore, only in the presence of belatacept, a reduced number of alloactivated Tfh cells was measured (5.6 fold reduction, $p = 0.002$; Fig. 2). Anti-IL21R inhibited the differentiation of B cells in this T cell dependent system. During blockade in the MLR, we observed a dose-dependent inhibition of B cell proliferation along with a reduction in CD19+CD27^{hi}CD38^{hi} plasmablast formation. In addition, costimulation blockade by belatacept inhibited B cell proliferation and differentiation.
Conclusion: These findings demonstrate that the interaction between human germinal center Tfh and B cells can be differentially blocked by IL21R antagonists and agents targeting the CD28−CD80/86 costimulatory pathway resulting into affected B cell differentiation. This may pave the way for more effective therapeutic approaches in ABMR.





035 TOLERANCE

O341 CONTINUOUS IL-2 EXPOSURE BREAKS RENAL ALLOGRAFT TOLERANCE INDUCED VIA MIXED CHIMERISM PROTOCOL IN NONHUMAN PRIMATES

Yohei Yamada[1], Neal Smith Rex[2], Kiyohiko Hotta[3], Tetsu Ohura[3], Svjetlan Boskovic[3], Joren Madsen[3], Robert Colvin[2], Gilles Benichou[3], Ben Cosimi[3], Tatsuo Kawai[3]

[1]Department of Pediatric Surgery/KEIO University School of Medicine; [2]Massachusetts General Hospital/Pathology; [3]Massachusetts General Hospital/Tranplant surgery

Background: Tolerance of allografts achieved in mice via stable mixed hematopoietic chimerism relies essentially on continuous elimination of developing alloreactive T cells in the thymus (central deletion). Conversely, while only transient mixed chimerism is observed in non-human primates and patients, it is sufficient to ensure tolerance of kidney allografts. In this setting, it is likely that tolerance depends on peripheral regulatory mechanisms rather than thymic deletion.
Methods: A series of monkeys underwent donor bone marrow transplantation and kidney transplantation with nonmyeloablative conditioning as the mixed chimerism protocol. Among animals achieved long term survival (370–4120 days) without maintenance of immunosuppression, seven recipients were treated with daily subcutaneous injections of IL-2 (0.1–3 million IU/m^2) and serum creatinine level was monitored. To evaluate involvement of CD8+/CD3+ T cells in breakdown of tolerance, some animals were treated with anti-CD8 mAb or CD3-Immunotoxin to delete CD8+ or CD3+ T cells during the IL-2 treatment.
Results: Kidney graft functions were impaired following daily administration of IL-2 (1 million IU/m^2) after 3 to 15 days of IL-2 treatment, one animal required 3 million IU/m^2. IL-2 administration was associated with an expansion of alloreactive CD4+/CD8+ effector T cells producing IFNγ cytokine in the kidney grafts. Interestingly, kidney functions were restored once IL-2 treatment was discontinued early and the animals remained tolerant. In addition, IL-2 mediated kidney allograft rejection could be partially prevented via CD8+ or CD3+ T cell depletion. No denovo donor specific antibody was observed. Finally, no evidence of autoimmune pathology was found in any of the monkeys' native organs.
Conclusions: Our finding further supports the view that maintenance of tolerance induced via mixed chimerism relies on T cell regulatory rather than deletional mechanisms.

O342 IL-2-BASED *IN VIVO* EXPANSION OF TREG IN COMBINATION WITH CO-STIMULATION BLOCKADE PROMOTES MAJOR MHC-MISMATCHED ALLOGRAFT TOLERANCE

Lerisa Govender, Rajesh Kumar, Jean-Christophe Wyss, Manuel Pascual, Dela Golshayan
CHUV

Over the past few years, the potential of regulatory T cells (Treg) has been highlighted in immunotherapeutic strategies for autoimmune diseases and after allogeneic transplantation. The first hurdle for the therapeutic use of Treg is their insufficient numbers in non-manipulated individuals, in particular when facing strong immune activation and expanding effector cells, such as in response to an allograft. In this study, we aimed to expand Treg directly *in vivo* and determine their suppressive function, efficacy and stability in promoting donor-specific tolerance in a stringent murine transplantation model. Our data suggest that IL-2-based therapies such as IL-2/JES6-1 immune complexes (IL-2c) lead to a significant increase of Treg *in vivo*. IL-2c expanded Treg not only suppressed Teff proliferation but also Teff cytokine production and allowed prolonged graft survival of major MHC-mismatched skin grafts in wild-type non-lymphopenic recipients. Since the expanded Treg alone were however not sufficient to induce tolerance in stringent experimental conditions, we investigated whether a combination of co-stimulatory blockade with IL-2c-based treatments would be an efficient method for expanding functional Treg *in vivo*, thereby favorably shifting the pool of alloreactive T cells towards regulation in response to an allograft. Indeed, the combination of IL-2c with short-term co-stimulation blockade led to tolerance of major MHC-mismatched skin grafts by hindering the activation and function of not only donor-specific T cells but also B cells, which play a major role in chronic allograft rejection.

O343 DONOR MHC-DERIVED PEPTIDE RECOGNIZED BY TCR BIASED-CD8+ TREGS SUPPRESSES ORGAN REJECTION

Carole Guillonneau
INSERM UMR1064

We previously reported that in a rat MHC mismatched heart allograft model, treatment with CD40Ig leads to indefinite allograft survival mediated by CD8+CD45RClow Tregs, through interaction with pDCs. Although essential, the exact role of TCR/MHC/peptide interaction in Treg activity is still unknown. We therefore studied the Tregs' TCR fine specificity and its impact on Tregs function and allograft survival.

Among 82 unique 16 aa donor-derived allopeptides, we identified one dominant allopeptide, called Du51, that led to a strong activation of Tregs, as shown by the upregulation of several markers. By generating a MHC-I RT1. Aa/Du51 tetramer (tetDu51), we showed that tetDu51+ CD8+ Tregs were enriched in spleen of CD40Ig-treated long-term surviving recipients compared to naive rats. Interestingly, tetDu51+ Tregs were the most suppressive subset among the total Treg population in both direct and indirect pathways of allorecognition. *In vivo*, we showed that Du51 infusion alone resulted in indefinite allograft survival in 80% of the recipients treated with the highest dose of Du51 peptide. The combination of Du51 therapy with either blockade of MHC-I or depletion of CD8+ cells abrogated allograft survival. Moreover, Du51 infusion did not protect against third-party graft acute rejection. Finally, long-term surviving Du51-treated rats displayed a higher number of Tregs in spleen compared to naive ones, an inhibition of alloantibody responses and no sign of chronic rejection. Altogether, these results demonstrated that Du51 treatment alone induced highly suppressive donor-specific CD8+ Tregs capable of *in vivo* tolerance induction.

This study showed that CD40Ig-induced CD8+CD45RClow Tregs recognize Du51 allogeneic peptide with strong therapeutic potential and exert a dominant tolerance, highlighting the importance of the TCR/peptide/MHC interaction for Treg generation and function.

O344 CD8+CD45RCLOW T CELLS: A PROMISING CELL-BASED THERAPY TO INDUCE IMMUNE TOLERANCE

Severine Bezie, Laetitia Boucault, Veronique Daguin, Ignacio Anegon, Carole Guillonneau
INSERM U1064

Background: We previously reported the suppressive properties of rat CD8$^+$CD45RC^{low} T cells. To date, human counterparts have never been studied for their relevance as a cell-based therapy.
Methods: Cell populations were sorted from healthy volunteers PBMCs by FACS Aria. CD8+CD45RC^{low} T cells were cultured with syngeneic CD4+CD25− T cells and allogeneic APCs. Tregs were expanded for 14 days with cytokines and allogeneic APCs or polyclonal stimulation. PBMCs with or without expanded Tregs were injected in NSG mice previously irradiated for xenogeneic GVH reaction study, or previously grafted with allogeneic human skin for allograft survival study.
Results: We proved for the first time that human CD8+CD45RC^{low} T cells are natural regulatory cells. In contrast to CD45RC^{high} subset, CD45RC^{low} cells inhibited allogeneic T cell proliferation *in vitro*, even after thawing and more efficiently than classical CD4+Tregs. Their suppressive mechanisms are complex, involving cytokines, IL-2 deprivation, and contact requirement, but not cytotoxicity, and their regulatory capacity is promoted by pDCs rather than cDCs, as we observed with rat cells. Considering their potential for cellular therapy, we developed a process to expand human Tregs in short-term culture and obtained until 1055 ($\pm$131) fold expansion. Compared with natural Tregs that display a distinct phenotypic profile including all memory cells, expanded Tregs are enriched in effector memory cells and regulatory cytokines secreting cells and are biased in their TCR repertoire. Furthermore, expanded Tregs are highly suppressive *in vitro*, and above all, *in vivo* significantly delay GVH development and allogeneic skin graft rejection in humanized mice.
Conclusion: We identified and characterized a new natural regulatory T cell population as a promising candidate for cellular therapy.

O345 TRANSCRIPTOMIC MICROARRAY ANALYSIS OF REGULATORY B LYMPHOCYTE POPULATIONS IDENTIFIED IN PATIENTS TREATED WITH BELATACEPT

Jérémy Bigot[1], Caroline Pilon[1], Marie Matignon[2], Abdel Aissat[1], José Cohen[3], Philippe Grimbert[4]

[1]INSERM U955 IMRB; [2]INSERM U955 IMRB, Service de Nephrologie et de transplantation, CHU Mondor; [3]INSERM U955 IMRB, CIC Biothérapie CHU Henri Mondor; [4]INSERM U955 IMRB, Service de Nephrologie et de transplantation, CIC-Biothérapie,CHU Mondor

Background: The regulatory B lymphocytes are involved in the establishment of self-tolerance but currently, no specific phenotypic or transcriptional markers are clearly described. In humans, immature transitional B cells defined by

CD24hiCD38hi secrete IL-10 and can inhibit the T cell response *in vitro*. Recently, we have shown that kidney transplant recipients treated with Belatacept (co-stimulatory blocking agent) have a significantly higher rate of CD24hiCD38hi B cells. We present the first transcriptomic and consequently the phenotypic analysis associated of this human population.
Methods: B cells were obtained from human PBMC by magnetic bead selection. Three populations were sorted by flow cytometry: transitional CD24hiCD38hi population and two control populations CD24+CD38-(memory) and CD24intCD38int (mature). A transcriptomic analysis was performed by Agilent Whole human genome oligo microarrays on five healthy donors. Phenotypic analysis and qPCR was performed to confirm some candidate markers identified by the transcriptome data.
Results: Transcriptome bioinformatic analysis revealed a particular signature for the interest CD24highCD38high population with 338 genes over-expressed and 789 genes under-expressed compared to the two other populations. Genes involved in signaling pathways, apoptosis, cell cycle arrest and metalloendopeptidase activity were specifically expressed. Phenotypic analysis showed that CD24hi CD38hi transitional B cells have higher percentage of CD9, CD10, CD1b and lower percentage of CD25, CD58, CD39 and CD73 markers confirming the transcriptome data. qPCR analysis on 13 genes also confirmed the transcriptome profile.
Conclusion: Transcriptomic analysis of these cells enables the identification of specific candidate markers available in flow cytometry and shortly a correlation between selected markers and functional activity and regulatory properties will be analyzed. Moreover, those markers will be analyzed in Belatacept treated patients.

O346 PHENOTYPE CHARACTERIZATION, MIGRATION, ENGRAFTMENT AND SAFETY EVALUATION OF HUMAN CORD BLOOD DERIVED *EX-VIVO* CREATED DI-CHIMERIC CELLS IN NOD SCID MOUSE MODEL: A PRELIMINARY STUDY

Joanna Cwykiel[1], Natalia Filipek[1], Malgorzata Cyran[1], Wojciech Malewski[1], Aleksandra Sklodowska[1], Ewa Bryndza Tfaily[1], Medhat Askar[2], Maria Siemionow[1]
[1]University of Illinois at Chicago; [2]Cleveland Clinic

Background: The aim of this study was to evaluate the safety, migration, and engraftment of *ex-vivo* fused human cord blood derived di-chimeric cells (DCC) in the NOD SCID mouse model.
Methods: A total of 22 fusions of human umbilical cord blood cells (UCB) were performed. UCB from two unrelated donors were separately stained with PKH26 and PKH67 dyes. Fused with polyethylene glycol, double (PKH26/PKH67) stained DCC were sorted and subjected to the following *in vitro* evaluations (10 fusions): lymphocytotoxicity (LCT) test, PCR-SSOP, STR-PCR, viability (Annexin-V, Tunel, LIVE/DEAD staining), colony forming unit (CFU) assay, phenotype, and COMET assay. DCC (3-5x106 cells) from 12 fusions ($n = 4$/Group) were delivered: Group 1: intraosseous, Group 2: intramuscular, and Group 3: subcutaneous to the NOD SCID recipient mice. Control mice in Groups 4, 5, and 6 ($n = 4$) received 3×106 UCB utilizing the same three delivery methods. Mice were evaluated daily by palpation for tumor growth. To assess the migratory pathways of DCC, peripheral blood, bone marrow, lymph nodes, spleen, lung, liver, and skin were assessed at 3 months after delivery using immunofluorescent staining, and PCR. Furthermore, H&E staining was performed to assess harvested tissues for tumor growth.
Results: The presence of HLA class I and II from both UCB donors were confirmed by LCT, PCR-SSOP, and STR. CFU determined proliferative properties of DCC comparable to the UCB. COMET assay confirmed no damage to the DNA of DCC. Migratory properties of DCC were confirmed at 24 and 72 h after cell delivery. Immunofluorescent, histological, and PCR analysis are currently performed.
Conclusions: Phenotype, viability, proliferation, safety, and migratory properties of DCC were characterized. This unique concept of DCC supportive therapy, introducing cells presenting phenotype characteristic of both transplant donor and recipient, is a new approach in the development of transplant tolerance induction.

012 HISTOCOMPATIBILITY

O347 C1Q-BINDING ABILITY OF DONOR-SPECIFIC ANTI-HLA ANTIBODIES FACILITATES THE IDENTIFICATION OF HARMFUL ANTIBODIES ONLY PRE-TRANSPLANT

Teresa Kauke[1], Cornelia Oberhauser[2], Viviane Lin[3], Michaela Coenen[2], Martin Angele[4], Markus Guba[4], Jens Werner[4], Michael Fischereder[5], Bruno Meiser[3], Manfred Stangl[4], Antje Habicht[3]

[1]Laboratory of Immunogenetics, University Hospital Munich; [2]IBE, University Hospital Munich; [3]Transplant Center, University Hospital Munich; [4]Department of Surgery, University Hospital Munich; [5]Renal Division, Department of Internal Medicine IV, University Hospital Munich

Background: The impact of donor-specific (DSA) and non donor-specific (nDSA) anti-HLA antibodies detected only by solid phase assay on graft outcome after renal transplantation is still a matter of debate. Differentiating HLA-antibodies by their ability to bind the complement product C1q might enable a better risk assessment. Therefore we investigated the clinical relevance of pre- and posttransplant C1q binding HLA-antibodies on graft outcome in our center.
Methods: We analyzed the sera of 611 renal allograft recipients who were transplanted in our center between January 2005 and December 2011. The presence of HLA-antibodies and their C1q binding capacity was studied by Luminex Assay prior and after transplantation. Acute rejection (AR) episodes, graft dysfunction (20% increase in creatinine in yearly intervals) and graft survival were assesed within a median follow-up of 4.9 years.
Results: 109/611 (17.9%) patients were immunized at time of transplantation with only 2.6% showing pre-existing DSA und 15.2% nDSA. After transplantation 39/611 (6.4%) recipients developed *deNovo* DSA and 68/611 (11.1%) *deNovo* nDSA. While neither pre-existing nor deNovo nDSA significantly influenced the rate of AR, graft function and/or survival, DSA significantly impaired renal function.

	No HLA antibodies	Pre-existing DSA	Pre-existing nDSA	DeNovo DSA	DeNovo nDSA
Acute rejection (%)	27.6	37.5	30.1	59.0	32.3
Allograft loss (%)	20.1	37.5	28.0	51.3	22.1

However, pre-existing DSA influenced AR rates (C1q− versus C1q+: 33 vs. 40%) and graft survival (C1q− versus C1q+: 30 vs. 50%) only if they were C1q binding while the development of deNovo DSA was associated with a significant increase in AR (C1q− versus C1q+: 50 vs. 63%), and overall graft loss (C1q− versus C1q+: 42 vs. 55%%) independently of the of their ability to bind C1q.
Conclusion: Distinguishing HLA-antibodies by their C1q-binding ability facilitates the identification of renal transplant recipients at immunologic risk only in DSA, which are pre-existing but not in those which develop deNovo post transplant.

O348 COULD C1Q−SCREENING CHANGE THE ANTIBODY MONITORING PROTOCOLS AFTER KIDNEY TRANSPLANTATION?

Wettstein Dániel[1], Döhler Bernd[2], Morath Christian[3], Ruhenstroth Andrea[2], Scherer Sabine[2], Tran Thuong H.[2], Gombos Petra[2], Schemmer Peter[4], Wagner Eric[5], Fehr Thomas[6], Zivcic-Cosic Stela[7], Balen Sanja[7], Weimer Rolf[8], Slavcev Antonij[9], Bösmüller Claudia[10], Norman Douglas J.[11], Zeier Martin[3], Opelz Gerhard[2], Süsal Caner[2]

[1]Department of Transplantation and Surgery, Semmelweis University; [2]Department of Transplantation Immunology, University of Heidelberg; [3]Department of Nephrology, University of Heidelberg; [4]Department of Transplantation and General Surgery, University of Heidelberg; [5]Immunology and Histocompatibility Laboratory, CHU de Quebec, Laval University; [6]Division of Nephrology, University Hospital Zurich; [7]Departments of Nephrology and Dialysis and Immunology, University Hospital Centre, Rijeka; [8]Department of Internal Medicine, University Clinic of Giessen and Marburg, Campus Giessen, Giessen; [9]Department of Immunogenetics, Institute for Clinical and Experimental Medicine (IKEM), Prague; [10]Department of General and Transplant Surgery, Innsbruck Medical University; [11]Oregon Health and Science University, Portland, OR

The widespread use of sensitive antibody detecting methods, especially the Single Antigen Bead (SAB) technique raised several questions regarding the clinical relevance of in these techniques detected antibodies. The Transplantaion Society has formulated clear guidelines for pretransplant and posttransplant antibody monitoring, however, the clinical experience and more recent publications indicate that the application of the C1q-linked SAB test could lead to important changes in the present antibody monitoring algorithms.

We examined the C1q fixing ability of the HLA antibodies detected in 83 patients with failed kidney transplants who were matched with controls with functioning grafts. We then reviewed the recommendations and algorithms regarding the management of kidney transplant patients.

Posttransplant HLA antibodies (*de novo* and persistent) could be detected more frequently in the graft loss group than in the control group with functioning grafts, however, the difference was only significant at high cut-off mean fluorescence intensity level (MFI) ≥5000 (total antibodies: 59% vs. 36%; $p = 0.013$). Interestingly, when C1q binding ability in sera of rejectors and non-rejectors with DSA was compared, none of the non-rejectors demonstrated C1q positivity, whereas 43% of rejectors showed C1q positive antibodies, although not necessarily donor-specific ($p = 0.005$).

Combining solid phase techniques with complement detection is a promising new approach in the field of antibody testing. The present guidelines recommend antibody eliminating treatment only when DSA was present in the serum, and antibody mediated rejection is proven by kidney graft biopsy. Our recent findings suggest that the C1q SAB assay provides additional information to the IgG SAB assay, and is a valuable tool in the decision process for the initiation of rejection therapy. It seems that C1q positive DSA alone indicates the necessity for antibody eliminating therapy.

O349 SPECTRUM OF ANTI-HLA SENSITIZATION AFTER KIDNEY ALLOGRAFT NEPHRECTOMY

Marie Matignon[1], Claire Leibler[2], Olivier Moranne[3], Laurent Salomon[4], Dominique Charron[5], Philippe Lang[6], Christian Jacquelinet[7], Caroline Suberbielle[5], Philippe Grimbert[2]

[1]Nephrology and Renal Transplantation and CIC-BT 504 Department, Henri Mondor Hospital, AP-HP, INSERM U955, ParisEst University; [2]Nephrology and Renal Transplantation and CIC-BT 504 Department, Henri Mondor Hospital, AP-HP, INSERM U955, Paris Est University; [3]Nephrology & Public Health Department, CHU Nice, Nice, France; [4]Urology Department, Henri Mondor Hospital, AP-HP, Paris XII University, Créteil, France; [5]Histocompatibility and Immunology Department, Saint Louis Hospital, AP-HP, Paris, France; [6]Nephrology and Renal Transplantation and CIC-BT 504 Department, Henri Mondor Hospital, AP-HP, INSERM U955, Paris XII University; [7]Agence de la biomédecine

The issue of allograft nephrectomy after kidney transplant failure remains unresolved. An accurate description of both features and outcome of anti-HLA sensitization at the time and following allograft nephrectomy in different clinical context may allow clinicians to better define clinical indication and preventive therapeutic strategy.

We carried out a retrospective analysis of 63 clinically indicated allograft nephrectomies performed from January 2005 to January 2010. Groups were defined on time elapsed since transplantation (6 months) and clinical background. Anti-HLA antibodies (Luminex assays) and calculated panel reactive antibodies (cPRA) were determined at baseline and three and 12 months after kidney transplant.

Fifteen (24%) nephrectomies were performed less than 6 months after transplantation (early nephrectomies). Among late nephrectomies performed more than 6 months after transplantation (48 (76%)), 14 patients (22%) were considered asymptomatic and 34 (54%) as having graft intolerance syndrome. Anti-HLA sensitization and cPRA at baseline were significantly lower in patients undergoing early and late asymptomatic nephrectomies. However, both increased considerably within the 3 months following surgery, reaching similar levels to those observed in the graft intolerance syndrome group.

In populations undergoing clinically indicated nephrectomy, the spectrum of anti-HLA sensitization depends on both the clinical and immunological context, potentially requiring different immunomodulatory preventive strategies.

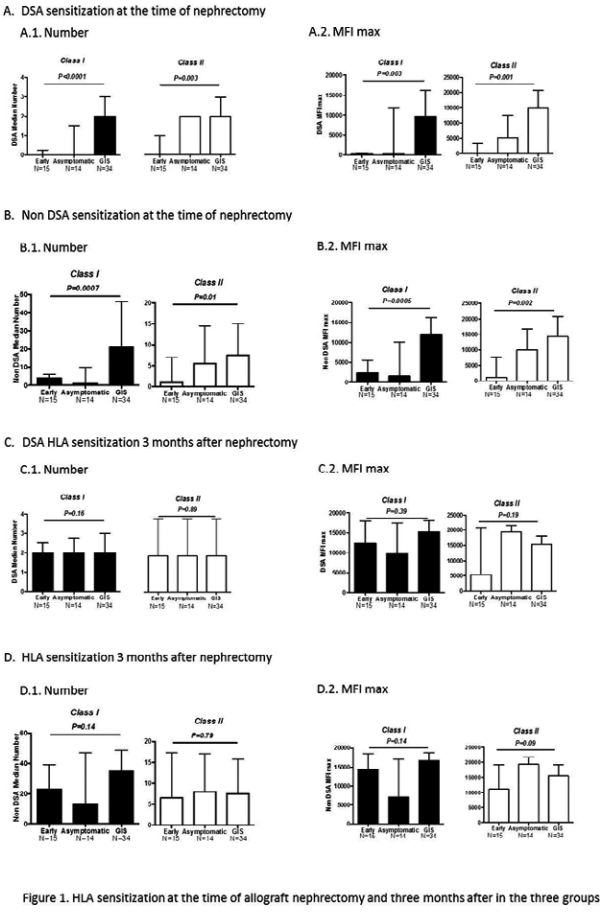


Figure 1. HLA sensitization at the time of allograft nephrectomy and three months after in the three groups

O350 ALLOANTIBODY FORMATION FOLLOWING FAILURE OF A FIRST RENAL TRANSPLANT CAN BE BETTER PREDICTED BY THE NUMBER OF DONOR AMINO-ACID MISMATCHES AND THEIR PHYSIOCHEMICAL MISMATCH SCORE THAN CONVENTIONAL HLA

Kosmoliaptsis Vasilis, Mallon Dermot H, Chen Yining, Bolton Eleanor M, Andrew Bradley J, Taylor Craig J
University of Cambridge

Background: We reported previously that allosensitisation following a failed kidney transplant increases with each mismatch at HLA-A,-B,-C,-DRB1,-DRB3/4/5 and -DQB1 loci. Equal weighting was assigned to HLA-mismatches and no account was taken of differences in donor HLA immunogenicity according to recipient HLA type. Using intra- and inter-locus amino-acid subtraction we have assessed the influence of donor amino-acid mismatches (AMM), eplet mismatches (EMM) and amino-acid electrostatic mismatch score (EMS) on the development of HLA-specific alloantibodies in this cohort.
Methods: Serum samples from 131 consecutive patients were obtained pre-transplant and every 3 months after graft failure, and screened using HLA-single-antigen-beads. The effect of AMM, EMM and EMS on the development of HLA-specific alloantibody (calculated reaction frequency, cRF) and donor HLA-specific antibody (DSA) was determined.
Results: On univariate analysis, donor AMM, EMM and EMS all correlated with increasing HLA-class I and class II cRF. Other significant variables were time waiting and dual immunosuppression, but not graft nephrectomy. Multivariate analysis, controlling for time waiting and dual immunosuppression, showed the odds ratio (OR) for developing sensitisation (cRF>15%) was independently correlated with AMM (OR = 1.37 per 10 mismatches), EMM (OR = 1.35 per 10 mismatches) and EMS (OR = 1.24 per 10 units). Using linear-regression models to subtract the impact of HLA-mismatches, only AMM and EMS had significant additional strength in predicting cRF>15%. Increasing AMM, EMM and EMS all correlated with a higher risk of development of HLA-class II DSA, but only EMS predicted the risk of HLA-A and -B DSA development.
Conclusion: HLA-specific allosensitisation can be better predicted using AMM and EMS than conventional HLA-matching.

O351 PREFORMED AND *DE NOVO* ANTI-ENDOTHELIAL ANTIBODIES IN RENAL TRANSPLANT PATIENTS: EFFECT ON ALLOGRAFT EVOLUTION

Elena Sánchez Zapardiel, Esther Mancebo, María Díaz-Ordoñez, Lucía Jorge-Huertas, Lara Ruiz-Martínez, Antonio Serrano, José Castro-Panete María, Pablo Morales, Amado De Andrés, María Morales José, Sara Domínguez-Rodríguez, Estela Paz-Artal
Hospital Universitario 12 de Octubre

Background: Anti-endothelial antibodies (AECA) have been related to autoimmune diseases and humoral alloresponses. Our aim is to determine the prevalence of AECA among kidney recipients, characterize their pattern and study their relationship with renal transplant evolution.
Methods/Materials: We selected 334 renal transplanted patients without pre- and post-transplant anti-HLA and anti-MICA antibodies and retrospectively tested the presence of AECA in their first post-transplantation (Tx) serum sample by indirect immunofluorescence (IFI) on human vein endothelial cells. By analyzing the pre-Tx sample of AECA+ patients, we determined whether AECA were pre-existing or *de novo*. Anti-HLA, anti-MICA and ANA were studied by multiplexed bead assay.
Results: Seventy (21%) patients had post-Tx AECA, 44% *de novo* AECA and 56% preformed AECA. Preformed AECA were more frequent among female recipients ($p = 0.055$) whereas *de novo* AECA were associated with peritoneal dialysis ($p = 0.025$), longer dialysis time ($p = 0.023$), patient's weight ($p = 0.002$) and cyclosporine use ($p = 0.025$). We found six different IFI patterns: anti-cytoskeleton (31%), cytoplasmic discrete speckles (29%), anti-nuclear (19%), mitochondrial-like (15%), rod and rings-like (4%) and anti-Golgi apparatus (1%). Kaplan-Meier curves for negative-, preformed- or *de novo* -AECA status showed global allograft rejection differences ($p = 0.049$), with significantly less survival for grafts in *de novo* AECA+ recipients ($p < 0.0001$). In a multivariate analysis, *de novo* AECA emerged as an independent risk factor for allograft rejection (HR 5.75, $p < 0.0001$).
Conclusion: The development of preformed AECA is more common among female renal transplant recipients whereas *de novo* AECA might be associated with cyclosporine use, type and length of dialysis. Six different IFI patterns support the reported heterogeneity of AECA. The presence of *de novo* AECA is associated to shorter graft survival and allograft rejection.

O352 HLA INCOMPATIBLE LIVING DONOR RENAL TRANSPLANT OUTCOMES: A SINGLE CENTRE EXPERIENCE

Vijaya Ramasamy[1], Pramod Nagaraja[1], Emma Burrows[2], Frances Edwards[2], Sandra Lloyd[2], Rhian Cooke[1], Argiris Asderakis[1], Tracey Rees[2], Siân Griffin[1]
[1]University Hospital of Wales, Cardiff; [2]Welsh Transplantation and Immunogenetics Laboratory (WTAIL)

Background: The optimal desensitization regimen and induction immunosuppression for renal transplant recipients with a positive cross match due to preformed donor specific antibodies (DSAs) is unclear.
Methods: Data was collected prospectively for 25 recipients of a living donor (LD) transplant performed following removal of HLA donor specific antibodies (HLAi). Prior to treatment, four recipients had a positive cytotoxic cross match and 21 had a positive flow cytometric cross match, due to the presence of class I HLA DSA ($n = 7$, 28%), class II HLA DSA ($n = 9$, 36%) or both ($n = 9$, 36%). The comparator group were 81 immunologically low risk, cross match negative LD renal transplants recipients (HLAc). The desensitization regimen comprised of rituximab 4 weeks prior to transplantation, double filtration plasmapheresis (DFPP) with low dose intravenous immunoglobulin (IVIG) and induction with T cell depletion (either alemtuzumab or anti-thymocyte globulin). The group without DSAs received induction with basiliximab. All patients received triple oral immunosuppression with tacrolimus, mycophenolate mofetil and prednisolone.
Results: The mean age of the two groups was similar (HLAi 47 $\pm$ 11 years, HLAc 45 $\pm$ 14 years, $p = 0.525$). The HLAi group were more likely to be female (HLAi 72%, HLAc 35%, $p = 0.001$), to have received a previous transplant (HLAi 64%, HLAc 15%, $p < 0.001$), and have been receiving dialysis for longer period prior to the current transplant (median HLAi 18 months, median HLAc 11 months, $p = 0.026$). Similar proportions in each group received pre-emptive transplants (HLAi 16%, HLAc 33%, $p = 0.096$) and were diabetic (HLAi 8%, HLAc 14%, $p = 0.457$). Graft outcomes and complications in the first year are shown in Table 1. The 5-year cumulative Kaplan-Meier death censored graft survival curve is depicted in Fig. 1. All 5 cases of antibody-mediated rejection were successfully treated with no graft loss and subsequent good function. One graft failed in the HLAi group due to recurrent FSGS.
Conclusion: By tailoring induction immunosuppression to immunological risk, excellent medium term outcomes can be achieved, despite the presence of HLA DSAs prior to transplantation. We are currently defining the post transplant course of HLA DSA levels following this desensitization and induction regime.

	HLAi group (n = 25)	HLAc group (n = 81)	p Value
Patient survival at 1 year (%)	100	96	0.329
Death censored graft survival at 1 year (%)	96	94	0.681
Mean eGFR ± SD ml/min/1.73 m^2			
1 month	60 ± 17	60 ± 20	0.929
6 months	56 ± 13	56 ± 16	0.973
12 months	54 ± 13	63 ± 18	0.053
2 years	54 ± 12	60 ± 18	0.191
3 years	50 ± 13	60 ± 16	0.092
4 years	49 ± 13	57 ± 18	0.275
5 years	54 ± 6	54 ± 20	0.975
No. of patients with rejection in 1st year (%)	6 (24)	16 (20)	0.647
Type of rejection in 1st Year (%)	3 borderline acute cellular rejection(33)@1 acute cellular rejection(11)@5 acute antibody mediated rejection(56)	12 borderline acute cellular rejection(55) @10 acute cellular rejection(45)	
No. of patients experienced neutropenia in 1st year (%)	16 (64)	22 (27)	0.001
No. of patients with CMV infection in 1st year (%)	1 (4)	1 (1)	0.374
No. of patients with BK viraemia in 1st year (%)	3 (12)	1 (1)	0.014
No. of patients with bacterial infection in 1st year (%)	7 (28)	28 (35)	0.542

025 LIVER

OLB01*

DUAL HYPOTHERMIC OXYGENATED PERFUSION IN LIVER TRANSPLANTATION WITH DONATION AFTER CIRCULATORY DEATH GRAFTS: FIRST CLINICAL SERIES

Rianne Van Rijn, Negin Karimian, Alix Matton, Laura Burlage, Andrie Westerkamp, Janneke Wiersema-Buist, Ruben De Kleine, Marieke De Boer, Robert Porte

University Medical Center Groningen

Background: Animal studies have demonstrated that dual hypothermic oxygenated machine perfusion (DHOPE) via hepatic artery and portal vein is a promising method to restore hepatic energy status and reduce reperfusion injury in donation after circulatory death (DCD) liver grafts. Aim of this clinical study was to assess the safety and feasibility of DHOPE in DCD liver transplantation.
Methods: In 10 patients undergoing DCD liver transplantation, the liver was treated with 2 hr of DHOPE after transportation with conventional static cold storage (SCS). Livers underwent DHOPE with Machine Perfusion Solution Belzer - UW at 12°C using a pressure controlled device (Liver Assist, Organ Assist). During DHOPE, mean arterial pressure was 25 mmHg and portal pressure 5 mmHg. Outcome was compared with a matched control group of 20 DCD liver transplantations preserved with SCS only.
Results: There were no technical problems during DHOPE. Median preservation time including DHOPE was not longer compared to controls (8.7 h [IQR 7.8–9.9] vs. 8.4 h [IQR 7.9–8.8]; p = 0.448). During DHOPE, median hepatic ATP content increased >10-fold from 5.5 (IQR 3–10) to 65.5 μmol/g protein (IQR 41–87); p = 0.005. All DHOPE preserved livers showed excellent early function. Postoperative median peak ALT was lower compared to controls (966 U/l [IQR 718–1631] vs. 1856 U/l [IQR 1086–2380]; p = 0.006). At a median follow up of 7.3 months (range 4.9–12.2) one of the DHOPE preserved livers had developed non-anastomotic biliary strictures (NAS). In contrast, incidence of early NAS was 30% in controls (p = 0.372).
Conclusion: This first clinical study of end-ischemic DHOPE in DCD liver transplantation demonstrates that this technique is safe, can restore cellular energy levels, and reduce reperfusion injury. Our data suggest that DHOPE may reduce the incidence of NAS after DCD liver transplantation.

OLB02*

10 YEAR NATIONAL EXPERIENCE OF LIVER TRANSPLANTATION AND RESECTION OF CHOLANGIOCARCINOMA

Sheehan Mark[1], O'farrell Naoimh[1], Lavelle Lisa[2], Saeed Nina[1], A. Cooney[1], N. Swan[3], O. Traynor[1], Justin Geoghegan[1], Donal Maguire[1], Emir Hoti[1]

[1]St Vincent's University Hospital; [2]St Vincent's University Hospital Radiology Department, Elm Park, Dublin; [3]St Vincent's University Hospital Pathology Department, Elm Park, Dublin

Introduction: Cholangiocarcinomas have a dismal prognosis, with only 20% amenable to surgical resection. For unresectable cholangiocarcinomas, initial studies demonstrated poor results with liver transplantation (LT). However, the Mayo Clinic published 5-year survivals >60% in cases undergoing neoadjuvant chemoradiotherapy + LT. This approach is novel and not yet adopted internationally. Here, we examine different management approaches for cholangiocarcinoma.
Methods: Data were collected between 2005 and 2014, from a prospectively maintained LT database and retrospectively retrieved from the hospital's pathology database.
Results: Sixty-two cholangiocarcinomas were treated with curative intent. Twenty-four (39%) unresectable cholangiocarcinomas, predominantly hilar, completed the Mayo-protocol. The remaining 38 (61%) underwent resection, predominantly right extended hepatectomy (63%). LT group was significantly younger (51 years vs. 61 years) (p = 0.05). Eighteen (75%) transplants had an underlying diagnosis of primary sclerosing cholangitis compared with 11% of resections (p < 0.0001). There were four (16.7%) in-hospital deaths in the LT group compared with three (7.9%) resections (p = 0.288). Excluding these from long-term survival analysis; there was a trend towards increased survival in Mayo-protocol patients (8.8 years [3.6–14.0 years] vs. 4.4 years [0.7–8.1]) (p = 0.082). LT tumors were significantly smaller (p < 0.0001), with 65% demonstrating a complete pathologic response to neoadjuvant chemoradiation. Survival was significantly increased in T0 tumors compared with patients with residual disease (p = 0.005).
Conclusions: This is the first European study to mirror the Mayo Clinic experience. We demonstrated 55% 5-year survival rates with the Mayo protocol, with 65% having a complete pathologic response to neoadjuvant therapy. This in itself is associated with a significantly increased survival in patients previously classified as unresectable.

033 TISSUE ENGINEERING

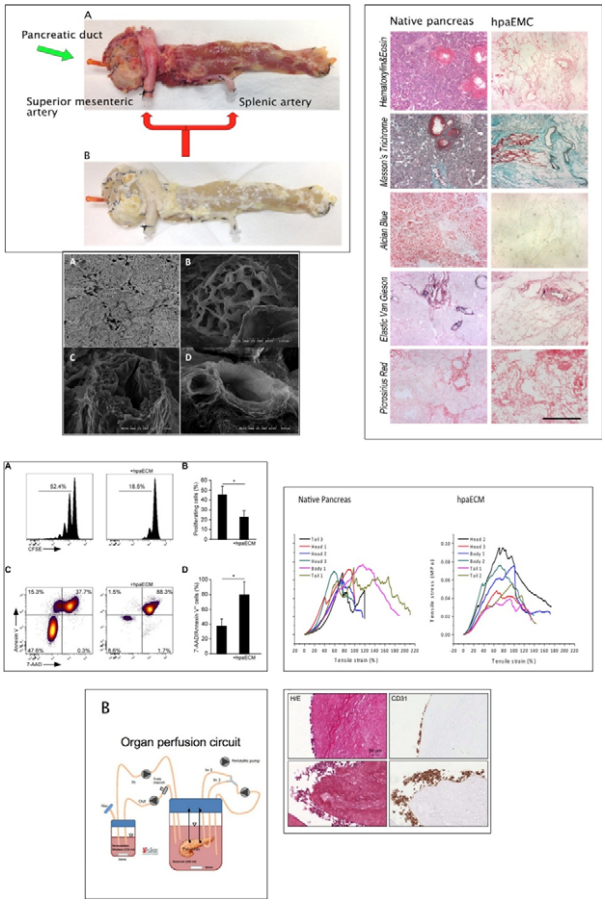
OLB03

THE HUMAN PANCREAS AS A SOURCE OF PRO-TOLEROGENIC EXTRACELLULAR MATRIX SCAFFOLD FOR A NEW GENERATION BIO-ARTIFICIAL ENDOCRINE PANCREAS

Giuseppe Orlando[1], Andrea Peloso[2], Luca Urbani[3], Paolo Cravedi[4], Ravi Katari[5], Panagiotis Maghsoudlou[6], Enrique Mario Alvarez Fallas[6], Valeria Sordi[7], Carolina Purroy[8], Guoguang Niu[9], John P. Mcquilling[9], Sittadjody Sivanandane[9], Alan C. Farney[5], Samy S. Iskandar[5], Jeffrey Rogers[5], Robert J. Stratta[5], Emmanuel C. Opara[9], Lorenzo Piemonti[7], Shay Soker[9], Paolo De Coppi[6]

[1]WFSOM; [2]IRCCS Policlinico San Matteo - Dept. of General Surgery, University of Pavia, Pavia, Italy; [3]2Surgery Unit, Stem Cells and Regenerative Section, UCL Institute of Child Health and Great Ormond Street Hospital, London, UK; [4]Department of Medicine, Recanati Miller Transplant Institute and Immunology Institute, Icahn School of Medicine at Mount Sinai; [5]Wake Forest School of Medicine; [6]Surgery Unit, Stem Cells and Regenerative Section, UCL Institute of Child Health and Great Ormond Street Hospital, London, UK; [7]Diabetes Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy; [8]Renal Division, Department of Medicine, Icahn School of Medicine at Mount Sinai, NY, USA; [9]WFIRM - Wake Forest Institute for Regenerative Medicine

Background: Our study aims at producing acellular extracellular matrix scaffolds from the human pancreas (hpaECMs), as a first critical step towards the production of a new generation, fully human-derived bio-artificial endocrine pancreas (BAEP). In this BAEP, the hardware will be represented by hpaECMs, while the software will consist in the cellular compartment generated from patient's own cells.
Methods: To achieve our goal, human pancreata were decellularized with Triton-based solution and thoroughly characterized. Primary endpoints were: complete cell and DNA clearance, preservation of ECM components, analysis of growth factors (GFs) and stiffness, ability to induce angiogenesis, conservation of the framework of the innate vasculature, and immunogenicity. Secondary endpoint was hpaECMs' ability to sustain growth and function of human islet and human primary pancreatic endothelial cells (hPPEC).
Results: HpaECMs can be successfully and consistently produced from human pancreata, maintain their innate molecular and spatial framework and stiffness, as well as vital GFs. Importantly, hpaECMs inhibit human naïve CD4+ T cell expansion in response to polyclonal stimuli by inducing their apoptosis and promoting their conversion into regulatory T cells. hpaECMs are cytocompatible and supportive of representative pancreatic cell types.
Discussion: hpaECMs has the potential to become an ideal platform for investigations aiming at the manufacturing of a regenerative medicine-inspired BAEP.



023 KIDNEY

OLB04

DE NOVO DONOR SPECIFIC ANTIBODIES AND ASSOCIATION WITH KIDNEY ALLOGRAFT REJECTION AFTER EARLY SWITCH FROM CALCINEURIN INHIBITOR TO EVEROLIMUS: ANALYSIS OF THE ELEVATE TRIAL

Johan W. De Fijter[1], Hallvard Holdaas[2], Patricia Lopez[3], Cesar Escrig[3], Zailong Wang[4], Frans H.J. Claas[1], Wolfgang Arns[5], Josep M. Cruzado[6], Markus Van Der Giet[7]

[1]Leiden University Medical Centre, Leiden; [2]Oslo University Hospital, Oslo; [3]Novartis, Basel; [4]Novartis Corporation, East Hanover, NJ; [5]Cologne Merheim Medical Center, Kohl; [6]Bellvitge University Hospital, Barcelona; [7]Charite - Universitätsmedizin Berlin, Berlin

Background: Formation of *de novo* donor specific antibodies (dnDSA) is a risk factor for antibody-mediated rejection (AMR) and increased risk of graft failure. The ELEVATE study has monitored prospectively, the status and formation of DSA at M12 and M24 using a central lab assessment.
Methods: ELEVATE a 24M, multicenter, open-label trial, in which *de novo* RTxRs were randomized at 10–14 weeks post-Tx to convert from CNI to EVR (n = 360: C0 6–10 ng/ml) or continue standard CNI (n = 357: C0, tacrolimus: 5–10 ng/ml, cyclosporine: 100–250 ng/ml); all received enteric-coated mycophenolate sodium (MPS) + corticosteroids. Blood samples were collected for all patients at baseline, randomization (RND), M12 and M24 or at time of rejection episodes. Samples were processed and analyzed by Luminex assay in one central lab. dnDSA was defined by a MFI value ≥500 any time post-RND in patients with MFI <500 at RND, other cut-off levels were explored.
Results: Baseline characteristics of this population have been previously described. PRA <20% was observed in EVR: 89.0% and CNI: 87.9%. HLA mismatches ≥3 were observed in 64.3% and 66.6% of patients in the EVR and CNI group, respectively. Overall, a low incidence of dnDSA HLA class-I was observed at M12 and M24 for both groups when using different cut-off levels. Higher incidence of dnDSA HLA class-II (mainly *de novo* DQ antibodies) in comparison to dnDSA HLA class-I was observed in both groups at M12 and M24 (with a trend towards higher incidence within the CNI group) [Table1 A]. No association between *de novo* DSA (either class-I or -II) and diagnosis of AMR or BPAR was evident; neither at M12 nor at M24 [Table1 B,C].
Conclusion: Early conversion to EVR/MPS therapy at 3M post-Tx did not increase the risk of *de novo* DSA formation compared to continuation on CNI/MPS. Moreover, no association between *de novo* DSA formation and a diagnosis of AMR or BPAR was observed, even using the lowest detection thresholds.

TABLE 1: Outcomes on *de novo* DSA at M12 and M24

A) Incidence of de novo DSA (%)

HLA Class I		M12		M24		HLA Class II		M12		M24	
		EVR	CNI	EVR	CNI			EVR	CNI	EVR	CNI
MFI ≥ 500	HLA A	6.3	2.8	8.4	2.1	MFI ≥ 500	HLA DR	3.4	0	3.4	1.7
	HLA B	4.5	2.7	2.7	0.7		HLA DQ	16.7	14.3	8.3	14.3
MFI ≥ 1000	HLA A	4.2	0.7	7.4	0.7	MFI ≥ 1000	HLA DR	1.1	0	1.1	0
	HLA B	2.7	0.7	1.8	0		HLA DQ	11.1	10.2	2.8	14.3
MFI ≥ 2000	HLA A	3.2	0.7	5.3	0.7	MFI ≥ 2000	HLA DR	1.1	0	1.1	0
	HLA B	0.9	0.7	0.9	0		HLA DQ	5.6	10.2	2.8	10.2
MFI ≥ 3000	HLA A	2.1	0	3.2	0	MFI ≥ 3000	HLA DR	0	0	1.1	0
	HLA B	0.9	0	0.9	0		HLA DQ	2.8	8.2	0	6.1

B) AMR in patients with *de novo* DSA (N/n)*

HLA Class I		M12		M24		HLA Class II		M12		M24	
		EVR	CNI	EVR	CNI			EVR	CNI	EVR	CNI
MFI ≥ 500 at time of event and <500 at RND	HLA A	6/0	4/0	8/0	3/1	MFI ≥ 500 at time of event and <500 at RND	HLA DR	3/1	0/0	3/0	2/1
	HLA B	5/1	4/0	3/0	1/0		HLA DQ	6/0	7/0	3/0	7/0

* N; number of patients with corresponding DSA category, n number of patients that developed AMR

C) BPAR in patients with *de novo* DSA (N/n)^

HLA Class I		M12		M24		HLA Class II		M12		M24	
		EVR	CNI	EVR	CNI			EVR	CNI	EVR	CNI
MFI ≥ 500 at time of event and <500 at RND	HLA A	6/0	4/1	8/0	3/2	MFI ≥ 500 at time of event and <500 at RND	HLA DR	3/0	0/0	3/0	2/1
	HLA B	5/0	4/0	3/0	1/0		HLA DQ	6/0	7/1	3/0	7/1

^ N; number of patients with corresponding DSA category, n number of patients that developed BPAR

027 LUNG

OLB05*

THE ORGAN CARE SYSTEM (OCS™) LUNG INSPIRE INTERNATIONAL TRIAL RESULTS

Gregor Warnecke[1], Dirk Van Raemdonck[2], Jasleen Kureja[3], Michael Smith[4], Gabiel Loor[5], Gilbert Massard[6], Federico Rea[7], Andre R. Simon[8], Javier Moradiellos[9], Andres Varela[9], Igor Tudorache[1], Christian A. Kuehn[1], Murat Avsar[1], Wiebke Sommer[1], Bettina Wiegmann[1], Hermann Reichenspurner[10], Joren Madsen[11], Jaya Nagendran[12], Pascal A. Thomas[13], Nicola Santelmo[6], Pierre E. Falcoz[6], Anne Olland[6], Marco Schiavon[7], Christoph Knosalla[14], Roland Hetzer[14], Steven Tsui[15], Christian Bermudez[16], Kumud Dhital[17], Ken Mccurry[18], Marshall Hertz[5], Axel Haverich[1], Abbas Ardehali[19]

[1]Hanover Medical School; [2]University of Leuven; [3]UCSF, CA; [4]St. Joseph's Hospital, Phoenix, AZ; [5]University of Minnesota, Minneapolis, MN; [6]University of Strassburg; [7]University of Padua; [8]Royal Brompton and Harefield Trust, London; [9]Hospital Puerto de Hierras, Madrid; [10]UKE, Hamburg; [11]Harvard Medical School, Boston; [12]University of Alberta, Edmonton; [13]University of Marseille; [14]DHZB, Berlin; [15]Pap worth Hospital, Cambridge; [16]University of Pittsburgh Medical Center; [17]St. Vincent's Hospital, Sydney; [18]Cleveland Clinic; [19]UCLA, CA

Purpose: The OCS Lung INSPIRE Trial is a prospective randomized trial comparing portable *ex-vivo* machine perfusion versus standard cold storage in routine lung transplantation.
Methods: Donor lungs are preserved with OCS-Lung perfusion device (OCS) or Standard of Care (SOC) Cold flush and storage. Donors: age 300 mmHg.

Recipients: ≥18 years old; double lung transplant. 21 centers in U.S., Europe, Australia and Canada randomized 320 patients. Primary effectiveness endpoint: composite of early patient survival and freedom of primary graft dysfunction (PGD) grade 3 within first 72 h. Primary safety endpoint is the rate of lung graft related SAEs. Primary hypothesis: OCS treatment is non-inferior to SOC regarding the primary effectiveness endpoint, patient survival post-transplantation and freedom of PGD Grade 3 within T72 hours.
Results: 306 subjects were treated per protocol, 141 OCS group and 165 SOC group patients. While the total out of body time was longer in the OCS group, the total ischemia time was significantly shorter versus SOC group ($p < 0.001$). At day 30, survival and freedom of PGD 3 within 72 h was 79.4% of the OCS vs. 70.3% of SOC patients (Primary effectiveness endpoint: Non-inferiority $p = 0.0045$ and superiority $p = 0.09$). For the composite of in hospital survival and freedom of PGD 3 within 72 h, 80.1% of OCS patients met the endpoint vs. 66.7% of SOC arm (Non-inferiority $p = 0.0003$ and superiority $p = 0.01$). Freedom from PGD 3 within the first 72 h post-transplant was 82.3% in OCS arm vs. 70.3% in the SOC arm (superiority test $p = 0.016$). The trial met the primary safety endpoint under the non-inferiority margin of 0.075; $p = 0.0035$.
Conclusions: This trial meets all its non-inferiority endpoints and multiple important superiority endpoints including a significant reduction of PGD grade 3 using the OCS Lung technology. These results provide evidence for new portable EVLP strategies in routine lung preservation to improve outcomes.

011 HEART

OLB06* LONG-TERM THERAPY WITH EVEROLIMUS IN HEART TRANSPLANT RECIPIENTS: RESULTS FROM THE CERTIC REGISTRY

Luciano Potena[1], Roberto Fiocchi[2], Ciro Maiello[3], Andrea D'armini[4], Mauro Rinaldi[5], Gino Gerosa[6], Massimo Maccherini[7], Ugolino Livi[8], Maria Frigerio[9], Delia Colombo[10], Rossella Brusa[10], Francesco Parisi[11]

[1]Sant'Orsola Malpighi Hospital Bologna; [2]Papa Giovanni XXIII Hospital, Bergamo; [3]Monaldi Hospital - II Università, Napoli; [4]San Matteo Hospital, Pavia; [5]San Giovanni Battista Hospital, Torino; [6]University Hospital of Padova; [7]Policlinico Santa Maria alle Scotte, Siena; [8]Santa Maria della Misericordia Hospital, Udine; [9]Niguarda - Ca' Granda Hospital, Milano; [10]Novartis Farma; [11]Bambin Gesù Hospital, Roma

Everolimus (EVR) displays multiple effects potentially associated with benefits in patients with long-term complications, such as malignancies, allograft vasculopathy or renal insufficiency. We designed the CERTIC registry to collect prospectively multicenter data on safety and efficacy of long term *de novo* and converted patients.

Between 2008 and 2010, 781 recipients of renal or HT on therapy with EVR for at least 6 months entered this 5 year registry. We report the results from the analysis after 5 year of observation about survival, malignancies and renal function in HT recipients' cohort.

401 HT recipients were enrolled; 95 patients (24%) started EVR as a *de novo* strategy, while 306 (76%) started EVR for clinical reasons 8 ± 5 year after HT, mainly because of renal dysfunction (n = 173, 57%), allograft vasculopathy (n = 65, 21%), malignancies (n = 41, 13%) or other reasons (n = 27; 9%). Overall, including the period of EVR treatment before study entry, patients had been on EVR for 7 ± 1 year. Survival rate was 93% in *de novo*, with 7 fatal events, and 83% in conversion patients with 53 fatal events. Incidence rate of post-transplant malignancies, excluding non-melanoma skin cancers, was 0.9% in *de novo* and 2.1% in conversion patients considering tumors occurring after EVR introduction. Renal function was overall stable during EVR treatment, with 32 patients out of the whole cohort, all in convertion group, returning to dialysis (8%) at 13.5 ± 5.5 year from transplant. Estimated glomerular filtration rate (eGFR, ml/min/1.73 m^2) at study enrollment and at 5 year was: 65.7 ± 46.8 and 68.4 ± 43.8 in *de novo*, p = ns; 56.4 ± 42.1 and 56.5 ± 38.9 in conversion patients, p = ns. EVR introduction in patients with renal function impairment stabilized eGFR levels (44.5 ± 27.4 at conversion and 51.0 ± 34.3 at 5 year, n = 173, p = ns).

By picturing real-life clinical practice, this large prospective registry on EVR shows promising survival rates, low incidence of malignancies and renal function seems to be effectively preserved.

FO001 EUROPE'S FIRST SUCCESSFUL DCD HEART TRANSPLANT WITH FUNCTIONAL ASSESSMENT

Simon Messer[1], Richard Axell[2], Paul White[2], Simon Colah[1], Ayyaz Ali[1], Steven Tsui[1], Stephen Large[1]

[1]Papworth Hospital; [2]Addenbrookes Hospital

Aim: The demand for heart transplantation has never been greater. With falling numbers of suitable brain dead (DBD) donors and an exponentially increasing waiting list, attention has fallen on the Donation After Circulatory Determined Death (DCD) Donor. Unfortunately even although it is almost 50 years since the world's first successful DCD heart transplant there still remains no method of functional assessment of the DCD donor heart following the mandatory warm ischaemic period. We sought to determine whether function could be restored and assessed within the donor using normothermic regional perfusion (NRP).

Methods: Local Regional Ethics Committee, National Health Service Blood and Transplant and donor next of kin consent were obtained. Following declaration of death, the donor was transferred to theatre where a sternotomy was performed and cerebral blood flow excluded by clamping the aortic arch vessels. The donor was then placed upon NRP and perfusion restored to the heart. The trachea was then intubated and the lungs ventilated. After 60 min the donor heart was weaned from NRP upon an infusion of Dopamine 5 μg kg min and Vasopressin at 4 units/h. A Swan Ganz catheter was then floated and direct pressure measurements and cardiac outputs obtained. Hearts then underwent cardioplegic arrest before instrumentation upon the TransMedics OCS before placing them in working mode.

Results: Function was restored to all five donor hearts successfully using NRP with 3 donor hearts suitable for transplantation. Following the instigation of a clinical programme the final heart studied was transplanted into a consented recipient who was discharged from intensive care on post operative day four with good biventricular function.

Conclusion: NRP can be used to successfully restore function to the DCD heart. This reduces the warm ischaemic time, restores control to the DCD donation process and facilitates safe transplantation with reduced risk of primary graft dysfunction.

	1	2	3	4	5
CI (l/min/m^2)	2.2	2.7	3	2.1	3.2
CO (l/min)	4	5.2	4.6	4.5	6.0
HR (bpm)	–	99	90	100	85
CVP (mmHg)	4	8	6	16	10
PCWP (mmHg)	9	9	14	14	12
MAP (mmHg)	31	51	70	50	85

027 LUNG

FO002 **LUNG TRANSPLANTATION OUTCOME IS MAINLY DRIVEN BY ANTIBODY MEDIATED REJECTION**

Antoine Roux[1], Lan Ines Bendib-Le[1], Sonia Holifanjaniaina[2], Kimberley Thomas[3], Hamid Abdul Monem[1], Clément Picard[1], Dominique Grenet[1], Sandra Demiranda[1], Benoit Douvry[1], Laurence Beaumont-Azuar[1], Edouard Sage[4], Jerome Devaquet[5], Elise Cuquemelle[6], Caroline Suberbielle[7], Marc Stern[1], Magali Colombat[2], François Parquin[6]

[1]Hopital Foch, Pneumology Department; [2]Hopital Foch, Pathology Department; [3]Department of Pathology & Laboratory Medicine David Geffen School of Medicine UCLA; [4]Hopital Foch, Thoracic Surgery Department; [5]Hopital Foch, Intensive Care Unit; [6]Hopital Foch, Thoracic Intensive Care Unit; [7]Hopital Saint-Louis, Service d'histocompatibilité

Background: Graft failure in both kidney and heart transplantation are mainly driven by antibody mediated rejection (AMR). In the context of lung transplant (LT), because of diagnostic difficulties, this clinical entity remains a matter of debate.

Methods/Materials: In order to validate the current AMR diagnostic criteria in LT and demonstrate the impact of AMR on LT prognosis, we conducted a retrospective analysis of our LT cohort (January 2010-December 2013). AMR was defined by association of clinical symptoms, DSA presence,and either C4d+staining and/or histological pattern consistent with AMR. Patients (pts) were categorized in four groups by their DSA: (i) with DSA and AMR (DSA+AMR+), (ii) with DSA and no AMR (DSA+AMR-), (iii) with non significant DSA (DSAns = one specificity, with an MFI = 500–1000 once), and (iv) without DSA (DSA-). The three latter were grouped as AMR- pts. Pre-LT and peri-operative clinical data, cumulative number of acute cellular rejection (ACR) episodes by month 12, freedom from Chronic Lung Allograft Dysfunction (CLAD),and graft survival were reported.

Results: Among 206 transplanted patients, 11% were DSA+AMR+ ($n = 22$), 41% were DSA+AMR-($n = 84$), 6% were DSAns ($n = 13$), and 42%were DSA-($n = 87$). Every AMR+ patient had clinical abnormalities associated, and only 3/22 had histological abnormalities with C4d-staining. Every AMR+ pts were treated with combination of plasmapheresis, rituximab and intravenous immunoglobuline. Comparison showed higher cumulative numbers (mean $\pm$ SD) of ACR at month12 in the DSA+AMR+ group (2.1 $\pm$ 1.7) vs. DSA+AMR- (1 $\pm$ 1.2), DSAns (0.75 $\pm$ 1), DSA-(0.7 $\pm$ 1.23) groups. AMR+ pts had higher frequencies of CLAD and worse graft survival than AMR-patients (HR = 53.24 $p < 0.0001$ and HR = 5.4, $p < 0.006$ respectively).

Conclusion: Our results clearly prove the negative impact of AMR on LT clinical course, and advocate for early active diagnosis approach and specific treatment of AMR.