BOOK OF ABSTRACTS

LIVRE DES RÉSUMÉS

CANADIAN SOCIETY OF TRANSPLANTATION
SOCIÉTÉ CANADIENNE DE TRANSPLANTATION

ANNUAL SCIENTIFIC MEETING
RÉUNION SCIENTIFIQUE ANNUELLE

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Lake Louise, Alberta

Dr. Kathryn Tinckam, Conference Chair
Dr. Deepali Kumar, Conference Co-Chair

CLINICAL

2412

ESTIMATING DIFFERENTIAL RENAL FUNCTION USING ELLIPSOID APPROXIMATION OF RENAL VOLUME ON CT SCAN

Nguyen, Laura, Kamal, Fadi, Blew, Brian (University of Ottawa/The Ottawa Hospital, Ottawa, ON)

Background: Living renal donors must undergo extensive medical investigations in order to be approved for the donor program. Among other testing, this includes both computed tomography (CT) scans to evaluate vascular anatomy and nuclear medicine renal scans to assess for differential renal function.
Extensive research has been done using complex models to calculate precise radiographic measurement of renal volume on CT in order to estimate differential renal function based on differential renal volumes. Thus, the necessity of the nuclear medicine renal scan can be eliminated, reducing the radiographic burden and time commitment of the potential donor and addressing the ongoing scarcity of the necessary radionucleotide. However, these models are rarely used as they are often cost-prohibitive due to the need for proprietary software and they are labor-intensive for radiologists.
Methods: In this study, we examined whether a simplified estimation of differential renal volumes based on the ellipsoid formula (renal volume = $\pi ldw/6$, where l, d, and w represent three dimensions of the kidney) using CT scans, may also adequately estimate differential renal function.
Results: Charts of 79 consecutive living renal donors were reviewed retrospectively. The differential renal volumes measured on CT scans were reliable between operators ($p<0.05$). We found that the volume-based estimations of differential renal volume were in fact correlated to differential renal function on nuclear medicine scans ($r=0.29$, $p<0.01$). We were able to identify the kidney with the greater function in 53 (67%) of the 79 cases, and in all 8 (100%) of 8 cases in which the difference in differential renal function was clinically significant (>10% difference between kidneys).
Conclusions: These findings support removal of the nuclear medicine scan from routine assessment of potential kidney donors without the need for expensive radiologic software, but further research looking specifically at potential donors with clinically significant differential renal function between kidneys is required to confirm our findings.

2413

SURVEY OF CANADIAN CENTRES: THE IMPACT OF MORBID OBESITY AND THE ROLE OF BARIATRIC SURGERY IN KIDNEY TRANSPLANT PATIENTS

Chan, Gabriel, Tran, Duy Paul, Senecal, Lynne (Hôpital Maisonneuve-Rosemont, Mont-Royal, QC)

Morbid obesity is commonplace in the dialysis population and weight loss is difficult due to fatigue and time. Post-transplantation, obesity is further exacerbated due to corticosteroids and the unshackling of the dietary restrictions. Numerous studies have demonstrated worse outcomes associated with obesity, including complications, long term graft-survival and recipient-survival. At present, there are no national guidelines regarding obesity in transplantation.
Methods: A PDF survey was sent to the e-mail list of the CST Adult Kidney Group and Comité Rein-Pancréas of Transplant-Québec. Responses were returned by fax or e-mail.
Results: Twenty-four responses represented 13 centres. Eleven have an official policy. The maximum BMI varied between 32 - 40 kg/m^2. Interestingly, when responses from the same centre differed, the surgeon responded “yes” and the nephrologist “no”. Only 7 centres have a weight loss programme.

The majority counselled patients to lose weight, at minimum through diet and exercise, judging that there was an increased risk to the peri-operative period, worse graft survival and overall survival. Few felt that weight loss while on dialysis was too risky or futile.
Most centres have had an experience with bariatric surgery although only half were directly referred by the transplant centre. Even fewer details of the surgery were known.
Almost all indicated that the risk was significantly high enough to warrant a BMI limit, that patients should lose some of the weight prior to transplantation and that they would refer to a bariatric surgeon if conservative measures failed.
Discussion: Local policies vary widely and are usually "soft". Only a minority have a treatment algorithm for such patients, whose effectiveness is largely unknown. A national policy would be essential to ensure fair and equitable treatment of morbidly obese patients and to optimize future transplant outcomes.

2416

DEVELOPMENT OF A HEART TRANSPLANT KIDS CAMP

Dodd, Bernadette (Stollery Children's Hospital, Edmonton), Nicholas, David (University of Calgary, Edmonton), Urschel, Simon (University of Alberta, Edmonton, AB), West, Lori (University of Alberta, Edmonton, AB)

The pediatric heart transplant team at the Stollery Children's Hospital in Alberta sought programming to address psycho-social complications post-transplant. The development of a weekend family camp seemed like an excellent way to provide opportunities for social support and peer mentoring while promoting physical activity, self-esteem and knowledge about transplant. This report examines the development, execution and evaluation of the Heart Transplant Kids Camp.
Development: The multidisciplinary team worked together using knowledge translation concepts to develop an event that offered education and formalized support groups for both children and adults from across Western Canada. A standard evaluation of learning and support would be necessary to quantify success.
Execution: Banners welcoming guests and clothing distributed to camp participants bore a camp logo designed to illustrate membership in this unique group. Team events were developed to ensure campers would work together for a common goal while having fun; a soccer game was led by one of the physicians and mini Olympics were designed where kids would compete to win their home clinic the coveted Transplant Cup. Several teens ready to transfer to the adult program were recruited to attend camp as young adult mentors to help younger campers navigate growing up with a transplant. "Transplant School" paralleled camp activities and provided learning opportunities.
Evaluation: Satisfaction surveys were universally in support of future family camps. Families reported that the transplant team became more approachable, families rediscovered fun in life that had become too serious, and feelings of camaraderie and new relationships were described.
Conclusion: Transplant camp has served to provide heart transplant recipients and their families with opportunities for learning, peer support, and a sense of community. Staff participation has reinforced to recipient families that we are all part of a larger community invested in making pediatric heart transplant successful.

2417

IMPACT OF RESISTANCE TRAINING ON THE MECHANISMS INVOLVED IN THE DEVELOPMENT OF NEW ONSET DIABETES AFTER TRANSPLANTATION (NODAT) IN RENAL TRANSPLANT RECIPIENTS: A PILOT STUDY

Rakel, Agnès (CHUM, Montréal, QC), Hébert, Marie-Josée (CHUM, Montréal, QC), Rabasa-Lhoret, Rémi (IRCM, Montréal, QC), Karelis, Antony (UQAM, Montréal, QC)

Introduction: New-onset diabetes after transplantation (NODAT) following kidney transplantation is associated with adverse patient and graft outcomes. Lifestyle modification is recommended as a first line therapy to manage NODAT. However, no data currently demonstrate the efficacy of resistance training in the prevention of NODAT. Resistance training might be of special benefit in this population since underlying disease and treatment favour muscle loss and insulin resistance. The purpose of this pilot study was to assess the feasibility and the efficacy of a resistance training program on the mechanisms implicated in the development of NODAT in a population of kidney transplantation recipients.
Methods: Twenty ambulatory adults who received a kidney transplantation in the preceding month were randomized to an exercise (E) or a control (C) group for a duration of 4 months. Both groups had baseline and 4 months follow up oral glucose tolerance tests, body composition studies, anthropometry measures, VO2 max, strength tests, step count and quality of life questionnaires.
Results: E intervention resulted in 1-) a 20% decrease in the proportion of patients with abnormal glucose metabolism versus a 10% decrease in the control (C) group 2-) a 1 cm decrease in the waist circumference (91.5 cm to 90.5 cm) versus a 0.5 cm increase (92.8 to 93.3 cm) in the C group 3-) in a stable fat mass versus a 2% increase in the fat mass in the C group, 4-) a 9.4% increase in VO2 max versus a 3.4% increase in the C group 5-) a significantly improved quality of life versus the C group.
Conclusions: Improvements in glucose metabolism occur post-transplant with steroid and immunosuppressive drug withdrawal. However, this study suggests that resistance training could accentuate glucose metabolism improvements and bring added benefit on body composition, physical fitness and quality of life.

2418

ABO INCOMPATIBLE KIDNEY TRANSPLANTATION USING ABO IMMUNOABSORPTION COLUMN

Zaltzman, Jeff, Pavenski, Katerina, Prasad, Ramesh, Gottfried, Meagan, Cheatley, Patty, Meliton, Galo, (St. Michael's, Toronto, ON), Chua, Mui (UHN, Toronto, ON)

ABO incompatibility is the most common barrier to living donor kidney transplantation. Various protocols for transplantation of ABO incompatible (ABOI) kidneys exist, and involving a combination of intense immunosuppression and antibody removal by plasmapheresis, selective immunoadsorption (double-filtration plasmapheresis, protein A immunoadsorption) or specific immunoadsorption (antigen-specific immunoadsorption).
We report on the first 4 cases in North America of ABOI live donor kidney transplantation using Glycosorb ABO immunoabsorption column and immunosuppressive regimen as per Karolinska University Hospital protocol (Genberg et al, 2008, Kumlien et al 2006). All 4 recipients were ABOi, and 1/4 had DSA.
Protocol: Preoperative immunosuppressive regimen consisted of a single dose of Rituximab 375 mg/m^2 IV on day -28; Tac, MMF and Prednisone, day -7; a single dose of IVIG 0.5g/kg IV at day -1; Basiliximab 20 mg IVday 0 and 4, and Solumedrol 2mg/kg IV on day 0. Post-operative regimen included: Tac, MMF, streroids. (pt #4 with DSA received thymoglobulin).
Immunoabsoprtion with Glycosorb B column was performed beginning on Day -16 with patients requiring between 2-5 treatments. Procedure was performed on COBE Spectra apheresis system with~ 1.5 PV per exchange, over 4 hours. The patients tolerated procedure well, and there were no side effects observed.
ABO Titres were measured post-operatively with further immunoabsorptions only if titres by IAT exceeded 8 in post-operative week 1 and/or 16 in post-operative week 2.
Following transplant, all patients had immediate allograft function with no documented rejection events..At last follow-up Scr ranged from 116-132.
ABO column immunoadsorption is a good alternative to conventional plasmapheresis for ABOI solid organ transplantation. Its advantages include avoidance of exposure to blood products and a very favourable side effect

profile, and the ability to desensitize against high titres in a brief period of time; cost of the column is a major disadvantage at present.

Pt	ABO	Titres	dy -13	dy -10	dy -8	dy -6	dy -3	dy -2	dy 0	dy +	dy +2	dy +3	dy +4	5	6	7	wk2	wk3
1	AB-A	4	*			2		*	2	4			2		2		2	2
2	A-O	32				*8	4	*	4	4	8	8	*16		2		2	
3	B-O	128	*	*		*16	*8	4	4	8	8		4		2		4	4
4	A-O	16			*	4	*	2		*32	2	4	4	4	4	8		

*Glycosorb Rx

2421

REDUCTION IN NEW ONSET DIABETES AFTER TRANSPLANT (NODAT) WITH ERYTHROPOIETIN STIMULATING AGENTS, A CASE CONTROL STUDY.

Montada-Atin, Tess; Choi, Diana (Institute of Medical Science, Toronto,ON); Woo, Minna (St. Michaels Hospital, Toronto, ON); Retnakaran, Ravi (Mount Sinai Hospital, Toronto, ON); Huang, Michael; Prasad, Ramesh; Zaltzman, Jeffrey (St. Michaels Hospital, Toronto, ON)

Background: New-onset diabetes mellitus after renal transplantation (NODAT) adversely affects graft and patient survival. Approximately 10-15 % of post-renal transplant patients require an erythropoietin stimulating agent (ESA) for the treatment of anemia. Studies have shown that ESA protects mice against the development of diabetes through direct effects on pancreatic ß cells. However, the effect of ESA on the risk of diabetes in humans has not been well studied.
Methods: We performed a case control analysis of patients with NODAT who received a first live or deceased donor renal allograft between January 1, 2005 and December 31, 2010, comparing those with exposure to an ESA versus those without such exposure. Patients with a prior history of diabetes mellitus or more than one renal transplant were excluded. NODAT was defined based on the 2008 Canadian Diabetes Association criteria or need for anti-diabetic agents (oral or insulin). Multivariate logistic regression analysis was performed to determine factors independently associated with NODAT including age, body mass index (BMI), acute rejection (AR), donor source and random blood sugar (RBS) at discharge.
Results: 615 recipients met initial criteria. 153 were excluded. In the remaining 462, 62 (13.4%) had NODAT whereas 400(86.6%) did not. 21% of recipients with NODAT were exposed to an ESA compared to 79% who were not. By Fisher exact test, exposure to an ESA within the first 6 months post transplant reduced the risk of developing NODAT by 94% (OR=0.063, CI= 0.008-0.493, p= 0.012). Increasing age (OR=1.422, CI= 1.077-1.932, p < 0.0001) and RBS at discharge (OR 1.39, CI=1.167-1.658, p< 0.0001) were associated with an increased risk of NODAT, however even accounting for these factors by multivariate analysis, ESA exposure remained protective.
Conclusion: The risk for developing NODAT was significantly reduced in patients who were exposed to an ESA compared to those who were not exposed. There may be a role for ESAs in preventing NODAT particularly if given within the first 6 months of transplant, although this remains hypothesis generating.

2422

THE IMPACT OF PANCREAS ON CALCULATED CARDIAC RISK IN SIMULTANEOUS PANCREAS KIDNEY TRANSPLANTATION

Jiang, Alex (Western University, Schulich School of Medicine and Dentistry, London, ON), McGregor, Tom, Martin, Paul, McAlister, Vivian (Western University, Department of Surgery, London,ON), Sener, Alp, Luke, Patrick, (Western University, Department of Urology, London, ON)

Background. It is uncertain whether pancreas transplantation in type I diabetics with renal impairment improves overall patient health and survival. The aim of this study was to look at the impact of pancreas transplant on simultaneous pancreas kidney (SPK) recipients using the Framingham Risk Score (FRS) for 10-year cardiovascular disease (CVD) risk.
Methods. A retrospective review was performed on 55 SPK transplant patients. 20 solitary kidney (SK) recipients were analyzed as controls. The two groups were matched in terms of age and diabetes status. The FRS for 10-year CVD risk was calculated and compared between the two groups pre-operatively and one-year post-operatively. Individual risk factors were also compared separately to determine which factors conferred the most significant influence on cardiac risk reduction. This included systolic blood pressure (SBP), cholesterol levels and anti-hypertensive medications.
Results. Using the FRS calculator, we determined that pre-operative risk score for SPK transplant was 12.6±8.3% compared with 9.0±6.8% one-year post-operatively ($p = 0.001$). There was no statistical difference in SK transplant group when comparing the CVD risk pre-operatively and post-operatively. One year post-operatively, SPK patients had decreased their total number of antihypertensive medications by a mean of at least one agent ($p = 0.0000003$) and had reduced their dependence on statin ($p = 0.001$). Additionally, we determined that SPK transplant group had significantly lower LDL cholesterol compared to SK transplant group (2.16±0.79 vs 2.73±0.62 mmol/L, $p = 0.007$). However, there was no significant statistical difference between pre and post-operative SBP or HDL level.
Conclusion. SPK transplantation has a positive impact on patient health based on the analysis of FRS for 10-year CVD risk one-year post-operatively. It reduced the CVD risk from intermediate (10-20%) to low (<10%) risk range. Resulting impact of pancreas transplantation on cardiovascular complications including myocardial infarction, stroke and death continue to be studied.

2423

PHYSICAL THERAPY IN PEDIATRIC LEFT VENTRICULAR ASSIST DEVICE

Sollazzo, Stephanie, Lati, Jamil; Deliva, Robin; Humpl, Tilman; Conway, Jennifer; (The Hospital for Sick Children, Toronto, ON)

Purpose: Early initiation of physical rehabilitation for patients with ventricular assist devices (VAD) has been shown to improve exercise capacity, inspiratory muscle function and quality of life in adult patients. There is a paucity of pediatric literature regarding clinical practice and feasibility of physical therapy (PT) interventions for pediatric VAD recipients.
Methods: We report our initial experience with the HeartWare© HVAD pump in the pediatric setting following PT protocol initiation. A 13 year old previously healthy male presented with severe dilated cardiomyopathy and underwent placement of a left sided HeartWare© HVAD pump for ongoing circulatory support.
Results: The child was referred to PT post-operative day (POD) 4 in the critical care unit and therapeutic interventions included breathing and coughing exercises, range of movement and progressive mobility. He was transfer to the ward on POD 13. Important mobility milestones included: sitting in a chair for 60 minutes on POD 7, ambulation of 110 metres on POD 13 and treadmill ambulation on POD 20. There were no adverse events with PT interventions and VAD flows which were closely monitored demonstrated a response to exercise from 2.8L/min/m^2 at rest to 3.2-3.8 L/min/m^2 with activity. He underwent transplantation on POD 22 and was extubated the same day with no significant peri-operative

complications. He was discharged from hospital 7 days post-transplant and participated in an 8 week PT exercise rehabilitation program. Six minute walk distances were 582.4 and 782.5 metres at 3 and 9 weeks post-transplant.
Conclusion: This is the first pediatric case report of PT intervention with Heart Ware ©HVAD pump. PT exercise interventions are feasible and safe to initiate in the critical care unit in pediatric VAD patients and may contribute to earlier extubation, mobility and discharge in patients bridged to transplantation.

2424

15 YEARS EXPERIENCE OF PANCREAS TRANSPLANT AT THE ALTRA SOUTHERN ALBERTA TRANSPLANT PROGRAM

Wang, Wenjie (University of Calgary, Calgary, AB)

Diabetes results in microvascular and macrovascular complications such as nephropathy, retinopathy and cardiovascular disease. Pancreas transplantation restores metabolic derangement and reduces or halts some of these complications therefore improves patient survival. Simultaneous pancreas and kidney transplant (SPK) is the most common approach while pancreas transplant after kidney transplant (PAK) is increasingly performed. We report our 15 years experience with 87 pancreas transplant procedures in 81 diabetic patients at the ALTRA Southern Alberta Transplant Program in Canada (73 SPK and 14 PAK, 3 patients received repeat pancreas transplant). The patient characteristics between these two groups are similar. Pre-emptive kidney transplantation is a major advantage of living kidney donation as all except one patient in PAK group had pre-transplant dialysis comparing to an average of 849 days of pre-transplant dialysis in SPK group. There is good patient survival in two groups after a median follow-up of 8.3 years (90.0±9.5% in PAK vs 83.2±5.3% in SPK). Excellent 10-year graft survival is achieved in both groups in kidney (85.7±13.2% in PAK vs 71.2±6.3% in SPK) and pancreas (61.2±14.0% in PAK vs 62.6±6.8% in SPK). Biopsy proven acute rejection rate was low and similar between SPK and PAK. In conclusion, both SPK and PAK transplantation lead to excellent long term patient and graft survival in type I diabetic patients. Pre-emptive kidney transplantation is a major advantage of living kidney donation followed by pancreas transplantation.

2425

THE SIX-MINUTE WALK TEST, MELD-NA SCORE AND LENGTH OF STAY IN HOSPITAL FOLLOWING LIVER TRANSPLANTATION

Howes, Nancy

The Six Minute Walk Test (6MWT) is becoming more common during the assessment of candidates for liver transplantation. While the Model for End Stage Liver Disease Serum Sodium (MELD-Na) score is used to stratify patients to the list, information about the functional capacity of a candidate can perhaps help to guide preoperative care. Our purpose was to examine the relationships between the 6MWT, MELD-Na score and length of stay (LOS) in hospital following LT.
40 candidates for LT had 6MWT and MELD-Na score calculations preoperatively. LOS data were collected following discharge from hospital.
Bivariate correlations explored the relationships between 6MWT, MELD-Na, and LOS. Linear regression created a prediction for LOS.
There were statistically significant correlations between both 6MWT and MELD-Na score ($r = -0.38$, $p < 0.01$) and 6MWT and LOS ($r = -0.42$, $p < 0.01$). There was also a statistically significant correlation between MELD-Na and LOS ($r = 0.36$, $p < 0.05$).

Using linear regression, a prediction model was created, showing that twenty-four percent of the prediction of LOS could be accounted for by results from the preoperative 6MWT and the MELD-Na score.
The 6MWT provides important information about functional capacity and is a variable that could complement the MELD-Na scoring system.

2426

CONVERSION FROM TWICE-DAILY (PROGRAF®) TO ONCE-DAILY (ADVAGRAF®) TACROLIMUS FORMULATION IN STABLE PEDIATRIC KIDNEY TRANSPLANT RECIPIENTS: A COMPARATIVE PHARMACOKINETIC STUDY

Lapeyraque, Anne-Laure; Kassir, Nastya (Université de Montréal, Montréal, QC); Théorêt, Yves; Kajinovic, Maya; Clermont, Marie-José; Litalien, Catherine; Phan, Véronique (CHU Sainte Justine, Montréal, QC)

Introduction: Compliance to immunosuppressive therapy is critical to prevent organ rejection. Advagraf® is an extended release formulation of tacrolimus (Tac) which may offer better adherence. In this study, the Tac pharmacokinetic (PK) parameters were evaluated in pediatric kidney transplant patients during the conversion from Prograf® to Advagraf®.
Material and methods: Stable patients transplanted for at least 6 months were converted from Prograf® to Advagraf® on a mg:mg daily dose. All patients underwent 2 steady-state 24 hours PK profiles (14 concentration-time points), the second one at least 2 weeks after the switch. Tac blood concentrations were determined by HPLC MS-MS. CYP3A5 genotype was assessed for each patient and correlated to PK parameters.
Results: Thirty-eight PK profiles from 19 patients (12 males) aged between 7.0 and 18.9 years were obtained at a median post-transplantation time of 43.7 months (9.5-128.5). Median Tac daily dose was 0.11 mg/kg (0.06-0.19). After the conversion from Prograf® to Advagraf® on a mg:mg daily dose, mean C 0 and C min were consistently decreased, particularly in patients with CYP3A5 *3/*3 genotype. No statistically significant difference was observed for mean C max between the two formulations in this population. Time to maximum concentration (T max) was delayed with Advagraf®, consistent with its prolonged-release characteristics. Despite statistically significant difference in AUCs, the ratio of the least square means for AUC 0-24h was 90.8% with 90% CI limits between 80% to 125% (85.3% to 96.7% in the present study), which is the most commonly accepted definition of bioequivalence.
Conclusions: The steady state Tac exposure of once daily Tac formulation (Advagraf®) is equivalent to Prograf® twice a day after a mg:mg conversion in stable pediatric kidney transplant recipients. Because of the decrease in mean C 0 and C min observed after the conversion to Advagraf®, patients must only be switched from one Tac formulation to another under the control of Tac therapeutic drug monitoring.

2428

SERUM FGF-23 LEVELS ARE INDEPENTLY ASSOCIATED WITH MARKERS OF IRON METABOLISM IN KIDNEY TRANSPLANT RECIPIENTS

Mucsi, Istvan (McGill University Health Centre, Montréal, QC), Molnar, Miklos (Semmelweis University, Budapest), Czira, Maria (Semmelweis University, Budapest, Hungary), Rudas, Anna (Semmelweis University, Budapest, Hungary), Wolf, Myles (University of Miami Miller School of Medicine, Miami, USA)

Background: Recent reports suggested association and even a mechanistic link between iron metabolism and circulating levels of FGF-23. Here we wanted to assess if markers of iron metabolism are associated with circulating levels of C-terminal FGF-23 (cFGF23) in a prevalent cohort of stable kidney transplant (KT) recipients.

Methods: We collected socio-demographic parameters, medical and transplant history and laboratory data from 984 stable prevalent Tx recipients (mean age 51±13 years, 57% males, mean eGFR 51±21 ml/min/1.73m2, median Tx vintage 72 months [interquartile range 74 mo] and 21% diabetics). Serum FGF-23 was measured using a C-terminal enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA, USA). Iron metabolism was characterized by frequently used markers, such as serum iron, transferrin saturation, serum transferrin and ferritin; but also using percentage of hypochromic reticulocytes (HyRet) and serum soluble transferrin receptor (STFR) levels, which are the least influenced by inflammation. eGFR was calculated using the 4-variable equation derived from the Modification of Diet in Renal Disease Study.
Results: Age, gender and eGFR adjusted cFGF23 levels were weakly and negatively correlated with serum iron and transferrin saturation (r=-0.079 and r=-0.083, respectively; p<0.05). Serum ferritin was weakly-moderately and negatively (r=-0.170, p<0.001), whereas STFR positively (r=0.262, p<0.001) correlated with cFGF23 concentrations. In a multivariable linear regression model STFR remained significantly associated with logarithmically transformed cFGF23 levels (beta=0.200, p<0.001) (model was adjusted for age, gender, serum albumin, eGFR, Charlson Comorbidity Index, BMI, serum Ca, PO4, PTH, CRP, interleukin 6, TNF-alpha, Hb, serum erythropoietin levels and erythropoietin treatment). The same association was seen even when the analysis was restricted to patients with eGFR>60 ml/min and serum PO4 <1.5 mmol/l (n=271).
Conclusions: Markers of iron metabolism are associated with serum C-terminal FGF23 levels in stable kidney transplanted patients. Iron deficiency is an independent predictor of higher serum FGF23 levels. Iron deficiency may modulate FGF23 metabolism in kidney transplant recipients.

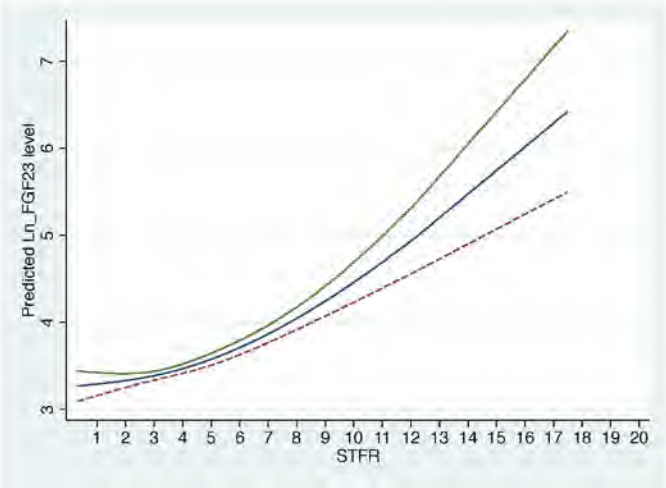

Figure : The association between soluble transferrin receptor and ln_FGF23 levels in kidney transplanted patients (n=964) based on a cubic spline model. The blue line represents the estimated Ln_FGF23 values, the green and red lines represent 95% confidence interval.

2429

CHANGES IN DXA MEASURES OF BONE MINERAL DENSITY AND BODY COMPOSITION AFTER PEDIATRIC RENAL TRANSPLANTATION

Tsampalieros, Anne, Griffin, Lindsey; Zemel, Babette S. (Children's Hospital of Philadelphia, Philadelphia, USA); Kalkwarf, Heidi (Cincinnati Children's Hospital, Cincinnati, USA); Leonard, Mary B. (Children's Hospital of Philadelphia, Philadelphia, USA)

Background: Children with CKD have multiple risk factors for impaired bone accrual. Successful renal transplantation (Txp) may improve some of the metabolic abnormalities but high steroid exposure and persistent hyperparathyroidism can impair bone recovery. The ability of DXA measures to capture disease and treatment effects on trabecular and cortical BMD in pediatric Txp has not been well characterized.

Methods: DXA scans were obtained in 56 Txp recipients (age 6 -21 yr) at Txp, and 3, 6, and 12 months later. Sex- and race- specific Z-scores were generated for DXA whole body bone mineral content (WB-BMC-Z), lumbar spine bone mineral density (LS-BMD-Z) and WB fat mass and lean body mass relative to age and adjusted for height-Z based on >900 healthy reference participants. Changes in Z-scores were assessed using quasi-least squares regression (QLS) models.
Results: At enrollment, LS-BMD-Z was significantly elevated in younger Txp recipients only. In all Txp recipients combined, LS-BMD-Z decreased significantly and decreases were independently associated with greater mean glucocorticoid (GC) dose (p=0.001) and greater declines in PTH levels (p=0.002). At enrollment, WB-BMC-Z scores were not different compared to reference participants. And although WB-BMC-Z scores on average did not change significantly over the year, greater GC doses (p<0.001) was independently associated with declines in WB-BMC-Z scores. Both WB fat mass and lean body mass Z-scores increased significantly over the study interval. QLS models demonstrated greater GC doses were significantly associated with increases in fat Z-scores (p=0.000).
Conclusions: These data demonstrated that greater GC exposure following Txp was associated with decreases in LS-BMD-Z and WB-BMC-Z scores. The association with declines in WB-BMC-Z may reflect impaired expansion of cortical dimensions with growth. These data suggest that DXA captures GC and PTH effects on trabecular and cortical bone following Txp. Future studies are needed to correlate with fracture risk.

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SERUM FGF-23 LEVELS ARE INDEPENDENTLY ASSOCIATED WITH SERUM ASYMMETRIC DIMETHYLARGININE (ADMA) LEVELS IN KIDNEY TRANSPLANT RECIPIENTS

Mucsi, Istvan; Czira, Maria E.; Rudas, Anna; Ujszaszi, Akos (Semmelweis University, Budapest, Hungary); Wolf, Myles (University of Miami Miller School of Medicine, Miami, USA); Kielstein, Jan T. (Medical School Hannover, Hannover, Germany); Molnar, Miklos Z. (Semmelweis University, Budapest, Hungary)

Background: Animal and clinical studies reported that FGF-23 interferes with vascular reactivity induced by the nitric oxide (NO) system. We assessed if serum FGF23 is associated with serum ADMA in stable kidney transplant recipients.
Methods: Data from 258 kidney transplant recipients (age 54+12, mean eGFR 42+21 ml/min/1.73m2) followed at a single transplant center were analyzed. Serum FGF-23 was measured using a C-terminal enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA, USA). ADMA was determined using liquid chromatography-mass spectrometry. Variables with non-normal distribution were natural log-transformed for the analyses.
Results: Serum FGF23 levels positively correlated with serum ADMA in bivariate Pearson correlation analysis (r=0.385, p<0.001). In a multivariable linear regression model serum FGF23 remained independently associated with serum ADMA levels (beta=0.157, p=0.013) (adjusted for age, gender, serum albumin, eGFR, Charlson Comorbidity Index, systolic blood pressure, serum phosphate, BMI, total cholesterol and serum PO4). Similar independent association was seen in a model using L-arginine/ADMA as the dependent variable.
Conclusions: Higher serum FGF23 is independently associated with higher serum ADMA levels in stable kidney transplant recipients. These results are compatible with the hypothesis that higher serum FGF23 levels may be associated with endothelial dysfunction in this patient population.

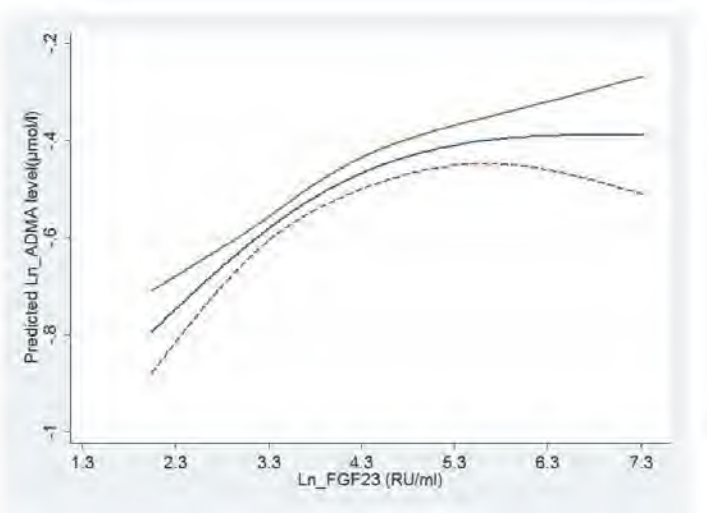

Figure: The association between ln_FGF23 levels and ln ADMA in kidney transplanted patients (n=258) based on a cubic spline model. The blue line represents the estimated Ln_FGF23 values, the green and red lines represent 95% confidence interval.

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DUCT-TO-DUCT BILIARY ANASTOMOSIS YIELDS SIMILAR OUTCOMES TO ROUX-EN-Y HEPATICOJEJUNOSTOMY IN SELECT PATIENTS UNDERGOING LIVER TRANSPLANTATION FOR PRIMARY SCLEROSING CHOLANGITIS

Hernandez-Alejandro, Roberto; Al-Judaibi, Bandar; Wall, William; Marotta, Paul; Uhanova, Julia (University of Manitoba, Winnipeg), Levstik, Mark; Quan, Douglas; McAlister, Vivian; Chandok, Natasha, (Western University, London, ON)

Background: While Roux-en-Y choledochojejunostomy is the default anastomotic technique for liver transplantation (LT) for primary sclerosing cholangitis (PSC), duct-to-duct (D-D) reconstruction is used in the setting of a preserved recipient common bile duct. Historically, the success rate of D-D reconstruction has been interior to Roux-en-Y loop. However, recent observational data suggest that in well-selected recipients, D-D reconstruction imposes no added adverse risk. The aim of this study was to assess the safety and efficacy of D-D anastomosis compared to Roux-en-Y reconstruction among adults transplanted for PSC.
Methods: A retrospective cohort of all patients aged ≥18 years who underwent primary LT for PSC from 1990 to 2011 were examined; subjects were stratified by type of biliary reconstruction (D-D versus Roux-en-Y). Recipient and graft survival, postoperative medical and surgical complications, and postoperative resource utilization rates were compared between the two groups.
Results: 72 patients fulfilled inclusion criteria; 58 had Roux-en-Y loop and 14 had D-D reconstruction. 54 subjects (73.0%) were male with mean /- standard deviation age at LT of 43.3 /- 14.4 years. Rates of recipient mortality, graft failure, biliary complications, acute cellular rejection, and reoperative rates were similar in both groups. Postoperative cholangiography was required more frequently in patients with D-D reconstruction (p=0.04).
Conclusion: In select recipients with PSC, D-D reconstruction is a safe and efficacious technique with comparable long-term clinical outcomes to Roux-en-Y reconstruction.

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INCIDENCE, DETERMINANTS AND MANAGEMENT OF NEOPLASIA AFTER KIDNEY TRANSPLANTATION: A POPULATION-BASED STUDY FROM 1985-2009

Cardinal, Hélois (Centre Hospitalier de l'Université de Montréal, Montréal, QC), Verhave, Jacobien (Centre Hospitalier de l'Université de Montréal, Montréal QC), Baran, Dana (McGill University Health Center, Montréal,QC), Boucher, Anne (Hôpital Maisonneuve-Rosemont, Montréal, QC), Dubé, Genevieve (Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC), Houde, Isabelle (Centre Hospitalier Universitaire de Québec, Québec, QC), Lapointe, Isabelle (Centre Hospitalier Universitaire de Québec, Québec, QC), Luke, L (McGill University Health Center, Montréal, QC), Masse, Mélanie (Centre Hospitalier de l'Université de Sherbrooke, Sherbrooke, QC), Reinhardt, Caroline (CHUM, Montréal, QC), Renoult, Edith (CHUM, Montréal QC), Sénécal, Lynne (Hôpital Maisonneuve Rosemont, Montréal, QC)

Introduction: Although the incidence of neoplasia after kidney transplantation has been reported in prior studies, their evolution and management have not been described extensively. Our aims were to determine the incidence, risk factors, management and evolution of non-cutaneous neoplasia in kidney transplant recipients (KTR).
Methods: We performed a retrospective cohort study in the 5 adult kidney transplant centers in the province of Québec. All patients who received a first kidney transplantation between January 1st, 1985 and January 1st, 2009 were included in this study. We calculated the cumulative incidence of non-skin cancer at 1, 5 and 10 years, and performed a Cox regression to identify risk factors for neoplasia.
Results: We identified 3708 patients who met the inclusion criteria. The cumulative incidence of non-skin cancer was 1%, 4% and 7% at 1, 5 and 10 years post-transplant. The most frequent cancer types were native kidney carcinomas (14%), prostate (11%), lung (9%), lymphoproliferative disorders (8%), breast (6%), bladder (6%), colon (5%) pancreas (3%) and thyroid (3%). At the time of presentation, 25 % of cancers were invasive (8% locally and 16% metastatic). In patients who developed a cancer, 46% died over the study period, and their death was related to cancer in 73%. Immunosuppression was decreased over the year following the cancer diagnosis in 43%, and graft rejection supervened in 12%. On multivariate analysis, the factors associated with the development of neoplasia were age (hazard ratio (HR) per 10-year increase: 1.51, 95% confidence interval (CI) 1.30-1.75), caucasian race (HR: 3.78, 95% CI 1.65-8.65), and previous rejection episodes (HR: 2.07, 95% CI 1.34-3.20). Induction with lymphocyte-depleting agents was not associated with the development of cancer (HR: 1.18, 95% CI 0.82-1.68).
Conclusion: In KTR, post-transplant neoplasia is frequent, associated with an elevated rate of mortality and supervenes especially in patients who are older, Caucasian and have experienced prior rejection.

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INFERIOR SURVIVAL IN LIVER TRANSPLANT PATIENTS WITH HEPATOCELLULAR CARCINOMA RECEIVING DONATION AFTER CARDIAC DEATH LIVER ALLOGRAFTS

Croome, Kris; Marotta, Pau; Wall, William; Hernandez-Alejandro, Roberto; (Western University, London, ON)

Background: Previous reports have shown that Donation after Cardiac Death (DCD) livers are used more frequently in patients with Hepatocellular Carcinoma (HCC) (20.2%) compared to donation after brain death (DBD) organs (13.0%). The adverse effects of Ischemia Reperfusion Injury (IRI) resulting in increased rates of ischemic cholangiopathy and decreased graft survival in recipients of DCD livers have been well documented. IRI has been previously shown to stimulate growth of micrometastases and an increase in the adhesion of tumour cells in the non-transplant setting. The impact of IRI in the setting of transplantation for HCC has not been thoroughly investigated.

Methods: The present study examined UNOS data from the Scientific Registry of Transplant Recipients (SRTR) on all deceased donor liver transplant recipients performed from January 1 1995 to October 31 2011.
Results: A total of 5638 patients in Group 1 (HCC DBD), 68651 patients in Group 2 (Non-HCC DBD), 242 patients in Group 3 (HCC DCD) and 2117 in Group 4 (Non-HCC DCD) were identified. On unadjusted Kaplan-Meyer survival analysis patients with HCC receiving DCD organs had inferior long-term survival compared to patients with HCC receiving DBD organs (p<0.001). Group 3 (HCC DCD) recipients had significantly lower graft and patient survival compared to Group 1 (HCC DBD) recipients at 1,3 and 5 years (66% vs 81%, 54% vs 69%, 45% vs 60%) respectively. On m ultivariate Cox analysis significant predictors of graft and patient survival included a diagnosis of HCC (p<0.001), receiving a DCD allograft (p<0.001), HCV+ status (p<0.001), recipient age (p<0.001), donor age (p<0.001), and MELD score (p<0.001). An interaction term created between receiving a DCD allograft and a diagnosis of HCC to examine for potentiation of effect was statistically significant (p=0.048). In a subgroup survival analysis on HCC recipients receiving a DCD allograft, recipients with Cold ischemia time (CIT) ≤380min (6h 20min) had significantly better survival than recipients with CIT>380min (p<0.001). In addition recipients with a total ischemic time (TIT) ≤420min (7h) had significantly better survival than recipients with a TIT >420 minutes.
There is an inferior patient and graft survival in HCC recipients of DCD allografts co.
Conclusion: Compared to those receiving DBD allografts. This potentiation of effect of inferior survival remains even after adjustment for the inherent inferiority observed in DCD allografts as well as other known risk factors. Also there is demonstrated inferior survival in DCD-HCC patients with prolonged TIT and CIT. It is hypothesized that this difference could reflect an increased rate of recurrence of HCC.

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THE CHANGING DONOR CHARACTERISTICS IN LIVER TRANSPLANTATION OVER THE LAST 10 YEARS IN CANADA

Sela, Nathalie; Croome, Kris P.; Marotta, Paul; Chandok, Natasha; Wall, William; Hernandez-Alejandro, Roberto (Western University, London, Canada)

Background: Liver organ donor characteristics have a significant impact on graft quality and in turn recipient outcome. In this study, we examined cadaveric liver donor characteristics and Donor Risk Index (DRI) trends in Canada over the past decade.
Methods: Data were extracted from the Canadian Organ Replacement Register (CORR) for the 10 year period between 2000-2010. Trends in the DRI and donor characteristics were examined including: age, race, height, cause of death (COD), location, cold ischemia time (CIT), and type of donation.
Results: 2660 cadaveric liver donors were evaluated between the 10 year from 2000-2010. Donor age, proportion of African American donors, proportion of CVA as COD, and proportion of donation after cardiac death (DCD) donors all increased over the aforementioned time period. Donor height remained unchanged throughout the decade. The proportion of transplants classified as local is increasing and, as a result, the CIT for donor livers is decreasing. Although many of the parameters that have a negative impact on DRI increased in Canada over the study period, the DRI only showed a small non-significant trend in increasing value. The slight increase in these parameters has been counteracted by a decrease in modifiable risk factors such as CIT and distance traveled. Recipient survival rates have increased from 71.43% in 1999-2001 to 75.5% in 2005-2007, however this trend is non-significant.
Therefore although there is an increase in the utilization of extended criteria organs, this has not compromised recipient survival. Patient survival rates continue to improve in the past decade.
Conclusions: The liver donor demographic trends in Canada suggest an increase in utilization of higher risk donors. However, overall graft quality is not compromised due to a decreasing trend in CIT and increase in local transplants. Better coordination and allocation practices across Canada are minimizing the risk for graft failure and leads to continued excellence in recipient outcomes.

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PSYCHOSOCIAL NEEDS ASSESSMENT POST-KIDNEY TRANSPLANT: FEASIBILITY OF A POST-TRANSPLANT SPECIFIC SUPPORT GROUP

Mitchell, Margot; Famure, Olusegun; Brijmohan, Angela; Sihota, Kiren; Shea, Mary (University Health Network, Toronto, ON)

Background: Lifestyle changes accompanied with transplantation may have implications for medication adherence over time. The use of social support groups have correlated with increased medical adherence in patients with chronic conditions. The objective of this study was to conduct a psychosocial needs assessment of post-transplant patients, to determine the utility of a support group, and identify barriers to attending one in a Canadian urban renal transplant centre.
Methods: A likert scale was used to assess the degree of patients' concern about specific psychosocial needs. Questions were grouped into "domains of transplantation" which addressed: *medical complications of transplantation return to normalcy, financial costs of transplant, psychological impact, social support post-transplant,* and *relating to other transplant patients*. Patient information regarding time since transplantation was used to stratify the results.
Results: Patients who were >2 years post-transplant were significantly more concerned about medical complications than patients in the other time groups ($\chi^2(4, n= 42)= 22.05$, $p<.001$), returning to normalcy (χ^2 (4, n= 28) = 10.21, $p<.04$) and had a greater desire to talk with other transplant patients (χ^2 (4, n= 28) = 12.08, $p<.02$). Patients who were 3-6 months post-transplant were significantly less concerned about complications following transplant ($\chi^2(4, n=42) = 22.04$, $p<.001$). Patients would not attend a social support group at TGH due to transportation barriers.
Conclusion: The main area of concern for patients was the medical complications of transplant, particularly for patients who had their graft for greater than two years. Specifically, patients identified side effects of medication as their leading concern, likely due to the chronic physical and psychological impact of their medication regimen. Studies have found that implementing social support groups have led to major increases in medication adherence over time due to continual informational support. For patients unwilling to attend a support group on site, alternative means of support are proposed.

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DUAL VERSUS SINGLE SITE LIVING DONOR KIDNEY TRANSPLANTATION

Kolozsvari, Nicoleta; Feldman, Liane; Kaneva, Pepa; Castelino, Tanya; (Steinberg-Bernstein Centre for Minimally Invasive Surgery and Innovation, Department of Surgery, Montréal, QC), Dumitra, Sinziana, (Section of Transplantation, Department of Surgery, Montréal, QC), Anidjar, Maurice (Division of Urology, Department of Surgery, Montréal, QC); Paraskevas, Steven, (Section of Transplantation, Department of Surgery, Montréal, QC); Chaudhury, Prosanto, (McGill University Health Centre, Montréal, QC); Metrakos, Peter; Tchervenkov, Jean, (Section of Transplantation, Department of Surgery, Montréal, QC)

Introduction: Although allograft transport is common in deceased donor transplantation of solid organs, concern persists regarding transportation in live donor kidney transplantation (LDKTx). At our institution, laparoscopic live donor nephrectomy (LLDN) initially occurred at a separate site 1km away from the recipient transplant operation, but was later moved to the same site. Our objective was to evaluate outcome differences in a dual-site LDKTx compared to a single-site setting.
Method: A medical record audit of all LLDNs between January 2004 and April 2010 and the corresponding LDKTx was performed. The move to a single-site setting occurred in May 2007. Demographic data, operative information and postoperative outcomes were collected from the patients' medical record and a prospectively maintained transplant database.

Primary outcomes were early graft function and creatinine clearance (CrCl). Secondary outcomes were 30 day donor and recipient postoperative complications, acute rejection and long term graft survival. Data are reported as n(%), mean(SD) or median [interquartile range]. Statistical significance was defined as $p<0.05$*.
Results: Fifty-three LDKTx using LLDN were performed with the two-site model and 51 operations followed in a single-site setting. The two groups were similar in terms of gender, BMI, preoperative CrCl and ASA but the single-site donors were older (42(11) vs 48(11) years*). The extraction (168(73) vs. 127(54) sec*) and perfusion times (232(80) vs. 191(69) sec*) were higher in the dual-site donors reflecting the use of the hand-port for kidney extraction beginning in 2009. After the move to the single site, donor intraoperative complications increased (2(4%) vs. 5(10%)*) potentially reflecting the learning curve in a new environment. There were no differences in postoperative course between the donors. At baseline, the recipients were similar in age, gender, BMI, comorbidities and dialysis, but the single-site recipients were more sensitized (max PRA I 3.22(12.3) vs 3.08(5.3) *). Cold ischemia times were similar in the dual-site and single site approaches (140[108-170] vs. 108[64-213]min). Recipient postoperative CrCl improved similarly with similar rates of DGF (4(8%) vs. 1(2%)) and SGF (3(6%) vs. 9(18%)). There were no differences in postoperative outcomes including biopsy proven acute rejection (11(22%) vs. 12(24%) and graft survival (90% vs. 95% 36-month DCGS).
Conclusion: There were no differences in outcomes after live donor kidney transplant between a dual and single site program. Establishing the safety of a dual-site LDKTx program may offer support for other institutions where donor surgeries may be encumbered by geographic limitations or availability of expertise.

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NEURODEVELOPMENT OUTCOMES FOLLOWING INFANT/TODDLER HEART TRANSPLANT

Deliva, Robin; Sollazzo, Stephanie; Graham, Ashley; Harkness, Maggie; Manlhiot, Cedric; Dipchand, Anne I. (Hospital for Sick Children, Toronto, Canada)

Background: Developmental delay has been described in pediatric heart transplant recipients with limited attention to motor outcomes. The purpose of this study was to examine neurodevelopmental outcomes in a cohort of infant/toddler recipients.
Methods: Single centre review of assessments completed since the initiation of a neurodevelopmental follow-up protocol at ages 1, 2 and 4 years which included the Alberta Infant Motor Scales (AIMS) (age 1), the Peabody Developmental Motor Scales (PDMS-II) (all ages), the Beery-Buktenica Developmental test of Visual Motor Integration (VMI) (age 4), and parent report using the Ages and Stages Questionnaire (ASQ).
Results: For the 1, 2 and 4 year assessments respectively, average ages of the 14 children were 15.4±2.4, 26.3±3.3 and 48.3±1.21 months and time post-transplant was 10.6±1.52, 15.0±4.36 and 37.7±15.2 months. Patient diagnoses included congenital heart disease (n=8) and cardiomyopathy (n=6). Average gestational age was 38.0±2.55 weeks and birth weight was 3.19±0.59 kgs. Six had a history of a neurologic complication. Three out of 4 children had AIMS scores below the 5th percentile. One year PDMS-II percentiles were 25.7±17.0, 41.4±34.6 and 36.7±27.1 for gross, fine and total motor scores respectively. Two year and four year percentile scores were 6.3±4.7, 16.0±11.0 and 7.0±8.5 and 14.2±15.4, 13.8±16.2 and 10.6±15.9 for the same. Univariate analysis revealed higher number of post-transplant admissions ($p=0.04$) and lower birth weight ($p=0.06$) correlated with lower gross motor scores, whereas only number of post-transplant admissions correlated with reduced fine ($p=0.01$) and total ($p=0.01$) motor scores. Need for mechanical support ($p=0.001$) and lower gestational age ($p=0.001$) correlated with lower VMI scores. There were no differences based on underlying diagnosis. Parent questionnaire results were consistent with standardized assessments.
Conclusion: Delayed motor skill acquisition is common in young heart transplant recipients. Further study is warranted to examine the long-term implications as they reach school age.

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THE FREQUENCY OF AND RISK FACTORS FOR LATE PULMONARY CONDITIONS FOLLOWING PEDIATRIC HEART TRANSPLANT

Deliva, Robin; Steinfeld, Tova; Preston, Heather; Mach, Kat; Teskey, Julia; Mathur, Sunita (University of Toronto, Toronto, Canada); Sollazzo, Stephanie; Dipchand, Anne I. (Hospital for Sick Children, Toronto, Canada)

Purpose: Survival following pediatric heart transplant continues to improve, however the development of late pulmonary complications (LPC), of multifactorial etiologies, poses a threat to quality of life and function. The purpose of this study was to describe development of LPC and use of cardiopulmonary physical therapy (CPT) interventions in pediatric heart transplant recipients.
Methods: Single centre review of pediatric patients who underwent heart transplant between January 1997 and June 2010 for the development of LPC.
Results: 128 subjects (71 males) met inclusion criteria with a median age at transplant of 2.2 years (range 0-17). Sixty-five (51%) had an underlying diagnosis of congenital heart disease (CHD). Seventy-six patients (59.4%) had ≥1 comorbidity at time of transplant, most commonly developmental delay (29.7%). Fifty subjects (39%) developed LPC: recurrent pneumonia (n=37, 28.9%), bronchiectasis (n=9, 7.0%), pleural effusion/chylothorax (n=6, 4.7%), asthma (n=13, 10.2%), interstitial lung disease (n=5, 3.9%), and other (n=20, 15.6%). Risk factors for developing LPC included age < 4 years (OR 2.85, CI 1.33-6.10, p=0.007), CHD (OR 4.11, CI 1.91-8.86 p<0.001), presence of comorbidities (OR 3.64, CI 1.70-7.79, p=0.001), cyclosporine use (OR 2.84, CI 1.31-6.16, p=0.008), phrenic nerve damage (OR 2.86, CI 1.02-7.97, p=0.044), acute pneumonia (OR 3.31, CI 1.48-7.41, p=0.004), failed extubation (OR 3.73, CI 1.58-8.79, p=0.003) and longer time on ventilator (OR 1.06, CI 1.02-1.11, p=0.003). Previous surgery and history of aspiration were not significantly linked with development of LPC. Sixty-two percent of the LPC group (24% of sample) were receiving CPT greater than 6 months post-transplant .
Conclusion: LPC develop in a significant portion of pediatric heart transplant recipients. Risk factors include young age, CHD, comorbidities, phrenic nerve damage, prior pneumonias and longer need for mechanical ventilation. CPT for LPC is indicated to assist with impaired airway clearance, however the impact on long term outcomes remains to be determined.

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PHARMACOGENETIC VARIABILITY IN RESPONSE TO AMLODIPINE IN HYPERTENSIVE PEDIATRIC CARDIAC TRANSPLANT RECIPIENTS

Mital, Seema; Fazalullasha, Fatima; Manickaraj, Ashok; D'Alessandro, Lisa; Manase, Dorin; Binesh, Tina; Safi, Mina; Daljevic, Tanya; Kantor, Paul; (Hospital for Sick Children, Toronto, ON)

Background: Systemic hypertension is common in pediatric cardiac transplant patients . Amlodipine, a calcium channel blocker, is the most commonly used first line anti-hypertensive agent. We analyzed pharmacogenetic factors influencing amlodipine response in this cohort.
Methods: Pediatric cardiac transplant recipients prospectively enrolled through the transplant centre biobank were studied. 24 hour, daytime and nighttime mean systolic and diastolic blood pressure (BP) were captured from serial ambulatory BP measurements. Hypertension was defined as BP above the 95th percentile for gender, age, and height. Amlodipine dose and BP indexed to amlodipine dose (in mg/kg/mmHg/10) was assessed. DNA was genotyped for SNPs in 3 genes: CYP3A4, CYP3A5 and NPPA.

Results: Of 124 heart transplant patients in the biobank. 97 received amlodipine during follow-up of whom 54 had ambulatory BP measurements available for analysis. Mean age at transplant was 8.2 yrs and maen follow-up of 6.9 yrs post-transplant. Mean dose of amlodipine was 0.163 mg/kg/day (range 0.04-0.6). The BP indexed to amlodipine dose was highly variable as shown in the Table. CYP3A5-GG genotype was associated with higher systolic and diastolic 24 hour and daytime BP compared to AA/AG genotypes ($p<0.05$) (Table).
Conclusions: There was large variability in amlodipine dose requirements and dose-adjusted response to amlodipine in hypertensive pediatric transplant recipients. Pharmacogenetic variation in CYP3A5 was associated with response to amlodipine. CYP3A5 genotype may provide an important marker to guide appropriate choice and dose of anti-hypertensive drug therapy in this cohort.

BP indexed to amlodipine dose (mg/kg/mmHg/10)	Daytime Systolic	Daytime Diastolic	Nighttime Systolic	Nighttime Diastolic	24 hours Systolic	24 Hours Diastolic
Overall mean	87±55	55±35	79±29	48±29	84±53	52±33
CYP3A5 GG	92±48	58±36*	83±51	50±30	88±55	55±34*
CYP3A5 AG/AA	72±44	45±28	66±42	40±26	70±43	43±27

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PROTEINURIA AND CHRONIC KIDNEY DISEASE IN LUNG TRANSPLANT RECIPIENTS

Partovi, Nilu; Huang, S; Zalunardo, N; Espino, G; Swiston, J; Yee, John; Levy, Robert D; (Vancouver General Hospital, Vancouver, BC)

Purpose: Lung transplant (LT) recipients are at risk for chronic kidney disease (CKD). Proteinuria is a risk factor for CKD and mortality in the general population but has not been studied in LT recipients. The purpose of this study was to determine the prevalence of, and risk factors for, CKD and proteinuria in LT recipients in British Columbia, Canada. **Methods:** Retrospective study of LT recipients followed at Vancouver General Hospital as of August 1, 2011. Logistic regression to determine factors associated with CKD (estimated GFR < 60 mL/min/1.73 m2) and proteinuria (urine albumin to creatinine ratio (ACR) 3.0 mg/mmol). Those on dialysis (N=1) or with a kidney transplant (N=3) excluded. Patients were treated with a calcineurin inhibitor (cyclosporine (N=4) or tacrolimus), mycophenolate or azathioprine, and prednisone. All received sulfamethoxazole prophylaxis.

Results: There were 71 (of 91) patients with urine protein assessments. Reasons for LT included chronic obstructive lung disease (38%), pulmonary fibrosis (32%), and cystic fibrosis (20%). Median time from LT was 5.4 (2.4-10.3) years; 56% were male; mean age at LT was 50 (/-13) years. 2 (3%) subjects had diabetes pre-transplant; 5 (7%) developed diabetes post-transplant. At most recent follow-up, CKD was present in 39 subjects (55%) (5 (13%) stage 3; 34 (87%) stage 4 CKD). 19 patients (27%) had proteinruia, with median urine ACR 4.95 (3.70-11.95) mg/mmol. Female sex (OR=6.33; CI:1.76-22.82), cardiovascular complications (OR=9.88; CI:1.94-50.48), and CMV disease (OR=3.79; CI:1.13-12.74), but not proteinuria (OR=0.80; CI:0.21-3.04), were associated with CKD. Higher systolic blood pressure (OR=1.04; CI:1.00-1.07) was associated with proteinuria, but there was no significant association with CKD (OR=1.59; CI:0.52-4.85). **Conclusions:** CKD was common among LT recipients and associated with cardiovascular and CMV disease. Proteinuria was present in 27% of patients, most of which was mild (microalbuminuria). Proteinuria was associated with higher systolic blood pressure but not CKD.

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PREDICTORS OF REVERSIBILITY OF RENAL FUNCTION AFTER ACUTE REJECTION AND ITS IMPACT ON GRAFT SURVIVAL IN KIDNEY TRANSPLANT RECIPIENTS

Trinh, Emilie; Tchervenkov, Jean; Mucsi, Istvan; Baran, Dana; Cantarovich, Marcelo; (McGill University Health Center, Montréal, QC)

Introduction: Acute rejection (AR) episodes negatively correlate with long-term graft survival after kidney transplantation (KTx). However, studies have shown that not all AR episodes have the same impact on graft survival depending on the degree of return to baseline renal function, but factors determining renal function recovery still need to be elucidated.
Purpose: To determine if a recovery of renal function after treatment for AR predicts better long-term death censored graft survival (DCGS). We also evaluated predictors of renal function recovery after AR in KTx pts.
Methods: We studied 729 pts (52±13 yr-old), who received a KTx at a single center between 01/1997 and 10/2010. Renal function was assessed according to the estimated glomerular filtration rate (eGFR) using the abbreviated MDRD equation. 87 pts who experienced a first biopsy-proven AR were divided into two groups, based on whether the eGFR returned to within 10% of pre-AR values within 3 months (responders) or not (non-responders). A further analysis was performed to evaluate predictors of eGFR recovery after an episode of AR.
Results: DCGS was similar in non-rejectors and in responders, and was significantly worse in non-responders (Figure 1). The proportion of pts with grade of ≥2 or with elements of antibody-mediated rejection (AMR) on biopsy was higher in non-responders vs. responders (43.4% vs. 22.9%, p=0.049, Figure 2). In addition, there was also a higher proportion of pts with >30% eGFR decrease from baseline at the time of AR among non-responders vs. responders (53.3% vs. 24.6%, p=0.005). Multivariate analysis showed that pts whose eGFR at the time of AR decreased >30% from baseline had an odds ratio of 8.673 [CI: 2.13-35.27] of non-recovery post-AR. Other variables, such as age, gender, living vs. deceased donor, DCD vs. SCD vs. ECD, immediate vs. slow vs. delayed graft function, HLA mismatch, time to AR, and baseline immunosuppression were not significantly associated with eGFR recovery post-treatment of AR.
Conclusion: Our results suggest that pts undergoing AR who recovered eGFR to within 10% from pre-treatment value have a similar long-term DCGS than pts without AR. The eGFR recovery after treatment of AR may therefore be a useful predictor of long-term DCGS. Additionally, the degree of change in eGFR from baseline at the time of AR and the severity of pathological changes on biopsy could be useful in assessing reversibility of eGFR after AR.

Figure 1

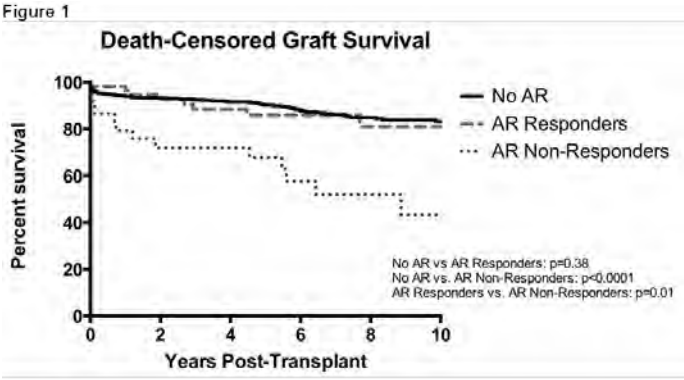
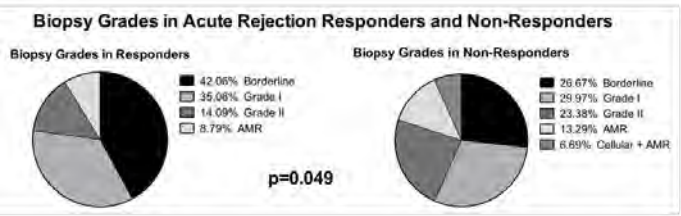


Figure 2



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WEIGHT GAIN AFTER ORTHOTOPIC LIVER TRANSPLANTATION: IS NASH CIRRHOSIS A RISK FACTOR FOR GREATER WEIGHT GAIN?

Rakel, Agnès; Kouz, Jasmine; Vincent, Catherine; Montréal, QC; (CHUM-HSL, Montréal, QC)

Introduction: Excessive weight gain is frequently observed among orthotopic liver transplant (OLT) patients. Obesity increases cardiovascular events in this population, and might also affect the new liver. We aimed to determine whether weight gain after OLT was greater in patients with NASH cirrhosis, compared to other types of cirrhosis, and to evaluate predictors of greater weight gain.
Methods: We conducted a retrospective study in 126 liver transplant recipients from 2005 to 2007. Several data were collected, including: age at transplant, sex, BMI, ethnic group, smoking, hepatoma or ascites before OLT, type of cirrhosis, length of hospitalization post OLT, presence of diabetes before and during follow up, lipid level before and during follow up, corticosteroids doses, type of immunosuppressive medication as well as the incidence of complications such as reject after OLT.
Results: We identified 17 patients with NASH cirrhosis and 109 patients with other types of cirrhosis. When the weight at transplantation was used as the baseline weight, no significant difference in weight gain was observed between NASH and non NASH patients at one or two years. However, when the 3 month post-transplant weight was used as the baseline (which is a weight free of ascites), weight gain was significantly higher in the NASH patients. In multivariate linear regression analyses (adjusted for age, sex, BMI before OLT, NASH, ascites, and corticosteroids), weight gain at one and two years was significantly greater in the NASH group ($p<0,0001$ and $p=0,042$, respectively) and in patients who had ascitis ($p=0.004$ and $p=0.002$).
Conclusion: Patients with NASH cirrhosis gain more weight in the first year post transplant compared to other types of cirrhosis. This difference still prevails at two years post-transplant. This study emphasizes the need for an early and tight follow-up with a nutritionist and a kinesiologist in OLT patients, particularly NASH patients.

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THE POWER OF YOUTH: A HIGH SCHOOL OUTREACH INITIATIVE EDUCATING YOUTH ABOUT ORGAN AND TISSUE DONATION AND TRANSPLANTATION

So, Stephanie; (Hospital for Sick Children, Toronto, ON), Norton, Andrea; (University Health Network, Toronto,ON), Meliton, Galo; (St. Michael's Hospital, Toronto,ON), Breckbill, Kathryn; (Hospital for Sick Children, Toronto, ON), Grant, David; Ng, Vicky; (Hospital for Sick Children, Toronto, ON)

Background: Social change is driven and achieved by the power of youth. Broadening their exposure to the topic of organ donation and transplantation (ODT) could spark their passion and social conscience and positively impact their support for organ donation. Over 10% of Canada's population is between the ages of 15-24. Most youth lack the needed knowledge about ODT to make informed, personal decisions about registering consent.
Goal: To increase knowledge and awareness about ODT to youth within a large Canadian city through presentations in classrooms and assemblies at secondary schools. To engage teachers in the use of curriculum on ODT within the classroom setting.
Method: During the 2011-2012 school year, a pilot project was initiated in joint partnership with health care providers (HCP) from 3 large organ transplant programs within adult and paediatric academic health science centers and the provincial organ donation agency (ODA). An engaging presentation was created with content on OTD. Presentations were given by a trio

comprised of a HCP, an ODA representative and an organ recipient or donor. Teachers were encouraged to supplement the presentations with use of the developed curriculum, "One Life…Many Gifts".

Results: Over 90 HCP's volunteered to participate in training sessions to be guest speakers. These HCP's included representation from physicians (27), nurses (25) and allied health professionals (44) across all organ groups. A total of 55 presentations were given at 35 schools between March-June 2012, reaching over 6000 students. The project was also profiled by leading news networks. Pre-presentation survey results showed that 23% of students were very knowledgeable about ODT. Post-survey results saw this number increase to 69%. Before the presentation only 33% of students knew about the online provincial registration site. Post presentation, 82% of students said they were likely/very likely to register. Reception was positive from teachers and students.

Conclusion: Educating youth within the school system increases their knowledge and awareness about ODT and has the potential to positively impact organ donation rates. Use of established curriculum coupled with engaging presentations from transplant HCP's, local ODA representatives and personal stories from a donor or recipient is key to the success of the outreach.

2451

GLOMERULAR SIZE ON TIME ZERO KIDNEY ALLOGRAFT BIOPSIES AND THE CHANGE IN KIDNEY FUNCTION AFTER LIVE KIDNEY DONATION

Yan, Howard; Hendren, Elizabeth; Dong, James; Hung, Tawny; (University of British Columbia, Vancouver, BC), Mengel, Michael; (University of Alberta, Edmonton, AB); Gill, John; (University of British Columbia, Vancouver, BC)

Identification of predictors of the decrease in kidney function after live kidney donation may help expand the living donor pool or identify donors for long-term medical follow up. In this study, we determined the association of demographic factors (donor age at transplantation, gender, body mass index), pre-donation kidney function (eGFR), and time zero kidney allograft biopsy findings (glomerular diameter and glomerular volume determined by the Weibel-Gomez method) with the percent decrease in pre-donation eGFR one year after live kidney donation among n=60 live kidney donors in our centre between 2000-9 with an adequate time zero kidney biopsy as well as an eGFR recorded immediately before and one year post-kidney donation.

The mean±std pre- and post-donation eGFR were 93±14 and 61±15 ml/min/1.73m^2, respectively. The mean±std (median, Q25,Q75) percent change in eGFR after donation was 34±12, (37,31,41)%, and 91% had ≥ 30% decrement in eGFR. There was a mean±std of 11±5 glomeruli available for analysis in each time zero implant biopsy: the mean glomerular diameter was 163±17 μm, while the mean estimated glomerular volume was 2.81±0.98•10^6 μm^3. No donors had glomerulomegaly (defined by volume ≥6.81•10^6 μm^3).

In regression analyses, age at time of kidney donation was associated with the percent drop in kidney function after donation (3% greater decrement in pre-donation eGFR for each decade older, p=0.018), but there was no association with pre-donation eGFR, glomerular diameter, glomerular volume, donor BMI, race, or gender (data not shown).

We conclude that the percent decrement in eGFR post-kidney donation is variable, and associated with donor age at transplantation. The absence of an association between glomerular size and volume with the change in kidney function after donation in this study may be due to conservative donor selection, and these parameters may still prove useful in the selection of donors with more marginal levels of pre-donation kidney function.

2452

THE PROGNOSTIC VALUE OF "TIME NEEDED ON DIALYSIS" IN PATIENTS WITH DELAYED GRAFT FUNCTION (DGF)

Marek, Caitlyn; (University of Saskatchewan, Saskatoon, SK); Thomson, Benjamin (Western University, London, ON); Bloch, Michael; Weernink, Corrine: (London Health Sciences Centre, London, ON); Shoker, Ahmed; (Saskatchewan Transplant Program, Saskatoon, SK); Luke, Patrick; (Western University, London, ON); Moser, Michael; (University of Saskatchewan, Saskatoon, SK)

Introduction: We hypothesize that in patients with DGF, the need for a longer period of dialysis post-transplant is associated with poorer long term function and an increase in complications.
Methods: This chart review involved collaboration between two Canadian Transplant Centres. A total of 774 patients (567 and 207) received kidney transplants between 2004 and 2011, of which 83 patients (59 and 24) developed DGF, defined as the need for dialysis in the first week post-transplant.
Results: A regression model including rejection on initial admission identified donor age (B=-0.31, p=0.034) and length of time needed on dialysis (TND) post-transplant (B=-0.37, p=0.008) as the most significant predictors of CrCl at one year in these patients with DGF. These patients were divided into three groups depending on TND (group 1: 7 days or less (n=52), group 2: 8-14 days (n=13), and group 3 (n=18): more than 14 days). CrCl at 30 days (42.4, 33.8, 20.0cc/min; p<0.001), 6 months (50.9, 49.8, 37.8 cc/min; p=0.037), and 1 year (51.2, 47.3, 37.3 cc/min, p=0.035) were all significantly different between the 3 groups. The proportion of patients having one or more complications (wound infection or leakage, reoperation, readmission, or graft loss within less than a year) increased with increasing time that dialysis was needed: group 1: 15/52 (28.8%), group 2: 7/13 (53.8%), group 3: 13/18 (72.2%), (p=0.004).
Discussion: Our study suggests that increased TND is associated with increased complications and worse long-term CrCl.

2453

THE LONG-TERM SIGNIFICANCE OF DE NOVO DONOR-SPECIFIC ANTIBODIES IN PATIENTS WITHOUT HUMORAL REJECTION

Lan, James; Keown, Paul; Landsberg, David; Gill, John; (University of British Columbia, Vancouver, BC)

Background: We previously reported results of a prospective study of 70 patients without pre-formed anti-HLA antibodies who were screened for the development of de novo anti-HLA antibodies at 0, 10, 20, 30, 60, 90, 180 and 365 days post kidney transplantation. Although development of de novo anti-HLA antibody in 11 patients (8 with DSA) was associated with rejection, screening for antibodies did not predict development of acute rejection (AR) before clinical evidence of allograft dysfunction (Transplantation 2010: 89(2):178-84). The current analyses reports long-term follow up outcomes on the study participants who were alive with a functioning transplant at one year post transplant (n = 9/11 with de novo anti-HLA antibody and 58/59 without de novo antibody formation). The outcomes assessed included graft survival, late humoral or late cellular rejection after the first post-transplant year, MDRD eGFR, the change in eGFR after the first post-transplant year, and proteinuria (>1000 mg/day) after a median follow up of 8.6 years (Table 1).
Results: Among the 9 patients with de novo Ab formation in the first year, 2 developed late humoral rejection versus 1/58 among those without de novo antibody. Among the n = 5 patients with de novo DSA formation but did *not* develop late humoral rejection on follow up the change in eGFR was similar to that in patients who never developed anti-HLA antibodies (-4 ml/min/1.73m^2). In contrast, among the n = 2 patients with de novo DSA formation *and* subsequently developed late humoral rejection, one suffered graft loss, and the other had a 45 ml/min/1.73m^2 drop in eGFR.
Conclusion: These findings suggest that the long-term significance of de novo DSA in the first year appears to be primarily as a risk factor for late humoral rejection. Independent of humoral rejection there was little clinical impact of de novo DSA in these low risk patients without pre-transplant anti-HLA antibody. Larger studies are needed to define the clinical significance of de novo DSA in patients without humoral rejection.

Table 1: Long-term allograft outcomes in non-sensitized kidney transplant patients with and without de novo antibody formation in the first year post transplant

	Did not develop de novo anti-HLA Ab during first year (n = 58)	Developed de novo anti-HLA Ab during first year (n = 9)	Developed de novo DSA during first year (n=7)
Median follow-up (Q25, Q75)	8.6(8.1, 9.0)	8.6(8.3,8.9)	8.6(8.2,8.9)
Patient deaths after 1 yr	3(5%)	0	0
Graft loss after 1 year	4(7%)	1(11%)	1(14%)
Acute rejection after 1 yr Acute Cellular Rejection Acute Humoral Rejection	2(4%) 1(2%)	0 2(22%)	0 2(29%)
eGFR (ml/min/1.73m^2) at last follow-up mean ± std*	52(29)	43(24)	41(27)
eGFR (ml/min/1.73m^2) at last follow-up mean ± std**	56(26)	49(19)	48(22)
Change in GFR after 1 yr*	-7(23)	-14(22)	-16(25)
Change in GFR after 1 yr**	-4(22)	-12(23)	-13(26)
Proteinuria > 1000 mg/day**	5(9%)	2(22%)	2(29%)
*eGFR of zero imputed for those with allograft failure **among those with a functioning graft			

2454

EVIDENCE OF ENHANCED SYSTEMIC INFLAMMATION IN STABLE KIDNEY TRANSPLANT PATIENTS WITH LOW FRAMINGHAM RISK SCORES

Mansell, Holly; Rosaasen, Nicola; (Saskatchewan Transplant Program, Saskatoon, SK); Dean, Jonathan; Gifeng, Zhou; Hassan, Abubaker; Mainra, Rahul; Shoker, Ahmed (University of Saskatchewan, Saskatoon, SK)

Background: Framingham risk scores (FRS) underestimate cardiovascular events in renal transplant recipients. Inflammation is a hallmark of both diminished renal function and vascular injury. Whether inflammatory chemokines more accurately predict CV risk compared to FRS has not been studied.
Objective: To explore the relationship between chemokines (CCL family) and FRS in a stable kidney transplant population.
Methods: The modified FRS (2009) was used to calculate the 10-year probability of developing cardiovascular disease in 150 kidney transplant recipients. Of these, eighty (53%) were enrolled in a cross-sectional study to measure plasma levels of fourteen CCLs (1,2,3,4,5,8,11,13,17,15,21,26 and 27) by Luminex technique. Statistical analyses were performed between FRS, patient demographics, immunosuppressant and eGFR. CCL levels in 30 normal subjects served as a control.
Results: 43.3% of patients were classified as low, 16% moderate and 40.7% high FRS. FRS correlated significantly with age, hemoglobin, systolic blood pressure, LVEF and urine microalbumin ($p<0.05$). FRS did not correlate with BMI, diastolic blood pressure, eGFR, CNI agent, hsCRP or PTH. Compared to controls, CCL 1, 4, 8, 15, and 27 were equally

increased in both the high and low FRS groups (p= 0.04, 0.03 respectively). There was a significant inverse correlation (p<0.04, R>-0.4) between CCL 8, 15 and 27 and eGFR. The number of low FRS-patients with CCL 15 and 27 values above the 95% cutoff control levels were 69% and 48% respectively.
Conclusion: CCL levels do not correlate with FRS. While CCL levels are inversely related to transplant eGFR, FRS is not, suggesting that chemokines more accurately reflect CV risk. Over one-half of stable kidney recipients (including those with low FRS) have increased chemokine levels, compared to normal controls. CCL-15 and 27 have potential as a biomarker to predict progressive allograft dysfunction and future CVD events.

2455

ASSESSING PATIENTS' PERCEPTIONS TOWARD POST-KIDNEY TRANSPLANT PRIMARY CARE: A PRELIMINARY ANALYSIS

Famure, Olusegun; Rivera, Laura; (University Health Network, Toronto, ON); Li, Anna (University of Toronto, Toronto, ON); Adcock, Lesley; Schiff, Jeffrey; Kim, Joseph S. (University Health Network, Toronto, ON)

The growing number of long-term kidney transplant survivors has led to an increased demand for resources at transplant centres. As a result, referring stable kidney transplant recipients (KTRs) to their primary care physicians (PCPs) for routine medical care has become more common. However, there have been concerns about insufficient guidelines provided to PCPs from transplant centres to manage the long-term care of these patients.
To ascertain patient perceptions regarding their post-transplant primary care, a survey was developed and administered to a cohort of KTRs at a large Canadian transplant program. The survey assessed patients' views on PCP performance, comfort level with their PCP, support received for health self-management, and barriers to better care.
A total of 237 patients completed the survey (76% response rate). Eighty percent indicated that a family physician was part of their health care team. While ≥ 75% of KTRs felt comfortable with their PCPs managing non-transplant related issues (e.g., vaccinations, periodic health exams, and specialist referrals), only 21% felt comfortable with PCPs managing transplant-related issues (e.g., immunosuppressive therapy). Fifty-five percent felt their PCP was above average to excellent in providing health education, encouraging behavioural interventions, and identifying issues related to care. Thirty percent felt PCPs were above average to excellent in providing referrals to community resources, and psychosocial support. Respondents also gave qualitative feedback on ways their care could be improved.
These initial results provide insight into patients' perceptions of primary care post-transplant. Next steps include the dissemination of a "sister survey" to PCPs involved in the care of KTRs to elicit their views regarding their role in the care of KTRs. The findings from both surveys will inform transplant nephrologists and PCPs on how best to design shared care models that will improve the longevity and quality of life for KTRs.

2457

GOING WITH THE FLOW, CANADIAN CROSSMATCH STANDARDIZATION

Liwski, Robert; (Dalhousie University, Halifax, NS), Pochinco, Denise; (Diagnostic Services of Manitoba, Winnipeg, MB), Tinckam, Kathryn; (University of Toronto, Toronto, ON), Campbell, Patricia; (University of Alberta, Edmonton, AB), Nickerson, Peter; (University of Manitoba, Winnipeg, MB)

Aim: Expeditious flow cytometry crossmatch (FCXM) assay giving consistent inter-lab results is critical for successful and safe national organ sharing. In order to understand the degree and nature of variability in FCXM testing across Canada, national proficiency testing (PT) program was established. In the 1st PT survey, the focus was on studying the factors that contribute to FCXM result variability. The 2nd survey examined the effects of assay standardization and optimization.

Methods: Both surveys consisted of 3 sera, 2 donor cells and MESF beads. Donor HLA typing and serum HLA antibody profiles were provided to aid XM interpretation. Questionnaire was sent to obtain specific protocol information. XM data (channel & MESF values) and questionnaire responses were collected from 13 participating labs and analyzed.
In survey 1, labs were asked to perform FCXM as per local lab protocol. In survey 2, labs were provided with optimized FCXM protocol (Liwski et al. ASHI 2011), pronase/DNase procedure and common reagents (pronase, DNase, & a-IgG-FITC). Labs were asked to test FCXM using their own protocols with specific modifications vs optimized FCXM protocol.
Results: In survey 1, significant inter-lab FCXM result variability was seen. MESF scale was helpful in result comparison. Technique, cell number and serum volume were main factors contributing to observed variation. Pronase use was critical for B cell XM. In PT part 2, the findings showed that standardization (cell number/serum volume and use of pronase) significantly improved result consistency. Use of optimized protocol and common a-IgG-FITC further improved results. Optimized assay compared favourably with conventional methods, while decreasing XM time by 1h.
Conclusions: Significant variability in FCXM methods and results were seen. Standardization of FCXM protocol improved result concordance. Optimized FCXM protocol will improve risk assessment precision, facilitate equitable national organ sharing and optimize low risk transplant allocation.

2458

PERITONEAL DIALYSIS (PD) VERSUS HEMODIALYSIS (HD) IN PATIENTS WITH DELAYED GRAFT FUNCTION (DGF)

Thomson, Benjamin; (Western University, London, ON), Marek, Caitlyn; Shoker, Ahmed; (University of Saskatchewan, Saskatoon, SK), Bloch, Michael; Weernink, Corrine; (London Health Sciences Centre, London, ON), Moser, Michael; (University of Saskatchewan, Saskatoon, SK) Luke, Patrick; (Western University, London, ON)

Introduction: Delayed graft function (DGF) in kidney transplantation increases adverse outcomes, including poorer 5 year graft function, long term allograft survival and future acute rejection episodes. Although several risk factors have been identified for DGF, it remains unclear if the dialysis modality in kidney transplant recipients with DGF alters perioperative or long term graft outcomes.
Materials and Methods: We performed a retrospective observational quality initiative at two Canadian renal transplant centers, of kidney transplants (2004-2011) in which DGF occurred in the recipient (n=77). We assessed the effect of demographic and clinical factors, including dialysis modality, on several hospitalization, infection and graft function related outcomes.
Results: Whether the post-transplant dialysis modality was PD (n=14) or HD (n=63), there was no difference with regards to any demographic factor, nephrotoxic drug exposure, cold ischemic time, or pre-transplant urinary volume. The use of peritoneal dialysis increased the risk of wound infection or leakage (PD 5/14 versus HD 6/63, p=0.024), but was associated with decreased length of hospitalization (13.7 versus 18.7 days, p = 0.009) and the duration of time requiring dialysis post-operatively (6.5 vs. 11.0 days, p=0.043). There was no difference in readmission to hospital within 6 months (4/14 vs. 23/63, p=0.759), and no differences in graft loss (0/14 vs 2/63, p=1.000) or acute rejection episodes (1/14 vs. 4/63, p=1.000) at one year, even when controlling for known confounders using linear regression analysis. . Serum creatinine did not differ between the peritoneal or hemodialysis groups, at 30 days (226.6 vs 231.4 mmol/L, p=0.899), 6 months (150.7 vs 166.4 mmol/L, p=0.433) or 1 year (153.1 vs 173.8 mmol/L, p=0.381).
Discussion: Prospective trials are needed to determine when renal transplant recipients on peritoneal dialysis should have their catheter removed, to decrease risk of wound infection or leakage while limiting duration of renal replacement and hospitalization.

2459

CANADA-WIDE EVALUATION OF RAPID OPTIMIZED FLOW CROSSMATCH (ROFCXM) PROTOCOL

Liwski, Robert; (Dalhousie University, Halifax, NS), Pochinco, Denise; (Diagnostic Services of Manitoba, Winnipeg, MB), Tinckam, Kathryn; (University of Toronto, Toronto, ON), Gebel, Howard; (Emory University, Atlanta), Campbell, Patricia; (University of Alberta, Edmonton, AB), Nickerson, Peter; (University of Manitoba, Winnipeg, MB)

Aim: Pre-transplant flow cytometric crossmatch (FCXM) detects donor specific antibodies. Standard 3-color FCXM, may be time intensive and contribute to transplant delays and cold ischemia time. We compared a rapid optimized (RO) FCXM protocol (Liwski et al. ASHI 2011) with <1h turn-around time to the conventional protocols used in 12 Canadian HLA laboratories.

Methods: 3 sera, 2 donor cells and common reagents/supplies including pronase, DNase, anti-IgG-FITC, MESF beads and 96-well trays were sent to participating labs. ROFCXM protocol along with instructional video, and pronase/DNase procedure were also provided. Laboratories performed FCXM by own lab procedure vs ROFCXM protocol. Sera were tested neat and diluted (1:2-1:16). XM channel and MESF values were collected.

Results: ROFCXM gave significantly higher delta MCF and MESF values than lab own method, in 4/4 predicted positive T cell XM, and there were no false positive results in the predicted negative T cell XM. ROFCXM gave higher delta MCF and MESF values in 6/6 predicted positive B cell XM. XM precision was improved when ROFCXM protocol was used (relative standard error of delta MCF was reduced by 28% for T cell and 37% for B cell XM). Importantly, ROFCXM decrease average XM time by 1h.

Conclusions: ROFCXM gave similar results, with superior precision in comparison to conventional FCXM methods and will decrease average XM time by >1h. National implementation of ROFCXM protocol will expedite pre-transplant testing and facilitate equitable national organ sharing.

2461

PREDICTORS OF LENGTH OF ICU STAY AND MORTALITY AFTER LIVER TRANSPLANT

Dumitra, Sinziana; Chaudhury, Prosanto; Barkun, Jeffrey; Alabbad, Saleh; Zogopoulos, George; Paraskevas, Steven; Hassanain, Mazen; Metrakos, Peter; Tchervenkov, Jean; (McGill University Health Center Royal Victoria Hospital, Montréal, QC)

Aim: Intensive Care Unit (ICU) care is required for all liver transplant(LT) recipients, however its length and outcome vary widely. In the new area of judicious resources allocation, predicting the length of ICU stay is paramount.

Methods: Patients having undergone a LT at our institution from 1996 to 2012 were reviewed (n=645). Recipient demographics, donor risk index (DRI), Model for End-stage Liver Disease (MELD) score, operative characteristics, complications within 90 days, the number of transplants, the ICU length of stay(LOS) (including step-down) and if death occurred during the initial ICU stay (n=79) were all collected. Statistical analysis was performed using a multivariate regression to determine predictors of the length of ICU stay and a COX multivariate analysis was performed to determine predictors of ICU mortality.

Results: The mean ICU LOS was 9.45(±13.5) days and the mean MELD score was 22.7(±10.8). In the multivariate regression, only the natural MELD (coef0.26 95%CI(0.17-0.34),p=0.000), pre-LT dialysis(coef4.52,95%CI(0.97-8.08),p=0.013) and re-transplantation(coef3.05,95%CI(0.39-5.70),p=0.013) were significant predictors of length of stay while cold ischemia, warm ischemia, cause of cirrhosis, recipient age, DRI, intraoperative transfusions (PRBC, FFP, platelets) as well as postoperative complications (acute renal failure and sepsis) were not. When looking at predictors of ICU mortality, only cold-ischemia (HR1.06,95%CI(1.01-1.11),p=0.014), diagnosis of HCC(HR2.25,95%CI(1.28-3.96),p=0.005) and pre-transplantation dialysis(HR1.94,95%CI(1.16-3.24),p=0.012) were significant.

Conclusion: Pre-transplant dialysis is a significant predictor of both ICU LOS and mortality, suggesting that extra resources should allocated to these patients. Re-transplantation and the natural MELD were also predictors of LOS while cold-ischemia predicted mortality.

2462

PRE-KIDNEY TRANSPLANT BIOPSY IN EXPANDED CRITERIA DONORS DO NOT CORRELATE WITH OUTCOMES

Rabin, Carly; Dumitra, Sinziana; Chaudhury, Prosanto; Paraskevas, Steven; Metrakos, Peter; Hassanain, Mazen; Tchervenkov, Jean; (McGill University Health Center Royal Victoria Hospital, Montréal, QC)

Introduction: Over the past years biopsy has become more common especially in deceased donors(DD) aged 60 and older. The impact of biopsy results among kidneys that are transplanted(KT) remains uncertain. In this study, we examined graft survival and function depending on biopsy findings.
Methods: Patients who underwent a DDKT(n=574) at our institution from 2002 to 2011 were reviewed. Demographics, death-censored graft survival, graft function subdivided into delayed(DGF), slow(SGF) and immediate graft function(IGF), GFR(at 30,60,365 days), transplant characteristics (pump, cold and warm ischemia) were collected. Biopsy results, exclusively performed on ECD(n=134) were analyzed for interstitial fibrosis, vascular changes and glomerular sclerosis(20%).Statistical analysis was done using COX for survival, logistic regression for graft function and Chi2 or Fishers exact test for univariate analysis.
Results: Interstitial fibrosis had no impact on the incidence of DGF(21%vs23%, p=0.88)or SGF(22%vs22%,p=0.889). Similarly, vascular changes also had no impact on DGF or GRF. In the COX analysis, vascular changes(HR=2.62,95%CI(0.62-11.2),p=0.192), interstitial fibrosis(HR=0.08,95%CI(0.07-1.32),p=0.251) and percent of sclerosed glomeruli(HR=1.02,95%CI(0.97-1.07),p=0.358) did not predict graft survival. In the logistic regression DGF did not correlate biopsy findings, coldtime ichemia or use of a pump. Non-ECD KT(OR=0.72,95%CI(0.39-1.34),p=0.301) did not impact DGF of graft survival in the overall cohort.
Conclusion: Biopsy findings did not correlate with graft survival or with graft outcomes, suggesting their poor predictive value in kidneys selected for transplantation. Expanded criteria donation had no deleterious impact in this cohort.

2466

OLDER LIVING-DONOR KIDNEY TRANSPLANTATION VS. DIALYSIS AND STANDARD CRITERIA DECEASED DONOR TRANSPLANTATION: A DECISION ANALYSIS

Sapir-Pichhdze, Ruth; (Toronto General Hospital, Toronto, ON), Farrokhi, Farhat; (University of Toronto, Toronto, ON), Naimark, David; Fowler, Rob; (Sunnybrook Health Science Center, Toronto, ON), Kim, Joseph; (Toronto General Hospital, Toronto,ON)

Background: Living donor (LD) kidney transplantation provides improved outcomes compared with deceased donor (DD) kidney transplantation. Little is known about health gains associated with older LD versus standard criteria DD kidney transplantation after waiting on dialysis.
Study Design: We conducted a decision analysis from the perspective of a transplant recipient using a Markov model. Probabilistic analysis was used to incorporate parameter uncertainty into the model. We carried out 5000 simulations across known parameter distributions in a cohort of dialysis patients followed from wait-listing to death. The base case was a 40-year-old wait-listed hemodialysis patient in the United States. Scenario analyses were performed to identify important determinants of the optimal strategy.

Intervention and outcomes: We compared life expectancy (LE) and frequency of strategy selection for transplantation from a 60 year-old LD or intermittent hemodialysis followed by transplantation with a standard criteria (30 year-old) DD (alternative strategy).
Results: In our 40 year-old candidate base case, the older LD strategy and the alternative strategy provided comparable projected survival (LE: 16.97±0.52 vs. 16.75±0.20 years), yet older LD was the preferred approach in 62.2% of simulations. In 20- and 60-year-old candidates, intermittent hemodialysis followed by standard criteria DD was preferable in 91.1% (LE in 20-year-old candidate: 22.76±0.60 vs. 23.50±0.39 years) and 100% (LE in 60-year-old candidate: 11.97±0.49 vs. 13.76±0.36 years) of the samples, respectively. These findings were robust to the length of waiting time until transplantation with a standard criteria DD. In contrast to older LD, using a 20 year-old LD, prolonged life expectancy by 4.5 (LE: 27.24±0. 24), 4.8 (LE: 21.70±0.33), and 3.8 (LE: 15.75±0.39) years in 20-, 40- and 60-year-old candidates, respectively.
Conclusions: The survival advantage associated with LD kidney transplantation is less pronounced with older LD. The importance of LD and recipient age matching is accentuated in younger recipients.

2469

SHOULD TRANSPLANT PROGRAMMES ENABLE CONTACT BETWEEN LIVING ANONYMOUS DONORS AND RECIPIENTS?

Campbell, Michael, Wright, Linda (University Health Network, Toronto, ON)

Transplant Recipients sometimes wish to express gratitude to their Living Anonymous Organ Donors (LADs). Transplant programmes may be uncertain about what kind(s) of contact they should enable between willing LADs and Recipients. LADs and Deceased Donors (DDs) are unknown to their Recipients. Recipients of DD organs are encouraged to write anonymous letters of thanks to the DD's family. In traditional living donation (e.g. donation from a family member or friend) Recipients can thank Donors in person, and Donors benefit from seeing the Recipient's health improve post-transplant. Should transplant programmes extend this benefit to LADs by facilitating meetings between LADs and Recipients? LADs agree to donate anonymously, but they may not be committed to this condition after donating, resulting in requests to meet Recipients. Meetings may result in disappointment, unrequited wishes for continued contact, or problematic behaviours. Transplant programmes are limited in their ability to protect LADs and Recipients from one another after they have been introduced.
This presentation will explore the potential benefits and risks of: (1) permanent anonymity, (2) facilitating meetings before surgery, and (3) facilitating meetings after surgery. It will review relevant literature and summarize the practices and experiences of centres in North America and Europe. We contend that programmes that facilitate meetings between LADs and recipients should develop policies and standard operating procedures to ensure consistent practice and clarify responsibilities of everyone involved. We propose ways to: inform LADs and Recipients about the option to meet, enable them to express their desire to meet, and determine the appropriate time to meet. We will describe staff roles in: (1) determining parties' readiness to meet, (2) preparing parties to meet, (3) facilitating meetings, and (4) post-meeting debriefings. We conclude that, in some cases, it is ethically acceptable to facilitate meetings between LADs and Recipients post-transplantation.

2473

MONITORING THE SAFETY OF ORGANS FOR TRANSPLANTATION: AN UPDATE FROM HEALTH CANADA

Goulet, Marie; Légaré, Carole; Vu, Duc; (MHPD, Ottawa, ON)

Health Canada is responsible for monitoring the safety of Cells, Tissues, and Organs (CTO) for transplantation in Canada. Since 2007, 17 case reports of suspected adverse reactions involving organs have been received. One report was

referred to the Health Products and Food Branch Inspectorate because it involved an "accident" that did not result in an adverse reaction (AR). Of the remaining 16 reports, 11 concerned a suspected infectious AR and 5 a non-infectious AR. Kidney and liver were the two organs more frequently implicated.
Microorganisms (more than one can be involved in a single case) involved in suspected infectious AR included: Candida albicans (2 cases: 1 involving kidney, 1 involving liver); Escherichia coli (2 cases, both involving kidney); Pseudomonas aeruginosa (1 case involving kidney); Cryptococcus neoformans (1 case involving kidney); Clostridium perfringens (1 case involving kidney); "Gram +bacillus" (1 case involving kidney); Mycobacterium tuberculosis (2 cases, both involving lungs); Hepatitis B virus (1 case involving liver); Hepatitis C virus (1 case involving kidney); Respiratory Syncitial Virus (1 case involving heart).
Suspected non-infectious AR included thrombosed liver (1 case), pulmonary infiltrates (1 case), death due to persistent anoxic encephalopathy (1 case), hypotension (1 case) and anaphylaxis (1 case).
Ten (out of 16) cases of suspected AR were found to be at least possibly related to the donor.
The current Regulations stipulate that "an unexpected serious adverse reaction that is thought to involve the transmission of an infectious disease or disease agent" is subject to mandatory reporting experience has shown the value of reporting any serious unexpected adverse reaction.

2474

ACUTE HYPOTENSIVE REACTION DURING ISLET CELLS INFUSION WITH CONCOMITANT USE OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS: A CASE REPORT

Goulet, Marie; Légaré, Carole; Vu, Duc (MHPD, Ottawa, ON)

As part of its vigilance activities, Health Canada conducts assessment of case reports of suspected adverse reactions following transplantation.
The occurrence of hypotension during a procedure can be the manifestation of a number of conditions, each of which is usually associated with other characteristic signs and symptoms. We report a case of acute isolated hypotension during islet cells infusion in a patient on angiotensin-converting enzyme (ACE) inhibitor therapy, which was submitted to Canada Vigilance. While we were unable to find similar cases in the literature, acute hypotensive reactions have been reported in some patients on ACE inhibitors during transfusion of blood products, especially platelets. Bradykinin is thought to play a major role in these reactions. Assessment of this case revealed that a causal link was probable.
More research is needed in order to confirm such an association and, if appropriate, develop recommendations to mitigate the risk.

2478

IMMUNIZATION GUIDELINES PRE AND POST PEDIATRIC SOLID ORGAN TRANSPLANT: A QUALITY IMPROVEMENT PROJECT IN ORGAN TRANSPLANTATION

Babu, Anita; Breckbill, Kathryn; Casas, Rebecca; Martin, Kathy; Seifert-Hansen, Mirna; Stunguris, Jennifer; Williams, Angela; Battiston, Christopher; Allen, Upton; Avitzur, Yaron; Hebert, Diane; (Hospital for Sick Children, Toronto, ON)

Background: Comprehensive vaccination guidelines for pediatric transplant candidates and recipients are limited. Vaccinations are often missed or delayed due to limited time from listing to transplant, acute illness, hospitalization and lack of standardized guidelines. Chronic disease and immunosuppression may also contribute to inadequate vaccine response.

A review of our centre transplant database revealed suboptimal documentation of vaccinations given. Variation in vaccination practices among organ groups highlighted the need for a standardized process to vaccination pre and post solid organ transplantation.
Project aim: To create a standardized immunization protocol for pre and post pediatric solid organ transplant patients and ensure adherence to an optimal vaccine schedule in preventing vaccine related infections.
Project description: A review of current transplant literature, national, and provincial guidelines, including provincial funding, was performed by a multidisciplinary team. A standardized immunization protocol was developed for pre and post-transplant patients.
Pre transplant principles: Vaccination before transplant is recommended to achieve an optimal immune response prior to immunosuppression. Earliest recommended age for vaccination, dose requirements, and accelerated scheduling were highlighted. All routine live vaccinations should be given prior to transplant when possible. A minimum transplant wait list hold time of 4 weeks is recommended post administration of live vaccines.
Post-transplant principles: In general, vaccinations should be resumed at 6 months to 1 year post transplant to optimize response in the presence of immunosuppression. Live vaccines are currently not recommended post-transplant at our centre due to limited safety data. Vaccination guidelines for patients not vaccinated, incompleted series, and for supplemental doses if vaccinated prior were highlighted. Protocols were distributed in pamphlet and electronic format for transplant centre staff and primary health care practitioners involved in transplant care. Follow up will assess adherence and optimization of vaccinations pre and post-transplant.

2479

TOTAL TUMOR VOLUME/ALPAH FETOPROTEIN SCORE FOR THE SELECTION OF LIVER TRANPSLANT CANDIDATE WITH HEPATOCELULAR CARCINOMA; APROSPECTIVE VALIDATION

Kneteman, Norman, (University of Alberta, Edmonton, AB)

Liver transplant candidacy for patients with hepatocellular carcinoma (HCC) is currently validated based on Milan criteria; extended criteria remain a matter of debate. The present prospective multicentric study recruited patients for liver transplantation according to the Total Tumor Volume (TTV ≤115 cm^3)/ alpha fetoprotein (AFP ≤400 ng/ml) composite score. From January 2007 to March 2012, 162 patients with HCC were listed for liver transplantation (28 females-134 males; 56.8±6.2 years). Of them, 134 patients were within Milan criteria, and 28 beyond Milan. The average follow-up from listing was 35.4 ±21.1 months. The risk of drop-out was higher for patients beyond Milan but within TTV/AFP (14/28, 50%), than for patients within Milan (19/132, 14.4%, p<0.001). Similarly, intent-to-treat survival from listing was lower in the patients beyond Milan but within TTV/AFP (49.6% vs. 79% at four years, p<0.001). After a median waiting time of 8 months (mean: 11.9 ±12.1), 88 patients were transplanted. Patients within Milan, and those beyond Milan but within TTV/AFP demonstrated similar post-transplant survivals (86.8 vs. 77.5 at four years, p=0.378) and recurrence rates (4.5% vs. 10.5%, p=0.319).
Conclusion: Based on the present prospective study, HCC liver transplant candidate selection should be expanded to the TTV (≤115 cm^3)/ AFP (≤400 ng/ml) score in centers with at least 8-month waiting times. An increased risk of drop-out on the waitlist can be expected for patients in the expanded group, but with very good post-transplant survival, and the ability to help 20% more patients.

2481

PARENTAL HEALTH-RELATED QUALITY OF LIFE (HRQOL) AND FAMILY FUNCTIONING WITHIN PEDIATRIC THORACIC TRANSPLANTATION

Andrews, Krysta; Dipchand, Anne; Solomon, Melinda; Zhao, Xiu Yan; Anthony, Samantha; (The Hospital for Sick Children, Toronto, ON)

Purpose: While transplantation (Tx) is a well-established treatment for patients with end-stage disease, an understanding of the biopsychosocial implications surrounding Tx is limited. This cross-sectional study assessed the impact of pediatric thoracic Tx on parents and family.
Methods: Parents of pediatric heart or lung Tx recipients completed the PedsQL Family Impact Module, an instrument designed to measure parental physical, emotional, social and cognitive functioning, as well as communication and worry. Parent-reported family daily activities and family relationships are also included. Higher scores indicate better perceived functioning and psychosocial well-being. Multiple regression were utilized to evaluate risk factors associated with PedsQL Family Impact domains (e.g. gender, diagnosis, age at Tx, time since Tx, presence of allograft vasculopathy, etc.).
Results: Parents (75 mothers and 46 fathers) of 108 heart and 13 lung transplant recipients participated. Mean age of recipients (61 female, 50.4%) at the time of study participation was 10.1 years (range 1 – 18 yrs), with a mean time since transplant of 4.3 years (range 0.21 to 16 yrs). Results reflect a moderate level of family functioning, with the subscales of emotional functioning and worry reflecting the greatest negative impact. The Family Impact Module Total Score was 68.5, with Parental HRQOL and Family Functioning Summary Scores of 71.2 and 70.5 respectively. These scores correlated positively with increased time post-transplant, older, female recipients and no diagnosis of posttransplant lymphoproliferative disease. Results from additional analyses indicated that mothers perceived a higher level of family functioning across all dimensions than fathers, and reported scores on overall psychosocial well-being were lower than those of other chronic disease populations.
Conclusions: Further research exploring differences between parental HRQOL and perceived family functioning is warranted. Future studies to evaluate psychosocial interventions aimed at enhancing the overall health and well-being of Tx families are needed.

2482

DICHOTOMOUS T-CELL REPOPULATION AFTER ANTI-THYMOCYTE GLOBULIN INDUCTION THERAPY IN HIGH IMMUNOLOGICAL RISK AND DCD RENAL TRANSPLANT RECIPIENTS

Telfer, Siobhan; (Department of Surgery, London Health Sciences Centre, London, ON), Mok, Amy; (Department of Microbiology and Immunology, Western University, London, ON), Ullah, Sha; (Schulich School of Medicine and Dentistry, Western University, London, ON), Jevnikar, Anthony; Luke, Patrick; Sener, Alp; (Department of Surgery, London Health Sciences Centre and Department of Microbiology and Immunlogy, Western University, London,ON)

Background: T-cell suppression, through antibody-mediated induction therapy is routinely used to promote graft survival in high immunological risk (HR) and donation after cardiac death (DCD) renal transplant recipients. Depletional therapy with anti-thymocyte globulin (ATG) initially leads to lymphopenia. The immune system however, has the capacity to repopulate the T-cell compartment. The T-cell response post ATG induction has not been characterized in HR and DCD recipients, who often have increased rates of rejection despite adequate immune suppression. Understanding this response is critical for optimizing early immune therapies in these patients.
Objective: To establish the dynamics of T-cell reconstitution in DCD and HR recipients after ATG induction.

Methods/Results: Reconstitution and proliferation rates of various recipient (HR n=10 and DCD n=10) T-cell subsets were analyzed by 13-colour flow cytometry for up to 6 months post induction therapy with ATG. Following ATG administration, there was a rapid decline in the frequency of T-cells in both groups. These levels remained depressed for over 28 days. In HR patients, there was a rapid surge in the frequency of CD4 naive T-cells, which was not observed in DCD recipients. Although we observed significant proliferation in the effector memory phenotype in both the HR and DCD patients, the response in the DCD cohort was blunted. Regulatory T-cells showed a surge in proliferation rates, however the magnitude of change was not as great in the DCD group. Immune reactivity, assessed using the ImmuKnow® assay, revealed that CD4 T-lymphocytes appeared to be less immune-reactive in the DCD group.
Conclusion: This preliminary study provides perspective on the effects of ATG on the T cell response at an early period following renal transplantation in two important and rapidly growing populations of recipients (HR and DCD). Initial data suggests there is a lack of significant homeostatic proliferative response in DCD recipients following ATG. Moreover, data suggests that CD4-lymphocytes may be less reactive in the DCD group. These observations suggest that ATG may be an overly aggressive approach in DCD recipients.

2483

FUNCTIONAL HETEROGENEITY AND UNIQUE TARGET-INDUCED IMMUNE RESPONSES OF NATURAL KILLER (NK) CELL SUB-POPULATIONS: IMPLICATIONS FOR ASSESSMENT OF POST-TRANSPLANT RECOVERY OF THE RELEVANT NK CELL SUBSETS AND THEIR IMPACT ON HEMATOPOIETIC CELL TRANSPLANT (HCT) OUTCOME

Faridi, Rehan; Kemp, Taylor; Liacini, Abdelhamid; Dharmani, Poonam; (University of Calgary, Calgary, AB), Lewis, Victor; Storek, Jan; (University of Calgary, Calgary, AB), Berka, Noureddine; (Calgary Laboratory Services, Calgary), Khan, Faisal; (University of Calgary, Calgary, AB)

Background: A normal, fully reconstituted immune system after hematopoietic cell transplantation (HCT) is critical for the control of post-transplant infections, establishment of graft tolerance and, in some cases, mediation of graft-versus leukaemia effect. Natural Killer (NK) cells, being the first in line of defence against tumours and infections, are also the earliest among lymphocyte populations to reconstitute and achieve functional maturity after transplantation. However, many HCT recipients with normal recovery of NK cells still suffer from complications including infections and disease relapse suggesting that different NK cell subsets may be responsible for anti-leukemic or anti-viral immune responses. Here, we set out to determine, in healthy individuals whether different NK cell subsets (cytolytic or regulatory) elicit unique immune responses against different targets (leukaemia cells or herpes viruses).
Methods: Peripheral blood mononuclear cells (PBMNCs) from 25 healthy donors were stimulated with different targets including a leukaemia cell line (K562) and herpesviral (Epstein Barr Virus, EBV) infected cell lysate. A 5-colour flow cytometry based estimation of cytotoxicity (expression of CD107a, a surrogate marker for degranulation) and cytokine (IFN-γ) production was performed for both $CD56^{bright}CD16^{neg}$ regulatory and $CD56^{dim}CD16^{pos}$ cytolytic NK cell subsets.
Results: Different NK cell subsets were immunodominant against different targets. Leukaemia (K562) – specific response includes both degranulation and IFN-γ production and was mediated by both cytolytic and regulatory NK cells. On the contrary, EBV specific NK cell response was primarily characterized by degranulation and was dominated by cytolytic NK cells. A consistent shedding of CD16 was found associated with degranulation of cytolytic NK cells in response to EBV but not to K562 cells. Cytolytic NK cells in general exhibited a bifunctional immune response against both targets while regulatory NK cells were primarily IFN-γ producers.
Conclusions: NK cell subsets elicit a unique immune response against different targets (leukemia cells or herpesvirus). Assessment of posttransplant recovery of these target specific functional NK cell subsets will be more relevant for the prediction of transplant outcomes and may have future implications for the cellular therapy/prophylaxis of herpesviral disease or leukemia relapse.

2485

BERLIN HEART RIGHT VENTRICULAR ASSIST DEVICE AS A BRIDGE TO RECOVERY POST HEART TRANSPLANTATION FOR FAILED FONTAN-CIRCULATION.

Seitz, Stefanie; (University of Alberta, Edmonton, AB)

Background: Outcome of the 3-stage surgery for bicavopulmonary anastomosis (Fontan circulation) has improved and allows long term survival of children with univentricular hearts. However mostly associated to increased pulmonary vascular resistance failure is common leaving heart transplantation as sole therapeutic option. The most common indications for transplantation include protein-losing enteropathy (PLE) leading hypoalbuminemia and secondary renal failure. Post transplantation these patients are particularly at risk of right ventricular failure due to elevated pulmonary resistance, causing a high early mortality of 35-50% of patients. Supporting the RV with a mechanical device in this phase may allow the ventricle to recover and the pulmonary vasculature to remodel.
Clinical case: We report a 4 year old girl with a history of Hypoplastic left heart Syndrome with failing Fontan circulation and PLE. She was evaluated and listed for heart transplantation. She required urgent Fontan take down for clinical deterioration with chronic pleural effusions, ascites and generalized edema refractory to medical management. She received orthotopic ABO incompatible heart transplantation after 1 year on the wait list. Her immediate postoperative course was complicated by acute right ventricular failure due to increased pulmonary pressures and aortic anastomosis stenosis. Despite escalating inotropic support her right ventricle became increasingly dysfunctional within the initial 72 hours post-transplant, requiring the implantation of a Centrimag® right ventricular assist device (RVAD). After reconstruction of the aortic arch several attempts to reduce the continuous flow RVAD support for explantation failed at the time of chest closure. The patient remained on a Levitronix® RVAD and was switched to a Berlin Heart® when she was considered a suitable candidate for heart transplantation due to absence of cardiac recovery. She was subsequently listed for re-transplantation. The patient gradually stabilized clinically with improvement of right ventricular function despite chronic renal failure requiring renal replacement therapy for 7 months. Her right ventricular function improved, she showed no evidence of pulmonary hypertension on Sildenafil and the PLE resolved 8 months after transplantation. Consequently the RVAD support was gradually weaned.15 months post-transplant the assist device was successfully explanted and she was discharged from the hospital one month after explantation of the device.
During a 6 months follow-up without mechanical support she has remained clinically stable with no signs of heart failure or PLE. RV function remained normal with pulmonary pressures in the upper normal range.
Conclusion: Prolonged transient RVAD support may lead to gradual recovery of the right ventricle and normalization of the pulmonary resistance after heart transplantation for failed Fontan.

2486

AN ASSESSMENT OF THE TIME-DEPENDENT EFFECT OF BLOOD PRESSURE ON THE RISK OF GRAFT FAILURE IN KIDNEY TRANSPLANT RECIPIENTS USING A MARGINAL STRUCTURAL MODEL

Fozdar, Nishant; Famure, Olusegun; Li, Yanhong; Kim, Joseph; (Toronto General Hospital, University Health Network, Toronto, ON)

Background: Due to the involvement of the kidneys in blood pressure (BP) regulation, the relationship between BP, kidney function, and their joint influence on graft failure is complex. This study aims to determine the independent and time-varying effect of BP on the risk of graft failure in a contemporary population of adult Canadian kidney transplant recipients.
Methods: A total of 933 patients who underwent a deceased or living donor kidney transplant (DDKT and LDKT, respectively) at the Toronto General Hospital from 1 Jan 2000 to 31 Dec 2010 with follow-up until 31 Dec 2011 were included. Systolic BP

(SBP), diastolic BP (DBP), mean arterial pressure (MAP), and pulse pressure (PP) were recorded at baseline, 1-month, 3-months, 6-months, 9-months, 12-months, 18-months, 24-months, and then yearly post-transplant. The primary outcome was the time to total graft failure (graft loss or death). The impact of BP on outcomes was assessed adjusting for other covariates (including kidney function as a time-dependent confounder affected by prior exposure) in a marginal structural Cox proportional hazards model.

Results: Over 3,928 person-years at risk (median follow-up of 3.6 years), 128 patients achieved the primary outcome. Time-varying BP was not significantly associated with total graft failure in the overall cohort or DDKT recipients (see Table). However, there was a significantly increased risk of total graft failure in LDKT recipients as a function of SBP, MAP, and PP. Moreover, donor type significantly modified the relationship between BP and outcome ($P < 0.05$).

Conclusions: Higher BP significantly increases the risk of total graft failure in LDKT but not DDKT recipients. The lack of measurable effect among DDKT recipients may relate to the overriding influence of other competing risk factors and/or residual confounding.

Table: Time-varying effect of blood pressure on total graft failure assessed using a marginal structural Cox proportional hazards model*

Blood Pressure Measure	Whole cohort	DDKT recipients	LDKT recipients	P value for interaction
SBP (per 10 mmHg)	1.07 (0.91, 1.25)	0.95 (0.77, 1.16)	1.52 (1.24, 1.87)	< 0.001
DBP (per 10 mmHg)	0.86 (0.67, 1.10)	0.77 (0.57, 1.03)	1.00 (0.66, 1.52)	0.29
MAP (per 10 mmHg)	0.96 (0.76, 1.22)	0.86 (0.61, 1.20)	1.48 (1.08, 2.04)	0.02
PP (per 10 mm Hg)	1.07 (0.92, 1.25)	0.97 (0.80, 1.17)	1.49 (1.19, 1.86)	0.002
SBP ≥ 140 or DBP ≥ 90	1.15 (0.75, 1.75)	0.87 (0.51, 1.47)	1.66 (0.88, 3.14)	0.11

*Renal function and ACE-inhibitor/angiotensin-receptor-blocker use included as time-varying covariates. Values expressed as hazard ratios (95% confidence interval)

2488

EARLY SUCCESS WITH INITIATIVES TO INCREASE DECEASED DONATION IN BC

Gill, Jagbir; (University of British Columbia, Vancouver, BC), Ferre, Ed; Appleby, Amber; Grant, Greg; (BC Transplant, Vancouver, BC)

Background: Between 2000 and 2009, deceased organ donation rates in BC had been amongst the lowest in Canada, with a rate of 7.2 donors per million population (pmp) as of 2009. In 2010, BC Transplant undertook a mandate to implement a series of outreach initiatives to increase deceased organ donation, including targeted education on organ donation in hospitals across the province, expansion of donation after circulatory death (DCD) protocols, and a trial of full time in-house organ donation specialists in selected hospitals. We present the preliminary results of these initiatives.

Methods: Using data captured prospectively by BC Transplant, we report the total number of deceased donors in BC, the proportion of deceased donors that were DCD, and the conversion of referred to actual donors (donor conversion rate) per year between 2009 and 2012. To examine the impact of in-house organ donation specialists, these metrics were also specifically determined in centers where organ donation specialists were placed.

Results: Between 2009 and 2012, deceased donation rates in BC increased from 7.2 to 13.6 donors PMP (Figure 1). As outlined in Figure 2, DCD activity increased during this time, with DCDs comprising over 15% of all donors in BC in 2012 (as of October 30, 2012).

The total number of referrals of potential donors remained relatively stable with 159 referrals in 2009, 144 referrals in 2010, and 147 referrals in 2011. However, the proportion of these potential donors that ultimately donated increased substantially from 20.1% in 2009, to 31.8% in 2010, and to 38.1% in 2011.

In-house donation specialists were initially introduced at a single tertiary care hospital in BC in 2010 and donation statistics for this site are outlined in the Table. Between 2009 and 2011, the number of potential donors referred more than doubled, while the conversion of these potential donors to actual donors increased nearly 3 fold, resulting in a 6-fold increase in the number of

deceased donors at this site. In 2011, DCD activity was especially high at this site with DCDs accounting for 25% of all deceased donors. By comparison, DCDs accounted for only 9.8% of all deceased donors in all remaining hospitals in B.C in 2011.
Conclusion: Systematic implementation of initiatives to increase deceased organ donation in BC has resulted in a near doubling of deceased donation rates and significant expansion of DCD activity to levels comparable to those in Ontario and the United States. In house donation specialists may be particularly effective at increasing referrals of potential donors, improving conversion of potential to actual donors, and at increasing DCD activity.

Figure 1:

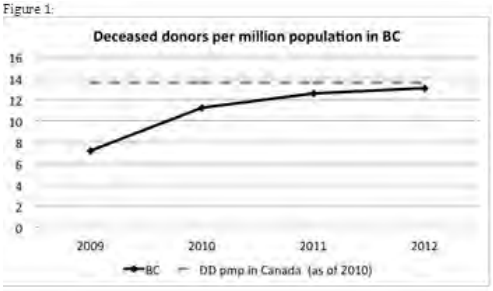
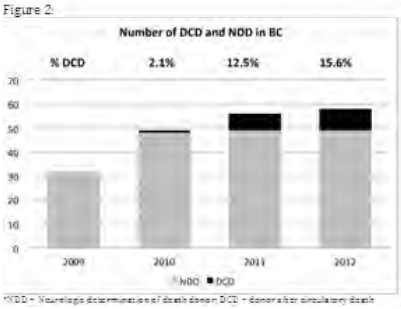


Figure 2:



*NDD = Neurologic determination of death donor; DCD = donor after circulatory death

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2490

IS URIC ACID ON INDEPENDENT RISK FACTOR FOR GRAFT FAILURE IN KIDNEY TRANSPLANT RECIPIENTS?

Kim, Esther; Famure, Olusegun; Li, Yanhong; Kim, Joseph; (Toronto General Hospital, University Health Network, Toronto, ON)

Background: Hyperuricemia is frequently observed in kidney transplant recipients and it has been implicated in poor allograft function and survival. However, this association remains controversial since time-varying confounders such as kidney function have not been properly addressed in prior studies.
Methods: This study examined 1,169 kidney transplant recipients who were transplanted between 1 Jan 2000 and 31 Dec 2010 (followed until 31 Dec 2011). Hyperuricemia was defined as serum UA > 416 µmol/L (> 7.0 mg/dL) in males and > 357 µmol/L (> 6.0 mg/dL) in females. Serum UA and kidney function (using the CKD-EPI formula) were measured after kidney transplantation at 1-month, 3-months, and every 3-months thereafter. Recipient, donor, and transplant characteristics were collected at baseline. The primary outcome was time to total graft failure (i.e., graft loss or death). The association of UA and total graft failure was assessed in a marginal structural Cox model accounting for kidney function as a time-varying confounder.
Results: When UA was considered a time-fixed exposure at 1-month, it was associated with an increased risk of total graft failure regardless of how it was measured, i.e., continuous or binary (see Table). UA as a time-varying exposure showed an increased risk of total graft failure when measured as a continuous (but not binary) variable. Finally, simultaneously accounting for the time-varying effects of UA and kidney function showed a modestly protective association of UA when measured as a continuous (but not binary) variable.
Conclusions: UA was generally associated with an increased risk of total graft failure when considered as a time-fixed or time-varying exposure. However, accounting for the time-varying effects of kidney function reduced this association to

null or modestly protective. The latter may be an indicator of nutritional status, especially within the range of normouricemia, but this is speculative and requires further study.

2494

FIRST CANADIAN EXPERIENCE IN PEDIATRIC EN-BLOC RENAL ALLOGRAFT DONATION AFTER CARDIAC DEATH

Dion, Marie; (University of Western Ontario, London, ON) Rowe, Neal; Shum, Jeffrey; Weernink, Corinne; Sener, Alp; Luke, Patrick; (London Health Sciences Centre, London, ON)

Introduction: The use of small pediatric kidneys obtained from donors less than four years of age after cardiac death (DCD) has been very limited. This is based on concerns regarding allograft function and growth, as well as limited experience in issues of consent for organ donation and withdrawal of life support in infants. A limited number of such transplants have been reported. Despite the success of small en-bloc grafts from neurologically determination of death (NDD) infant donors, DCD en-bloc allografts remain underutilized. In this cohort, we are excited to report our outcomes and first experience with transplantation of DCD en-bloc kidneys obtained from donors less than 4 years of age.
Methods: We reviewed all renal transplants at our institution from 2000 to 2012 to identify recipients who received an en-bloc pair of kidneys from pediatric donors less than 4 years of age. We examined recipient characteristics, perioperative characteristics, surgical complications, and allograft outcomes. The outcomes of DCD en-bloc allografts were compared with NDD en-bloc allografts.
Results: 20 recipients were identified with heterogeneous causes of end-stage renal disease. The mean age at transplantation was 50.7 ± 16.0 years. Four of the en-bloc kidney pairs were obtained by DCD and the remainder was procured from NDD donors. The mean donor age was 20.4 ± 12.0 months and mean donor weight was 12.3 ± 3.7 kg. In the DCD cohort the mean donor age was 15.6 ± 12.0 months with weight of 9.9 ± 2.4 kg as compared the NDD group with donor age 21.6 ± 12.0 months and weight 12.9 ± 3.8 kg. Surgical complications were minimal in both groups. All DCD allografts are currently functioning with mean GFR of 79.7 ± 21.5 mL/minute which was the similar as the recipients of NDD allografts (81.5 ± 38.7 mL/minute) at one year follow-up (see Figure 1). Delayed graft function (DGF) was higher in the DCD group affecting two out of four (50%) recipients as compared to 12.5% of the NDD group (RR 4.0, p = 0.162).
Conclusions: We are pleased to report successful transplantation of a small cohort of en-bloc DCD kidneys from donors less than 4 years of age. Outcomes at 1 year are comparable to NDD recipients. Recipients of DCD allografts demonstrated higher rates of DGF (numbers prohibitively small to make an accurate statistical comparison).

2495

HEALTH-RELATED QUALITY OF LIFE IN LONG-TERM SURVIVORS OF PEDIATRIC LIVER TRANSPLANTATION

Konidis, Stacey; Nightingale, Scott; Lilly, Leslie; Therapondos, George; Fu, Ann; Avitzur, Yaron; Ng, Vicky; (The Hospital for Sick Children, Toronto, ON)

Background: Long-term survival after pediatric liver transplantation (LT) is now the rule rather than the exception. Improving long-term outcomes after transplantation must consider not only the quantity but also the quality of life years survived. The objective of this study was to characterize health-related quality of life (HRQOL) of recipients 15 years after pediatric LT.
Patients and Methods: Patient inclusion criteria were all recipients of a first isolated, pediatric LT performed in a single program prior to December 1996 with continuous follow-up at either the pediatric or adult-partner centre. Patient exclusion criteria included severe developmental or neurological impairment. HRQOL was assessed with the Medical

Outcomes Study Short Form 36 version 2 (SF-36v2) for participants aged ≥18 years, and the Pediatric Liver Transplant Quality of Life Tool (PeLTQL) for participants aged <18 years. All participants completed an age-appropriate Pediatric Quality of Life Inventory 4.0 Generic Core Scale (PedsQL4.0), as well as a general medical status questionnaire.
Results: A total of 47 patients met inclusion criteria, with 5 patients ineligible based on exclusion criteria. The remaining 42 patients were contacted for an overall participation of 64.3%. Amongst the final cohort of 27 (67% male) participants, mean age was 24.6±6.7 yr (range 16.9-40.6). Median age at the time of pediatric LT was 1.7 yr (range 0.5-17.0). Indications for LT included biliary atresia (15), metabolic liver disease (4), liver tumour (2), and other (6). Five (18.5%) participants underwent re-transplant, 3 of which were concurrent with a kidney transplant. 17 (63.0%) subjects were Caucasian, 4 were Sub-Continental Indian, and 6 were of other ethnicity. The mean score for the PedsQL (n=27) was 72.1±16.1. The SF-36v2 (n=20) scores were divided into the physical and mental component scores with respective means of 49.6±11.1 and 45.3±12.5, compared to a norm-based score of 50±10. The PeLTQL score mean was 93.0±15.5 out of a possible 130 points. 12 (44.4%) subjects reported good adherence in missing medications less than once a month. One subject had been weaned off immunosuppression. 17 subjects (63.0%) reported a current situation of full-time work or study and four subjects reported part-time work or study. 13 (50.0%) subjects reported that the school/ employer wasn't aware of their condition.
Conclusion: Mean SF36v2 and PedsQL4.0 scores for 15-year pediatric LT survivors were not significantly different than that for a healthy population. Most subjects were able to function in a full-time work or study situation 15 years after LT, and half of the subjects had not shared their health condition with their school or employer. Good long-term survival is achievable after pediatric LT.

2496

TEN YEARS EXPERIENCE WITH EXTRA CORPOREAL LIFE SUPPORT FOR ADULT RESPIRATORY FAILURE: EVOLUTION OF INDICATIONS, TECHNIQUES AND OUTCOMES

Cypel, Marcelo; Paraghamian, Armen; dePerrot, Marc; Pierre, Andrew; Yasufuku, kazuhiro; Arce, Chantel; Ferguson, Niall; Fan, Eddy; Waddell, Tom; Keshavjee, Shaf;(University of Toronto, Toronto, ON)

Objectives: The use of Extracorporeal Life Support (ECLS) in patients with severe respiratory failure (RF) has been applied for over 30 years. Recent advances in technology and a better understanding of patient physiology while on support have expanded the use of ECLS. We set out to review our single center experience with ECLS for adults with severe RF, focusing on the change in indications, ECLS configuration, and outcomes.
Methods: Retrospective review of ECLS experience in adult patients from a single centre (2002 to 2012), divided into 2 cohorts: (I) 2002-2007, and (II) 2007-2012. Patients were considered for ECLS if they presented with severe hypercapnic and/or hypoxemic RF, or hemodynamic failure due to severe pulmonary hypertension.
Results: In the study period 75 patients underwent ECLS for severe RF. Indications included acute respiratory distress syndrome (ARDS; n=17), bridge to lung transplantation (LTx; n=30), and post-LTx grade III primary graft dysfunction (PGD; n=28). Device configuration included veno-arterial (VA, n=36), veno-venous (VV, n=17), veno-veno-arterial (VVA, n=3), arterio-venous (AV, n=11), and pulmonary artery to left atrium (PA-LA, n=8). The first and second cohorts consisted of 24 and 51 patients respectively. Time on device was significantly longer in cohort II (median 180h vs. 90h; p=0.01). Significant changes in indications, ECLS mode and outcomes were observed between cohorts I and II. Whereas PGD was the most common indication for ECLS in cohort I (75%), bridge to LTx and ARDS (80%) were the most common indications in cohort II. VA ECLS was the most common approach in cohort I (90%), whereas VV ECLS and pumpless modes such as AV and PA-LA were the most common modes in the second cohort (72%). No patients were extubated while on ECLS in cohort I whereas 9 patients (18%) were extubated in cohort II (p=0.05). Survival to decanulation (68% vs. 45%; p=0.04) and hospital discharge (55% vs. 28%; p=0.04) were significantly higher in cohort II.
Conclusion: We observed a sharp increase in the use of ECLS for ARDS and as a bridge to LTx in the past 5 years, while the number of patients requiring ECLS for PGD after LTx has remained stable. VV and pumpless modes have been the

main ECLS configurations in more recent years. These changes in practice patterns were associated with an increasing number of patients extubated on ECLS and improved mortality.

2497

SHOULD WE TRANSPLANT KIDNEYS IN RECIPIENTS >70 YEARS OLD?

Shapiro, R. Jean, Sun, Yvonne (BC Transplant, Vancouver, BC)

We examined the impact of increasing older organ donor and recipient age on transplant outcomes in our renal transplant recipients. 1004 adults 18-69 yo (Group1, mean age 49) received renal allografts between 2005 to 2011, and results were compared to 49 patients ≥70 yo (Group 2, mean age 72). 62% group 1 were male compared to 78% in group 2, but were similar for other characteristics: Caucasian (65% for both); first transplant (92 vs 90%); PRA >19 (14 vs 18%); living donor (56 vs 57%); donor age (45 vs 53 yo); cold ischemia time (416 vs 547 min); donor race (73 vs 89% Caucasian). Graft fail rate was significantly higher for group 2 compared to group 1 (26 vs 10%, p=0.059) and average fail date was shorter (280 vs 831 days). Fewer grafts in group 2 survived beyond 1 yr (31%) compared to 68% in group 2. There were 51 deaths in group 1 (5%) compared to 10 in group 2 (20%), and the deaths tended to occur later 903 vs 353 days post transplant. Death with functioning graft occurred in 50% of group 1 and 80% in group 2. Average initial length of stay was shorter in group 1 compared to group 2 (7.4 vs 13.9 days), and the frequency of delayed graft function was less in group 1 (20 vs 35%). The readmission rate was higher for group 2 with 32% requiring more than 2 admissions in the first year compared to 24% in group 1. In those with functioning graft at one year, renal function was no different in group 1 compared to group 2 (average creatinine 126 vs 134 umol/L) and percent with creatinine <130 umol/l was similar (66% for both). Patient and graft survival were improved in the younger cohort compared to older recipients (93 vs 80%; 87 vs 75%). There were no significant differences within group 2 in patients with failed graft vs functioning graft except there were no deaths in the subgroup that did not fail. This suggests that graft failure in the older age group is a predictor of death.

2499

COMPARISON OF PEDIATRIC EN-BLOC RENAL ALLOGRAFT GROWTH AND FUNCTION FOR DONORS LESS THAN ONE YEAR OF AGE AS COMPARED TO OLDER PEDIATRIC DONORS

Dion, Marie; (University of Western Ontario, London, ON) Rowe, Neal; Shum, Jeffrey; Weernink, Corinne; Langford, Sarah; Sener, Alp; Luke, Patrick; (London Health Sciences Centre, London, ON)

Introduction: The use of pediatric en-bloc kidneys obtained from infant donors of very small size has previously been investigated. Some transplantation centers have avoided the utilization of very small kidneys due to functional concerns (allograft function and growth) and technical concerns (vascular anastomoses, vascular thrombosis and ureteral implantation). In this study we report our outcomes of small en-bloc renal allografts obtained from infant donors less than one year of age.
Methods: We reviewed all kidney transplants at our institution from 2000 to 2012 to identify recipients of en-bloc kidney allografts from donors less than 4 years of age. We examined recipient characteristics, transplant characteristics, surgical complications, and allograft outcome. En-bloc allograft outcomes were compared for donors less than one year of age and one to four years of age. Renal growth was assessed using post-transplantation ultrasounds.
Results: 20 recipients were identified with heterogeneous causes of end-stage renal disease. The mean age at transplantation was 50.7 ± 16.0 years. Six of the en-bloc kidney pairs were obtained from donors less than one year of

age (mean age 8.3 ± 2.4 months, mean weight 8.7 ± 1.7 kg), the remainder being obtained from donors between one and four (mean age 25.8 ± 10.3 months, mean weight 11.7 ± 6.1 kg). Surgical complications were minimal in both groups.
Five out of six allografts obtained from donors less than one year of age are currently functioning (one recipient died of respiratory causes with a functioning graft 34 months after transplantation). The younger allografts had a mean GFR of 90.0 ± 30.2 mL/minute which was comparable to the mean GFR of older allografts (77.3 ± 39.1 mL/minute) at one year follow-up (see Figure 1). Delayed graft function (DGF) was similar between the younger and older allograft groups (16.7% and 21.4 % respectively). Starting volume was significantly smaller in the less than one year of age grafts (35.2 ± 10.5 cm^3 versus 55.8 ± 17.9 cm^3, p = 0.0011). Both groups demonstrated remarkable volumetric growth within the first year of transplantation (253.2% for younger grafts and 198.3% for older grafts; not statistically significant).
Conclusions: In this analysis we demonstrate successful transplantation of pediatric en-bloc kidneys from very young recipients less than 1 year of age. Outcomes at 1 year are comparable to recipients of older en-bloc donors. Both groups show excellent graft survival while maintaining acceptable complication and DGF rates.

2500

"STOCK MARKET BETA" PREDICTS RENAL TRANSPLANT REJECTION

Schmid, Sarah; (University of Saskatchewan College of Medicine, Saskatoon, SK), Reeder, Bruce; Bryce, Rhonda; Rana, Masud; Pahwa, Punam; Shoker, Ahmed; Moser, Michael; (University of Saskatchewan, Community Health and Epidemiology, Saskatoon, SK)

Introduction: An elevated coefficient of variation of tacrolimus (CV%) has repeatedly been shown to be associated with non-adherence. The "Stock Market Beta" (SMB) is a measure of a stock's volatility relative to an index such as the Dow Jones Industrial Average. We hypothesize that greater volatility (SMB) of Creatinine (Cr) relative to tacrolimus levels [Tac] is associated with an increased odds of rejection.
Methods: This was a retrospective chart review of all single-kidney only transplants in our Renal Transplant Program from March 2004 to March 2010 (n=117) whose primary immunosuppressive agent was tacrolimus (n=81). We collected all available Cr and [Tac] over the first two years post-transplant. SMB was calculated for each patient, while excluding the rise in Cr immediately preceding a diagnosis of rejection.
Results: Rejection was seen in 28 of 81 patients. Univariate analysis revealed that Absolute (unsigned) SMB (|SMB|) but not SMB (which can be positive or negative) was associated with increased odds of rejection. Average |SMB| for patients with rejection was 16.4% while for patients without rejection, it was 7.2% (p=0.02). A logistic regression model including donor type (deceased vs. living) and number of HLA matches revealed that |SMB| was strongly associated with rejection (OR=1.181 [1.08,1.29], p=0.0003).
Discussion: Increased volatility of Cr relative to [Tac] is associated with increased odds of rejection. Whereas an increased CV% suggests non-adherence, an increased |SMB|, which takes into account both Cr and [Tac], may be a signal of immunologic unrest.

2501

IS HYPOMAGNESEMIA A NOVEL RISK FACTOR FOR THE DEVELOPMENT OF NEW-ONSET DIABETES MELLITUS AFTER KIDNEY TRANSPLANTATION?

Huang, Johnny; Famure, Olusegun; Li, Yanhong; Kim, Joseph; (Toronto General Hospital, University Health Network, Toronto, ON)

Background: New-onset diabetes mellitus (NODM) after kidney transplantation increases the risk of cardiovascular events and graft failure. Several studies have suggested a link between hypomagnesemia and NODM but this association remains controversial.
Methods: A cohort study was conducted in 838 non-diabetic patients who received a kidney transplant from 1 Jan 2000 to 31 Dec 2010 (with follow-up to 31 Dec 2011). Hypomagnesemia was defined as a serum magnesium (Mg) < 0.74 mmol/L. NODM was diagnosed based on the American Diabetes Association criteria. Serum Mg was measured post-transplant at 1-month, 3-months, and every 3-months thereafter. Recipient, donor, and transplant characteristics were collected at baseline. Cox proportional hazards models were fitted to examine the association of baseline Mg (at 1-month), time-varying Mg (every 3-months), and rolling average Mg (over each 3-month interval) and time to NODM while adjusting for other covariates.
Results: Over 2,709 person-years of follow-up (median follow-up 3.5 years), 166 NODM events were observed. The Cox models suggested an inverse relation between baseline serum Mg and NODM (HR 0.89 per 0.1 mmol/L increase in Mg [95% CI: 0.74, 1.06]). Similar results were observed for time-varying and rolling average Mg levels (HR 0.89 [95% CI: 0.76, 1.04] and HR 0.83 [95% CI: 0.69, 1.01], respectively). Patients with hypomagnesemia (vs. normal Mg levels) showed a modest increased risk of NODM in time-fixed (HR 1.21 [95% CI: 0.82, 1.81]) and time-varying (HR 1.22 [95% CI: 0.87, 1.71]) Cox models.
Conclusions: Our results suggest that lower serum Mg is consistently associated with a quantitatively increased risk of NODM across different measures of Mg but the hazard ratios did not reach statistically significance. The analysis will be repeated with an updated dataset (with follow-up to 31 Dec 2012) to increase the precision of the estimated associations and verify the relation between serum Mg and NODM.

2503

MEDICATION ADHERENCE IS TIED TO SOCIAL SUPPORT IN RENAL TRANSPLANT RECIPIENTS

Shapiro, R. Jean; (Vancouver, BC) Paterson, Theone; Thornton, Wendy; (Simon Fraser University, Burnaby, BC) Hussaini, Trana; Partovi, Nilufar; (Vancouver General Hospital, Vancouver, BC), Vishloff, Jen; (Simon Fraser University, Burnaby, BC) Mooney, Maureen; (BC Transplant, Vancouver, BC)

Support, psychosocial variables, and cognitive functioning are important in predicting medication adherence. We examined the impact of tacrolimus formulation on medication adherence comparing once daily Advagraf ® to usual twice daily Prograf ® in a 4 month long randomized study in stable renal transplant recipients. Medication was dispensed utilizing MEMS caps technology; other methods of determining medication adherence included pill count, and patient recall. Subjects also completed a battery of questionnaires testing cognitive and psychosocial parameters. 35 patients (22 males) so far have been enrolled, of whom 7 have withdrawn, 5 within the first few days after randomization. One unanticipated reason for withdrawal was patient concern regarding failure to cope with a new medication administration strategy. 16 subjects have been randomized to Advagraf ®, 19 to Prograf ®; 20 have completed the study and 8 remain to complete thus far.
Pearson correlations were used to examine possible relationships between variables related to social support, transplant events (years on dialysis prior to transplant and type of transplant received: cadaveric vs. living). Living situation (alone vs. with others) was positively correlated with time on dialysis (r=.522, p=.046, 2-tailed), indicating that it is more likely for patients who live alone to have spent longer on dialysis than those with immediate support networks (including spouses and non-spouses) in the home; they were also more likely to have received a deceased donor than those living with others (r=-.509, p=.044, 2-tailed). Living situation and number of years on dialysis were both negatively correlated with the percentage of medication adherence. These relationships indicate that patients who live alone were more likely to have somewhat lower rates of proper adherence (r=-.423, p=.040, 1-tailed for percent overall adherence; r=-.446,

p=.032, 1-tailed, for percent days with correct number of doses taken). Additionally, patients who spent longer on dialysis prior to transplant had somewhat lower rates of adherence as measured by MEMS caps (r=-.580, p=023, 2-tailed, for percent overall adherence; r=-.581, p=.023, for percent correct daily dose).
These results show support for a potentially important role for social support in medication adherence post renal transplant. Given findings in our previous investigations, the role of cognitive function is likely contributory, but remain to be analyzed in this study.

2506

TO DP OR NOT DP THAT IS THE QUESTION: CLINICAL RELEVANCE OF HLA DPA AND DPB IN 10/10 HLA MATCHED STEM CELL TRANSPLANTS

Berka, Noureddine; Koo, Desmond; Lamb, Kasia; Zeeban, Sharon; Faridi, Rehan; Kemp, Taylor; Liacini, Abdelhamid; Storek, Jan; Lewis, Victor; Khan, Faisal; (Calgary Laboratory Services, Tissue Typing Laboratory, Calgary, AB)

The association between HLA-DP and hematopoietic stem cell transplantation (HCT) outcomes is still questionable. In the present study, we examined 80 patients who underwent first allogeneic HCT between the years of 2005-2008 and were followed up for at least 2 years post-transplant. These patients were 10/10 matched for HLA-A, -B, -C, -DR and –DQ at high resolution using Sequence Specific Priming (SSP) and/or Sequence Based Typing (SBT). We analyzed the impact of donor/recipient (D/R) mismatches at HLA-DPA1 and HLA-DPB1 on different HCT outcomes including aGVHD, cGVHD, and risk of disease relapse in a single center study. Additionally, we classified HLA-DPB1 mismatches as permissive or nonpermissive according to a previously described TCE4 algorithm and attempted to confirm its efficacy. Each categorical testing variable were compared with the different clinical end points by 2-tailed Fischer's exact test. The magnitude of effect was estimated by risk ratios and their 95% confidence intervals. "p" value ≤0.05 (two-sided) was considered significant.
Results: A total of 160 alleles were observed at HLA-DPA1 and 159 alleles were observed at HLA-DPB1 locus. HLA-DPA1 and DPB1 allelic frequencies in the study population were not significantly different from those previously observed in other Caucasian populations. At DPA1 locus, D/R mismatching was observed in 15% of D/R pairs, however almost all of them were mismatched at the level of single allele. Since only one patient was mismatched for both DPA1 alleles, the effect of DPA1 mismatches were not analyzed for single or double mismatches. At the DPB1 locus, 44% D/R pairs were found mismatched, including 24% for single allele and 20% for both alleles. No variables analyzed (allelic or nonpermissive mismatches) were significantly associated with any transplant outcomes evaluated in this patient cohort.
Conclusion: Our study did not confirm the association between HLA-DP mismatches and HCT outcomes. The TCE4 algorithm also did not yield significant advantageous results. Our results are in accord with a recent French study but disconcordant with various studies conducted elsewhere, including the United States and the United Kingdom. Further molecular study of HLA-DP in the clinical setting is warranted to further elucidate the role of this gene and its mismatching on HCT outcome.

2508

PRANCREAS RETRANSPLANTATION OFFERS EXCELLENT SHORT TERM RESULTS DESPITE SURGICAL CHALLENGES

Marquez, Max; Bazerbachi, Fateh; Selzner, Markus; Norgate, Andrea; Schiff, Jeffrey; McGilvray, Ian; Cattral, Mark; (University Health Network MOT, Toronto, ON)

Improved outcomes and graft survival in pancreas transplantation has increased the number of pancreas re-transplants performed. However, pancreas retransplantation presents significant surgical and medical management challenges. Aim: To compare the outcomes of pancreas retransplants and primary pancreas after kidney transplants at a single center. Methods: Between January 2003 and May 2012, 70 pancreas after kidney transplantations have been performed at our institution. Of those, 56 (80%) were primary pancreas transplants, while 14 (20%) received a retransplant graft. All patients received identical maintenance therapy. Pancreas graft function was determined by fasting blood glucose levels (FBG), HbA1c, and annual oral glucose tolerance testing. Serum creatinine was used to assess kidney graft function. Graft survival, and patient survival were calculated by log rank analysis. Results: 14 First graft failures were retransplanted for rejection (7), duodenal leaks (4), thrombotic episodes (3). First grafts had similar pre-transplant characteristics to retransplants with respect to male sex (55% vs. 78% p=0.14), donor age (23.7±13.1 vs. 21.8±9.4 p=0.62), recipient age (44.4±7.9 vs. 40.4±6.2 yrs p=0.09). Donor and recipient weight were comparable (p=0.11, p=0.19). Pancreas warm ischemic time (26.76 vs. 31 min p=0.51), cold ischemic time (7.75 vs. 8.25 h p=0.76), and Total length of hospital stay (12.7±11.3 vs. 10.7±6.6 days p=0.54) were also comparable. Retransplants had shorter graft follow-up (49.7±29.4 vs. 35.3±26.1 months p=0.13). There was no difference in the rate of post-operative complications in the first 6 months, except for duodenal leaks (p=0.03). Graft function 1, and 3 years were comparable (p=NS). Pancreas graft survival at 1 and 3 year was similar between first grafts and re-transplant at 91% vs. 100% and 88% vs. 100% respectively. Creatinine levels were higher in the primary grafts at 1 and 3 years (p=0.07 and p=0.06). Conclusion: Despite technical and immunological challenges, pancreas retransplantation has excellent short term results. More detailed studies are required to better assess the effect on long term kidney graft function.

2509

LIVER TRANSPLANTATION FOR RURAL RESIDENTS: ACCESIBILITY AND OUTCOMES

Marquez, Max; (University Health Network MOT, Toronto, ON) Morillo, Andrea; (UHN, Toronto, ON) Wiersma, Elaine; (Lakehead University, Thunder Bay, ON), Bazerbachi, Fateh; Mahdi, Zainab; Selzner, Markus; Renner, Eberhard; McGilvray, Ian; (UHN, Toronto, ON)

Management of chronic disease such as end stage liver disease and subsequent liver transplantation may only be available in large urban areas. Rural residence poses significant challenges for the patient seeking care, as well as the transplant centre providing the care. Research studies have suggested that barriers for rural residents can have a negative impact in outcomes and quality of care.
Aim: To determine the impact of place of residence on post liver transplantation outcomes and complications. Methods: We performed a retrospective review of 1411 liver transplants performed at a single institution from Jan. 2000 to Dec. 2011. 1033 deceased donor grafts recipients and 378 live donor liver transplants . Survival was calculated using Log-Rank, while clinical data was analyzed using Chi-square, and Student[apos]s t-test or Mann-Whitney U-test. Rural residence was determined using the Canada Post Conversion file and a cut-off point of less than 1000 inhabitants for rural qualification. Linear distance to transplant center was calculated using Google maps.
Results: 9.3% (131) transplants were from rural recipients (RR) vs. 90.7% (1280) urban recipient (UR). Linear distance from place of residence to transplant center is not significantly different between RR and UR. Demographics were similar between the groups in terms of age (59.0±11.9 vs. 51.3±12.1 years), gender (69.2% vs. 68.8% male), and most underlying liver disease (Hep C, Hep B, Fulminant Hepatitis, Alcohol, HCC and other). Only autoinmmune hepatitis was significantly higher in rural recipients 8.4% vs 3.8% (p=0.02). Donor demographics were comparable in for RR and UR terms of age, as well as cold and warm ischemic times. Total length of stay (23 vs. 24 days RR vs. UR) and post-transplant ICU stay (3.3 vs. 4.5 respectively) were comparable. No difference in mediate or immediate transplant complications were observed between the two groups in terms of type, or degree of severity in the complications (Clavien score). Retransplant rate was similar between RR and UR at 4.5% vs 5.1%. (p=0.16). Graft survival at 1, 3, and 5 years was 90%

vs 90%, 86% vs. 82%, and 80% vs 78% for RR and UR respectively (p=0.79), patient survival was also comparable at 93% vs 92%, 83% vs. 85% and 81% vs. 79% respectively (p=0.76). No change in the RR to UR ratio was observed over 10 years time.
Conclusion: Etiology for end-stage liver disease may significantly vary according to place of residence beyond autoimmune hepatitis. Rural recipients have remained steadily below 10% of our transplant population over the last decade despite excellent outcomes and similar complication rates.

2510

THE DEVELOPMENT OF A PROCESS TO FACILITATE CONTACT BETWEEN DONOR FAMILIES AND RECIPIENTS IN ALBERTA, CANADA

Worton, Kim, Brown, Pam (Alberta Health Services, Edmonton, AB)

Historically, Canadian legislation has prohibited direct contact between organ and or tissue donor families and transplant recipients. Although anonymous correspondence has been supported, organ procurement organizations (OPO) and transplant programs have felt an obligation to protect the confidentiality and interest of donor families and recipients.The Human Tissue and Organ Donation Act replaced the Human Tissue Gift Act in Alberta in August 2009. This new legislation no longer prohibits direct contact between recipients and donor families.
In preparation for the development of a program policy a literature review was conducted which revealed that many US transplant organizations have been successfully facilitating contact between donor families and recipients for many years. Success has been influenced by the work transplant programs do to prepare donor families and recipients about the potential benefits and risks of direct contact. Legal, ethical and communication issues were considered in the development and implementation of the AHS policy and procedure, disclosure letters and agreement forms.
To date we have facilitated one recipient and donor family meeting. The meeting was successful and the feedback from the donor family and recipient was positive. Currently, the donor family and recipient have maintained direct contact. Based on the success we have had to date other direct contacts are in progress.
This new legislation impacts transplant and organ donor programs at a local, provincial and national level. As the legislation changes in other provinces a coordinated provincial and inter-provincial approach that meets the legal, privacy and legislative requirements of each province will be necessary.

BASIC

2414

PRECLINICAL EVALUATION OF TRIPTOLIDE IN PREVENTION OF ACUTE ALLOGRAFT REJECTION OF HIND LIMB TRANSPLANTATION IN RATS

Wu, Z.; Barama, A.; Xu, J.; Daloze, P.; Qi, S[5]; (CRCHUM, Dept. of Surgery CHUM, Montréal, QC)

Abstract: The composite tissue allotransplantations (CTA) are not routinely performed for tissue reconstruction due to the potentially harmful adverse effects associated with the immunosuppressive agents, which last lifelong administration. Therefore the development of appropriate pre-clinical animal model and novel strategy is critical, which allows an understanding of antigenicity of complex tissue transplants and mechanisms to promote graft acceptance. The hind limb transplant in rat is an ideal model for CAI, which contains numerous different types of tissues including skin, fascia, muscle, tendon, bone, blood vessel et all. Triptolide, the active component extracted from the Chinese herb Triperygium wilfordii hook. F is shown the effect in responsible for the immunosuppressive, anti-inflammatory and anti-proliferative effects in vitro; however the effectiveness of Triptolide still needs further study. The hide limb transplantation rat model was established to explore the mechanism of Triptolide in vivo.
Method: the generation of hide limb transplantation rat model was made, in which the Lewis rats with a RT-11 MHC locus was employed as recipient and the Brown Norway rats was treated as donor. Rats were separated by five groups: isograft control, allografts untreated control, allografts treated with Triptolide; allografts treated with Tacrolimus and allografts with Triptolide and Tacrolimus in addition the recipients pre-treated two reagent around1-14 days. The effect of Triptolide on MLC was examined; the proliferation of mitogens was analyzed, the end point of study was set at day 50 after surgery.
Result: all allograft controls rejected the grafts within one week, in which the rats didn't receive any treatment. The mean survival times (MST) of Triptolide and tacrolimus mono-therapy groups (10.5± 0.5 days and 14.6±0.5 days) revealed extending allograft survival time, compared with the untreated allograft group (6±0.5 days). The therapy of Triptolide combinated with tacrolimus shown a prolongation allograft survival time and was superior to the Triptolide or tacrolimus (17.6±0.5 days vs 10.5± 0.5 and 14.6±0.5).
Conclusion: results revealed that synergistic interaction between Triptolide and tacrolimus was produced in prevention acute limb allograft rejection.

2415

SPECIFIC INHIBITION OF PROTEIN KINASE C-D REDUCES ISCHEMIA-REPERFUSION INDUCED LUNG INJURY IN VIVO

Zhao, Jinbo; Kim, Hyunhee; Lee, Daiyoon; Keshavjee, Shaf; Liu, Mingyao; (Latner Thoracic Surgery Research Laboratories, Toronto General Keshavjee Research Institute, University Health Network; Department of Surgery, U of T, Toronto, ON)

Purpose: Ischemia-reperfusion (IR) induced lung injury is an important clinical issue owning to its contribution to the development of primary graft dysfunction in lung transplantation, cardiopulmonary bypass, and etc. However, no effective drugs exist in clinic for its treatment. PKCδ is suggested to modulate inflammatory response and cell death in lung epithelium cells. Thus, we tested whether pharmacological inhibition of PKC-δ can ameliorate IR-induced lung injury.
Methods and Materials: A rat pulmonary IR model was established by clamping the left hilum for 60 min and reperfused for 2 h. δV1-1, a specific PKCδ inhibitor, was conjugated with TAT (a cell penetration peptide) and was administrated (I.V) before and after the clamping. TAT peptide alone was used as a negative control. After reperfusion, the left lung

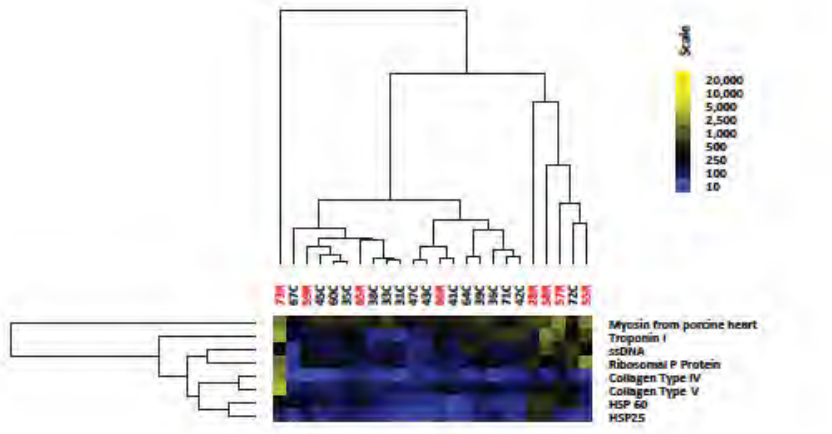
was ventilated with pure O_2 for 5 min by clamping the right hilum. Arterial blood gases and the left lung function were assessed. The left lung tissue and serum were collected for wet/dry ratio and histological study.
Results: Compared with TAT control, TAT-δV1-1 treatment significantly increased the oxygenation after IR (522.40±19.20 vs. 325.30 ± 70.20 mmHg, $p<0.05$). Lung function of TAT-δV1-1 treated rats were improved in peak airway press, elastance and compliance compared to the control ($p<0.05$). The IR-induced alveoli septal thickening, edema, hemorrhage, inflammatory cell infiltration and cell apoptosis were attenuated after TAT-δV1-1 treatment.
Conclusions: PKCδ inhibition has protective effects against IR-induced lung injury by improving lung oxygenation and lung function, and reducing inflammatory response and cell apoptosis in vivo. Therefore, inhibition of PKCδ could be a therapeutic target for drug development to prevent IR-induced lung injury in human.

2419

AUTOANTIBODIES AND REJECTION AFTER HEART TRANSPLANTATION: PROFILING WITH ANTIGEN MICROARRAYS

Chruscinski, Andrzej; Lioe, Jocelyn; Huang, Flora; Rao, Vivek; Levy, Gary; Ross, Heather; (Toronto General Hospital, Toronto, ON)

Introduction: Autoantibodies in pre-transplant serum have previously been implicated in heart transplant rejection. In order to more fully profile autoantibodies, we generated custom antigen microarrays comprising 60 autoantigens and probed the microarrays with pre-transplant serum. Our hypothesis is that autoantibodies are elevated in patients who experience significant rejection after heart transplantation
Methods: Patients who developed significant rejection (two or more episodes of ISHLT 2R rejection over the first year) where classified as rejectors (n=8) while patients who did not experience rejection were classified as non-rejectors (n=16). Antigen Microarrays were constructed by spotting proteins, peptides, and lysates onto FAST slides using a microarrayer. After blocking the slides, they were probed with diluted pre-transplant serum. In order to visual binding of autoantibodies, the slides were then probed with a fluorescently labelled secondary antibody. The Significance of Analysis of Microarrays (SAM) algorithm was used to detect significant changes in antigen reactivities. Patients in the rejector group were younger, more likely to be female and were more likely to have a non-ischemic cardiomyopathy.
Results: After antigen microarrays were performed, SAM analysis revealed eight antigen reactivities that were significantly elevated (Q value < 0.05) in the rejector group. Patients in the rejector group are indicated by red numbers in the vertical columns in the heatmap. Higher reactivity is indicated by yellow and lower reactivity is indicated by blue. Interestingly, we identified enhanced reactivity to cardiac myosin in the rejector group as has been described previously. In addition, we observed reactivity to other antigens such as troponin I, single-stranded DNA, heat shock proteins, and Ribosomal Protein P to be elevated in the rejector group.
Conclusions: Patterns of autoantibodies in pre-transplant serum may identify patients at increased risk of rejection after heart transplantation. We are now in the process of verifying these results in a second cohort of heart transplant recipients.



2420

HOST APCS, NOT VASCULAR ENDOTHELIUM, CONTROL INDIRECT REJECTION BY CD8 T CELLS

Al-Adra, David; Thangavelu, Govindarajan; Ewen, Catherine; (University of Alberta, Edmonton, AB) Anderson, Colin; (Alberta Diabetes Institute, Edmonton, AB)

Background: Indirect activation of T cells occurs when alloantigens are presented in recipient MHC. How CD8s mediate indirect graft rejection is an important unresolved issue. Previous studies using male antigen (HY) specific CD8 T cell receptor-transgenic (MataHari) mice showed that non-vascularized skin grafts but not vascularized heart transplants could be indirectly rejected. Circumstantial evidence suggested that the origin (host vs. donor) of graft vasculature determined the outcome, rejection being dependent on recognition of HY in MHC I on *recipient* vascular endothelium. This view predicts that all non-vascularized grafts (e.g. islets) should be susceptible to indirect CD8 rejection. However, alternative mechanisms, such as indirect presentation by APCs, were not ruled out. We re-examined the role of recipient vascular endothelium and MHC I expression in CD8 T cell mediated indirect allograft rejection.
Methods and Results: Part 1- We transplanted MHC mismatched (to eliminate the direct pathway) non-vascularized male islets, skin, or heart into female MataHari mice. CD8 T cells were unable to reject islets or heart but could reject skin via the indirect pathway ($p < 0.0001$, skin vs. islets). Even previously HY sensitized MataHari recipients were unable to reject male islets indirectly. **Part 2** - To examine whether host vasculature or host APCs need to present HY for indirect rejection we created reciprocal bone marrow chimeras such that MHC I was only expressed on either recipient radiosensitive cells (e.g. APCs) or radioresistant cells (e.g. vascular endothelium). Our preliminary data indicates that CD8 mediated indirect rejection depended on the presence of MHC I on host APCs and not on host vasculature.
Conclusions: Taken together, our results suggest that alloantigen presentation by recipient MHC I on vasculature ingrowth into non-vascularized allografts cannot be the primary explanation for indirect rejection by CD8 T cells, and that instead, presentation by host APCs may be critical.

2427

CORRELATION OF HLA ALLOEPITOPES WITH THE DEVELOPMENT OF DE NOVO DONOR SPECIFIC ANTIBODIES AFTER RENAL TRANSPLANTATION

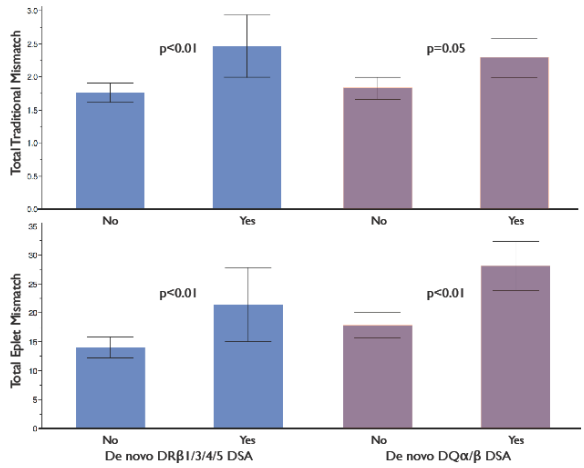
Wiebe, Chris; Pochinco, Denise; Blydt-Hansen, Tom; Gibson, Ian; Rush, David; Nickerson, Peter; (University of Manitoba, Winnipeg, MB)

Aim: To investigate the correlation of HLA alloepitopes with the development of *de novo* donor specific antibodies (*dn*DSA) after renal transplantation.
Hypothesis: An epitope based HLA matching approach will be predictive of *dn*DSA development after renal transplantation.
Methods: 315 consecutive renal transplants were performed between Jan. 1999 and Dec. 2010. High resolution typing of 256/315 (81%) donor recipient pairs was performed. HLAmatchmaker software (version 3.0) was used to determine eplet mismatches (MM).
Results: Prospective monitoring revealed the development of *de novo* HLA-DR or HLA-DQ DSA in 39 recipients. Specificities included HLA-DR (n=15), HLA-DQ (n=30), or both (n=6). Mean traditional HLA mismatch was predictive of *dn*DSA development for HLA-DRβ1/3/4/5 (1.7 MM vs. 2.6 MM, $p<0.01$, Figure 1) and HLA-DQ α/β (1.8 MM vs. 2.3 MM, $p=0.05$). Eplet mismatch was also predictive for HLA-DR (13.9 MM vs 22.4 MM, $p<0.01$) and seemed to perform better than traditional antigen mismatching for HLA-DQ (16.7 MM vs 28.3 MM, $p<0.01$). Twenty-six HLA-DR and 38 HLA-DQ eplets were significant univariate predictors of *dn*DSA (Figure 2). Three HLA-DR and five HLA-DQ eplets showed

independent significance in a multivariate model (Figure 3). Furthermore, the locus specific mismatched eplet load was significant in a multivariate model which included known clinical predictors (ie non-adherence).

Conclusion: Eplet load better predicted *dn*DSA development at the HLA-DQ loci and was equivalent for HLA-DR when compared to traditional high resolution matching. Immunodominant eplets were characterized by their ability to predict which specific eplet mismatches lead to *dn*DSA development. This information could be used to identify high risk patients who may benefit from more intensive monitoring or immunosuppression.

Figure 1



2431

CONTROL OF IL-18 INDUCED PRO-INFLAMMATORY CYTOKINE EXPRESSION BY HUMAN TUBULAR EPITHELIAL CELL (HTEC) PRODUCTION OF IL-37

Yang, Yunbo; Yin, Ziqing; (Matthew Mailing Centre for Translational Transplantation Studies, London Health Sciences Centre, London, ON), Ma, Shengwu; (Department of Biology, University of Western Ontario. London Ontario, Canada, London, ON) Zhang, Zhu-xu[4]; Jevnikar, Anthony M; (Matthew Mailing Centre for Translational Transplantation Studies, London Health Sciences Centre, London, ON)

IL-37 is a newly described member of the IL-1 family, which is anti-inflammatory by its inhibition of pro-inflammatory cytokine production. While human IL-37 has no murine homologue, shared amino acid sequences with IL-18 allows binding to both human and mouse IL-18 receptors as well as IL-18 binding protein (IL-18BP). IL-37 binds to IL-18Rα with low affinity and also can form a trimeric complex with IL-18BP and IL-18Rβ to block IL-18 activity. IL-37 is expressed by mononuclear cells, dendritic cells and breast carcinoma cells, but to date no report has described IL-37 expression in renal tubular epithelial cells (TEC) nor any capacity to attenuate kidney ischemia reperfusion injury (IRI). We have found that human and mouse TEC have basal expression of IL-18R [illegible]-18Rβ and IL-18BP, and TNF [illegible]-induible [illegible] and hypoxia expression of IL-18. Exogenous IL-37 (300 ng/mL) down-regulated the IL-18 induced expression of IFN-□, □6 and IL-1β in murine NG TEC ($p < 0.05$) and human PT2 TEC ($p < 0.01$). Importantly, we found that LPS, IFN-□□ and-□8 induced the expression of IL-37 in human PT2 TECs. Consistent with an inhibitory role, mRNA silencing of IL-37 expression in TEC resulted in an augmented expression of TNF-□, □6 and IL-1β induced by IL-18. Collectively these data suggest IL-37 production by TEC may be a previously unrecognized intra-renal control mechanism to attenuate inflammation. In support of this, enhanced expression of IL-37 by transfection of human PT2 TEC with IL-37 plasmid decreased pro-inflammatory cytokine expression of TNF-□, □6 and IL-1β induced by IL-18 ($p < 0.05$). Our results confirm that TECs express IL-18 and IL-18R, and for the first time report that TEC express the IL-18 contra-regulatory proteins IL-37 and IL-

18BP which may function as an important anti-inflammatory control mechanism. These data may form a basis for new strategies to prevent renal inflammatory injury and IRI during transplantation.

2433

CD4+ BUT NOT CD8+ MEMORY T CELLS ESCAPE GRANZYME B MEDIATED REGULATION BY DN-TREG

Su, Ye; Huang, Xuyan; Lian, Dameng; Min, Weiping; Jevnikar, Anthony; Zhang, Zhu-Xu; (Matthew Mailing Centre for Translational Transplant Studies, London Health Sciences Centre; Departments of Medicine and Pathology, Western University, London, ON)

Memory T cell (Tm) homeostasis is poorly understood and is a critical challenge to controlling transplant rejection. At present there are no effective clinical strategies to control Tm and contradictory reports exist as to their control by regulatory T cell (Treg). We previously found that TCRαβ+CD3+CD4-CD8-NK1.1- (double-negative, DN)-Treg could effectively suppress CD4+ and CD8+ effector T cells and thus mitigate transplant rejection. We utilized this model to test whether DN-Tregs could similarly suppress Tm. Tm was generated by immunizing mice with allogeneic spleen cells. Interestingly DN-Tregs suppressed CD8+ Tm by >53% but had no effect on CD4+ Tm cells. As well DN-Tregs express very high levels of granzyme B (GzmB), suggesting a potential control mechanism and indeed no suppression was observed using perforin null DN-Tregs. In BALB/c (H-2d) to B6-Rag1-/- mice (H-2b) skin allograft transplantation, DN-Tregs suppressed CD8+CD44high Tm mediated rejection and significantly prolonged graft survival over CD8+ Tm cell transfer alone (63.0±4.7 days, vs. 21.5±1.7 days, p<0.05). In contrast, co-transfer of DN-Tregs had no effect on CD4+CD44high Tm mediated rejection (25.3±1.4 days, vs. 20.0±0.7 days, p>0.05). DN-Tregs co-transferred with CD8+ Tm had significantly reduced total CD44high CD8+ Tm (10.9×10^4 vs. 27.9×10^4), CD44high CD62Lhigh central Tm (6.2×10^4 vs. 13.1×10^4), and CD44high CD62Llow effector Tm (4.7×10^4 vs. 14.8×10^4) in spleens (n=3, p0.05). There was no effect with co-transferred CD4+ Tm. CD4+ Tm cells express higher levels of GzmB inhibitory Serpin Protease Inhibitor 6 (SPI-6) mRNA (7.7-fold) and have higher intracellular expression of SPI-6 protein than CD8+ Tm (5.2-fold) (p0.05, n=3), suggesting CD4+ Tm may escape DN-Treg control by resistance to GzmB. We show for the first time, that DN-Treg can control CD8+ Tm and suggest that DN-Treg along with targeting SPI-6 may be useful to limit the expansion of Tm in transplantation.

2434

MECHANISMS OF RENAL TUBULAR CELL SURVIVAL IN HYPOXIA

Alnasser, Hatem; (Department of Urologic Sciences, University of British Columbia, Vancouver, BC)

Background: Hypoxia-ischemia injury in kidney donors causes poor transplant outcomes and is one of the persistent concerns in the use of donation after cardiac death (DCD) for transplantation. However, the molecular pathways for controlling kidney cell survival/death after hypoxic or ischemic insult remains unclear. Clusterin (CLU) is a chaperone-like protein with cytoprotective activity that renders the kidney resistance to ischemia-reperfusion injury. This study was designed to investigate the role of autophagy in CLU-dependent kidney cell survival in response to hypoxia.
Methods and Materials: Ischemia-reperfusion injury (IRI) in the kidneys of mice was induced by clamping renal pedicles at 32°C of body temperature for 45 min. Hypoxia in renal tubular epithelial cell (TEC) cultures was induced by exposure to 1%O2 for 24 hours. Autophagy was determined by either the increase in LC3-BII expression in Western Blot, or the LC3-GFP aggregation in confocal microscopy. Cell apoptosis was determined by FACS analysis.
Results: Here, we showed that autophagy was significantly activated in wild type (WT) but not CLU deficient kidneys following IRI. Similar results were seen in kidney cell cultures, indicated by activation of autophagy both in human

proximal tubular epithelial cell line (HKC-8) cells and wild type mouse primary TECs following exposure to hypoxia, but was impaired in CLU null TECs. Hypoxia-induced autophagy activation was CLU dependent and was positively correlated with cell survival. Further studies demonstrated that incubation with an autophagy inhibitor bafilomycin Al significantly promoted hypoxia-mediated cell death both in HKC-8 cells and mouse primary TECs.
Conclusion: These data suggest that CLU-dependent autophagy may protect the kidney from hypoxia/ischemia-mediated cell death.

2436

MTORC2 CONTROLS MYOFIBROBLAST DIFFERENTIATION THROUGH CTGF REGULATION

Bernard, Monique, Dieudé, Mélanie; Hamelin, Katia; Hébert, Marie-Josée (CRCHUM, Montréal, QC)

Chronic fibrosis is a major complication of transplantation. In the kidney, microvascular rarefaction precedes and predicts classical fibrogenic changes such as accumulation of interstitial myofibroblasts involved in extracellular matrix accumulation and fibrosis. Microvascular rarefaction generates tissue hypoperfusion characterized by lower oxygen, nutrients and growth factor levels. We have recently demonstrated that persistent growth factor deprivation induces a CTGF-dependent and TGF-β-independent autocrine loop leading to sustained myofibroblast differentiation. Here, we aimed at further delineate the mechanisms fuelling fibroblast production and secretion of CTGF by focusing on the mTOR/Akt pathway.
WI-38 human fibroblasts were exposed to serum free medium as a model of microvascular rarefaction. Akt activation (serine 473 phosphorylation), CTGF synthesis and secretion and myofibroblast differentiation (αSMA) were evaluated by Western Blot. The importance of CTGF as an autocrine factor favoring myofibroblast differentiation was studied with CTGF siRNA. The implication of the mTORC2 complex in sustaining CTGF synthesis was evaluated by silencing Rictor.
Serum deprivation increased the expression of the myofibroblast differentiation marker αSMA and increased intra- and extra-cellular levels of CTGF. CTGF siRNA blocked αSMA synthesis, identifying CTGF as a central factor for myofibroblast differentiation in this system. Serum deprivation for 2 days or more induced Akt phosphorylation. Rictor silencing (82%, p<0,0001) lowered Akt activation (60%, p=0,0002) leading to decreased CTGF synthesis (45%, p=0,0286) and secretion (52%, p=0,0390) and lower αSMA synthesis (74%, p=0,0003).
Collectively these results demonstrate that prolonged growth factor deprivation induces myofibroblast differentiation through mTORC2 and CTGF-dependent pathways. These results provide novel insights into the mechanisms supporting the fibrogenic response downstream of prolonged reduction of nutrient and growth factor input.

2448

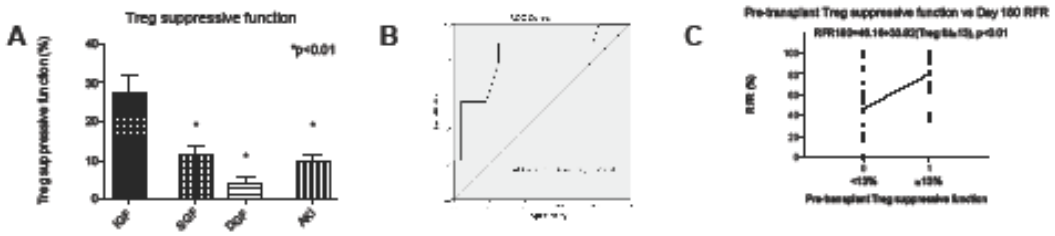
RECIPIENT PRE-OPERATIVE REGULATORY T CELL FUNCTION: A PREDICTOR OF ACUTE KIDNEY INJURY AFTER KIDNEY TRANSPLANTATION

Nguyen, Minh-Tr; Sahakian, Sossy; Fryml, Elise; Liu, Shuqing; Tchervenkov, Jean; Paraskevas, Steven;
(McGill University Health Centre, Montréal, QC)

Background: Acute kidney injury (AKI) complicates >20% of kidney transplantation, and in both its most severe (delayed graft function, DGF) and milder (slow graft function, SGF) forms, increases the risk for acute rejection and 5-year graft loss. Diagnosis of AKI is currently made too late after irreversible graft damage. Regulatory T cells (Tregs) are lymphocytes that are critical for self and transplant tolerance. Recent evidence suggests that they also attenuate murine

AKI. Their implication in AKI after kidney transplantation, however, remains unknown. We hypothesized that recipient pre-operative Treg frequency or function could predict AKI after kidney transplantation.
Methods: Consecutively enrolled deceased donor kidney transplant recipients (n=53) were divided into AKI (n=37; DGF, n=10; SGF, n=27) and immediate graft function (IGF, n=16) groups based on post-transplant dialysis requirement and 24-hour serum creatinine. Donor, organ procurement, and recipient characteristics were similar between all groups except for donor age/extended criteria donors (collinear variables) and cold ischemic time. Pre-transplant recipient peripheral blood CD4 $CD25^{hi}$FoxP3 Treg frequency was quantified by flow cytometry analysis. Treg suppressive function was measured by suppression of autologous CD4 CD25- CFSE-labeled effector T cell proliferation by CD4 CD25 Treg in vitro.
Results: Pre-transplant Treg suppressive function, but not frequency, was decreased in the DGF (3.86 ± 1.86%), SGF (11.71 ± 2.11%), and AKI (9.75 ± 1.76%) in comparison to the IGF (27.33 ± 5.00%) recipients (one-way ANOVA post-hoc Tukey $p<0.01$; Fig. 1A). When accounting for significantly different variables between our groups (donor age, cold ischemic time), a higher pre-transplant Treg suppressive function decreased the odds of suffering from DGF in multinomial logistic regression (OR=0.79, $p<0.03$). Moreover, pre-transplant Treg suppressive function predicted AKI in ROC curve analysis (AUC = 0.82, $p<0.01$; Fig. 1B) with a sensitivity of 89% and specificity of 75% at a cut-off value of 13%. This cut-off value also predicted 180-day percent donor renal function recovery (RFR, [observed recipient GFR – pre-transplant recipient GFR]/[0.5 x donor GFR]) in linear regression (B=33.82, $p<0.01$; Fig. 1C).
Conclusion: Recipient Treg suppressive function, measured prior to kidney transplantation and without prior knowledge of donor or organ procurement characteristics, predicts AKI and 180-day renal function recovery. This could guide peri-transplant clinical decisions and lead to novel Treg-targeted therapies for AKI.

Figure 1



2450

RESVERATROL ATTENUATES STIMULATED T-CELLS ACTIVATION AND PROLIFERATION: A NOVEL ADJUNCT THERAPY AGAINST CELLULAR REJECTION IN CARDIAC TRANSPLANTATION

Nagendran, Jeevan; West, Lori; Dyck, Jason; Nagendran, Jayan; (Edmonton, AB)

Objective: Resveratrol is the major bioactive phenol found in red wine and red grapes. Recently, this drug has been shown to directly inhibit cardiac hypertrophy. Resveratrol mediates these benefits through inhibition of protein kinase B (PKB/Akt), leading to inhibition of nuclear factor of activated T-cells (NFAT)-dependent transcription in the cardiomyocyte. Therefore, because NFAT dependent transcription is critical for cellular rejection by T-cells in cardiac transplantation, we hypothesize that resveratrol can inhibit human T-cell response under stimulation.
Methods: Human peripheral blood mononuclear cells (PBMC) were isolated by Ficoll method and cultured in RPMI1640 medium with 10% human serum and pen/strep in a 37^0C, 5%CO_2 incubator. The cells were pre-treated with resveratrol (50μM) overnight (18hrs) before stimulation with cell stimulation cocktail (PMA/Ionomycin, eBioscience) for 4 hours. The cultured medium was collected for ELISA analysis and the cells were harvested for western blot analysis. Cells were stained with CellTrace Violet (CFSE) proliferation dye and cultured for 5 days in PMA/Ionomycin and 0 and 50μM resveratrol for flow cytometry analysis.

Results: In non-stimulated PBMCs, markers of T-cell activation, tumor necrosis factor-α (TNF-α), interferon γ (INF-γ), and cell proliferation were comparable between resveratrol treated and controls. However, when PBMCs were stimulated, resveratrol treated cells displayed a significant decrease in TNF-α and INF-γ when compared to controls ($p<0.01$). As well, when compared to controls, T-cell proliferation was significantly reduced in cells treated with resveratrol. Furthermore, our data also indicate that activated phosphorylation levels of Akt were significantly reduced following resveratrol treatment in stimulated PBMCs ($p<0.01$), suggesting that resveratrol is able to inhibit calcineurin activity indirectly by significantly decreasing Akt activity.
Conclusions: Taken together, our data suggest that resveratrol is able to decrease the immune response of stimulated T-cells by inhibiting Akt, thus suppressing NFAT mediated gene transcription. Therefore, resveratrol may be a possible adjunctive therapy for patients undergoing cardiac transplantation, as this drug has a very low side-effect profile, along with pleiotropic effects against cardiac hypertrophy.

2456

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

Knaak, Jan M.; Boehnert, Markus; Louis, Kristine; Spetzler, Vinzent; Grant, David; Levy, Gary; (Multi Organ Transplant, Toronto, ON), Keshavjee, Shaf; (Division of Thoracic Surgery, Toronto General Hospital, Toronto, ON), Selzner, Markus; (Multi Organ Transplant, Toronto, ON)

Background: Cold static preservation is poorly tolerated by livers retrieved after cardiac death (DCD). We compared the impact of cold static liver preservation vs subnormothermic (33°C) ex vivo liver perfusion (NEVLP) on outcome of DCD pig liver transplantation.
Methods: 45min cardiac arrest was induced in pigs as a model of DCD organ retrieval. We compared 10 hr cold static preservation with 3hr NEVLP plus 7hr cold storage. After 10hr of preservation orthotopic pig liver transplantation was performed. Hepatocyte, endothelial cell (EC), and bile duct injury were assessed by H&E staining and immunohistochemistry. AST was determined as a marker of liver injury. Thrombus formation was assessed by MSB staining.
Results: NEVLP vs cold storage resulted in decrease serum AST levels (486 vs 934 Units, p<=0,000084) and reduction of hepatocyte necrosis on H&E staining (15% vs 45% p=0.000193). Similar NEVLP vs cold preserved grafts had less cleaved caspase 3 (16 cells/HPF vs. 46 cells/HPF, p= 0,000056) and TUNEL (14 cells/HPF vs. 47 cells/HPF, p=0,018) positive cells as markers of apoptosis. Microthrombosis within the peribiliary arteries was only observed in cold stored organs (3/4 livers) but not in NEVLP treated grafts (0/4 livers). CD31 staining of EC demonstrated severe EC injury in cold stored DCD grafts, while EC lining was preserved in NEVLP treated DCD livers.
Conclusion: NEVLP decreases hepatocyte, endothelial cell and biliary injury in DCD grafts and prevents thrombosis in peribiliary arteries.

2460

ANTI-PERLECAN ANTIBODIES ARE NOVEL ACCELERATORS OF IMMUNE-MEDIATED VASCULAR INJURY

Dieudé, Mélanie; Qi, Shijie; Beillevaire, Déborah; (Research Centre, Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, QC) Patey, Natalie; (Research Centre, Hôpital Ste-Justine, Montréal, QC) Hébert, Marie-Josée; (Research Centre, Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, QC)

Acute vascular rejection (AVR) is characterized by immune-mediated vascular injury and heightened endothelial cell (EC) apoptosis. We previously reported that apoptotic ECs release a bioactive C-terminal fragment of perlecan referred to as LG3 and that anti-LG3 IgG titers are increased in renal transplant patients with acute vascular rejection compared to subjects with Banff grade I rejection or normal allografts. Hence, we hypothesized that anti-LG3 antibodies represent novel accelerators of alloimmune vascular injury of potential importance in shaping the severity of rejection episodes.
Methods: We performed passive transfer of 50 ug of anti-LG3 IgG or 50 ug of control IgG every other day during three weeks in an animal model of vascular rejection based on orthotopic aortic transplantation between fully MHC-mismatched mice. When indicated, warm ischemia was induced by clamping the aorta for 15 min in the donor before transplantation. Transplanted aortas were harvested at 3 weeks post-transplantation. Aortic sections samples were H/E stained or immunohistochemistry was performed for the detection of NK cells (GM1 Asialo), T cells (CD3) or C4d deposition.
Results: Passive transfer of anti-LG3 IgGs in mice transplanted with a non-ischemic allograft led to a modest increase in neointima formation (p=0.17). In ischemic allografts however, passive anti-LG3 transfer induced striking NK and T cell infiltration (p=0.003 and 0.03 respectively), transmural C4d deposition and obliterative vascular remodelling(p=0.04).
Conclusion: These results suggest that anti-LG3 antibodies significantly increase vascular inflammation and obliterative remodelling in allografts exposed to an initial ischemic insult and identify anti-LG3 antibodies as novel accelerators of immune-mediated vascular injury.

2463

LG3, A C-TERMINAL PERLECAN FRAGMENT BIOACTIVE ON MESENCHYMAL STEM CELLS

Pilon, Eve-Annie; Soulez, Mathilde; Groleau, Jessika; Dieude, Melanie; (CRCHUM - Université de Montréal, Montréal, QC), Durocher, Yves; (Biotechnology Research Institute, Montréal, QC), Hébert, Marie-José; (CRCHUM - Université de Montréal, Montréal, QC)

Introduction: Transplant Vasculopathy (TV) is a leading cause of longterm allograft loss. TV is characterized by vascular narrowing due to obliterative neointima formation caused by accumulation of alpha smooth muscle actin positive cells, which include mesenchymal stem cells (MSC). Sustained apoptosis of endothelial cells (EC) is associated with neointima formation at the site of injury. Apoptotic EC release LG3, a C-terminus perlecan which in turn favors neointima formation (Soulez M et al, Circ Res 2012). We hypothesize that LG3 induces a pro-migratory phenotype on MSC, therefore favoring obliterative remodelling.
Methods: Human MSC are exposed to increasing doses of recombinant LG3 and migration is evaluated by Wound Assay (WA) and transmigration in Boyden chamber. The involvement of ERK1/2, Src and b1 integrins in LG3-induced MSC migration are evaluated by WA and Western Blot (WB) using selective biochemical inhibitors (PD98059 and PP2 respectively) or a blocking antibody against b1 integrins.
Results: Migration and transmigration of MSCs are significantly increased when MSC are incubated in the presence of LG3 compared to vehicle ($p \leq 0.05$ and $p \leq 0.02$ respectively). In addition, LG3 induces phosphorylation of ERK1/2 at a concentration of 2.5µg/mL ($p \leq 0.01$). Specific inhibitors of ERK1/2 and Src (PD98059 and PP2 respectively) reduce the LG3-induced migration of MSCs ($p \leq 0.01$ and $p \leq 0.001$ respectively). Incubation of MSC with a neutralizing antibody against the β1 integrin receptor reduces the migration of MSCs ($p \leq 0.001$) as well as the phosphorylation of ERK1/2.
Conclusion: These results suggest that LG3, through interactions with b1 integrin receptors, induce an ERK 1/2 and Src-dependent migratory phenotype in MSC. These results identify LG3 as a new mediator contributing to the migration of mesenchymal stem cells to the site of vascular injury.

2464

RIP3 MEDIATED NECROPTOSIS REGULATES CARDIAC ALLOGRAFT INFLAMMATION AND SURVIVAL

Pavlosky, Alexander; Zhang, Zhuxu; Jevnikar, Anthony; Lau, Arthur; Huang, Xuyan; Yin, Ziqin; (Matthew Mailing Centre for Translational Transplant Studies, London Health Sciences Centre, London, ON)

Despite recent advances in immunosuppression, patients who have undergone allogeneic cardiac transplantation still suffer from poorly understood chronic graft loss. Previous studies have shown that Tumour Necrosis Factor Alpha (TNFα) contributes to cell death by activating apoptotic and newly identified Receptor Interacting Protein 1 and 3 (RIP1/RIP3) mediated necroptotic death pathways. These variations in cell death may be important for graft survival as necroptosis can lead to the release of chemotactic danger molecules which have been shown to activate host immune cells. This pathway has yet to be studied in transplantation.
Our data shows that sirolimus treatment (9 days) markedly prolongs cardiac allograft survival of B6-RIP3 null as compared to B6 wild type donor heart grafts into Balb/c recipients (95 5.8 vs 24 2.6 days $p<0.001$). *In vitro*, murine vascular endothelial cell (VEC) cell death is reduced by the RIP1/RIP3 inhibiting small molecule necrostatin-1 (Nec-1) following TNFα treatment (68.5% vs 32.8%, PI positive at 48 hours). As well, necrosis and release of the pro-inflammatory danger molecule HMGB1 are attenuated *in vivo* in RIP3 null heart allografts and *in vitro* with VEC after RIP1/RIP3 inhibition. Finally, quantitative blinded scoring of necrotic cell death, vascular endothelial cell damage and graft infiltration was attenuated in RIP3 null hearts compared with wildtype allografts (0.8 /- 0.4 in RIP3 null vs 1.8 /- 0.4). These data suggest that RIP1/RIP3 contribute to inflammatory injury in cardiac allografts through VEC necroptotic death and the release of danger molecules. The ability of immunosuppression to provide rejection protection or permit tolerance is influenced by the level of cell death and inflammation. We suggest targeting RIP1 and RIP3 mediated necroptosis may be an important therapeutic strategy in solid organ transplants.

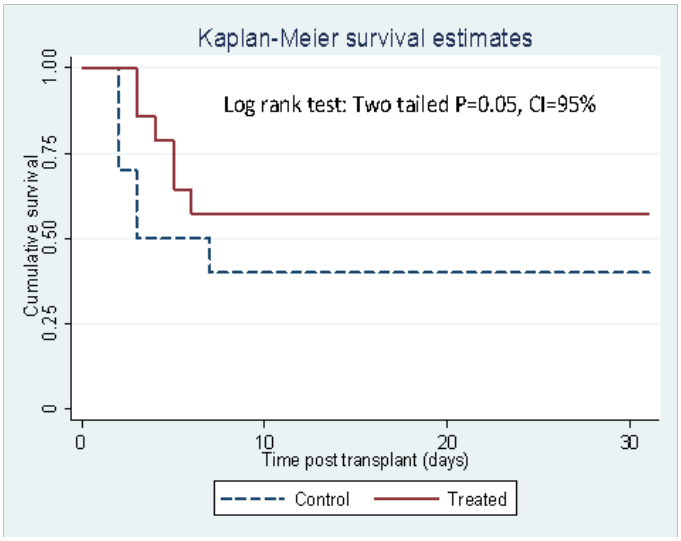
2465

THE CASPASE INHIBITOR IDN-6556 REDUCES INJURY DUE TO COLD ISCHEMIA AND REPERFUSION IN KIDNEY TRANSPLANT

Gala-Lopez, Boris; Pepper, Andrew; Zhu, Lin-Fu; Pawlick, Rena; Shapiro, AM James; (Alberta Diabetes Institute, University of Alberta, Edmonton, AB)

Background: Ischemia-reperfusion injury (IRI) has a profound influence on early and late function of the transplanted kidney. Apoptosis contributes to parenchymal cell loss and plays a major role in graft dysfunction due to IRI. Previous work demonstrated that IDN-6556 is an irreversible pan-caspase inhibitor reducing apoptosis, which is currently being clinically evaluated in a number of diseases, including transplant. This study examined the efficacy of the caspase inhibitor IDN-6556 in reducing the damage associated to IRI in kidney transplant
Methods: kidneys from male Lewis rats were perfused and preserved in University of Wisconsin solution at 4ºC for 24 hours, with or without 100 μmol/L IDN-6556. Three different groups were designed with treated and control animals. In one group, kidneys were transplanted in recipient rats, which underwent nephrectomy of the native kidneys at the time of transplant. Whole blood creatinine levels were measured at 0, 1, 3, 7, 14 and 30 days post-transplant in recipient rats, at which time kidneys were retrieved for histopathology analysis. A second group consisted in 24hr preservation of the kidney followed by histopathology analysis, including TUNEL and KIM-1 staining, and a final group received transplant and animals were sacrificed after 24 hr for similar analysis.
Results: Post – transplant creatinine reduction was observed after day 3 and was not significant when comparing both transplanted groups (p=0.142, CI=95%, 16.62 – 86.53). Thirty-day survival was significantly higher in the treated group

(p=0.05). Grafts from the IDN-6556–treated groups had markedly reduced caspase activities and TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling)-positive cells, suggesting reductions in apoptosis.
Conclusions: Animals receiving kidneys preserved in IDN-6556 showed decreased injury due to cold ischemia and reperfusion. Today, when organ shortage has imposed the use of extended criteria donors, agents like this may become a rational therapeutic approach to reduce the risk of renal ischemia in the clinical setting.



2467

INHIBITION OF RECEPTOR INTERACTING PROTEIN 3 (RIP3) PROMOTES LONG TERM RENAL ALLOGRAFT SURVIVAL

Lau, Arthur; Wang, Shuang; Haig, Aaron; Yin, Ziqin; Zhang, Zhu-xu; Jevnikar, Anthony; ([1]Matthew Mailing Centre for Translational Transplant Studies, London, ON)

Background: Death of kidney parenchymal cells during transplantation occurs through counterbalanced mechanisms of cellular death, apoptosis and necrosis. Members of the receptor interacting protein family (RIP1, RIP3) are regulators of newly described pro-inflammatory programmed necrosis, termed 'necroptosis', which can be upregulated by blocking caspase-8 mediated apoptosis. We therefore tested the effect of caspase-8 inhibition and RIP3 in kidney allograft injury.
Results: Previously we have shown that caspase-8 shRNA silencing reduced kidney ischemia-reperfusion injury at 48h. To test caspase-8 shRNA silencing on alloimmunity, in the present study we transplanted C57BL/6 (H-2^b) kidneys into fully nephrectomized Balb/c (H-2^d) recipients without immunosuppression. Surprisingly, donor kidney inhibition of apoptosis using caspase-8 shRNA reduced allograft survival compared to controls (33.3±8.7 vs 68.3±10.9 days, p=0.0003, n=7-9/group). Quantification of necrosis by ethidium homodimer (ETH) staining showed increased necrotic death in caspase-8 shRNA kidneys at day 4 post-transplant compared to controls. To test whether enhanced necrosis was linked to caspase-8 inhibition, we exposed renal tubular epithelial cells (TEC) to pro-inflammatory cytokines and assessed cell death by Annexin-V/PI co-labeling. Indeed, Annexin-V/PI co-positivity increased in TNF-α/IFN-γ treated and caspase-8 inhibited (zVAD) TEC at 48h (from 9% to 17%) which was blocked by RIP3 inhibition using Nec-1. Similarly, TNF-α/IFN-γ treated $RIP3^{-/-}$ TEC had decreased Annexin-V/PI co-positivity compared to wild type controls (22% vs. 51%). We then directly tested the role of RIP3 in allograft survival using donor kidneys from $RIP3^{-/-}$ mice. Absence of donor RIP3 increased spontaneous acceptance and prolonged allograft survival compared to wild type control recipients (94.6±2.0 vs 43.0±10.4 days, p=0.04, n=11-17/group). As well, $RIP3^{-/-}$ transplants had lower serum creatinine levels compared to controls (31±6.6 vs 86.2±24.1 µmol/l, p=0.03, n=7-9/group) at study end (day 100 or earlier termination). Interestingly,

while a trend to reduced tubular injury was noted in RIP3$^{-/-}$ allografts, reduced vasculopathy was the most prominent difference in histological injury (0.5±0.4 vs. 1.6±0.2, p=0.02,n=4-5/group).
Conclusion: These data demonstrate for the first time that loss of RIP3 mediated necroptosis improves kidney allograft survival in non-immunosuppressed recipients, but does not eliminate graft injury. As various forms of cell death are inter-regulated, inhibition of both apoptosis and necroptosis may be required to more fully attenuate graft injury and prolong allograft survival.

2468

ENDOTHELIN-1 AND BIG-ENDOTHELIN-1 AS POTENTIAL BIOMARKERS IN CLINICAL EX VIVO LUNG PERFUSION

Machuca, Tiago; Cypel, Marcelo; Liu, Mingyao; Zhao, Yidan; Yeung, Jonathan; Chen, Manyin Hsin, Michael; Saito, Tomohito; Guan, Zehong; Grasemann, Hartmut; dePerrot, Marc; Waddell, Thomas; Keshavjee, Shaf; (University of Toronto, Toronto, ON)

Objective: Normothermic ex vivo lung perfusion (EVLP) is a preservation technique that allows reassessment and improvement of donor lungs prior to transplantation. We hypothesized that the endothelin-1 (ET-1) axis is associated with donor lung performance during EVLP and recipient outcomes after transplantation.
Methods: We assessed levels of ET-1, big ET-1 and endothelin converting enzyme 1 (ECE-1) in the perfusates of donor lungs enrolled in a clinical trial of 4-6 hours of EVLP. The trial included lungs from high-risk brain death donors (BDD) and lungs from donation after cardiac death (DCD). They were divided into three groups: I. Control: bilateral transplantation with good early outcomes (absence of PGD grade 3); II. PGD3: bilateral lung transplantation with PGD grade 3 within 72 hours; III. Declined: lungs rejected following EVLP. Single-lung transplants and patients bridged with extracorporeal life support were excluded.
Results: There were 25 cases in group I, 7 in group II and 15 in group III. At 1 hour of EVLP, perfusates of declined lungs had significantly higher levels of ET-1 (3.2±2.8 vs 1.8±2.3 pg/ml, *p*=0.01) and big ET-1 (16.5±14.4 vs 7.0±6.5, *p*=0.0009) compared to control lungs. At the end of EVLP, big ET-1 was also higher in declined lungs compared to controls (35.4±17.7 vs 21.5±10 pg/ml, *p*=0.005). In BDD lungs the ET-1 axis did not show significant differences between groups. However for DCD cases, groups II and III had higher ET-1 and big ET-1 levels at the end of perfusion when compared to group I (group II: ET-1 3.4±0.9 vs 1.3±1.4 pg/ml *p*=0.03, big ET-1 70.7±40.4 vs 20.2±9.8 *p*=0.01; group III: ET-1 3.7±2.7 vs 1.3±1.4 pg/ml *p*=0.007, big ET-1 44.8±17.1 vs 20.2±9.8 pg/ml *p*=0.003). There were no differences in ECE-1 levels in all groups.
Conclusion: In DCD lungs ET-1 and big ET-1 in perfusate predicted outcomes after lung transplantation. They were also associated with non-utilization of lungs after EVLP and thus could represent useful biomarkers to improve selection of donor lungs.

2470

NEBIVOLOL REVERSES THE DELETERIOUS EFFECT OF TACROLIMUS ON THE PANCREAS IN RAT

Jadhav, Ashok; Ahmed, Mohamed; Jadhav, Ashok; Barlas, Angeliki; Gopalakrishan, Venkat; (Dept of Pharmacology, University of Saskatchewan, Saskatoon, SK)

Background and objectives: Nebivolol is a selective ß1- adrenergic antagonist with endothelial and nitric oxide- (NO) dependent vasodilatation properties. Tacrolimus (Tac) increases the risk of diabetes mellitus. We investigated whether nebivolol reverses the deleterious effect of Tac on the pancreas in Sprague Dawley Rats.

Materials and methods: Animals were subdivided into 4 groups of six animals. group 1, control, received 0.5 mg/kg/day of castor oil (s/c), group 2 received 1.5 mg/kg/day of Tac (s/c), group 3 received 1 mg/kg/day of nebivolol (s/c), and group 4 received Tac and nebivolol. Insulin was measured by ELISA and insulin resistance (IR) was calculated from HOMA-IR. Blood flow was measured by conventional microsphere method. The duration of the study was 45 days.
Results: Pancreatic blood flow measurements in groups 1, 3 and 4 were similar. Flow in group 2 (0.08±0.02 ml/g) was significantly lower than that in groups 4 (1.1±0.4 ml/g), group 1 (0.6±0.2 ml/g), and group 3 (0.9±0.3 ml/g) *($p<0.05$).* Fasting plasma glucose (FPG) levels in groups 1, 3 and 4 were similar. FPG levels in group 2 (7.3±0.1 mmol/L) were significantly higher than those in groups 1 (6.6±0.1 mmol/L), group 3 (5.7±0.24 mmol/L) and group 4 (5.70±0.21 mmol/L) *($p<0.05$).* Plasma insulin levels in group 2 (0.069±0.02 µg/L) were much lower than those in groups 1 (0.22±0.06 µg/L), group 3 (0.268 - 0.2 µg/L) and group 4 (0.195±0.09 µg/L) *($p<0.05$).* HOMA- IR values were similar.
Conclusions: Nebivolol enhances pancreatic blood flow, reverses hyperglycemia and normalizes insulin secretion in animals treated chronically with high dose Tac.

2471

PREVENTION ALLOGRAFT REJECTION IN HEART TRANSPLANTATION THROUGH CONCURRENT GENE SILENCING OF TLR AND KINASE SIGNALING PATHWAYS

Zhang, Xusheng; (University of Western Ontario, London, ON) Lian, Dameng; (Multi-Organ Transplant Program, London Health Sciences Centre, London, ON), Zheng, Xiufen; Chen, Di; Siu, Kingsun; Johnston, Nathan; (University of Western Ontario, London, ON), Luke, Patrick; Jevnikar, Anthony; (Multi-Organ Transplant Program, London Health Sciences Centre, London,ON) Min, Wei-Ping; (University of Western Ontario; Multi-Organ Transplant Program, London Health Sciences Centre, London, ON)

Background: Toll-like receptors (TLRs) act as initiators and conductors responsible for both innate and adaptive immune responses in transplantation. The mammalian target of rapamycin (mTOR) is one of the most critical signaling kinases that affects broad aspects of cellular functions, including metabolism, growth, and survival. Concurrent gene silencing of multiple TLR adaptors, MyD88/TRIF and downstream kinases mTOR may enhance DC-mediated tolerance induction, thereby preventing graft rejection in heart transplantation.
Methods: Recipients (BALB/c) were treated with MyD88, TRIF and mTOR siRNA vectors, 3 and 7 days prior to heart transplantation and 7 and 14 days after transplantation. After siRNA treatment, recipients received a fully MHC-mismatched C57BL/6 heart. Control groups included untreated mice and the mice treated with scrambled siRNA or mTOR siRNA monotherapy.
Results: Treatment with mTOR siRNA significantly prolonged allograft survival (32.5±3.2 days) in heart transplantation. Moreover, the combination of mTOR siRNA along with MyD88 and TRIF siRNA further extended the allograft survival (89.5±5.6 days); Flow cytometric analysis showed an upregulation of FoxP3 expression in spleen lymphocytes and a concurrent downregulation of CD40, CD80 and MHC II expression in splenic dendritic cells in MyD88, TRIF and mTOR treated mice. MLR, using T cells isolated from long-survival recipients, showed a significantly decreased response to the donor original antigen. Tissue histopathology demonstrated an overall reduction in lymphocyte interstitium infiltration, hemorrhage and vasculopathy in mice treated with MyD88, TRIF and mTOR siRNA vector.
Conclusion: This study is the first demonstration of preventing immune rejection of allogeneic heart grafts through concurrent gene silencing of TLR and kinase signaling pathways, highlighting the therapeutic potential of siRNA in clinical transplantation.

2472

DIFFERENTIAL EFFECTS OF CALCINEURIN INHIBITORS ON THE IN-VIVO AND IN-VITRO VASCULAR REACTIVITY OF RAT AORTIC RINGS

Jadhav, Ashok; Ahmed, Mohamed; Gopalakrishnan, Venkat; Shoker, Ahmed; (University of Saskatchewan, Saskatoon,SK)

Aim: To compare the differential effects of Cyclosporin (CsA) and Tacrolimus (Tac) on nitric oxide (NO)-dependent and -independent vascular responses *in-vitro* and *in-vivo*.
Methods: We used the dose response curve (DRC) of acetylcholine (Ach) and sodium nitroprusside (SNP) on phenylnephrine (PE)-constricted thoracic and abdominal rat aortas in both sets of experiments (5-6 rats in each group). For the *in-vivo* experiments, CsA (15mg/kg, B Wt., n=6) or Tac (0.15 mg/kg, B wt., n=6) or control (vehicle, n=6) were administered subcutaneously for 15 days.
Results: In vitro CsA (1mg/ml) inhibition of Ach DRC in thoracic (IC_{50} 9.5 ± 2.5 µM vs. control IC_{50} 68 ± 18.7 nM, $p<0.01$) and abdominal aortas (IC_{50} 7.0 ± 3.14 µM vs. control IC_{50} 37 ± 15.3 nM, $p<0.01$) were complete. Tac (0.1mg/ml) induced partial inhibition in thoracic (IC_{50} 222 ± 60.2 nM vs. control IC_{50} 68 ± 16.4 nM, $p<0.05$) and abdominal (IC_{50} 215 ± 58.6 nM vs. control IC_{50} 50 ± 17.8 nM, $p<0.05$) aorta. *In-vitro* endothelium independent SNP-DRC was similar between CsA, Tac and control. *In-vivo* result showed that the NO-dependent pathway in thoracic aorta was significantly inhibited by Tac (E_{max} 39± 9.55 %** vs. control E_{max} 98± 2 %, $p<0.01$) compared to CsA (E_{max} 66± 8.79 %** vs. control, $p<0.01$). The rest of the *in-vivo* responses mimicked the *in-vitro* data.
Conclusions: There is a difference in NO-dependent responses. Tac has a milder vasoconstriction compared to CsA *in-vitro*, while *in-vivo* CsA vascular toxicity is lower than Tac.

2476

EVALUATING THE ROLE OF LYMPHOCYTES IN BK VIRUS NEPHROPATHY

Morsy, Deyaa El Deen; Tibbles, Lee Anne; (University of Calgary, Calgary, AB)

Reactivation of BK virus (BKV) is a major challenge in renal transplant recipients. The use of immunosuppression following transplantation causes disruption of the balance between host immunity and viral pathogenesis, which results in BKV reactivation with subsequent lytic injury of renal tubular epithelial cells followed by tubular inflammation, tubular cell atrophy, interstitial fibrosis and allograft destruction. The aforementioned changes comprise BKV nephropathy (BKN), which is now the leading cause of early renal allograft loss. Both cellular and humoral immunity were demonstrated to be involved in the early elimination of BKV. Nevertheless, the exact involvement of B and T cells in **pathogenesis** of BKN remains largely unknown. **Our hypotheses** are that host lymphocytes may become infected with BKV, therefore 1) becoming viral reservoirs enhancing viral dissemination; and 2) altering their gene expression and behaviour, contributing to BKN. Using real-time PCR and immunoblotting, our recent data show that BKV can infect human B and T cell lines, and peripheral blood lymphocytes, although the ability of BKV to use primary cells as natural reservoirs for viral reproduction is still under investigation. Our data also show that BKV infection causes a remarkable change in cell shape and behaviour, with B cells acquiring a refractile, fusiform appearance and becoming adhesive to culture plates. These changes in cell phenotype are unlikely to occur without associated changes in gene expression. We previously found that BKV causes activation of Akt/mTOR, and MAPK pathways in renal tubular cells. These pathways control the expression of several inflammatory cytokines such as IL-6, IL-17, IL-18, and TNF alpha. These pathways will be assessed in peripheral blood B and T cells, and the potential for expression of pro-inflammatory and pro-fibrotic mediators will be further explored using gene expression profiling. Immune cell activation triggered by BKV infection rather than viral recognition and antiviral responses would be a novel mechanism in pathogenesis of BKN. This project is

anticipated to establish a baseline for understanding host-viral interaction in patients with BKN. Together with other projects currently under investigation, it could help in designing tools for assessment of, and following-up progression of BKN in allograft recipients.

2477

INFLUENCE OF ACTIVATING KILLER IMMUNOGLOBULIN-LIKE RECEPTORS OF NATURAL KILLER CELLS AMONG RENAL TRANSPLANT RECIPIENTS ON PROTECTION AGAINST CYTOMEGALOVIRUS INFECTION/REACTIVATION

Dharmani, Poonam, Faridi, Rehan M.; Liacini, Abdelhamid; Doulla, Jagdeep; Sar, Aylin; Yilmaz, Serdar (University of Calgary, Calgary, AB); Berka, Noureddine (Calgary Laboratory Services, Calgary, AB); Khan, Faisal M. (University of Calgary, Calgary, AB)

Introduction: Reactivation of Cytomegalovirus is one most frequent viral complication that affects renal transplant recipients. In this regard, role of NK cells becomes important by virtue of their anti-viral activities as well as the fact that currently used immunosuppressive drugs significantly impair T cells. Functioning of these NK cells are regulated by series of receptors known as Killer Immunoglobulin like receptors (KIR) that exist in activating and inhibitory forms. Here, we assessed whether K IR gene profile of kidney transplant recipients influence the rate of CMV reactivation after transplantation.
Method: A total of 192 renal transplant recipients were genotyped for a set of 16 KIR genes by a Luminex based multiplex KIR genotyping assay. Humans have various combinations of these 16 genes that normally segregate in two haplotypic forms – 'A' (containing 1 activating receptor gene) and 'B" (containing 2-6 activating receptor genes). PCR based method was used for CMV reactivation assessment. Logistic regression analysis was performed to assess the association of recipient KIR gene profile with CMV reactivation.
Results: An activating KIR gene profile of the recipient was found significantly associated with protection from CMV infection. Renal transplant recipients carrying Haplotype B ($p=0.02$), ≥ 5 activating genes (0.01) or presence of 2DS2 activating KIR (0.01) were significantly associated with low rate of CMV reactivation.
Conclusions: Our findings strongly support the role of NK cells in general and that of activating KIR profile in particular in the immune surveillance against cytomegalovirus after renal transplantation.

2480

RIGHT VENTRICULAR DISTENSION IN DONOR HEARTS FOLLOWING CARDIOCIRCULATORY DEATH: IMPLICATIONS FOR POST-TRANSPLANT FUNCTION

White, Christopher; (Cardiac Sciences Program, St. Boniface General Hospital, Winnipeg, MB), Ali, Ayyaz; (Papworth Hospital, Cambridge), Hasanally, Devin; (Institute of Cardiovascular Sciences, St. Boniface Hospital, Winnipeg, MB), Wang, Fei; Xiang, Bo; (National Research Council Institute for Biodiagnostics, Winnipeg,MB), Mundt, Paul; (Cardiac Sciences Program, St. Boniface Hospital, Winnipeg, MB), Large, Stephen; (Papworth Hospital, Cambridge), Arora, Rakesh; (Cardiac Sciences Program, St. Boniface Hospital, Winnipeg, MB), Tian, Ganghong; (National Research Council Institute for Biodiagnostics, Winnipeg , MB), Freed, Darren; (Cardiac Sciences Program, St. Boniface Hospital, Winnipeg, MB)

Objective: Donation after cardiocirculatory death (DCD) has been proposed as a means to expand the donor pool for heart transplantation. Experimental studies have documented predominant right ventricular (RV) dysfunction in resuscitated DCD hearts that may preclude successful transplantation; however, the mechanism underlying this

observation has not been previously investigated. Therefore, we sought to characterize changes in RV pressure, volume, and function following withdrawal of life-sustaining therapy in a large animal model of DCD.

Methods: Thirteen female Yorkshire pigs (50kg) were anesthetized and mechanically ventilated with room air. Mechanical ventilation was discontinued, hypoxic cardiac arrest ensued, and a 10-minute warm-ischemic standoff period was observed. Changes in RV pressure, volume, and function following cessation of mechanical ventilation were continuously monitored via a conductance catheter placed in the RV. Changes in RV volume were further evaluated using cine cardiac magnetic resonance imaging (CMRI).

Results: Changes in the arterial partial pressure of oxygen (PaO_2), RV pressure, RV volume, and RV function following cessation of mechanical ventilation are displayed in Figure 1. The PaO_2 decreased rapidly and reached its nadir after 1.5 minutes. This corresponded with a significant increase in RV systolic pressure (baseline RVSP 24.1±0.9 vs. 35±2 mmHg, p<0.001), improvement in diastolic function (baseline $dPdt_{min}$ -172±24 vs. -267±24 mmHg/s, p<0.001), and improvement in systolic function (baseline $dPdt_{max}$ 254±32 vs. 378±32 mmHg/s, p=0.002) compared to baseline. However, subsequent RV function progressively declined leading to an 18±6% (p=0.006) increase in RV end-diastolic volume and a significant increase in RV end diastolic pressure (baseline 4.2±0.5 vs. 7.3±0.4 mmHg, p=0.001). Cardiocirculatory death occurred 8.5±0.8 minutes following cessation of mechanical ventilation. CMRI confirmed an 18±12% increase in RV end diastolic volume compared to baseline.

Conclusions: The RV is forced to accommodate the donor circulatory volume following withdrawal of life-sustaining therapy, and experiences significant distension that may exacerbate myocardial injury. Therefore, assessment of RV function prior to transplantation of the DCD heart is essential. This highlights the need for an *ex vivo* perfusion device capable of biventricular functional assessment.

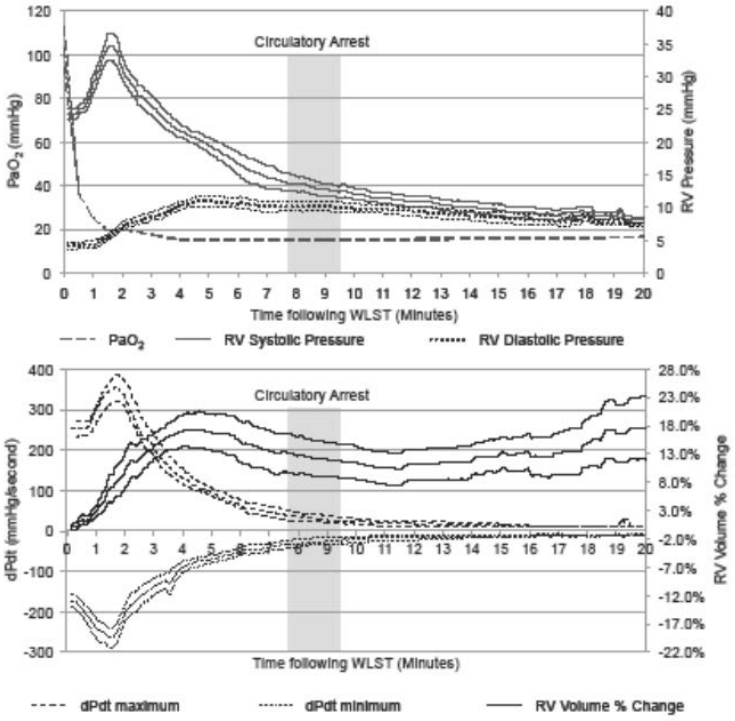


Figure 1. Changes in the arterial partial pressure of oxygen, and right ventricular pressure, volume, and function following cessation of mechanical ventilation. *dPdt, rate of pressure change in the right ventricle; PaO_2, arterial partial pressure of oxygen; RV, right ventricle; WLST, withdrawal of life sustaining therapy.*

2484

MICRORNA IN ISCHEMIA REPERFUSION INJURY IN HEART TRANSPLANTATION

Zhou, Larry, Johnston, Nathan; Zhang, Xusheng; Yao, Jijuan (London, ON); Chen, Di (University of Ontario, London, ON); Siu, Leo (University of Western Ontario, London, ON); Luke, Patrick (London Health Sciences Centre, London, ON); Jevnikar, Anthony; Min, Weiping; Zheng, Xiufen (London Health Sciences Centre, University of Western Ontario, London, ON)

Background: MicroRNAs (miRNA) are single-stranded RNAs containing 18-24 nucleotides in length and have emerged as a prominent class of gene regulators . Due to their capacity to silence multiple target mRNAs and their reversible regulation, miRNA regulatory activity has been demonstrated in ischemia reperfusion (I/R) injury induced in non-heart transplantation settings. We propose that miRNA may be involved in I/R injury in heart transplantation by regulating downstream gene expression.
Methods: Hearts were excised from C57/BL6 mice, and perfused and preserved in UW solution at 4 0 C for 18h to induce cold ischemia. After preservation hearts were implanted into syngeneic C57/BL6 recipients. Heart grafts were harvested for examination of the miRNA and gene expression by microarray assay. miRNA and gene expression were further confirmed by real time PCR. Histopathological change and apoptosis in heart grafts were examined by H&E staining and TUNEL.
Results: 18h cold ischemia caused graft dysfunction, histopathology changes and increased apoptotic cells in grafts. Using miRNA microarrays, we detected miRNA changes in the graft with prolonged cold ischemia (18h) on day 3 post transplantation. We found a total 14 miRNAs have been up-regulated by prolonged cold I/R as compared to grafts without cold ischemia, among which miR-711 was significantly increased up to 9 fold. In parallel, we analyzed for altered gene expression in heart grafts using gene expression microarrays. We found that 13 genes were up-regulated and 37 genes including a candidate downstream gene Angiopoietin 1 were down-regulated (>3 fold change, p<0.05). Work is in progress to assess the effect of altering miR-711 expression on candidate genes.
Conclusion: This is the first demonstration that prolonged cold ischemia reperfusion changes miRNA expression profiles and gene expression profiles in syngeneic cardiac transplants. Further work is needed to test if miRNA is similarly involved in I/R injury using allogeneic heart transplants and whether miR-711 is directly affected by therapeutic strategies that alter I/R injury.

2487

CD1D+CD5+ B CELLS PRODUCE INTERLEUKIN-10 AND HAVE A REGULATORY CAPACITY IN CHILDREN: IMPACT ON GRAFT ACCEPTANCE?

Ling, Ying; Dijke, Esmé; Motyka, Bruce; West, Lori; Urschel, Simon; (University of Alberta, Edmonton, AB)

Introduction: In mice, CD1d CD5 B cells have regulatory properties associated with interleukin-10 (IL-10) production. In humans, this phenotype is up to 10 times more frequent in infants than in adults. Infants show better heart transplant outcomes than older recipients, including acceptance of ABO-incompatible grafts. However, they also show increased severity of infections with polysaccharide-encapsulated bacteria. We hypothesize that CD1d CD5 B cells contribute to the altered immune response during infancy, particularly towards polysaccharides such as the ABO-antigens and bacteria capsules.
Methods: CD1d CD5 B cells were FACS-sorted from pediatric splenocytes and cultured parallel to non-CD1d CD5 B cells using T-dependent (α-IgM CD40L) and T-independent (CpG, α-IgM crosslinker) B cell stimuli. IL-10 was measured in supernatants by ELISA. The regulatory impact of CD1d CD5 B cells on other cells was assessed through proliferation of CFSE-stained peripheral blood mononuclear cells (PBMC) after stimulation with B cell stimuli or T cell stimuli (α-CD3 α-

CD28) in three conditions: 1) PBMC at the original proportion of CD1d CD5 B cells ($PBMC^{original}$), 2) PBMC to which CD1d CD5 B cells were added to double the original proportion ($PBMC^{double}$), and 3) PBMC from which CD1d CD5 B cells were depleted ($PBMC^{depleted}$).
Results: When stimulated with α-IgM CD40L, IL-10 levels were seen in CD1d CD5 B cells but not in non-CD1d CD5 B cells. However, CpG stimulation induced IL-10 production also in B cells not expressing CD1d and CD5. When stimulated with α-IgM CD40L, the median frequency of dividing B cells was 27% higher in absence of CD1d CD5 B cells compared to the original proportion (P=0.081). A similar trend was seen in all B cell stimulation conditions. In contrast, both absence and double proportion of CD1d CD5 B cells had little effect on T cell proliferation. Switched memory B cells (CD27 IgM-) showed the strongest alterations of proliferation in both unstimulated cultures and in stimulated cultures with CpG and α-IgM crosslinker associated with the presence or absence of CD1d CD5 B cells. For the T-dependent stimulation α-IgM CD40L, the strongest effect was an increase in proliferation of non-class switched memory B cells (CD27 IgM) in absence of CD1d CD5 B cells compared to the original proportion (P=0.059).
Conclusions: These results indicate that CD1d CD5 B cells in humans inhibit the proliferation of B cells, particularly when stimulated in a T-dependent manner. Evidence of IL-10 production in non-CD1d CD5 B cells suggests the existence of further phenotypes of regulatory B cells in humans. The high prevalence of this cell type in early childhood in conjunction with these functional capacities indicates a contribution to better graft acceptance in this age group.

2489

PROBABILITY OF A POSITIVE AHG-CDC CROSSMATCH IS DETERMINED BY THE NUMBER, STRENGTH AND SPECIFICITY OF ANTI-HLA ANTIBODIES

Lan, James; Gill, John; Keown, Paul; (UBC, Vancouver, British Columbia, Vancouver, BC)

Background: The factors determining the relationship between donor-specific antibodies (DSA) and a complement-dependent cytotoxicity (CDC) crossmatch are not clearly defined. Canadian consensus guidelines propose an MFI of 1000 as the clinically acceptable threshold for identification of anti-HLA antibodies via Luminex technology, but whether this value predicts crossmatch (XM) positivity is unknown. We hypothesize that this relationship may depend on the number, class, and strength of antibodies present in recipient serum.
Methods: Serum samples from 120 patients wait-listed for kidney transplantation who had DSA determined by single-antigen testing via the Luminex platform were selected for analysis. DSA strength was expressed as the mean florescent intensity (MFI). AHG-CDC T-cell XM was performed on all samples against 12 different donors and the results recorded as a score of 0 to 8.
Results: Of the 238 samples tested, 40 had a single Class I DSA (mean MFI[SD]: 4353[3925]). Two of the 3 samples (66%) with MFI > 10,000 (MFI 11592, MFI 18256) were associated with a positive AHG-CDC XM, while all 37 samples (100%) with MFI < 10,000 were associated with a negative XM. 31 samples contained multiple Class I DSAs: 13/16 of these samples (81%) with MFI > 5000 were associated with a positive AHG-CDC XM, while all 15 samples (100%) with MFI < 5000 were negative. The relationship between DSA strength and XM reactivity among samples containing a single versus multiple Class I DSA is shown in the graph below. Construction of a receiver-operator curve (ROC) also showed clear discrimination between sera containing a single versus multiple DSA with distinct separation of threshold values for prediction of a positive XM. 38 samples contained only Class II antibodies. None of these were associated with a positive CDC-AHG T-cell XM, independent of the MFI value or the number of DSAs.
Conclusion: For sera containing only a single Class I DSA the threshold for prediction of a positive AHG-CDC XM appears to be an MFI of 10,000, whereas this declines to an MFI of 5000 in sera with multiple anti-donor specificities. These values both differ markedly from the threshold for definition of DSA determined by Canadian consensus guidelines which serve as the basis for current transplant recipient selection.

2491

EXPANSION OF CD25+FOXP3+ REGULATORY T CELLS FROM PEDIATRIC THYMIC TISSUE: POTENTIAL FOR CELLULAR THERAPY?

Dijke, Esmé; McMurchy, Alicia; Levings, Megan; Larsen, Ingrid; Rebeyka, Ivan; Ross, David; West, Lori; (University of Alberta, Edmonton, AB)

Introduction: Infant heart transplant (HTx) recipients have better graft survival than patients transplanted at older ages. Nonetheless, due to need for lifelong therapy, infant HTx recipients carry a heavier immunosuppressive burden, resulting in substantial morbidities from adverse drug effects. Development of cellular therapy using regulatory T cells (Tregs) to suppress graft-directed immune responses would greatly benefit these infants. A major challenge however is difficulty generating a large quantity of stable, highly suppressive Tregs. During infant cardiac surgery, thymectomy is usually performed in order to gain exposure of the retrosternal operative field. We investigated the potential of explanted thymic tissue as a source for isolation and expansion of highly suppressive CD25 FOXP3 Tregs.
Methodology: Thymic tissue (n=8) was obtained from thymectomy during pediatric cardiac surgery; thymocytes were recovered through mechanical dissociation. FOXP3 cells were isolated by automated magnetic cell separation of CD25 thymocytes; CD25-depleted cells were used as controls. Cells were expanded for two weeks by stimulation with anti-CD3, IL-2, rapamycin and CD32 L-cells. FOXP3 and intracellular cytokine staining was performed to define characteristics of expanded cells. The suppressive capacity after expansion was determined by co-culturing expanded cells with anti-CD3/CD28-stimulated peripheral blood mononuclear cells (PBMC) and analyzing proliferative responses by BrdU incorporation followed by Cell Proliferation ELISA.
Results: FOXP3 cell frequency within the total thymocyte population ranged from 2.2 to 3.2%. Isolated CD25 cell populations were 72% positive for FOXP3 (median, range: 60 - 81%). After two weeks of culture, we observed a 16-fold expansion (median, range: 4 - 48-fold) of CD25 cells with >95% viability; 0.4 to 25-fold expansion was observed for control cells with 49–88% viability. The expanded CD25 cells were >90% FOXP3 and produced no IL-2 or IFN-gamma, whereas control cells were <14% FOXP3 and 58–65% produced IFN-gamma. Moreover, in contrast to control cells, expanded CD25 FOXP3 cells were highly potent suppressors, efficiently suppressing proliferating PBMC >50% even at a 1:10 ratio of Tregs:PBMC.
Conclusion: We demonstrated that highly suppressive FOXP3 Tregs can be expanded from CD25 thymocytes isolated from pediatric thymic tissue, indicating that explanted thymuses may be a potential source for isolation and expansion of Tregs for cellular therapy for infant HTx recipients. Future work includes defining the stability of expanded Tregs and their capacity to suppress allo-reactive immune responses.

2492

HYDROGEN SULFIDE HAS LONG-TERM PROTECTIVE EFFECTS AGAINST WARM RENAL ISCHEMIA-REPERFUSION INJURY

Zhu, Justin; (Western University, London, ON), Lobb, Ian; (Department of Microbiology and Immunology, Western University; Matthew Mailing Centre for Translational Transplant Studies, London, ON) Yang, Ethan; Liu, Winnie; Lan, Zhu; Garcia, Bertha; Sener, Alp; (Western University, Canada, London, ON)

Background: The number of patients with end-stage renal disease are expected to increase significantly with the predicted increases in obesity, hypertension and diabetes across the globe. Unfortunately, these patients continue to be at risk for renal cell carcinoma. The treatments offered for renal masses depend upon the size and location of the tumour, and lean towards nephron-sparing partial nephrectomy, which hold particular importance for patients who already have renal dysfunction. The nature of partial nephrectomy requires the clamping of the renal pedicles for up to

45 minutes resulting in warm renal ischemia and reperfusion injury (IRI) upon restoration of blood flow, which injures residual renal tissue and may lead to unnecessary premature dialysis. Hydrogen sulfide (H_2S) is a novel endogenous gasotransmitter that has recently gained reputation for its protective effects against IRI. We recently demonstrated that exogenous administration of H_2S is protective against prolonged cold and short-term warm renal IRI. In the current study, we examined whether exogenous H_2S has long-term protective effects against warm renal IRI to further investigate whether H_2S may be a therapeutic solution to minimize the damage associated with warm renal IRI in chronic renal failure patients undergoing renal oncological procedures.
Materials and Methods: Uni-nephrectomized Lewis rats underwent 1 hour of warm ischemia and 2 hours of reperfusion during intraperitoneal treatment with phosphate buffered saline (PBS; IRI group) (n=6) or PBS supplemented with 150 µM NaHS (H_2S group) (n=6), and were compared with sham-operated rats (n=4). Serum creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels were analyzed at post-operative days 3 and 7 to assess graft function and systemic inflammation. Animals were sacrificed at day 7 and kidneys were harvested for RT-PCR analysis.
Results: H_2S treatment improved long-term graft function as serum Cr at day 7 was significantly decreased in the H_2S group compared to IRI animals ($p<0.05$), though Cr levels in both treatment groups decreased with time. AST and ALT levels were initially elevated in both treatment groups, but subsided to baseline levels at day 3 and remained at baseline at day 7. There were no significant differences in the expression of inflammatory and apoptotic markers TLR4, CCR5, IL8, TNFα, IFNγ, IL2, ICAM1, BCL2, ERK1, ERK2, BID and KIM1.
Conclusion: At day 3, uninephrectomized rats have largely resolved their systemic inflammatory states as shown by resolved AST and ALT levels. At day 7, kidneys in both treatment groups have resumed to their comparable baseline inflammatory states as shown by similar genetic expressions. H_2S was able to confer a long-term renal protective effect against warm IRI, and may offer a novel therapeutic approach to minimizing the detrimental sequelae of prolonged warm renal IRI associated with renal surgery, especially in patients with chronic renal disease.

2493

BLOOD GROUP A TRANSGENIC MICE AS A MODEL FOR ABO-INCOMPATIBLE (ABOI) HEART TRANSPLANTATION (HTX)

Kratochvil, Annetta; Motyka, Bruce; Aizouki, Fadi; Wang, Sheila; Tao, KeSheng; Tollenaar, Stephanie; Bennett, Jody; Marshall, Thuraya; (University of Alberta, Edmonton, AB), d'Apice, Anthony; Cowan, Peter; (Immunology Research Centre, Melbourne, Australia), West, Lori J.; (University of Alberta, Edmonton,AB)

Introduction: Our group demonstrated that infant ABOi HTx results in development of immune tolerance to the donor ABO antigen(s). We recently developed mice transgenic for expression of A- and H-glycosyltransferases (AH-Tg, C57/BL6 (B6) background). By lectin staining, AH-Tg mice express the blood group A-antigen on vascular endothelium of solid organs including heart. Human blood group A into O heart transplantation can be approximated using AH-Tg mice as donors and B6 wild-type (WT) mice as recipients. This model will allow detailed study of mechanisms of B cell tolerance, AMR and accommodation. Herein we sought to characterize 'natural' and induced anti-A antibodies (Abs) in WT B6 mice and binding to A-expressing AH-Tg hearts, and to investigate AMR following transplantation of AH-Tg heart grafts into WT B6 mice with high anti-A titre. We hypothesize that serum from WT mice with high anti-A titre will bind to AH-Tg cardiac tissue, and that AH-Tg heart grafts will undergo AMR following transplantation into WT recipients with high anti-A Abs.
Methods: Juvenile WT B6 mice were induced to produce anti-A Abs by intraperitoneal injection of blood group A red blood cells. Sera from WT and AH-Tg mice of different ages were assessed for anti-A Ab titres by hemagglutination assay. Binding of serum anti-A Abs to AH-Tg hearts was determined by immunohistochemistry. AH-Tg heart grafts were heterotopically transplanted into WT recipients with high anti-A titre and AMR assessed by immunohistochemistry.
Results: We identified a trend of increasing anti-A Abs in WT mice with increasing age (n=108). No AH-Tg mice had detectable anti-A Abs (n=19). Using a commercial anti-A Ab, we detected A-antigen on vascular endothelium of hearts,

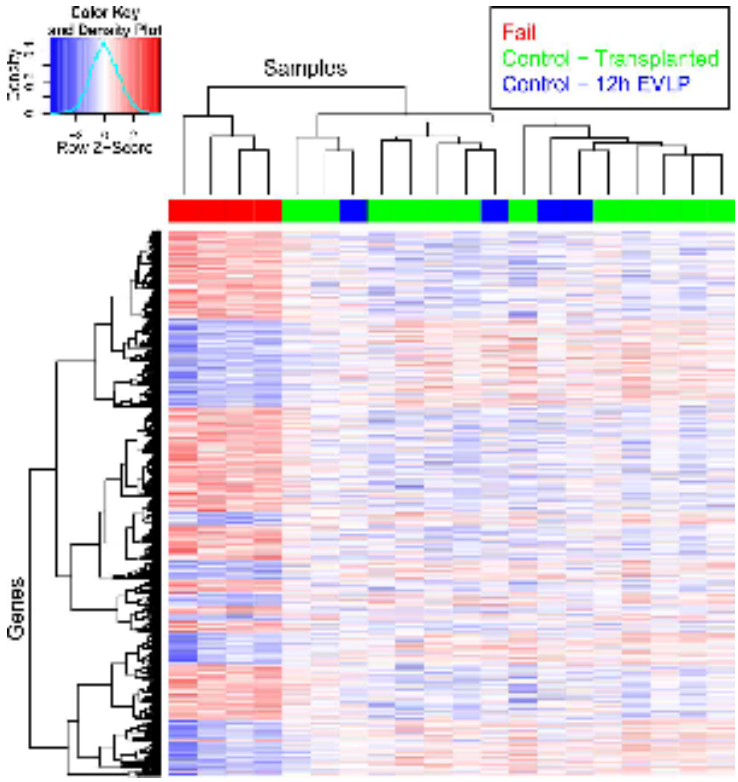
kidneys, livers, and lungs from AH-Tg mice. Serum from older WT mice with natural anti-A Abs or from younger A-antigen-sensitized WT mice bound to AH-Tg but not WT cardiac tissue.
Conclusions: This study provides insight into the development of anti-A Abs in WT B6 mice and confirms A-antigen expression on AH-Tg cardiac endothelium by serum anti-A Abs. Studies are ongoing to examine AMR following transplantation of AH-Tg heart grafts into WT mice with high anti-A titre.

2498

DISCOVERY OF MRNA BIOMARKERS PREDICTING DONOR LUNG FAILURE

Zamel, Ricardo; Machuca, Tiago; Yeung, Jonathan; Bonato, Riccardo; Bai, Xiao-Hui; Waddell, Thomas; Liu, Mingyao; Cypel, Marcelo; Keshavjee, Shaf; (University Health Network, Toronto, ON)

Introduction: Normothermic ex vivo lung perfusion (EVLP) is a new method to assess and improve the quality of donor lungs prior to committing to transplant. We have increased the time a lung can stably undergo EVLP from 4 to 12 hours in the laboratory. In order to discover biomarkers that can predict the likelihood of poor outcome from the donor lung, we have employed a genome-wide RNA expression study focused on different phenotypes related to EVLP performance.
Methods: We collected lung biopsies at end of cold ischemic time (CIT) of brain death donor lungs which were unable to stably complete an extended (12 h) EVLP (n = 4), which stably completed extended (12 h) EVLP and passed clinical criteria for transplant, despite not being transplanted (n = 4), and which stably completed 4 h EVLP, were transplanted and produced good recipient outcome (n = 12). RNA expression was analyzed with Affymetrix U133plus2 microarrays.
Results: Clustering and differential expression analyses suggest that lungs able to complete 12h EVLP are very similar to successfully transplanted lungs with good outcome. In contrast, lungs failing to complete 12 h EVLP form a distinct group. For further analysis we treated the former 2 groups as a single group (control) for comparison with the latter group (fail). After multiple testing correction and setting a significance cut-off of a false discovery rate (FDR) below 5%, we found over 1300 genes that displayed statistically significant expression differences between control and fail groups. Figure 1 shows a hierarchically clustered heatmap of these genes, demonstrating the distinctiveness of the fail lungs. Although the sample size of fail lungs is small, this level of significance is well above what would be expected by chance due to sampling error alone. Of these genes, 119 had relative fold differences above 2 fold, 278 had no overlapping intensity values between groups: area under ROC curve (AUC) = 1, and 29 genes had both greater than 2 fold differences and AUC = 1. These represent potentially highly predicative biomarkers of lung quality.
Conclusions: By selecting lungs with extreme EVLP performance phenotypes, we have found that extended EVLP may provide a good indication of transplant outcome. Analysis of expression profiles between these phenotypes indicates large differences at the transcript level and has provided several potential mRNA biomarkers of transplant lung failure or success.

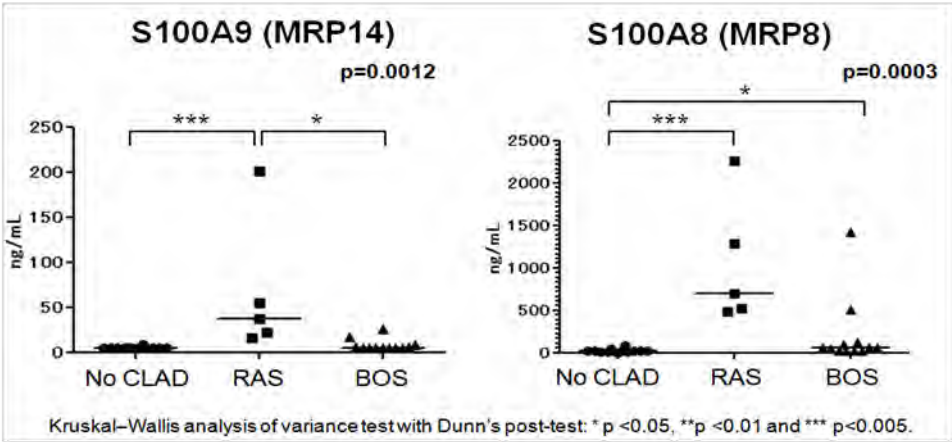


2502

DAMAGE-ASSOCIATED MOLECULAR PATTERN MOLECULES IN HUMAN CHRONIC LUNG ALLOGRAFT DYSFUNCTION: POTENTIAL MARKERS FOR BIOLOGIC SUBTYPING

Saito, Tomohito; Liu, Mingyao; (Latner Thoracic Surgery Research Laboratories, University Health Network, University of Toronto, Toronto, ON), Binnie, Matthew; (Division of Respirology, University of Toronto, Toronto, ON), Sato, Masaaki; (Department of Thoracic Surgery, Kyoto University, Kyoto, Japan), Azad, Sassan; N Machuca, Tiago; Matsuda, Yasushi; Cypel, Marcelo; Waddell, Thomas; Keshavjee, Shaf; (Latner Thoracic Surgery Research Laboratories, University Health Network, University of Toronto, Toronto, ON)

Purpose: Chronic lung allograft dysfunction (CLAD) remains a major cause of mortality and morbidity after transplantation. As a novel form of CLAD, restrictive allograft syndrome (RAS) demonstrates distinct clinical and pathological features from the previously identified CLAD known as bronchiolitis obliterans syndrome (BOS). Our previous study on proteomic analysis of bronchoalveolar lavage fluid (BALF) indicated that damage-associated molecular pattern molecules (DAMPs) such as S100 family proteins may be potential biomarkers for human CLAD. We aimed to further characterize human CLAD by quantifying S100 family in BALF.
Materials and Methods: 17 consecutively identified specimens from patients with CLAD (5 RAS, 12 BOS with no evidence of concomitant infection or acute rejection, median 44 months after lung transplantation) and 12 control recipients with no CLAD were included in this study. In total, 29 banked BALF specimens were analyzed. All the BALF specimens from CLAD cases had been taken after their clinical onsets. RAS was defined as CLAD (irreversible decline in FEV1 < 80% baseline) with irreversible decline in total lung capacity (TLC< 90% baseline). BOS was defined as CLAD without RAS. S100A8, S100A9, S100A12 and S100P expression were measured in BALF by enzyme-linked immunosorbent assay.
Results: All of S100A8, S100A9, S100A12 and S100P were upregulated in RAS compared with no CLAD patients. S100A9 was elevated in RAS compared to BOS. In contrast, expression of S100A8 was elevated in BOS compared with no CLAD patients.
Conclusions: In CLAD patients, we have noted a distinct difference in DAMPs such as the expression of S100 family proteins in BALF. It appears that S100A9 is associated with RAS and not BOS. Hopefully, further characterization of molecular pathways involved in the development of CLAD subtypes will help to develop more accurate diagnostics and specifically directed therapies for patients with underlying CLAD.



2504

MFG-E8 RELEASE BY APOPTOTIC ENDOTHELIAL CELL MICROENVIRONMENT REPROGRAMS MACROPHAGES INTO PRO-FIBROTIC CELLS

Brissette, Marie-Joëlle; Cailhier, Jean-Francois; (CHUM, Montréal, QC)

Chronic transplant vasculopathy (CTV) is an important cause of interstitial fibrosis and tubular atrophy in the renal allograft. It is characterized by the presence of apoptotic endothelial cells (EC), which generate a microenvironment that leads to the typical myointimal proliferation found in CTV. Macrophages play an important role in CTV. We previously demonstrated that this microenvironment reprogramed murine macrophages into anti-inflammatory, pro-repair phagocytes through Milk Fat Globule Epidermal Growth Factor-8 (MFG-E8) release. We now want to evaluate if these observations can be replicated in a human system and elucidate the role MFG-E8 in human macrophage reprogramming and in a CTV in vivo model.
To model the CTV environment, human EC were serum-starved for 4 h to create an apoptotic serum-starved conditioned medium. To evaluate the role of MFG-E8, we used human recombinant MFG-E8 to highlight the role of this protein in macrophage reprogramming specifically. We also studied the role of MFG-E8 in a fully mismatched murine aortic transplantation model.
We demonstrated that apoptotic human endothelial cells release MFG-E8 in a caspase-3-dependent manner. The anti-inflammatory phenotype of human macrophages was lost when caspase-3 was inhibited in EC prior to serum starvation. Recombinant human MFG-E8 recapitulated the anti-inflammatory reprogramming of human macrophages. Finally, transplantation of MFG-E8 KO aortas into BALB/c resulted in increased myointimal proliferation compared to MFG-E8 WT aortas.
Our study demonstrates an important role for the release of MFG-E8 from apoptotic endothelial cells in human macrophage reprogramming and the importance of the apoptotic microenvironment in an anti-inflammatory pro-repair macrophage response. These results suggest that MFG-E8 could be considered as a tissular analarmin that can attenuate the pro-inflammatory macrophage reprograming promoting in a balanced inflammatory microenvironment resulting in reduced tissue injury.

2505

A PHOSPHO-SPECIFIC FLOW CYTOMETRY ASSAY TO DEFINE THE ROLE OF THE INHIBITORY MOLECULE CD22 ON INFANT CD27+IGM+ B-CELLS, CONTAINING ABO ANTIGEN-SPECIFIC ANTIBODY-SECRETING CELLS, IN THE DOWN-REGULATION OF B-CELL SIGNALING

Derkatz, Kimberly; Dijke, Esme; Motyka, Bruce; West, Lori; (University of Alberta, Edmonton, ON)

Introduction: Immune immaturity allows ABOi HTx to be performed safely in infants and results in B-cell tolerance to donor ABO antigens (ags). ABO antibodies are thought to arise in a T-independent (TI) manner; in experimental settings, TI B-cell activation has been shown to be inhibited by binding of the B-cell co-receptor CD22 to CD22L (sialic acids), leading to B-cell tolerance. Since immunity to TI-ags (including ABO) is generally reduced in early childhood, we hypothesize a role for CD22, possibly contributing to ABOi HTx tolerance. Previously, we showed that CD22 expression is higher on CD27 IgM B cells in infants when compared to older individuals. In this study we performed functional assays with CD27 IgM B cells to assess the presence of ABO ag-specific IgM antibody-secreting cells (ASC). In addition, we established a PhosPho Flow assay (PPF) to assess B-cell signaling by examining intracellular protein phosphorylation of the B-cell receptor (BCR) and CD22 upon stimulation.

Methods: CD27 IgM B cells and CD27-IgM B cells were isolated from human splenocytes (n=3 infant, n=6 adult) by magnetic cell sorting, labelled with proliferation dye and stimulated with CpG plus IL-2,10 and 15. After one week, the frequency of ABO ag-specific ASC was detected by ELISPOT. The PPF was optimized using a Ramos B cell line. Intracellular protein phosphorylation of CD22(pY822), located downstream of CD22, and PLCγ2(pY759), located downstream of the BCR, were examined after stimulation with anti-IgM at various times (0-60min).
Results: Though the total number of ASCs was lower in infant samples compared to older individuals, ELISPOT analysis revealed that the majority of ABO ag-specific ASCs were derived from CD27 IgM B cells in both groups. A representative example is depicted in *Figure*. Preliminary results by PPF (n=8) showed PLCγ2(pY759) signaling beginning at 0.5min and peaking at 4min, with a 7-fold increase (median, range: 6-15 fold) in Median Fluorescence Intensity (MFI) of pY759. CD22(pY822) signaling began at 2min with a modest peak at 12min, with a 1.6-fold increase (median, range: 1-4 fold) in MFI of pY822.
Conclusion: The overall increased expression of CD22 on infant CD27 IgM B cells, which includes ABO ag-specific ASC precursors, may increase their susceptibility to decreased signalling, leading to inactivation. CD22 may therefore play an inhibitory role in infant immune responses to ABO ags after ABOi HTx resulting in B-cell tolerance. Future PPF studies will enable us to assess B-cell signaling within the CD27 IgM infant B-cell subset by examining phosphorylation of the intracellular proteins CD22(pY822) and PLCγ2(pY759) upon stimulation with anti-IgM in the presence/absence of synthetic CD22 ligands.

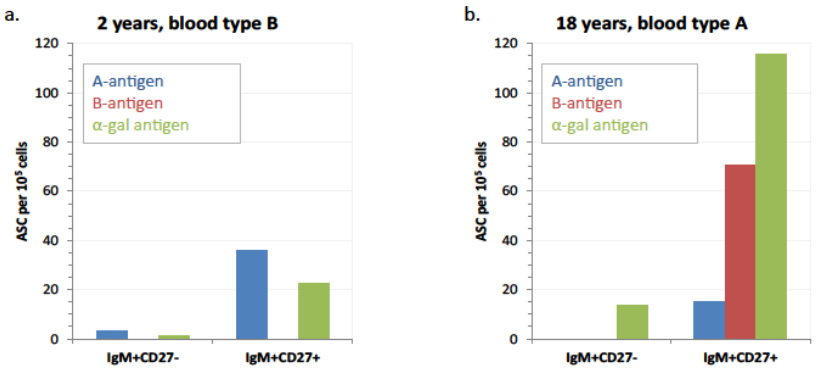


Figure: Presence of ABO antigen-specific IgM antibody-secreting cells (ASC).
IgM^+CD27^- and IgM^+CD27^+ splenocytes were isolated and cultured for 7 days with CpG and cytokines. ELISPOT analysis revealed that the vast majority of ABO antigen-specific IgM ASCs were derived from the $CD27^+IgM^+$ B cell population. Two examples are shown: **(a)** 2 year old, blood type B individual – we detected only A antigen-specific ASC. **(b)** 18 year old, blood type A individual – we detected only B antigen-specific ASC. In both samples, α-gal antigen-specific ASCs were detected as expected. The α-gal epitope is synthesized on glycolipids and glycoproteins of non-primate mammals and New World monkeys.

2507

FORMATION OF ENDOTHELIAL MICROPARTICLES IN ISCHEMIC ACUTE KIDNEY INJURY

Fairhead, Todd; Burger, Dylan; Boucher, Jonathan; Slatculescu, Andreea; (Ottawa Hospital Research Institute, Ottawa, ON)

Background: Ischemia-reperfusion injury (IRI) of kidney allografts is unavoidable at the time of transplantation and places the graft at risk for initial dysfunction and a poor long-term outcome. Endothelial injury in (IRI) is characterized by changes in microvascular bloodflow, coagulation, and permeability and contributes to tubular

epithelial injury and subsequent chronic kidney disease. Microparticles (MPs) are small, anuclear fragments shed from the cell membranes under conditions of stress/damage. MPs have procoagulant activity and may promote crosstalk between cell types. Endothelial MPs are found at low concentrations in the plasma of healthy subjects and at increased concentrations in conditions of chronic vascular injury such as vasculitis and chronic kidney disease. However, it is not known whether MP levels are acutely increased after IRI. We hypothesized that endothelial MP levels would be increased in AKI and would contribute to inflammation.

Methods: We examined MP formation in male C57BL6 mice subjected to bilateral renal ischemia-reperfusion (I/R) for 45 minutes. Blood was collected 2 hours after reperfusion and MPs were isolated from platelet-free plasma by differential centrifugation. MPs were then quantified by flow cytometry and distinguished as VE-Cadherin ve (Endothelial MPs) and/or Annexin V ve (Total MPs) events ranging between 100-1000 nm in size.

Results: Compared with sham-operated mice, mice subjected to I/R displayed significantly higher plasma levels of endothelial microparticles (3149±902 MPs/ml vs.1059 ±152, $P<0.05$). Conversely, the total number of circulating microparticles was not significantly altered (6009±2126 vs. 6804±2126, $P<0.05$). To assess whether endothelial MPs could activate neighboring endothelial cells, MPs collected from human microvascular endothelial cells (HMVECs) were incubated with healthy HMVECs in culture. After 12 and 24 hours, no difference in endothelial expression of CD31 (PECAM), or CD144 (VE-Cadherin) was observed.

Conclusions: Our results show that endothelial microparticles are increased early after reperfusion in an animal model of schema-reperfusion injury. Such increases may be indicative of underlying vascular injury, and could serve as an early biomarker of IRI in transplantation.