BOOK OF ABSTRACTS

LIVRE DES RÉSUMÉS

CANADIAN SOCIETY OF TRANSPLANTATION
SOCIÉTÉ CANADIENNE DE TRANSPLANTATION

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RÉUNION SCIENTIFIQUE ANNUELLE

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Dr. John Gill, Conference Chair
Dr. Kathryn Tinckam, Conference Co-Chair

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ABSENCE OF INCREASED ACUTE DIALYSIS RISK IN LIVING KIDNEY DONORS

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Reduced kidney function confers a higher risk of acute kidney injury at the time of an inciting event, such as sepsis. Whether the same is true in those with reduced renal mass from living kidney donation is unknown. We conducted a population-based matched cohort study of all living kidney donors in Ontario who underwent donor nephrectomy from 1992 to 2009. We manually reviewed the medical records of these living kidney donors and linked this information to provincial healthcare databases. Non-donors were selected from the healthiest segment of the general population. There were 2027 donors and 20,270 matched non-donors. The median age was 43 years (interquartile range, 34 to 50) and individuals were followed for a median of 6.6 years (maximum 17.7 years). The primary outcome was acute dialysis during any hospital stay. Reasons for hospitalization included infectious diseases, cardiovascular diseases, and hematological malignancies. Only one donor received acute dialysis in follow-up (6.5 events per 100,000 person years), a rate which was statistically no different than 14 non-donors (9.4 events per 100,000 person years). These results are reassuring for the practice of living kidney donation. Longer follow-up of this and other donor cohorts will provide more precise estimates about this risk.

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ORGAN AND TISSUE DONATION REQUIRED REFERRAL POLICY DEVELOPMENT IMPLEMENTATION AND EVALUATION

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Purpose: This report discusses the development, implementation and evaluation of a required referral policy for organ and tissue donation implemented in June 2010 in the district.
Methods: In 2007, a full time District Resource Nurse position was created in the District. The goal of this position was to provide education, develop policies, perform chart audits, and promote processes to thereby increase referrals for donation. In 2008 completed chart audits identified the majority of potential donors were not being referred and screened for donation. Therefore, a policy to guide this practice was developed over a two-year period, and approved in April 2010. An implementation plan rolled out between April and June. This included media and professional education strategies supported by tools such as posters, presentations, changes to the health record forms and clinical triggers.
Results: A year to year comparison identified an increase in tissue referrals (from 33 to 125) and actual donors (from 10-20) between 2009 and 2010. In addition 100% of potential organ donors were identified and screened with no missed organ donors. The number of facilities making referrals increased from three to seven. More work needs to be done to close the continuing gap between number of deaths and numbers of referrals. Staff feedback indicated lack of knowledge and fear as noncompliance factors. Barriers and challenges included low attendance for education, staff resistance, geography, and time. There were also challenges with physician engagement.
Recommendations: The district should add the absolute contraindications for tissue referrals to educational materials and offer ongoing education. Regular unit and site-specific reporting for managers and senior leaders on referral data and policy compliance should be implemented. Leanings can inform the roll out of the new Human Organ and Tissue Donation Act, which has a required referral provision, expected to be proclaimed in 2012.

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SMOKING INCREASES RECURRENT HEPATITIS AND DEPRESSION AFTER LIVER TRANSPLANTATION

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Aims: Smoking is a common behavior among transplant candidates. The aim of this study is to evaluate the effect of smoking on the incidence of a range of complications after liver transplantation.
Methods: The charts of liver transplant recipients were reviewed using the McGill University Health Centre liver transplant database over a 14-year period. Demographic characteristics such as age, sex, race, body mass index, smoking status, etiology of liver disease, Child-Pugh score

and MELD score at the time of transplant were recorded. Perioperative complications in both the short and long term were recorded. Chi-square analysis and multivariate analysis were performed using the SAS program version 9.2.
Results: 444 liver transplants had occurred between 1990 and 2004 (54.8±11.5 years, 66.4% male, mean BMI: 26.4±5.8, mean MELD score 25.2±10.7, mean Child-Pugh score 11.2±1.8, active smokers 22.3%, non-smokers 74.8%, ex-smokers 2.9%). Smokers were more likely to be male (79.8% vs 62.6%, p=0.0014) and to be of Caucasian race (87.9% vs 77.1%, p=0.0192). The etiology of liver disease was more likely to be alcohol among the smokers and ex-smokers as compared to the non-smokers (26.3% vs 13.9%, p=0.0037). Non-smokers survived an average of 6.05+/-0.24 years, and smokers survived 5.93+/-0.43 years (p=0.81).
Viral hepatitis tended to recur more often among those smokers who had been transplant for viral hepatitis (28.6% vs 11.7%, p=0.01). Depression was significantly more common among smokers and ex-smokers than among non-smokers (12.1% vs 6.1%, p=0.043). No other complications, including patient mortality, vascular complications, or rejection, were found be associated with smoking status. Multivariable analysis revealed that alcohol (OR 14.2; 95% CI [1.7-116.1]), hepatocellular carcinoma (HCC) (OR 14.9; 95%CI (1.69-130.85)), PBC (OR 10.8; 95% CI [1.1-109],), and hepatitis C (OR 10.1; 95%CI (1.3-81.2)) were particularly predictive of depression. Smoking (OR 2.77; 95%CI (1.14-6.73)) and Caucasian race (OR 3.97; 95%CI (1.48-10.63)) were predictive of recurrent viral hepatitis.
Conclusion: Our study shows that smokers had a higher rate of recurrent viral hepatitis after transplantation, which may be explained by the adverse effects of tobacco on both structural and immunologic host defenses. Depression was more common among smokers after liver transplant, although alcohol and hepatitis C were likely confounders for this association. There was a trend towards increased survival among non-smokers. These findings indicate the need to encourage smoking cessation in at least targeted populations prior to liver transplant.

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LATE CYTOMEGALOVIRUS TRANSMISSION AND IMPACT OF T-DEPLETION IN CLINICAL ISLET TRANSPLANTATION

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The epidemiology of Cytomegalovirus infection (CMV) in islet transplantation is not well defined. The current study defines incidence, transmission and clinical sequelae of CMV reactivation or disease in 121 patients receiving 266 islet infusions at a single institution. The donor (D)/ recipient (R) serostatus was D+/R- 31.2%, D+/R+ 26.3%, D-/R+ 13.2% and D-/R- 29.3%. CMV prophylaxis with oral ganciclovir or valganciclovir was given in 68%. CMV infection occurred in 14/121 subjects (11.6%); six had asymptomatic seroconversion and 8 others had positive viremia (6 asymptomatic and 2 with CMV febrile symptoms). Median peak viral loads were 1,755 copies/ml (range 625 – 9,100,000). Risk factors for viremia included lymphocyte depletion (thymoglobulin or alemtuzumab, p<0.001). Viremia was more common in D+/R+ vs. D+/R- (p=0.12), occurring mostly late after transplant (median 306 days). Presumed transmission from islet transplantation occurred in 8/83 of D+/R- procedures (9.6%). Of the 2 cases of CMV disease, one resulted from islet transmission from a CMV positive donor (D+/R-); the other was due to de novo exogenous infection (D-/R-). Therefore CMV transmission presents rarely after islet transplantation and with low incidence compared to solid organ transplantation, but occurs late posttransplant. The use of lymphocyte depleting therapies is a primary risk factor.

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DO PREOPERATIVE FUNCTIONAL MOBILITY AND MELD SCORE AFFECT LENGTH OF STAY FOLLOWING LIVER TRANSPLANTATION?

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Background: Functional capacity in end-stage liver disease patients is difficult to quantify. The Six Minute Walk Test (6MWT) correlates well to VO_2 max testing and is commonly used in the assessment of other organ transplants to predict clinical outcomes. Patients awaiting liver transplantation (LTx) have the Model for End Stage Liver Disease (MELD) score as an index of disease severity which is correlated to mortality. Our purpose was to examine the 6MWT in LTx candidates and correlate this to the MELD score and length of stay (LOS) in hospital post-transplantation.
Methods: Adult candidates for LTx completed the 6MWT and had a MELD score calculated pre-transplantation. LOS data were collected on discharge from hospital following transplantation.
Analysis: Simple bivariate correlations explored the relationships between 6MWT distance, MELD score, and LOS in hospital post-transplantation.

Results: The current sample of 16 subjects (mean age of 48.6, 32% female), had a mean MELD score of 18 (95%CI 15.4 to 20.5), and mean 6MWT of 466.5m (95%CI 396.9 to 526.6m). The correlations between 6MWT distance and MELD scores, and LOS and MELD scores were non-significant. There was a trend towards a negative correlation between 6MWT and LOS ($r = -0.48$, $p = 0.06$).
Conclusions: Traditional evaluation for liver transplantation has not routinely included an assessment of functional capacity. The results of this interim analysis suggest that MELD score does not predict the physical fitness of a liver transplant candidate, nor LOS following transplantation. The findings suggested that those performing better on the 6MWT tended to have shorter LOS following transplantation. A larger sample size is currently being evaluated. The 6MWT is an inexpensive and practical tool that measures functional mobility and may be utilized to optimize outcomes and cost and resource utilization.

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EVALUATING THE USE OF BASILIXIMAB IN LOW IMMUNOLOGICAL RISK TRANSPLANT PATIENTS

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Background: The 2009 Kidney Disease: Improving Global Outcomes practice guidelines for renal transplant recommended the use of basiliximab in all low to moderate immunological risk renal transplant patients. However, the evidence to support this in the era of triple immunosuppression with tacrolimus, mycophenolate and prednisone is lacking. This study evaluated the use of basilixmab in a large Canadian renal transplant program.
Methods: We retrospectively analyzed data from 112 low immunological risk renal transplant patients, 44 of whom received basiliximab induction and 68 received no induction. The primary outcome of interest was kidney function estimated by calculated creatinine clearance. Secondary outcomes included delayed graft function, biopsy proven acute rejection, de novo diabetes, opportunistic infections, and de novo malignancies.
Results: Calculated creatinine clearance was higher in the basiliximab group at one week versus the no induction group (68 mL/min versus 56 mL/min, $P = 0.039$). Calculated creatinine clearance at 1 month, 3 months, 6 months, 9 months and 12 months post transplant was similar between the two groups. Delayed graft function trended to favour the basiliximab group, but the difference was not significant (11.5% versus 20.6%, $P = 0.204$). However, biopsy proven acute rejection was significantly higher in the no induction group (2.3% versus 13.2%, $P = 0.047$, NNT: 9). The number of de novo diabetes cases was higher in the basiliximab group, but this difference was not significant (18.2% versus 11.8%, $P= 0.343$). The incidence of opportunistic infections and malignancies were similar between the groups and occurred infrequently.
Conclusions: Basiliximab induction in low immunological risk renal transplant patients concurrently receiving tacrolimus, mycophenolate and prednisone improves the estimated renal function at week 1 post transplant, but not at subsequent time points, and reduces the risk of biopsy proven acute rejection.

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ACUTE RENAL FAILURE IN CADAVERIC KIDNEY DONORS: IMPACT ON KIDNEY GRAFT FUNCTION

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The shortage of kidneys from cadaveric donors has led to an increase use of marginal donors. The purpose of this study is to evaluate if the utilization of kidneys from donors with acute renal failure (ARF) is safe and give acceptable renal function up to 1 year after transplantation. We reviewed all cadaveric kidney transplantations performed in our center from January 2006 to december 2010. Using the AKIN definition for ARF, we found 25 donors with acute kidney injury. These 25 donors gave kidneys to 29 recipients. Two recipients lost their grafts because of thrombosis and were excluded from subsequent analysis. The donors were mostly male (19/25), their mean age was 45 ± 13 years and the mean creatinine level at time of harvesting was 223 ± 145 µmol/L. The recipients comprised 14 woman and 15 men and their mean age was 53 ± 14 years. After the transplantation, 5 patients had delayed graft function and 11 patients had a slow recovery of function defined has a decrease of creatinine levels less than 10% of the pre transplant level for 3 consecutive days. Three patients had biopsy proven acute rejection. The mean creatinine levels at 1, 3. 6 and 12 months were 140 ± 43, 126 ± 37, 122 ± 38, and 123 ± 34 µmol/L respectively. In conclusion, the use of donors with acute renal failure can be safe and give good renal function up to one year after transplantation. Further studies are needed to evaluate long term function of these grafts and better characterized the baseline profile of the ARF donors. Comparison of this group of recipients with recipients transplanted in the same period with extended criteria donors not in ARF is underway.

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PHARMACOGENETICS OF TACROLIMUS IN PEDIATRIC SOLID ORGAN TRANSPLANT RECIPIENTS

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Achieving and maintaining therapeutic tacrolimus levels is challenging. Tacrolimus is metabolized by primarily by *CYP3A5*. A/G substitution (*CYP3A5*3/*3*) results in a non-expressor genotype with slow drug clearance. We assessed the association of *CYP3A5* genotype with tacrolimus bioavailability and clearance in pediatric transplant patients.

Methods: In a prospective cohort study of pediatric solid organ transplant recipients enrolled in the SickKids Transplant Centre Biobank, we assessed out-of-range levels and tacrolimus pharmacokinetic (PK) profile i.e. post-dose 12-hour area-under-the-curve (AUC) values between *CYP3A5* expressors and non-expressors.

Results: We assessed 37 cardiac transplant patients during 14 day post-transplant follow-up (8 expressors, 29 non-expressors). Mean tacrolimus level was higher (10.5 vs 7.2 ng/ml, p=0.003) while mean tacrolimus dose was lower (0.07 vs 0.13 mg/kg/dose, p=0.008) in non-expressors vs expressors. Time to reach therapeutic levels was 11 days in non-expressors and 14 days in expressors (p=0.34) with over 50% out-of-range levels in both groups 14 days after transplant. Seventeen patients (8 heart, 5 kidney, 2 liver, 1 lung, 1 mixed transplant) underwent PK testing on follow-up (median age 9.5 years; median time from transplant, 4.7 mos). 4 were *CYP3A5* non-expressors *(*3/*3*), 13 were expressors (**1/*3* or **1/*1*). Compared to expressors, *CYP3A5* non-expressors had higher median peak i.e. 2-hour post-dose levels (p=0.032). The AUC corrected for dose/kg was higher in non-expressors vs. expressors (0.032 vs 0.018 mcg*h/l/mg/kg, p=0.09), and was higher in patients >6 yrs vs < 6 yrs (0.024 vs 0.014 mcg*h/l/mg/kg, p=0.03). Taken together, *CYP3A5* non-expressors >6 years old had the highest AUC (0.042 mcg*h/l/mg/kg) compared to all other groups (p<0.05).

Conclusion: Age and *CYP3A5* genotype are important determinants of tacrolimus PK and should be used to individualize tacrolimus starting dose. A prospective genotype-guided trial of tacrolimus dosing in pediatric solid organ transplant recipients is underway with the goal of reducing out-of-range concentrations in the early post-transplant period.

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SUCCESSFUL OUTCOME OF TRANSPLANTATION FROM A MULTIORGAN DONOR WITH HERPES ENCEPHALITIS

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Introduction: Viral encephalitis is generally recognized as a contraindication to organ donation because of the risk of transmitting an unknown viral infection to the organ transplant recipients

Methods: We report a case of a multiorgan donor who died from herpes virus encephalitis in whom lungs, the heart, the liver and both kidneys were used for transplantation with a successful outcome for all recipients at short-term follow-up.

Results: A 30-year-old woman presented herself to the emergency room with nausea, vomiting, headache and fever of 39 degrees Celsius. Soon after, she became confused and developed seizures. A brain CT scan showed cortical and sub-cortical multiple hypodense lesions. A lumbar puncture showed herpes virus at PCR (polymerase chain reaction). She was treated with intravenous acyclovir (1100 mg every 8 hours). Unfortunately, she was declared brain dead 3 days later. Although, several centers declined the offer for transplantation because of the documented herpes encephalitis, the heart, both lungs, the liver and the two kidneys were used for organ transplantation.

All transplant recipients were treated with acyclovir and had an uneventful postoperative course.

Conclusion: We suggest that herpes virus encephalopathy should not be considered a contraindication for organ donation if an appropriate treatment is given to both the donor and recipients.

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DONOR AGE MODIFIES THE EFFECT OF DELAYED GRAFT FUNCTION ON LONG-TERM OUTCOMES

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Background: It is largely admitted that delayed graft function (DGF) has a deleterious effect on graft survival. However, recent analyses of data from donor after cardiac death (DCD) kidneys suggest that such kidneys from young donors tolerate DGF significantly better than standard-criteria donor (SCD) or extended-criteria donor (ECD) kidneys from donors after brain death (DBD). The aim of this study was to examine whether donor age influences the association between DGF and long-term outcomes in DBD kidneys.
Methods: 680 consecutive subjects who received a DBD transplant between January 1990 and July 2005 were reviewed in this single-center, observational, retrospective study. We stratified the cohort according to donor age (below 40yrs, 41 to 50yrs, 51 to 60yrs and above 60yrs) and studied the association between DGF and uncensored and death-censored graft survival using proportional hazard models, adjusted for recipient age, diabetes, induction therapy, donor eGFR and cold and warm ischemia times.
Results: Median follow-up was 106mo (range, 1-233mo). 122 transplant recipients needed postoperative dialysis (DGF+). Through July 2010, we identified 195 and 85 uncensored and death-censored graft losses, respectively. Compared to subjects who did not receive postoperative dialysis (DGF-), DGF+ were older, more likely to be diabetic, to have received induction therapy and suffered longer cold and warm ischemia times. Donors of DGF+ recipients were older (43±20 *vs.* 37±18yrs; p<0.01) and had lower eGFR (90±33 *vs.* 104±37mL/min; p<0.01). Adjusted HR (95% CI) of uncensored graft survival in DGF+ compared to DGF- recipients was 0.98 (0.51-1.78) for donors aged under 40yrs, 1.53 (0.67–3.53) for donors 41 to 50yrs, 0.83 (0.31–2.20) for donors 51 to 60yrs and 2.53 (1.13–5,64; p=0.02) for donors >60yrs. Analyses of death-censored graft survival yielded similar results for donor age below 60yrs (HR and 95% CI 0.80, 0.23–2.81; 1.92, 0.65–5.67 and 1.06, 0.31–3.57 for donor age <40, 41–50 and 51–60yrs respectively) and showed a substantially increased risk for DGF+ recipients of donors >60yrs (HR 6.06, 95% CI 1.82–20.17; p<0.01). In an adjusted model using donor age as a binary variable (below or equal 60yrs *vs.* above 60yrs), the P value for interaction between donor age and DGF was 0.02 for death-censored graft survival.
Conclusion: This analysis reveals that the impact of DGF on graft survival from DBD is modified by donor age. Results further suggest that kidneys from donors above 60 years old where DGF is expected might need to be used with caution.

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CARDIAC OPTIMISATION OF DONORS, RESULTS FROM THE FIRST 3 YEARS IN QUÉBEC.

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Shortage of organs is well documented and there are many potential recipients who die waiting for a heart each year. During brain death, cardiac dysfunction can occur preventing the use of the donor's heart for transplantation. Beginning in 2007, a standardized donor management protocol was implemented in Quebec donors with the goal of improving cardiac function in potential donors with low ejection fraction. This protocol includes use of a Swan-Ganz catheter and hormonal resuscitation with levothyroxine, methylprednisolone, vasopressin, and insulin.
The goal of this study was to determine the effect of the management protocol on cardiac function and its impact on transplantation. We also sought to evaluate its impact on survival of the heart recipients.
Methods: This project was a retrospective analysis of 42 brain dead donors between June 2007 and September 2010, in whom the standardized management protocol was applied. The characteristics of patients, hemodynamic data, management and echocardiographic parameters were analyzed.
Results: Causes of death were traumatic (38.1%), intra-cerebral hemorrhage (33.3%), anoxic (14.3%), gunshot (4.8%), ischemic stroke (7.1%), or cerebral edema (2.4%). Hormonal resuscitation was carried out in 90% of cases and Swan-Ganz catheter was used in 40%. This management protocol resulted in improvement of the ejection fraction of at least 10% in 14 cases (33%). It resulted in worsening of the ejection fraction of ≥ 10% in 3 cases (7%) and was neutral (variation of ± 9%) in 25 cases (60%). Application of the management protocol with hormonal resuscitation therapy resulted in 16 transplantations. No other organ was lost when the protocol was used. One-year survival of the 16 transplanted patients was comparable to that of cardiac transplant recipients published by the ISHLT.
Conclusion: This standardized management protocol with hormonal resuscitation was able to increase to number of heart transplantation of 2 to 5 hearts transplantations each year in the province of Québec without negative effects on survival of recipients of those hearts. No other organ was lost with the use of this management protocol.

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ACUTE VASCULAR REJECTION OF RENAL ALLOGRAFTS IS ASSOCIATED WITH INCREASED PERLECAN PROTEOLYSIS AND ANTI-PERLECAN ANTIBODIES

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Background: Acute vascular rejection (AVR) is characterized by immune-mediated vascular injury and heightened endothelial cell (EC) apoptosis. We reported previously that apoptotic ECs release cathepsin-L which, in turn, cleaves perlecan, generating a bioactive C-terminal fragment referred to as LG3. Here, we investigated whether serum cathepsin-L and LG3 are increased in renal transplant recipients with AVR and whether anti-LG3 antibodies could be formed in association with AVR.
Methods: We performed a retrospective case-control study in which we compared circulating levels of cathepsin-L, LG3 and anti-LG3 IgG titers in kidney transplant recipients with AVR (n=15) versus those with tubulo-interstitial rejection (ATIR) (n=15) or stable graft function (n=30). Subjects with AVR were matched with controls with regards to transplant date (± 2 years) and the time elapsed between transplantation and serum collection (± 1 week). Sera were collected before or on the day of biopsy in patients who experienced rejection.
Results: Patients who experienced AVR had elevated levels of cathepsin-L, LG3 and anti-LG3 titers compared to subjects with ATIR or stable graft function ($p<0.05$ for all 3 mediators). Circulating LG3 levels were positively correlated with anti-LG3 titers (ρ: 0.28, $p=0.03$). Patients with AVR also presented higher pre-transplant anti-LG3 titers than other subjects ($p=0.04$).Graft function 2 years after transplantation was lower in patients with cathepsin-L, LG3 or anti-LG3 titers in the highest 2 tertiles versus the lowest tertile ($p<0.05$ for all 3 mediators). Subjects who had cathepsin-L and LG3 levels above the median showed poorer graft survival ($p<0.05$).
Conclusion: Collectively, these data identify cathepsin-L, LG3 and anti-LG3 antibodies as biomarkers of immune-mediated vascular injury and unfavorable allograft outcomes.

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HUMAN CYTOMEGALOVIRUS INFECTIONS REACTIVATION FIVE YEARS AFTER RENAL TRANSPLANTATION.

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Cytomegalovirus (CMV) infection is associated with adverse clinical outcomes in immunosuppressed renal transplant patients, but the incidence and association of CMV reactivation with adverse outcomes in patients very late after transplantation (5 years) have not been well defined.

Objective: To assess the reactivation of cytomegalovirus (CMV) infection and CMV disease in renal transplant patients 5 years after operation and to evaluate potential risk factors in reactivation in this cohort.
To assess reactivated CMV plasma DNAemia by polymerase chain reaction (PCR) and clinical outcomes in a cohort of 72 CMV-seropositive, renal transplant patients for more than 5 years. Risk factors for CMV reactivation and association with hospital stay were assessed by multivariable logistic regression and proportional odds models.
Design: A prospective clinical study for five years.
Patients: Seventy two anti-CMV immunoglobulin G (IgG) seropositive renal transplant patients operated upon in a university hospital in the period from January 2001 till December 2004 were followed up for 5 years.
Interventions: Once a year or whenever indicated, the patients were examined for active CMV infection by polymerase chain reaction from blood and or lower respiratory tract secretions. Bimonthly, detailed clinical and laboratory examination for signs of CMV disease e.g. refractory diarrhea, pancytopenia was carried out. Risk factors for CMV reactivation and association with hospital stay were assessed by multivariable logistic regression and proportional odds models.
Measurements and Main Results: Twelve of the 72 seropositive CMV IgG positive renal transplant patients (16.6%) developed an active CMV infection as diagnosed by the detection of HCMV DNA in plasma, or respiratory tract secretions. In three patients, the virus was isolated in the respiratory tract secretions. Severe CMV disease appeared in 3 patients with pneumonia. In patients with active CMV infection, the mortality tended to be higher (75%) than in those without (38%); the number of late acute rejection episodes, and the use of tacrolimus rather than cyclosporine as main immunosuppressive treatment. Cytomegalovirus viremia greater than 1000 copies/mL occurred in 42% (5/12; at a median of 18 days (range, 6-59 days). By logistic regression, CMV infection at any level (adjusted odds ratio [OR], 3.3; $P = 0.01$) and at greater than 1000 copies/mL (adjusted OR, 10.5; $P < 0.02$) and the average CMV area under the curve (AUC) in log(10) copies per milliliter (adjusted OR, 2.5; $P < 0.01$) were independently associated with hospitalization or death by 30 days.
Univariate testing for factors associated with active CMV infection showed the importance of recurrent sepsis ($p = 0.02$) and number of admissions to intensive care units or hospital wards ($p = .001$); and the number of attacks of diarrhea ($p = 0.05$). Multiple regression analysis identified only sepsis to be independently associated with active CMV infection ($p = 0.05$; odds ratio, 3.5).
Conclusions: In a group of renal transplant patients five years after operation who were anti-CMV IgG seropositive and had active CMV infection occurred frequently (16.6%). Septic patients were affected thrice as often as the total study population. Further studies in a larger patient group should evaluate the influence of CMV on post transplant mortality.

1589

INCREASED DEATH FROM CARDIOVASCULAR AND INFECTIOUS CAUSES IN FAR LIVING RENAL TRANSPLANT (RTX) PATIENTS

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We investigated whether patients receiving RTx who live farther from their attending nephrologist are more likely to die than those who live closer. A random sample of 167 patients who undergone RTx between 1996 and 2004 was examined. We calculated the distance between each patient's residence location and the practice location of their attending nephrologist. We used Cox proportional hazards models to examine the adjusted relation between distance and clinical outcomes (death from all causes, rejection episodes, infectious causes and cardiovascular complications) over a follow-up period of up to 6 years. During the follow-up period (median 3.3 yr, range 1.0-6.5), (22%) patients died. Compared with patients who lived within 50 km of their nephrologist, the adjusted hazard ratio of death among those who lived 50.1-150 km away was 1.04, 1.16 for those who lived 150.1-300 km away and 1.19 for those who lived more than 300 km from their nephrologist (p for trend < 0.001). The risk of death from infectious causes increased with greater distance from the attending nephrologist (p for trend < 0.001). The risk of developing acute rejection episodes did not increase with distance from the attending nephrologist(p for trend =0.2). The risk of death from cardiovascular causes increased with distance from the attending nephrologist (p for trend < 0.05). Compared with patients who lived within 50 km of their nephrologist, the adjusted hazard ratio of death among those who lived > 300 km away was 1.75 for infectious causes and 1.39 for cardiovascular causes. We can conclude that mortality and morbidity associated with RTx was greater among patients who lived farther from their attending nephrologist, as compared with those who lived closer.

1590

DIABETES AS AN OUTCOME PREDICTOR AFTER KIDNEY TRANSPLANTATION

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Renal transplantation is a key therapeutic option in end-stage renal disease. In this study, we used a single-center database to examine the risks of renal transplantation in patients with diabetes mellitus (DM). The hyperglycemic effect of immunosuppressant therapy further complicates post transplantation management of diabetes and, although this is still unproven, could be responsible for a higher incidence of post-transplantation infection, rejection and mortality.
Objective: In this study, we aimed to compare one-year outcomes of survival and morbidity after renal transplantation among recipients with and without diabetes mellitus.
Methods: A retrospective review of 1211 adult patients submitted to renal transplantation from January 2001 to December 2010 (with a minimum follow-up of 1 year) was undertaken. The patients were divided into two groups: those with (33%) and those without (67%) pre transplantation diabetes. Unpaired Student's t tests and x(2) tests were used to compare outcomes between diabetic and non diabetic renal transplant recipients. Survival, renal function, development of proteinuria, rejection, and infection (requiring hospitalization) were analyzed.
Results: Those with diabetes were older (47.2±7.2 vs. 39.5±10.6 years; P=0.01), had greater body mass index (mean, 29.5 vs 25.3 kg/m(2), p < 0.05), and had lower creatinine clearance (44.2±11.4 vs. 56.0±18.2; P=0.01). Forty one patients died in hospital (3.4%; P=non-significant). Diabetic and non diabetic patients showed no differences in rates of clinically significant infection or rejection in the first three postoperative months. Patients with diabetes showed a significant deterioration in their LDL-cholesterol (128±36 vs.165±29 mg/dl; P=0.02). Furthermore, survival rates were similar between these two groups. During the first year, 21% patients previously free of diabetes developed new-onset diabetes. No significant differences were seen in rejection at one year (16% vs. 22%), infection (32% vs. 35%), or mortality (13% vs. 9%). One-year survival was not significantly different (82% vs. 89%). No significant difference was found in one-year survival or in terms of higher morbidity in the renal transplant patients with diabetes, but a longer follow-up showed a significant decrease in survival. We found a trend toward decreased survival for those with DM at 1 year (80.4% vs 88.7%; p = 0.20). Mean follow-up time was 3.2 years. Infection rate within 6 months was greater among those with DM (19% vs 5%, odds ration = 6.25). Freedom from rejection at 3 years was similar (75.2% vs 76.8%, p = 0.57). Mean serum creatinine concentration at 1 year after transplant was 1.6 mg/dl in patients with DM (vs 1.34, p = 0.17). Multivariate analysis showed increased baseline creatinine level as a significant risk factor for survival. Body mass index >30 was a significant risk factor for survival among patients with DM.
Conclusions: We found an increased risk of serious infections in patients with DM, particularly within the first 6 months. However, our data suggest that diabetes is not associated with worse 1-year survival or higher morbidity in renal transplant patients, as long as good blood glucose control is maintained.

1591

GI SIDE EFFECTS OF MMF VERSUS EC-MPS IN MAINTENANCE HEART TRANSPLANT PATIENTS

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Background: The use of mycophenolate mofetyl (MMF) in heart transplant (Htx) has been limited by unwanted side effects, mostly GI complications, including vomiting, diarrhea, gastritis and GI bleeding. Myfortic, an enteric-coated formulation of mycophenolate sodium (EC MPS), is an alternative that is equally effective as MMF at immunosuppression in Htx patients, but has the added benefit of less upper GI adverse effects.
Methods: Our study aims to evaluate the change in the severity of GI symptoms at 1, 3, 6 and 12 months in Htx patients converted to Myfortic compared to those remaining on MMF treatment using health-related Q.O.L questionnaires such as GSRS (GI Symptom Rating Scale), GIQLI (GI Quality of Life Index) and PGWBI (Psychological general well being index). Data were gathered from 32 patients in each cohort from 2 Htx clinic in Canada and analyzed using Student's t-test.
Results: From the GSRS, there was statistically significant improvement in the Myfortic cohort's lower GI symptoms. There was less diarrhea (2.38 vs 3.11, P<0.05), less loose stool (2.42 vs 3.05, P<0.05), and less urgent need to have a bowel movement (2.60 vs 3.71, P<0.05). This is further confirmed by the GIQLI lower GI tract symptoms survey in which the Myfortic cohort also complained of less diarrhea (1.66 vs 2.14, P<0.05), less defecation disturbances (1.83 vs 2.69, P<0.05) and less lower abdominal pain (1.42 vs 1.71, P<0.05). However, the upper GI symptoms didn't demonstrate any significant differences. On the PGWBI, the Myfortic cohort demonstrated that they felt healthier (4.20 vs 4.60, P<0.05), happier with self (3.98 vs 4.58, P<0.05), more relaxed (4.20 vs 4.38, P<0.05) and were more in control of their thoughts and emotions. (4.74 vs 5.19, P<0.05)
Conclusions: We demonstrated that Htx patients with Myfortic maintainance therapy had fewer GI complaints, specifically less diarrhea, constipation and abdominal pain than in patients with MMF maintenance therapy over the course of 12 months. The decrease in GI symptoms was further reflected in patient's better psychological well being.

1592

PREVALENCE OF ENDOSCOPIC COLIC LESIONS AMONG PATIENTS AWAITING KIDNEY TRANSPLANTATION

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Introduction: Current recommendations for colorectal cancer screening among kidney transplant candidates are the same as the general population's. However, few studies have established the prevalence and characteristics of colic lesions among this population. METHODS: Retrospective study of all patients transplanted from January 1st 2007 to December 31st 2009 at CHUM (Montreal, Quebec). Patients transplanted for a second time were excluded from the study. Data regarding demographics, lifestyle, comorbidities, primary renal disease characteristics were collected. For every colonoscopy performed in the five years preceding transplantation, the indication, endoscopic and pathologic results were also obtained.
Results: 159 patients were included. A pre-transplant colonoscopy was performed in 40,3% of patients (n=64). Patients who underwent colonoscopies were significantly older (55,6 vs 43,1 yrs; p<0,05), had a higher BMI (28,3 vs 24,9 kg/m2; p<0,05), a greater prevalence of diabetes (29,7 vs 6,3% p<0,05), dyslipidemia (60,9 vs 41,1%; p<0,05) and diabetic nephropathy (23,4 vs 6,3%; p<0,05). Among patients who underwent colonoscopies, polyps were present in 32,8% (n=21), 19% of which had large polyps (> or = 10mm) and 62% of which had proximal polyps (at the splenic angle or above). Histologically, 66,7% of patients with polyps had adenomas and one patient had dysplasia. No significant differences were noted between the groups with and without adenomas.
Conclusion: Polyp prevalence is high among renal transplant candidates. 62% of patients had proximal lesions, justifying the performance of a total colonoscopy. Our results emphasize the indication of a screening colonoscopy among the renal transplant candidates.

1593

PRACTICES INFLUENCING CONSENT FOR ORGAN DONATION

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Background: The gap between available deceased donated organs and those awaiting life saving transplants has been well documented, with over 1500 Ontarians currently waiting for organs. Early referral to Trillium Gift of Life Network (TGLN) and ensuring an effective request with every potential donor's family are important elements of maximizing the availability of organs in Ontario.
Method: In November 2010 TGLN began collecting specific data elements related to the donation process. The collected data included timing of imminent death referral to TGLN, whether a plan (pre-approach plan) for family approach and donation discussion was developed by the healthcare team in

conjunction with TGLN and followed, as well as the profession of the person approaching the family for consent.
Results: Initial data reveals significant differences between consent rates and the three aforemention practice variables. Namely, timing of referral to TGLN, pre-approach plan and implementation, and the profession of the person approaching the family for consent.
Conclusion: This presentation will illustrate the opportunities for improvement of consent rates when identified practices are used in a donation situation, leading to an increase in the number of available organs for those awaiting transplants in Ontario.

1597

DIAGNOSIS OF ACUTE REJECTION FOR KIDNEY TRANSPLANTATION USING A NOVEL BLOOD-BASED BIOMARKER ASSAY

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Background: Acute graft rejection following organ transplantation remains a significant barrier to long term graft survival. Effective monitoring strategies are necessary for patient outcome management. Currently the invasive tissue biopsy is the most accurate and common method for diagnosis of acute rejection in renal transplant patients. Blood-based biomarkers may provide an alternative option for post-transplant monitoring of graft function and thereby permit timely diagnosis and therapeutic intervention to minimize graft damage. Utilizing a genomics panel previously demonstrated to diagnose early graft rejection in kidney transplant patients [Transplantation 2009 88(7):942-951] and which was discovered and validated on the high performance Affymetrix U133 plus 2 microarray platform, we developed a novel multiplexed genomic assay comprising 20 of the 24 genes in the original panel. The qBead Gene Expression Assay employs HTG Molecular's (HTG) quantitative Nuclease Protection Assay (qNPA™) chemistry in combination with the Luminex xMAP® Technology multiplexing platform.
Methods: In this preliminary study, PAXgene blood samples from 4 subjects with acute kidney rejection (AR) at 1 week post-transplant and 10 matched non-rejection subjects (NR) were used to assess the ability of the assay to discriminate between the two patient groups. These samples were not from the original discovery cohort used to create the genomics panel. Background-corrected intensity values from each of the genes were normalized to the mean intensity value of several control "housekeeping" genes, which were included in the multiplexed assay. A weighted Linear Discriminant Analysis (LDA) model previously employed on the Affymetrix U133 plus 2 array data sets was then recalibrated and applied to the entire qBead data set to classify the 4 AR and 10 NR samples.
Results: Applying the LDA model to classify the 4 AR and 10 NR samples in the HTG data set, we plotted the ROC curve and an AUC of 0.825 was achieved. Selecting a cut-off for the scores that would correctly classify the 4 ARs, we obtain 100% sensitivity and 70% specificity (3 NRs are miss-classified).
Conclusions: Preliminary evidence indicates that this novel PROOF Centre/Luminex/HTG qBead kidney gene panel assay can provide a useful tool for the diagnosis of acute kidney failure following transplantation. The assay promises to provide an eventually viable diagnostic that can be implemented clinically across transplant centres in North America, where most centres already use the Luminex platform in the care of transplant patients. Additional patients are being evaluated to further validate this assay.

1598

COMPARISON OF IDENTITY DEVELOPMENT AND QUALITY OF LIFE (QOL) IN RENAL TRANSPLANT (RTX) PATIENTS IN COMPARISON TO TYPE 1 DIABETES (DM) AND HEALTHY ADOLESCENTS.

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Objectives: Identity development represents a central task of adolescence. In contrast to identity diffusion, identity achievement is characterized by a coherent sense of who one is following a period of exploration and can help navigate the challenges of adulthood. As part of a prospective longitudinal study on adjustment to transition to adult care, identity development and QOL of patients with RTx or DM and healthy controls were compared.
Methods: Patients with RTx (n=31, M= 19.48 years, SD= 1.02) or DM (n= 54, $Mean$= 18.03 years, SD= 0.26) were recruited from 3 Canadian pediatric hospitals and compared to 90 controls ($Mean$= 18.29 years, SD= 0.81). Participants completed the QOL Profile: Adolescent Version and The Extended Objective Measure of Ego Identity Status.
Results: Between group comparisons revealed no significant differences among RTx, DM, and controls in their ratings of importance of various QOL aspects. Similarly, no significant differences in QOL, perceived control over the QOL domains, and perceived opportunities for growth and development were found among the three groups. For identity development, we found significantly lower (p= .038) levels of ideological identity in male RTx patients (M= 31.00, SD= 6.65) in comparison to their healthy male counterparts (M= 25.82; SD= 6.83). There were no additional significant differences found in identity development between renal patients and the diabetic and control groups.

Conclusions: Our results suggest that RTx adolescents enjoy comparable QOL in comparison to DM and healthy adolescents. Similarly, other than minimal differences in some aspects of identity development, RTx patients were comparable to healthy adolescents. Additional longitudinal studies are required in order to better understand the identity development of RTx adolescents and its influence on their transition to adulthood.

1599

SINUS TACHYCARDIA POST CARDIAC TRANSPLANTATION: IS THERE AN EFFECT ON LONG-TERM CARDIAC FUNCTION?

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Cardiac transplantation results in autonomic denervation and a subsequent increase in heart rate. The long-term consequences of this are currently unknown, yet rate-limiting drugs such as beta-blockers and non-dihydropyridine calcium channel blockers are frequently prescribed in an attempt to control post transplant sinus tachycardia. We set out to observe the long-term effects of sinus tachycardia in post transplant patients in order to better establish a role or lack thereof for rate-limiting drugs in post transplant therapy. We reviewed the functional and hemodynamic profiles of 42 patients post cardiac transplant and followed them forward until the present date with observations lasting as long as 23 years.
Patients were grouped according to their resting heart rate at one year: **Group 1**: 59-87 bpm, **Group 2**: 88-96 bpm and **Group 3**: >96 bpm. Groups were then analyzed at years 1, 3, 5, 9 and the last or most current year post transplant. On average, patients in **Groups 1** and **2** at year one had slightly increased resting heart rates in the final year of observation and moved towards the upper limit of their respective group, whereas those with more pronounced sinus tachycardia in **Group 3** had significantly lower resting heart rates in the final year. Hemodynamic and cardiac function outcomes were as follows:

Final Year Post Transplant	**Group 1 n=14**	**Group 2 n=13**	**Group 3 n=15**	**P value**
Mean Time Post Transplant (yr)	13.4±4.3	13.2±4.5	13.9±3.5	0.89
Year 1 Resting Heart Rate	83±5	93±2	105±8	<0.0001
Final Resting Heart Rate	86±12	97±10	91±12*	0.08
Resting Ejection Fraction	64±9	60±6	59±8	0.25
Exercise Ejection Fraction	67±8	63±9	64±7	0.47
Cardiac Output	5.4±1.6	5.7±1.4	5.6±1	0.91
Pulmonary Wedge Pressure	13±5	11±4	12±5	0.63

*p<0.01 vs Year 1
In conclusion, post transplant sinus tachycardia appears to have no significant impact on long-term cardiac function and the use of rate limiting drugs in an attempt to lower heart rates in this patient population has no role.

1600

CHART AUDIT PROCESS AND RESULTS

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Since 2008 the Program has conducted ongoing provincial medical chart audit to assess the potential for organ and tissue donation and compliance with provincial required request legislation. Ministerial authorization was obtained for the chart review. Trained staff completed the audits. Ongoing review and revision of the process and tool have identified ways to refine the audit and decrease the number of death charts to be audited. 2700-4000 charts were reviewed annually. In 2009 and 2010, results showed that 5% and 14% of potential organ donors were not referred to the relevant donation program. In both 2009 and 2010, 83% of potential tissue donors were not referred for screening. The data was shared internally with regional leadership teams and improvement efforts have shown more referrals and less missed referrals in some regions of the province. The data supported the rationale to change the provincial legislation to include required referral in addition to required request. As well, accountability reporting from the regions to the Minister of Health has been proposed to improve accountability. Overall, organ donation referrals and actual donors have increased. The donor rate in 2010 for this province was 20.1 per 1,000,000 population (up from 15.8 donors per 1,000,000 population in 2006). Planning is now underway to develop a provincial policy, provide professional education, and to develop tools to support the roll out of revised legislation, and to continue to build on momentum.

1601

EDUCATION INITIATIVE VIA SOCIAL MEDIA

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Abstract: Background of Problem: Analysis of pancreas transplant awareness and referral patterns indicated that only a fraction of the dialysis centres and endocrinologists were referring patients for K/P transplantation. As a result, many patients who would benefit from K/P or pancreas transplantation were not offered this opportunity. A lack of knowledge among nephrologists and endocrinologists about the progress in pancreas transplantation and subsequent success rates inhibited patient referral. Patients with type 1 diabetes, even those on dialysis, were either unaware of

transplant options available to them – or believed that a transplant may make them worse.
Goals of Project: The goal of the campaign was to raise awareness of pancreas transplantation. We sought to enhance the quality of patient care by improving communication with referring centres and through public education using social and conventional media tools.
Interventions: Our program embarked on a campaign targeting 3 levels, physicians, dialysis nursing staff and patients. Our key components included a new brand identity using posters,/brochures, an e-blast targeting physicians and new templates for the program. Using social media such as twitter, blogging, Facebook and YouTube we sought to educate patients regarding options. Lunch and learn sessions were held in dialysis units to encourage advocacy for potential patients.
Outcomes: To date the media campaign has produced an increase in referrals to our program by over 400% over a 6 month period. Our campaign has been highly successful and has significantly improved our communications with patients and referring centres. To maintain this success we have designed a long-term strategy to promote awareness for pancreas transplantation and to establish ourselves as a leading pancreas transplant centre.

1605

EVALUATION OF A FAMILY CAMP INTERVENTION IN PEDIATRIC HEART TRANSPLANTATION

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This study evaluated a support and education-based weekend residential camp experience for children with a heart transplant and their families. Approximately 60 individuals including 13 transplanted children attended the intervention, which comprised a series of recreational, social and educational activities geared for children, youth, parents and siblings. Activities were developmentally-based and substantial choice of activities was provided to participants. The camp was facilitated for families across Alberta, Canada. Evaluation comprised: (I) pre- and post-camp measures, including the adapted *Children's Inventory of Social Support (*Wolchik, Teal & Sandler, 1989) evaluating sources and need for support, perceived and available support network, actual or utilized support, and satisfaction with support, and the *Adolescent Coping Orientation for Problem Experiences* (McCubbin & Thompson, 1991) on behaviours used in managing difficult situations and life changes; (II) knowledge and satisfaction assessments; and (III) post-camp qualitative interviews.
In total, 22 campers participated in the evaluation. Participants consistently appreciated the recreational, supportive and educational components of the camp, and the weekend long format was well-received. On average, participants rated the camp 4.21/5 (1=expectations not at all met; 5=expectations strongly exceeded), suggesting that the camp met or exceeded expectations (parents: 4.45; children: 4.33). Key outcomes include increased social support and transplant-related knowledge. Qualitative findings suggested participants liked the substantial variety of activity choices, the expansion of their transplant-related social network, and the opportunity have fun in a natural setting. Participants strongly valued the opportunity to develop relationships with peers and health care providers, and families actively engaged in support sharing. All participants reported that they would be interested in a future camp. Participant recommendations include increasing learning modules addressing topics such as transition and adolescent motivation; mentoring opportunities for children by older youth, and more activities targeted to adolescents. In summary, the transplant family camp was shown to be an effective and strongly appreciated intervention. Evaluation triggered various improvements in our program including continuous educational events for parents and patients, a sibling support group, an online parent support network, and offering the family camp as an annually repeated event.

1609

ASSOCIATION BETWEEN RESIDENCE LOCATION AND LIKELIHOOD OF TRANSPLANTATION AMONG PEDIATRIC DIALYSIS PATIENTS

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Background: Many Canadian children with end-stage renal disease (ESRD) reside far from a pediatric kidney transplant centre. It is unknown whether this geographical barrier affects likelihood of transplantation.
Methods: For this population-based cohort study, we used data from a national pediatric ESRD database. Patients ≤18 years old who started renal replacement in 9 Canadian provinces during 1992-2007 were followed until death or last contact. Primary outcome was kidney transplantation (living or deceased donor). Distance between nearest pediatric transplant centre and each patient's residence was categorized as: <50km, 50 to <150km, 150 to <300km, and ≥300km. Using Cox proportional hazards models, we compared likelihood of transplantation among whites and non-whites living in various distance categories.

Results: Among 728 patients included, 52.2% were males and 62.5% were white. Compared to white children living <50km from a transplant centre, white (hazard ratio [HR] 0.73, 95% confidence interval [CI] 0.56-0.95) and non-white (HR 0.66 95% CI 0.48-0.92) children living ≥300km away were less likely to receive a transplant. Non-white children living <50km away (HR 0.59, 95% CI 0.45-0.78) were also less likely to receive a transplant compared to otherwise similar whites living <50km away.
Conclusions: Although equitable access to transplantation by residence location is observed among remote-dwelling adults with ESRD, white and non-white children with ESRD living ≥300km from a transplant centre were less likely to receive transplants – suggesting a geographic barrier potentially amenable to intervention. Lower likelihood of transplantation among non-white children living <50km away requires study.

1611

DOES EXPANDED CRITERIA DONOR STATUS MODIFY THE OUTCOMES OF DONATION AFTER CARDIAC DEATH KIDNEY TRANSPLANTATION?

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Background: The outcomes of kidney transplants that simultaneously exhibit donation after cardiac death (DCD) and expanded criteria donor (ECD) characteristics has not been well studied to date. This study aims to examine the outcomes of DCD vs. non-DCD kidney transplants as a function of both ECD status and the Kidney Donor Risk Index (KDRI), which is a more granular measure of donor quality.
Methods: A retrospective cohort study of deceased donor kidney transplant recipients (KTR) from 1 Jan 2000 to 31 Dec 2009 was conducted using the Scientific Registry of Transplant Recipients. The Kaplan-Meier method was used to graphically assess time to total graft failure across ECD/DCD groups. Cox proportional hazards models were fitted to examine the independent association between DCD status and total graft failure (i.e., graft loss or death) by ECD group and across quintiles of the KDRI.
Results: There were 67,231 patients included in the cohort, of which 546 (0.8%) were combined ECD/DCD kidney transplants. Kaplan-Meier curves show that DCD KTR have slightly inferior graft survival when compared with non-DCD KTR regardless of ECD status (Figure). In multivariable Cox models, the modestly increased risk of total graft failure among DCD (vs. non-DCD) KTR was not significantly modified by ECD status (HR 1.08 [95% CI: 1.01, 1.15] for non-ECD vs. HR 1.20 [95% CI: 1.04, 1.39] for ECD, P value for interaction 0.18). Similarly, the hazard ratios for total graft failure for DCD KTR were not significantly different across quintiles of KDRI (Table).
Conclusion: This study demonstrates that the ECD status or KDRI score of the donor kidney does not appreciably increase the relative hazard of total graft failure in DCD KTR. These findings suggest that the wider use of ECD/DCD donor kidneys may be an appropriate strategy to expand the deceased donor pool.

1612

KIDNEY FUNCTION AT TIME OF PANCREAS TRANSPLANTATION PREDICTS SUBSEQUENT RISK OF END-STAGE RENAL DISEASE

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Background: A successful pancreas transplant in patients with Type 1 diabetes mellitus improves glycemic control, reduces progression of microvascular complications, improves quality of life, and may increase life expectancy. Recipients of a pancreas transplant alone (PTA) have varying levels of kidney function at the time of transplant but the role of kidney function in predicting the subsequent risk of end-stage renal disease (ESRD) is not well understood.
Methods: All adult recipients of a first PTA from 1 Jan 1994 to 31 Dec 2009 in the Scientific Registry of Transplant Recipients were eligible for study inclusion. Estimated glomerular filtration rate (eGFR), based on the CKD-EPI formula, was categorized as < 60, 60 to 89.9, and ≥ 90 ml/min. The Kaplan-Meier method was used to graphically assess time to ESRD by eGFR group. Multivariable Cox proportional hazards models were fitted to determine the independent association of eGFR and ESRD. Fractional polynomial and competing risk models were used to allow non-linearity and account for death as a competing risk, respectively.
Results: A total of 1,135 PTA recipients were included in the study cohort. Patients with lower eGFR tended to be older, Caucasian, have larger BMI, receive less induction therapy, and underwent PTA in 2000 to 2004. There were 114 ESRD events over 3,826.9 person-years of follow-up. Figure 1 shows that patients with eGFR < 60 and 60 to 89.9 ml/min at the time of PTA were significantly more likely to develop ESRD than patients ≥ 90 ml/min over follow-up (log rank $P < 0.0001$). Patients with eGFR < 60 and 60 to 89.9 ml/min were 7.90 (95% CI: 4.53, 13.78) and 3.15 (95% CI: 1.73, 5.75) times more likely to develop ESRD than patients with eGFR ≥ 90 ml/min. Alternatively, every 10 ml/min increase in eGFR at the time of PTA was associated with a 20% reduction in the adjusted risk of ESRD (hazard ratio 0.80 [95% CI: 0.75, 0.85]). The fractional polynomial model showed an approximately linear relation between eGFR and the log hazard ratio for ESRD (Figure 2). Accounting for death as a competing risk did not change the overall results.

Conclusions: Kidney function at the time of PTA is a strong independent predictor of the risk for subsequent ESRD. These results may inform patient selection and the use of targeted interventions to reduce the risk of progressive kidney impairment in this patient population.

1614

LIVING DONOR AGE AND KIDNEY TRANSPLANT OUTCOMES: AN ASSESSMENT OF RISK ACROSS THE AGE CONTINUUM

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Background: With the demand for kidney transplants exceeding organ availability, transplantation of kidneys from older living donor is gaining widespread acceptance. Previous studies concluded that advanced donor age negatively impacts post-transplant outcomes, but detailed data on the extent to which living donor age influences allograft and recipient outcomes are wanting.
Methods: We used the Scientific Registry of Transplant Recipients database (2000 to 2009, n = 49,589) to assess the effect of living donor age (modeled as categorical and continuous variables) on delayed graft function (DGF), total graft failure, death-censored graft failure, and death with graft function. Cox proportional hazards models were fitted to adjust for potential confounders. The fractional polynomial method was used to examine the continuous relation between living donor age and the outcomes of interest.
Results: There was a significant increase in the adjusted odds for DGF (OR 1.87 [95%CI: 1.49 to 2.35]) in the oldest (60+ years) versus the youngest (age 18 to 29.9 years) age groups. The 10-year adjusted hazard ratios for total graft failure, death-censored graft failure, and death with graft function increased across ascending donor age categories (*P* for trend < 0.001). Moreover, the hazard ratios for these endpoints were non-linear across the range of living donor age studied (Figure). Combinations of recipient and living donor age categories showed that younger recipients of older living donors are at greatest risk for death-censored graft failure while older recipient of older donors are at greatest risk of death with graft function.
Conclusions: Our findings confirm the important influence of living donor age on kidney transplant outcomes and provide detailed estimates of risk across the living donor age continuum.

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INCREASED TIME-DEPENDENT VARIABILITY IN TACROLIMUS LEVELS IS ASSOCIATED WITH INFERIOR OUTCOMES FOLLOWING KIDNEY TRANSPLANTATION

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Background: Wide variations in tacrolimus levels have been associated with inferior kidney allograft outcomes. However, the time-varying nature of this variability has not been properly addressed in most studies. The objective of our study is to assess kidney transplant outcomes as a function of tacrolimus level fluctuations using methods to capture changes in drug level variability over time.
Methods: We studied adult kidney transplant recipients (KTR) at the Toronto General Hospital from 2000 to 2009 treated with tacrolimus-based immunosuppression. Patients with multi-organ transplants, re-transplants, and primary non-function were excluded. A time-varying Cox proportional hazards model was used to examine the association of tacrolimus level variability (as measured by the standard deviation of tacrolimus levels starting 1-year post-transplant or TacSD) and the composite endpoint of graft loss, death, or transplant glomerulopathy.
Results: A total of 365 patients were included in the study cohort. Median age was 53 years, 56.4% were male, 65.5% were Caucasian, 18.6% had diabetic ESRD, mean BMI was 26 kg/m^2, 55.1% were deceased donors, and 10.4% had a history of acute rejection in the first-year post-transplant. Time-varying Cox models showed a 40% increase in the adjusted hazard of developing the composite endpoint for every 1-unit increase in TacSD beyond the first year post-transplant [hazard ratio 1.4 (95% CI: 1.1, 1.8); $P = 0.01$]. A fractional polynomial model showed that the relation between TacSD and the log hazard ratio for the composite endpoint was approximately linear and significantly increased beyond a TacSD of 2. Subgroup analysis showed a trend towards worse outcomes in non-diabetic and younger KTR.
Conclusions: Wide variations in tacrolimus levels over time are associated with inferior patient and allograft outcomes. Elevations in TacSD should trigger a search for reversible causes (such as medication non-adherence) with the goal of reducing variability and improving long-term kidney transplant outcomes.

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ANTI-HLA-DP ANTIBODIES IN A HEART-TRANSPLANTED KIDNEY GRAFT RECIPIENT: A CASE STUDY.

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The role of anti-HLA-DP antibodies in renal transplantation is not fully elucidated. However, they can be a risk factor for antibody-mediated rejection (AMR) of second transplants. A 54 year-old female received a 5/10 HLA antigen (A-B-Cw-DR-DQ)-mismatched heart transplant in 2002. She developed renal failure secondary to calcineurin toxicity and was transplanted with a 5/10 HLA antigen-mismatched cadaveric donor kidney in February 2011. There was no repeat mismatch between the two transplants. According to flow cytometric solid-phase single antigen testing, the patient had non-donor-specific anti-HLA class I and class II antibodies including non-fully characterized anti-HLA-DP antibodies. A positive B-cell flow cytometric crossmatch result was observed and a decision was made to transplant the patient, adding IVIg to her immunosuppressive regimen. A 1-month biopsy revealed diffuse C4d staining along the peritubular capillaries but no clear signs of acute AMR. A 6-month protocol biopsy showed rather similar findings but uncovered BK virus nephropathy. Otherwise, renal function remains excellent to this date following plasmapheresis and IVIg therapy. Retrospective HLA-DP typing of both the patient and cadaveric kidney donor demonstrated a mismatch at both the ƀ (DPB1*01:01) and α (DPA1*02:01) chains. Further characterization of pretransplant and posttransplant serum by Luminex single antigen testing identified multiple anti-HLA-DP antibodies sharing the DEAV structural epitope, comprising the mismatched DPB1*01:01 but not the self-expressed DPB1*04:01 and DPB1*04:02. Also, reactivity against the mismatched DPA1*02:01 was observed. To rule out potential non-specific antibody reactivity, serum samples were treated with DTT and re-assessed by Luminex single antigen testing. Anti-HLA-DP antibodies remained strongly positive whereas all anti-class I antibodies were lost to treatment, suggesting possible differences in affinity. These results highlight the importance of HLA-DP typing when assessing an organ donor. Detailed characterization of anti-HLA antibodies is also essential to fully appreciate immunological risk in transplant candidates. Long-term follow-up of our patient will be essential to assess the impact of donor-specific anti-HLA-DP antibodies on graft survival and whether desensitization therapies such as plasmapheresis and IVIg can allow maintenance of acceptable renal function.

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A RETROSPECTIVE ANALYSIS OF CARDIOVASCULAR RISK FACTORS IN A LIVER TRANSPLANT POPULATION

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Objective To evaluate the relationship between Framingham cardiovascular risk scores (FRS) and transplant-related factors in a liver transplant population.

Methods: Retrospective chart review of 54 post-liver transplant patients in the Saskatoon area. Demographics collected included weight, blood pressure, serum lipids, diabetes mellitus, transplant duration, estimated GFR (eGFR) by C-G and IDMS, smoking, medications, and history of cardiovascular events. The modified FRS (2009) were used to calculate the 10-year probability of developing cardiovascular disease. Study subjects were sorted according to FRS: low risk (<10%), moderate risk (10-20%), and high-risk (>20%). Standard statistical analyses were performed between FRS, patient demographics, eGFR and imunosuppressants.

Results: 40% of patients were classified as low FRS, 29.6% moderate FRS, and 29.6% high FRS (of which 50% had new-onset diabetes after transplantation, NODAT). Among the high and low FRS groups, 43.8% (7/16) and 18.2% (4/22) were on an antimetabolite respectively. Immunosuppressant use was similar between the high and low-risk groups for tacrolimus, cyclosporine and sirolimus. FRS inversely correlated with eGFR ($p<0.02$) measured by either equation. eGFR-IDMS in the high-risk group [(60.4+/-22.1)ml/min] was significantly lower than that in the low-risk group[(97.1+/-54)ml/min] ($P<0.02$). Height, weight, BSA and transplant duration were not significantly different. Age and NODAT were significantly higher while GFR was significantly lower in the high-risk group, in the multivariate analysis. Receiving operational characteristic (ROC) analysis identified eGFR-IDMS at 42.7 ml/min with a sensitivity of 92%, specificity of 19%, and positive predictive value of 72% to identify high-risk patients. Box-plot analysis of variance between eGFRs in the three risk groups showed a p value of 0.001.

Conclusion: One-third of liver transplant patients have a high FRS. Low eGFR predicts those with high FRS. Liver transplant patients with low eGFR should undergo close management of cardiovascular risk factors, particularly those with NODAT.

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GASTROINTESTINAL HEMORRHAGE IN CRITICALLY ILL PATIENTS REQUIRING EXTRACORPOREAL MEMBRANE OXYGENATION: INCIDENCE, ETIOLOGY, AND IMPACT ON CLINICAL OUTCOMES

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Background: Extracorporeal membrane oxygenation (ECMO) is an established therapy for patients with cardiac or respiratory failure refractory to conventional therapeutic options. Gastrointestinal (GI) hemorrhage is a serious complication occurring in this patient population; however, data documenting its incidence, etiology, and impact on clinical outcomes are lacking.

Objective: To determine the incidence and etiology of GI hemorrhage in patients undergoing ECMO at an academic, tertiary care hospital, and determine its impact on clinical outcomes.

Methods: A single-center, retrospective review of all patients requiring ECMO from December 2007 to July 2011 was conducted. The incidence, etiology, and outcome of patients developing GI hemorrhage were determined. GI hemorrhage was defined as patients with a >10 g/L decrease in hemoglobin and clinical symptoms of GI hemorrhage or a bleeding source identified on endoscopic evaluation.

Results: During the study period, 55 patients (mean age 50 ± 16 years, 67% male) required ECMO, of which 45 patients (82%) received veno-arterial ECMO and 10 patients (18%) received veno-venous ECMO. The indication for ECMO was postcardiotomy in 42%, respiratory failure in 23%, acute myocardial infarction in 20%, hypothermia in 7%, myocarditis in 4%, and other causes in 4%. GI hemorrhage occurred in 21 patients (38%), of which endoscopic evaluation of the gastrointestinal tract was performed in 16 patients (76). Erosive esophagitis or gastritis was identified as the cause of GI hemorrhage in 7 patents (44%), while peptic ulceration, ischemic gastritis, ischemic colitis, gastric angiodysplasia, and post-sphincterotomy were etiologic in 3 (19%), 1 (6%), 2 (13%), 2 (13%), and 1 patient (6%), respectively. Initial treatment involved therapeutic hemostasis at time of endoscopy in 6 patients (29%) and conservative therapy in 15 patients (71%); however, 4 patients (19%) did not respond to initial treatment, of which 3 required surgical intervention and 1 required fluoroscopic embolization. In-hospital mortality was 51%; however, the occurrence of GI hemorrhage did not significantly increase the risk of in-hospital mortality (p=0.40) or decrease the probability of ECMO decannulation (p=0.17). Intensive care unit (ICU) was significantly longer for patients with GI hemorrhage (17 vs. 7 days, p=0.015); however, when the analysis was limited to surviving patients the difference was no longer significant (19 vs. 14 days, p=0. 57). There was no significant difference in hospital LOS between patients with GI hemorrhage compared to those without (all patients: 28 vs. 11 days, p=0. 07; survivors: 37 vs. 34 days, p=0. 94).

Interpretation: GI hemorrhage is a common complication observed in patients requiring ECMO; however, it does not appear to negatively impact major clinical outcomes including hospital or ICU LOS, in-hospital mortality, or likelihood of ECMO decannulation. Major clinical outcomes may be driven primarily by the severity of cardiac or respiratory failure necessitating ECMO. Further studies are required to confirm these results.

1621

A COMPARISON OF THE ONCE-DAILY (ADVAGRAF®) VERSUS TWICE-DAILY (PROGRAF®) FORMULATIONS OF TACROLIMUS IN DE-NOVO KIDNEY TRANSPLANT RECIPIENTS: A 12 MONTH OUTCOME STUDY

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Background: In 2009 our program switched from twice-daily (Prograf®) to once-daily (Advagraf®) tacrolimus as the calcineuirin-inhibitor (CNI) first-line component of immunosuppressive therapy in de-novo kidney transplant recipients (KTR). Graft and patient-related outcomes with these two tacrolimus formulations used de-novo have not been directly compared in Phase IV.

Methods: In this Phase IV study we identified all de-novo KTR transplanted at our institution between July 2009 (our switch date) and July 2010 who were prescribed Advagraf®, and compared them to de-novo KTR transplanted between July 2008 and July 2009 who were prescribed Prograf®. Graft function (eGFR by MDRD-7 equation) at 12 months post-transplant (primary outcome); new-onset diabetes mellitus (NODM) defined by Canadian Diabetes Association 2008 guidelines, BK viremia incidence, acute rejection, and graft survival to 12 months (secondary outcomes) were compared by student t-test, chi-square, or Fisher exact test as appropriate by intent-to-treat analysis. In addition, we computed time-to-steady state tacrolimus concentration, defined as first attainment of consecutive trough levels 5-10 ng/ml and the number of dose adjustments required to attain such steady state for each tacrolimus formulation.

Results: There were no important demographic differences between the Advagraf ® (N=106)/Prograf® (N=95) cohorts (recipient age 53/52 y, donor age 45/44y, male gender 60/55%, live donor 33/37%, peak PRA 22/27%, CIT 10.8/10.4h, smoking 34-/42%, dialysis duration 4.3/5.3y, all p=NS). EGFR at 12 months was similar (58.8±17 v 59.2±18 ml/min/1.73m^2, p=0.30). There were also no differences in eGFR at earlier times (1,2,3,6 mo), NODM (17v20%, p=0.58), BK viremia (10v7%, p=0.45), biopsy-proven acute rejection (7v16%, p=0.067), or graft survival (97v95%, p=0.30).Time-to-steady state was similar (9.2±1.1 v 8.1±4.7 days, p=0.49) although Advagraf® required fewer dosage adjustments to attain steady state (1.2±1.7 [0-8]v1.7±1.5 [0-7], p=0.03) yet

similar dose requirement (6.8±2.2(1-15) v 7±2.7(2-16) mg/d, p=0.51).
Conclusions: Important 12-month graft and patient outcomes seen in de-novo KTR prescribed once-daily or twice-daily tacrolimus formulations are similar. The once-daily formulation required fewer dose adjustments to attain steady state tacrolimus concentrations. The importance of this finding requires further evaluation.

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URETER RELATED COMPLICATIONS IN LIVE RELATED TRANSPLANT RECIPIENTS- SINGLE CENTER EXPERIENCE WITH 1970 TRANSPLANTS

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Introduction and objective: Ureter related complications are an important cause of morbidity in renal transplant patients. Herein we present our experience and managment of such complications. The impact of laproscopic live donor nephrectomy on ureter related complications was also analyzed.
Material and Methods: Our practice of vesicoureteral anastomosis has evolved as phase I from 1989 to 1993 when stent was put only when felt necessary, phase II from January 1994 to April 1995, when stent placement was randomized and phase III from May 1995 to December 2010, where all anastomoses were stented. Kidney was removed by open method till 1998 and laproscopic nephrectomy (left side) was started from 2002 onwards.
Results: Incidence of ureteral leak without DJ stent was 6.1% and 0.4% with stent.
Overall incidence of ureteral stenosis was 0.8%. Incidence of stenosis in stented and non-stented group was 0.8% and 2.3% respectively. Treatment offered for ureteral stenosis was percutaneous nephrostomy and antegrade stent.
Ureteral leak was present in .3% and 1.5% in open and laproscopic method respectively and ureteral stenosis in .9% and .75% respectively.
Conclusion: Two major ureter related complications were leak and stenosis and three-fourth of them resolved by minimal invasion. Major surgical revision was needed in around one-fourth of cases. Stent use helps in reducing incidence of ureteral leak, but doesn't seem to have any impact on ureteral stenosis. Laproscopic technique doesn't seem to have any adverse effect on ureter-related complications.

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VASCULAR COMPLICATION IN LIVE RELATED RENAL TRANSPLANT: AN EXPERIENCE OF 1945 CASES.

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Introduction and Objective: Among the surgical complications in renal transplantation the vascular complications are probably most dreaded, dramatic and likely to cause sudden loss of renal allograft. We present our experience and analysis of the outcome of such complications in a series of 1945 live related renal transplants.
Methods: One thousand nine hundred and forty five live related renal transplants were evaluated reterospectively for vascular complications. Complications were recorded and analyzed for frequency, time of presentation, clinical presentation and their management.
Results: The age of patients ranged from 6 to 56 years (mean=42).vascular complications was found in 25 patients (1.29%). Most common among these was transplant artery stenosis found in 11 (44%), followed by transplant artery thrombosis in 9 (36%), transplant vein thrombosis in 3 (12%) and aneurysm formation in 2 (8%) patients. The time of presentation also varied amongst complications. All cases of arterial thrombosis had sudden onset anuria with minimal or no abdominal discomfort while venous thrombosis presented as severe oliguria associated with intense graft site pain and tenderness. Management of cases with vascular thrombosis was done by immediate surgical exploration and 2 patients of renal artery stenosis were managed with angioplasty and stent placement.
Conclusions: Major vascular complications are relatively uncommon after renal transplantation but still they constitute an important cause of graft loss in early post operative period. Timely diagnosis of transplant renal artery stenosis is amenable to correction by endovascular techniques, and aneurysm and vessel thrombosis requires graft nephrectomy.

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RITUXIMAB THERAPY IS EFFECTIVE FOR POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS AFTER HEART TRANSPLANTATION

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Background: Post-transplant lymphoproliferative disorders (PTLD) remains an uncommon complication of heart

transplantation with high mortality rate reported after conventional therapies. Alternative treatments such as rituximab have been explored.
Methods/Results: Four patients with CD20 positive PTLD , received an intravenous dose of rituximab, 375mg/m^2, weekly for 6 ± 2 weeks. The mean age was 50 years (range 24-62 years) and 3 patients were male. The mean time from transplantation to a PTLD diagnosis was 19 months (range 6-37 months). Diagnostic B cell histology was diffuse large cell lymphoma in all patients. No patients had bone narrow or central nervous system involvement. Primary extranodal disease was noted in 75% of patients. Immunosuppressive therapy was decreased at the time of diagnosis. The mean follow-up was 41 months (range 10-74 months). The overall response rate was 75% with 3 complete responses and 1 case of progressive disease. The mean survival after rituximab was 36 months (range 6-68 months). The patient with progressive disease is currently alive but on conventional chemotherapy.
Conclusions: Single agent rituximab may offer a response and survival advantage in patients with PTLD after heart transplantation. Rituximab, along with immunosuppression reduction, should be considered as a first line therapy for patients with CD20 positive PTLD. Further evaluation of rituximab in PTLD after heart transplantation alone and in combination with others therapy is warranted.

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IMPACT OF SILDENAFIL TREATMENT ON PULMONARY HEMODYNAMICS AND OUTCOMES IN PATIENTS WITH SEVERE PULMONARY HYPERTENSION RECEIVING HEART TRANSPLANTATION

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Background: Elevated pulmonary vascular resistance (PVR) in heart transplant candidates is associated with poorer post-transplant survival. The aim of this study is to assess the effect of perioperative sildenafil administration on pulmonary hemodynamics and clinical outcomes in patients with advanced heart failure who were considered high-risk patients for heart transplantation (HT) because of elevated PVR and transpulmonary gradient (TPG).
Methods: 119 consecutive patients receiving HT between 2004-2011 were included. Fifteen patients (group A) had severe pulmonary hypertension (PH) (defined as PAPm >25 mmHg and/or PVR>2.5 Wood units (WU) and/or TPG>12mmHg after vasodilator test or continuous administration of inotropics drugs) compared to 104 patients (group B) without severe PH. Group A received sildenafil therapy. Pulmonary hemodynamics were evaluated before HT with and without sildenafil therapy. Right catheterization was also analyzed early after HT with sildenafil therapy and late after HT without sildenafil. Post transplant survival was compared between two groups.
Results: Sildenafil dosage was 109±42mg/day during 163±116 days before HT. After sildenafil therapy PAPm, PVR and TPG decreased from 43.9±12.5mmHg to 33.4±5.8mmHg, 5.0±1.1 WU to 3.0±1.6 and 17.3±3.2mmHg to 10.2±4.1mmHg respectively ($p<0.01$). All patients underwent successful heart transplantation. Sildenafil dosage was 140±70mg/day during 43±45 days after HT. There were no differences in PVR and TPG on sildenafil therapy early after HT and without sildenafil six months after HT. Post-transplant survival was similar between groups.
Conclusion: Sildenafil therapy successfully decreases PVR and TPG in patients with severe pulmonary hypertension allowing successful heart transplantation without increased post-transplant mortality.

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THE LEARNING CURVE EFFECT IN A CANADIAN DONATION AFTER CARDIAC DEATH (DCD) KIDNEY TRANSPLANT PROGRAM.

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Introduction: Donation after cardiac death our province has led to a 25% increase in the number of donor organs available for transplantation. A high rate delayed graft function (DGF) of around 70% was noted in our early experience, consistent with that of other programs. The purpose of our study was to compare our early (n=31 grafts) experience with our more recent (n=32 grafts) experience.
Methods: We retrospectively examined the charts of all of our 63 single-kidney DCD transplants from 36 donors, from June 2006 to October 2011.
Results: Overall, there were no cases of primary non-function, and DGF was seen in 41/63 kidney recipients (65%). Four grafts were lost (3 due to rejection) and there was one death with a functioning graft, and currently 58/63 (92.1%)

recipients remain dialysis-free (for 23 to 1903 days). When comparing the more recent transplants to the early ones, there was a significant improvement in the time from asystole to initiation of cold flush (12.0 +/- 0.6 min vs. 16.0 +/- 0.8 min, p=0.003). Average Cold Ischemic Time increased in recent years by close to 4 hours (732 +/- 51 min vs. 498 +/- 58 min, p=0.02). The rate of DGF (19/32,59.4% vs. 22/31, 71.0%) as well as Creatinine Clearance at 1 week, 1 month, and 1 year did not change significantly in recent cases compared to earlier ones. Length of stay has decreased significantly to 16.0 +/- 2.0 days from 12.8 +/- 0.8 days (p=0.035).
Conclusions: As more experience is gained in our DCD kidney program, technical improvements are noted in time to cannulation. Perhaps due to an increase in comfort level, cold ischemic time has increased and patients are being discharged from hospital sooner. DGF remains a significant issue, but the long-term results justify the use of DCD kidneys in our program.

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FACTORS ASSOCIATED WITH DELAYED GRAFT FUNCTION (DGF) IN DONATION AFTER CARDIAC DEATH (DCD) KIDNEYS

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Introduction: The purpose of this study was to review the experience of our DCD kidney program to determine factors associated with an increased risk of DGF.
Methods: We retrospectively analyzed the charts of all 63 patients receiving single-kidney-only transplant of a DCD kidney since our DCD program's inception in 2006. DGF was seen in 41 of 63 patients (65%) and there were no cases of primary non-function.
Results: Indications for dialysis had some overlap and included fluid overload (18/41), hyperkalemia (10/41), and no indication recorded or simply 'uremia' in 17/41. Patients with DGF were noted to have received donor organs with significantly longer time with systolic blood pressure (SBP) < 55 mmHg (28.2 min vs. 21.1 min, p=0.049) and a trend toward a longer time to asystole (29.3 min vs. 16.6 min, p=0.063). In fact, SBP < 55 mmHg for more than 30 minutes resulted in only 2 of 13 grafts (15.4%) showing satisfactory early function, compared to 20 out of 50 (40%) if the time was under 30 minutes, although the difference was not statistically significant. Machine cold perfusion (n=36) of the donor organs did not significantly improve the rate of DGF, however, it was associated with improved creatinine clearance on days 3 (12.6 vs. 7.8 mL/min, p=0.036) and 7 (20.0 vs. 8.7 mL/min, p=0.003) and patients receiving DCD kidneys that had been machine perfused were discharged sooner (16 vs. 11.5 days, p=0.006).
Discussion: Our results are comparable to those seen at other institutions. DCD kidneys from donors having a SBP<55 for more than 30 minutes are very unlikely to show early graft function. Machine cold perfusion seems to improve kidney function in the early post-transplant period in these DCD kidneys.

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ETHICAL ISSUES AND TRANSPLANTATION IN SCIENTIFIC JOURNALS

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Background: Scientific journals are an important vehicle for disseminating information to health care professionals. These journals aim to inform physicians in order to improve patient outcomes, raise awareness around various topics, spark debate, and so on. No previous study has examined how scientific journals report on ethical issues related to organ transplantation (OT). By examining how these issues are covered in scientific journals, we can gain a better understanding of common perceptions and priorities in the transplant and medical communities.
Purpose: The aim of this study was to analyze ethical issues related to OT as they appear in internal medicine (IM) and transplantation journals.
Methods: Using the Pubmed database, we retrieved 1,120 articles from the top ten IM journals (journals with the highest impact factor) and 4,644 articles from the two main transplantation journals (*Transplantation* and *AJT*). Out of the IM journal articles, only those in which OT was the main topic were analyzed (349 out of 1,120 articles). A total of 333 articles were randomly selected from the transplantation journals for analysis.
Results: There was more frequent reporting on ethical issues in the IM journals than in the transplantation journals (24.1% cf. 5.4%). Organ allocation was the most important issue. Equity, egalitarian principles, fairness, medical urgency, efficacy, utility, transparency, scientific knowledge and hope were identified as important elements in the creation of a sound organ allocation system. There was also much discussion of how factors such as ethnic background, gender, geographic location and socioeconomic status affect eligibility for organ transplantation, access and medical outcomes.
Conclusion: Organ allocation procedures and socio-demographic factors were the most important ethical issues raised in the scientific journals studied. We were surprised to discover that ethical issues were discussed more frequently in

the IM journals than in the transplantation journals. Does this mean that ethical concerns, particularly those related to organ allocation, should be debated not only in the transplant community, but also in the medical community at large? We were also curious as to why issues such as gift exchange, the expansion of the pool of eligible recipients, and information given to patients about marginal organs did not receive much attention in the scientific journals studied.

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A COMPARISON OF ABSORPTION PROFILES BETWEEN TACROLIMUS AND CYCLOSPORINE IN DIABETIC CARDIAC TRANSPLANT RECIPIENTS

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Background: Diabetes mellitus (DM) is a common complication after cardiac transplantation with approximately 20% of patients developing post transplant diabetes mellitus In addition, 20% of cardiac transplant recipients have DM pre-dating their transplant.We sought to determine if previously validated measures of AUC remain reliable in diabetic cardiac transplant patients who may demonstrate altered subclinical gastric absorption profiles.
Study Design: A single center, prospective, open-label, conversion study was conducted on diabetic cardiac transplant patients. Patients served as their own controls. Pharmacokinetic profiles were conducted on steadystate cyclosporine and following conversion to steadystate tacrolimus. Pearson's correlation coefficient and a calculated coefficient determination were used for examining relationships between concentrations (e.g. C0, C2) and AUCτ. Wilcoxonsigned-rank was used for comparing paired mycophenolate pharmacokinetic indices between the cyclosporin and tacrolimus time periods.
Results: Six patients were enrolled. Mean age was 55.8 years and the majority were males. Average time post heart transplant was 10.14 years. Table 1 demonstrates the coefficients of determination (R^2) and direction of correlations between pharmacokinetic indices. C4 and not CO or C2 is best at estimating AUCτ for cyclosporine in this diabetic population with an R^2 of 93%. Both C0 or C2 provided reasonable correlations with the AUCτ for tacrolimus treated patients. Table 2 demonstrates AUCτ for mycophenolate is 2 times greater and clearance(L/h) is reduced by 60% when mycophenolate is combined with tacrolimus as compared to cyclosporine as has been previously reported in renal transplant patients. There was no increase in rejection or HgbA1c (.067 +/- .017 vs .072 +/-.015, p .23) following conversion to tacrolimus. In addition there were no significant differences in fasting insulin or c-peptide levels once converted to tacrolimus.
Conclusion: Cyclosporine pharmacokinetics are altered in diabetic cardiac transplant patients. Routine use of C0 and C2 levels may not accurately reflect AUCτ in this population due to altered subclinical gastric absorption profiles. Mycophenolate pharmacokinetics are altered with concurrent use of Tacrolimus in the cardiac transplant population and may favor consideration of dose adjustment or monitoring. Further studies in larger populations are warranted.

Table 1: Coefficients of determination (R^2) and direction of correlations between pharmacokinetic indices

	Cyclosporine	Tacrolimus	Direction of correlation
C0 and AUCτ	35%	71%	positive
C2 and AUCτ	57%	83%	positive
C4 and AUCτ	93%	-	positive

Table 2: Comparative mycophenolate pharmacokinetics while on concurrent cyclosporine or tacrolimus

Cyclosporine	**Tacrolimus**	**p-value**
C0 1.8 (1.4 – 3.1) mcg/mL	**3.4 (3.3 – 4.8) mcg/mL**	**0.01**
AUCt 46.8(40.0 – 57.5) mcg*h/mL	**113.9 (69.6 – 127.9) mcg*h/mL**	**0.001**
Cl/F 21.4 (17.5 – 25.0) L/h	**8.5 (6.6 – 14.5) L/h**	**0.001**

1636

COMPARISON OF VIRAL LOADS OF EPSTEIN-BARR VIRUS (EBV) IN HEALTHY SUBJECTS, ORGAN TRANSPLANT RECIPIENTS AND PATIENTS WITH POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD)

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Background: EBV can be associated with PTLD in immunosuppressed patients. Early diagnostic is critical to prevent this life-threatening disease. Measurement of EBV DNA in the blood of patients at increased risk has been suggested as a potential strategy to prevent PTLD. However, EBV is a latent virus and little is known about the association between the viral load and clinical disease.
Objectives: To determine whether EBV DNA can be detected in the blood of healthy subjects and to compare these viral

loads with those of transplant recipients with and without PTLD.

Methodology: 100 healthy volunteers, 85 bone marrow transplant (BMT) recipients and 57 renal transplant (RT) recipients for whom an EBV viral load was measured based on a clinical indication between 2007 and 2010 were included in this observational retrospective study. Six additional RT patients with proven PTLD diagnosed prior to 2007 were also included. Circulating anti-EBV antibodies and EBV viral loads were measured in peripheral blood by an ELISA and by a quantitative PCR.

Results: Among healthy volunteers, 9 of the 82 EBV seropositive subjects (11%) had a detectable viral load, ranging between $7.2X10E^2$ and $4X10E^3$ copies/mL. In contrast, 50 of 85 BMT recipients (59%) and 21 of 57 RT recipients (37%) evaluated between 2007 and 2010 had detectable DNA levels (both $P<0.01$ *vs.* healthy volunteers). Among the 50 BMT subjects with a detectable viral load, 8 had confirmed PTLD and 4 had suspected PTLD based on clinical grounds. Compared to the 38 BMT without PTLD, these 12 subjects had higher viral loads (confirmed PTLD: 4,6 x $10E^6$, suspected PTLD : 1,7 x$10E^5$ and without PTLD : 4,2 x $10E^3$; $P< 0.01$ and $P<0,05$ *vs.* without PTLD respectively). Among the 27 RT recipients with detectable viral load, 7 subjects developed primary infection, 12 EBV-seropositive subjects did not develop the infection and 8 developed PTLD ($1.9 \times 10E^4$ *vs.* $1.1 \times 10E^4$ vs. $1.0 \times 10E^4$; P=NS). Importantly, 9 D+/R- RT recipients developed primary EBV infection, among which the two subjects who were subsequently diagnosed with PTLD did not produce detectable EBV antibodies; in contrast, all of the 7 subjects who remained PTLD-free had detectable anti-EBV antibodies (P=0.03).

Conclusions: EBV DNA can be detected in a substantial proportion of seropositive healthy subjects. However, this percentage is much greater in transplant recipients. This analysis suggest that it could be possible to use EBV viral load to screen for PTLD among BMT recipients, but not among RT recipients, with the caveat that RT is more likely to have been influenced by an intervention bias. Among RT subjects, there seems to be an association between absence of seroconversion and development of PTLD. Studies with longitudinal follow-up are needed to better evaluate the clinical utility of this assay.

1637

MAXIMAL KIDNEY LENGTH PREDICTS NEED FOR NATIVE NEPHRECTOMY IN ADPKD PATIENTS UNDERGOING RENAL TRANSPLANTATION

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Background: Native nephrectomy (NX) in patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) is performed on a case-by-case basis. Relative indications include: recurrent infections, pain, bleeding, space requirements for transplantation, and other symptoms secondary to mass effects. The purpose of our study was to determine if kidney size can be used to predict need for NX (pre-, post-, or concomitant with transplantation).

Methods: We performed a retrospective analysis of all ADPKD patients who underwent renal transplantation (TX) at our centre between January 2000 and March 2010. Maximal and mean kidney length, kidney length:patient height ratio, predicted kidney weight: patient weight ratios, body mass: kidney mass index ratios were assessed for ability to predict need for NX. Kidney size was obtained from pre-TX imaging reports and corroborated with post-NX surgical pathology reports. Size parameters were assessed for their potential predictive ability by way of ROC curve analysis.

Results: Sixty-nine patients met our inclusion criteria, of which 15 (22%) underwent native NX within a mean of 1.15 years (95% CI 0.56 – 1.74) of TX. No significant differences were found between demographic variables of the NX and Non-NX groups. Maximal kidney length predicted need for NX to a greater degree than any other parameters. The median kidney length in the NX group was 25.0 cm (95% CI 21.0 – 30.0), while the corresponding length in the Non-NX group was 19.6 cm (95% CI 18.5 – 20.1) (P=0.001). An ROC curve analysis revealed an AUC of 0.772 (95% CI 0.665 – 0.864) (P<0.0001). A criterion of<25 cm revealed Specificity of 79.6% (95% CI 66.5 – 89.4) and Sensitivity of 40.0% (95% CI 16.3 – 67.7) for NX, while a criterion of <19.1 cm revealed a Specificity of 100% (95% CI 78.2 – 100.0) and a Sensitivity of 44.4% (95% CI 30.9 – 58.6) for Non-NX.

Conclusions: Maximal kidney length in patients with ADPKD is associated with eventual need for native NX and may be of clinical use in risk stratification.

1638

LITERATURE REVIEW OF CONTINUOUS FLOW LEFT VENTRICULAR ASSIST DEVICES AND THE CREATION OF A PROVINCIAL REGISTRY: A COLLABORATIVE INITIATIVE INVOLVING CLINICIANS AND HEALTH TECHNOLOGY EVALUATORS

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Introduction: Advanced heart failure is increasingly being managed with long-term support using left ventricular assist devices (LVAD), for both "bridge to transplant" (BTT) and "destination therapy" (DT) patients. Quebec clinicians have expressed the need for a provincial LVAD registry to monitor patient outcomes.
Objectives: (1) To perform a systematic literature review of the effectiveness, safety and cost-effectiveness of continuous flow LVADs for BTT and DT patients. (2) To collaborate with an expert committee of clinicians, representing each Quebec transplantation centre, to interpret the evidence and develop the registry structure.
Methods: A systematic literature search (2008-2011) was performed, selecting clinical studies and registry reports that provided data for HeartMate II (or all continuous flow devices combined) and reported 1-year survival (censoring patients when transplanted) and/or quality of life. The committee provided feedback on important quality indicators for a registry.
Results: 3 BTT studies and 3 DT studies met the selection criteria. INTERMACS registry data were available for both strategies. 1-year survival for both types of LVAD users appears to be improving. The clinical studies show improved functional status and quality of life for both types of LVAD users. Adverse events during support were common and included right heart and respiratory failure; the most frequent were bleeding and infections. More DT patients than BTT patients experienced complications. Effectiveness and safety results from the clinical studies were, in general, supported by the "real world" registry data. Cost-effectiveness data specific to HeartMate II were lacking. The proposed structure for the registry included (1) baseline patient, device and implantation procedure variables; (2) outcome variables including changes in strategy (transplant, recovery, conversion of BTT to DT), and (3) comprehensive economic variables.
Conclusions: The results of this review provide important information and will facilitate the creation of a provincial registry for this rapidly evolving technology.

1639

SYSTEM DESIGN IMPROVES OUTCOMES

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Patients with organ failure awaiting transplantation continue to die while wait times increase (1). Integrated systems using a multidisciplinary team approach that incorporates best practices have been successful (2). This province had a transplant program for 40 years, but no program to support donation. The provincial organ and tissue program began in 2006 to implement guidelines, provide professional education, recommend delivery models and surveillance. Stakeholders from critical care medicine, critical care donation and tissue community designed the system. An advisory council and working groups oversee strategic directions. The model funded 2.4 FTE resource nurse positions across the province to support goals. Health professional needs assessments were completed and workshops developed to educate front-line staff. Chart auditing began in 2008 to monitor compliance with required request legislation. Objectives for physician education included resident education during ICU rotations, involvement in undergraduate medical education, donation focused grand rounds, and regional donation-specific continuing education, incorporating local data. Nursing education was supported through resource nurse-led education sessions, involvement in hospital orientation, and development of required referral policies. The cadaveric donor rate in 2010 was 20.1 per million population (14 donors per million population in 2005) and is 20.1 for 2011 to date and on pace to increase to 23 per million. Intensivist-led donor management protocols have seen the organs per donor rate increase from 4.0 to 4.7; the highest in the country and one of the highest rates in the world (3). Tissue donation referrals increased from 223 in 2007 to 348 in 2010. In 2011 we are on track for 155 tissue donors from all sources. The projected number for 2011 donors from the DHAs in Nova Scotia is 123, 76% more than 2010. The employment of a collaborative model, professional education, communication strategies, and donor management supported by chart review improves performance of the donation system.

1640

VACCINATION AND VACCINE RESPONSES IN PEDIATRIC LUNG TRANSPLANT RECIPIENTS

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Implementation of guidelines for vaccination and vaccine response monitoring has not been well established in pediatric transplant patients. This small population may be considered a particularly vulnerable group given the high level of immunosuppression that is required to prevent acute cellular rejection. The present study aimed to evaluate the vaccine history and subsequent serologic status of pediatric lung transplant recipients. Eight patients were enrolled, with a mean age of 10.7 years at time of transplantation. Despite the fact that documentation of vaccine history is part of the transplant assessment protocol, 5 (62.5 %) had no immunization records and 3 (37.5 %) had incomplete immunization records documented on the assessment form prior to surgery. Only 4 (50%) had a complete set of pre-transplant vaccine serologies, which are performed routinely prior to transplantation. After transplantation, 1 patient (12.5%) was found to have equivocal serology to Varicella, 1 had inadequate immunity to Diphtheria and 3 (37.5%) showed suboptimal immunity to Measles, Mumps and/or Rubella. Half of the participants were revealed to have negative antibody titres to Hepatitis B surface antigen. Of these 4 individuals, 3 had received Hepatitis B vaccinations. Revaccination was subsequently offered to those with

inadequate titres to Hepatitis B, Tetanus or Diphtheria. As patients are ineligible to receive live vaccines, appropriate counselling was provided to those with insufficient titres to Measles, Mumps, Rubella or Varicella. Proper diligence to vaccine history and serology can help identify those who may be susceptible to vaccine-preventable infections.

1641

STEROID-FREE IMMUNOSUPPRESSION PROVIDES EQUIVALENT LONG TERM OUTCOMES IN SELECTED RENAL TRANSPLANT RECIPIENTS

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Background: Most centres performing renal transplantation incorporate steroids as part of immunosuppression therapy. Since mid-2003, our centre has utilized a steroid-free (SF) regimen in selected renal transplant recipients in the belief that long term outcomes with SF would be equivalent to a steroid-containing (SC) regimen .
Methods: Data from all adult renal transplant recipients transplanted between Jan 1, 2004 and Dec 31, 2009 were extracted from the provincial database. We included repeat transplant recipients, recipients of live (LD) and deceased donors (DD), but excluded recipients of multi-organ allografts. Primary outcome measures were patient (PS) and graft survival (GS), and renal function (creatinine, eGFR). Secondary outcome measures included biopsy-proven acute rejection (BPAR) rate and grades, delayed graft function (DGF), need for dialysis in the first week, conversion between SF and SC, and infection with BK and CMV.
Results: Of 825 recipients, 590 were initially SF; 467 (79%) remained SF over the study period. 189/235 (80%) of SC recipients remained SC. Immunosuppression consisted of tacrolimus and mycophenolate for the majority, and induction with basiliximab (98% SF; 54% SC), or thymoglobulin (2% SF; 25% SC). Recipient age, percent male, and Caucasian race were no different between the two groups. Baseline risk factors for rejection were significantly higher in SC compared to SF ($p<0.0001$ for all): $\geq 2^{nd}$ transplant 2% SF vs. 26% SC; peak PRA 2.8% in SF vs.13.8% in SC; DD 37% in SF vs.52% in SC; mean cold ischemia time 6.52 hrs in SF vs. 8.43 hrs in SC. Six year PS was better in SF compared to SC with hazard ratio (HR) of 0.42 (95% confidence interval (CI) 0.24, 0.75) in a model adjusted for age and sex; similar findings were noted for GS (HR 0.44; 95% CI 0.29, 0.68). Renal function at six years follow up was comparable (creatinine 116 ± 41umol/L SF; 129 ± 60 SC; mean ± SD p=0.31). More patients in the SC group experienced DGF and need for hemodialysis in the first week (34.5% and 31.1% respectively) compared to the SF group (10.4% and 8.8%) ($p<0.0001$ for both). At least one episode of BPAR was experienced by 26.8% of SC compared to 9.3% of SF ($p<0.0001$), and time to first BPAR was shorter in SC compared to SF (110 days vs 280 days; p=0.03). There were no differences between SF and SC in rate of BK or CMV infection.
Conclusion: We conclude that outcomes in a SF regimen in a large renal transplant cohort are at least equivalent to those in a SC regimen. Reasons for this may relate to significant selection and treatment bias at the time of transplant.

1642

EXTRA-CORPOREAL MEMBRANE OXYGENATION TEMPORARY SUPPORT TO TREAT EARLY GRAFT FAILURE AFTER CARDIAC TRANSPLANTATION

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Early graft failure is the major cause of mortality within 30 days after heart transplantation. We examined the impact of extracorporeal membrane oxygenation (ECMO) on graft recovery and early mortality.
Methods/Results: We retrospectively reviewed 13 patients with early graft failure unresponsive to ionotropic support at our institution between January 2007 and June 2011 treated with early (n=8) or delayed (n=5) ECMO. Eight patients (mean age 46.3 ± 19.5 years, male 75%) were weaned from cardiopulmonary bypass with peripheral arteriovenous ECMO. Five patients (mean age 38.4 ± 13.5 years, male 60%) treated with important ionotropic support for early graft dysfunction needed delayed ECMO support for acute hemodynamic collapse.
The 8 patients treated early were weaned after a mean support of 3.5 ± 1.3 days with full recovery of left ventricular function (ejection fraction 60 ± 12%). In this group the 30-day and 1-year survival was 87 % and 75 % respectively. The causes of mortality were respiratory failure in one patient and septic shock in the other.
All patients treated with delayed ECMO could not be weaned from mechanical support and died of multi organ failure.
Conclusions: In our experience ECMO support is a reliable therapeutic option for graft salvation in severe early graft failure if the support is initiated early. In this case complete recovery of cardiac function is frequent and usually occurs less than 4 days after ECMO installation with good survival. On the contrary delayed ECMO appears to be associated with poor outcome. This emphasizes the necessity to identify precociously graft dysfunction and to treat it aggressively.

1644

TOOL KIT: "PLANNING MY TRIP TO THE HOSPITAL." A GUIDE ON HOW TO PREPARE CHILDREN AND ADOLESCENTS WITH AUTISM FOR TRANSPLANT

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Children and adolescents with Autism Spectrum Disorder (ASD) have a "triad of impairment (Hartley, Sikora & McCoy, 2008, p. 819)," including deficits in communication, in social reciprocity and repetitive behaviours (Hartley, Sikora & McCoy, 2008). Sometimes they also display maladaptive behaviours and perseveration that interfere with medical procedures.
Non-adherence is a serious risk for children/adolescents undergoing transplant particularity if they are depressed or anxious (Berney-Martinet, Key, Bell, Lepine, Clermont & Fombonne, 2008). Non-adherence is an even greater risk for patients with cognitive deficits, challenging behaviours and poor social skills, as demonstrated in the literature.
Patients who have Autism and require a transplant are doubly at risk for challenging behaviours (e.g. aggression) that interfere with treatment. Furthermore, many have impaired comprehension and will not understand why such invasive procedures are necessary. The transplant process involves multiple invasive procedures that cause cumulative stress, with little opportunity for recovery in-between procedures. Busy transplant professionals who have not been trained to care for autistic children/adolescents do not have the skills required to handle severe agitation and/or aggression.
An interprofessional collaboration between Medical Psychiatry and the solid organ transplant teams at a quaternary care hospital has resulted in an innovative, evidenced-based approach to care. Since the inception of this clinical project, Planning My Trip to the Hospital Toolkit, eight children/adolescents with ASD ranging in ages from eight to sixteen have participated. This toolkit has now been used with patients with other complex mental illnesses. The goal of this presentation is to illustrate this program through case presentations and to discuss how a transplant team can prepare a child/adolescent with Autism for transplant.

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1645

EXCEPTIONNAL DISTRIBUTION: ANALYSIS, RESULT AFTER TRANSPLANTATION

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Subject: In the optic of respecting the Health Canada recommendations, Transplant Québec have in place a procedure of exceptional distribution. The outcome of the recipient who received an organ from such a donor was unknown.
Objectifs: 1. Analyse all cases of exceptional distribution of 2010. 2. Evaluate the health of recipients who received an organ from exceptional distribution one year after transplantation
Methodology: We revised all donors' files of 2010 who were under an exceptional distribution. We contacted the transplantation programs involved to obtain specific data related to the recepients' health following the transplantation. We wanted to know how they were doing, if they were alive or deceased, or if they had any complications related to the exceptional distribution.
Results: We found out that almost 30% of our recipients received organs from donor under exceptional distribution. Of 432 transplanted organs in 2010, 87 were transplanted from a donor in exceptional distribution. Of those 87 organs, there were 9 hearts, 7 lungs, 21 livers, 42 kidneys and 6 kidney-pancreas, one combined liver-kidney and a double kidney transplant.
It also showed that the most frequent reason for an exceptional distribution was the unknown risk of contamination with HIV, HCV and HBV.
Of the 85 transplanted patients, 7 died but the reason of their death had no link with the exceptional distribution. 7 patients had major complications with no link with exceptional distribution.
Conclusion: The exceptional distribution procedure works very well permitting to evaluate risks versus benefits for the recipient. There is no major complication for our patient one year post transplantation.
We know our results could be different but we trust that our transplant physicians take the best decision for their patients when they are confronted with a donor under exceptional distribution.

1646

HOW YOUNG IS TOO YOUNG TO BE A LIVING DONOR?

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Transplant centres are approached by people under the age of 21 indicating that they wish to be living organ donors. In several Canadian provinces the law permits people as young as 16 years to consent to living organ donation. Many health professionals have reservations about accepting young living donors (LDs) due to concerns about evaluating their ability to appreciate the potential risks and benefits of donation surgery, as well as the ethical acceptability of taking from those in the earlier stages of life. While the World Health Organization states that adolescence corresponds roughly to the ages of 10-19 years, parts of the brain associated with judgment continue to develop into the mid-20s. These challenges raise questions regarding the best way to evaluate the judgment of young potential LDs. Additionally, there may be a need to modify the evaluation process to address the personal values that influence decision-making at different stages in the life cycle.
This presentation will focus on how these issues impact on the ethical evaluation of young LDs, addressing donor autonomy and voluntariness, the informed consent process, donor motivation and expectations, and judgment of risks and benefits. It will explore social factors that may influence young LDs' decisions regarding donation such as family support and peer relationships. It will also discuss the importance of evaluating the relationships between young LDs and their recipients. Case-based material will illustrate how existing evaluation frameworks may be adapted to the assessment of young LDs. We contend that there are compelling ethical reasons to offer the opportunity of living donation to selected young people. We propose that living donor programs should develop policies and procedures to evaluate young LDs that respect the values of their respective organizations and communities and recognize that young people vary in maturity, life experiences and personal development.

1647

COST EFFECTIVENESS OF A STRATEGY OF PAYING TO INCREASE KIDNEYS FOR TRANSPLANTATION FROM LIVING DONORS: A DECISION ANALYSIS

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Background: Eligible patients with end-stage renal disease can be treated with a living or deceased donor transplant or dialysis. There are not enough kidneys for transplantation; thus the effectiveness and cost-effectiveness of strategies to increase the supply of kidneys requires consideration. Decision-analytic modeling was used to determine the cost-effectiveness of a strategy of paying people to give a kidney, assuming that financial incentives would increase the supply of kidneys for transplantation.
Methods: Data from the USRDS, CORR, along with published literature was used to estimate the cost and clinical outcomes associated with dialysis and transplantation over a lifetime horizon for patients waitlisted for transplantation. Decision analysis was then used to model the cost-effectiveness of financial incentives, assuming that kidney transplants would increase from 1 to 10% per year via incentives. Payment varied from $10,000 to $100,000, along with other clinical and costing parameters, within plausible ranges. The outcome was the incremental cost per quality-adjusted life year (QALY) gained.
Results: In the base case analysis (transplants increased by 1%; and payment of $10,000), the cost per QALY was $8846. If payment were to increase donation by 2%, the cost per QALY would be $1644; at an increase of 5%, the paid program is both more effective and cheaper. If the payment were to increase to $50,000 and $100,000 respectively (assuming a 1% increase in donation), then the cost per QALY would be $70,631 and $147,862, respectively.
Conclusions: Although the impact of payment on donation rates is uncertain, our model suggests that a strategy of incentives to increase kidneys could be cost-effective, despite small changes in transplantation rates. Further research on the acceptability and feasibility of payment to living donors, as well as studies investigating how payment would impact transplant rates are warranted.

1649

SHOULD THE WORST ORGAN GO TO THE LEAST SICK PATIENT?: MELD AND DONOR RISK INDEX AS PREDICTORS OF EARLY ALLOGRAFT DYSFUNCTION

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Background: There is a global tendency to transplant extended criteria organs (ECD) (Donor Risk Index DRI ≥1.7) ideally into recipients with a lower MELD score and to transplant standard criteria organs (DRI <1.7) into recipients with a high MELD score. This allocation strategy assumes that an increasing MELD score increases the probability of graft loss inherent to higher DRI allografts. There is a lack of evidence in the current literature for this assumption.
Methods: A review of our prospectively entered database for Donation after brain death (DBD) liver transplantation (N=310) between the dates of Jan 1 2006 and Sept 30 2010 was performed.DRI was dichotomized as standard livers (DRI <1.7) and ECD livers (DRI ≥1.7). Recipients were divided into 3 strata, those with high MELD (≥27) medium MELD (15-26) and low MELD (<15) scores. An updated definition of early allograft dysfunction (EAD) was recently validated as a

predictor of graft failure and death in the first 6 months.We analyzed EAD as it relates to both DRI and MELD scores.

Results: The overall incidence of EAD was 24.5%. Mortality in the first 6 months in recipients with EAD was 20% compared to 3.4% for those without EAD (RR 5.56 CI 1.96,15.73)($p<0.001$). Graft failure rate in the first 6 months in those with EAD was 27% compared to 5.8% for those without EAD (RR 4.63 CI 2.02,10.6)($p<0.001$). In multivariate adjusted logistic regression an interaction term was created for MELD score and DRI and this was significantly associated with the odds of EAD ($p<0.001$).

In patients with low MELD scores, a significantly increased rate of EAD was seen in patients transplanted with a high DRI liver (25%) compared to those transplanted with a low DRI liver (6.25%) ($p=0.012$). In medium MELD and high MELD patients, there was no significant difference in the rate of EAD in patients transplanted with a high DRI liver compared to those transplanted with a low DRI liver.

Conclusions: The use of high DRI livers (DRI $\geq$ 1.7) in low MELD patients results in higher levels of EAD, whereas the use of high or low DRI livers for recipients with medium or high MELD does not result in a significantly different rate of EAD. These results suggest that it is not appropriate to preferentially allocate organs with higher DRI to recipients with lower MELD scores.

1650

PRE-OPERATIVE STRATIFICATION OF PATIENTS UNDERGOING RETRANSPLANTATION OF THE LIVER

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Patients undergoing liver retransplantation (ReLT) are at higher risk of graft failure and mortality compared to patients undergoing their first liver transplantation, due to both the technical challenges of the procedure and the severity of illness in the recipient. Previously published ReLT predictive scores, in the post MELD era, have used both pre-operative and intra-operative variables, limiting their utility in pre-operative decision making. The present study aimed to develop a pre-operative score predicting graft survival in patients undergoing ReLT.

A total of 116 adult patients undergoing 134 ReLT, with donation after brain death organs, were identified from our prospectively entered transplant database between the dates of January 1982 and September 1 2011. Median follow-up was 4.3 years. Potential variables associated with graft survival following ReLT were identified a priori from review of the literature. A Cox proportional hazard model with backward stepwise selection was used predicting graft survival at 5 years post ReLT. Risk factors retained in the final model included: intubation prior to ReLT, MELD score, Serum creatinine umol/L, recipient age, Hepatitis C virus and Donor Risk Index (DRI). The final score was definied as:

$$\text{ReLT\_Score} = \exp\{(0.515 \text{ if intubated}) + (0.030 * Cr) + (0.039 * MELD) + (1.022 * DRI) + (0.958 \text{ if Hep C}) + (0.039 * age)\}$$

We validated the prediction score internally using the bootstrap method in the original derivation data set by sampling with replacement for 1000 iterations. The predictive accuracy of the final model using Harrell's C statistic was 0.8342. indicating that the model is good predictor of graft survival. This was more predictive than the value of previous models using both pre and intra-operative variables (C=0.774). When divided into quartiles the prediction score accurately predicted graft survival on Kaplan-Meier curves (logrank $p=0.008$).

The present study defines a pre-operative predictive score which accurately predicts graft survival following ReLT of the liver. Quantitative assessment of the risk of graft failure following ReLT using this predictive score is useful to inform patients and physicians of prognosis and outcome, as well as for patient stratification.

1652

PNEUMOCYSTIS PNEUMONIA IN SOLID ORGAN TRANSPLANT RECIPIENTS: NOT YET AN INFECTION OF THE PAST

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Background: Solid organ transplant (SOT) recipients are at risk of Pneumocystis pneumonia (PCP), especially in the first year post-transplant. Although trimethoprim-sulfamethoxazole prophylaxis substantially decreases this risk, there is little data and no consensus on optimal duration of prophylaxis. Consequently, there is lack of standardization of prophylaxis duration (3 months to lifelong, depending on organ group) in transplant programs across Canada.

Methods: We performed a retrospective chart review to identify all cases of PCP, confirmed by bronchoscopy, in our adult kidney, liver and lung transplant recipients from 2001-2011.

Results: Of 1241 patients followed by our clinic (657 kidney, 44 kidney/pancreas, 436 liver and 104 lung or heart/lung), a total of 14 PCP cases were identified in 2 kidney, 1 kidney/pancreas, 5 liver, 5 single lung, and 1 heart/lung transplant recipient. At the time of PCP diagnosis, immunosuppression consisted of prednisone, tacrolimus, and mycophenolate mofetil in 79% of patients, and 53% had

previously received trimethoprim-sulfamethoxazole for prophylaxis. Early PCP occurred in all 5 liver and 1 kidney transplant recipient who never received prophylaxis (17-204 days post-transplant). Of those who had received 6 months of prophylaxis (1 kidney, 1 kidney/pancreas), PCP occurred at 846 and 4778 days respectively. Late onset PCP occurred in lung recipients who received 12 months of prophylaxis (lung 645-1414 days; heart/lung 1583 days post-transplant). Five patients had experienced acute rejection and six patients had CMV viremia on average 59 and 204 days preceding PCP, respectively. There were 3 deaths (1 liver, 2 lung) thought to be directly related to complications of PCP.

Conclusion: Our experience supports the current American Society of Transplantation recommendation for lifelong prophylaxis in the lung transplant population, in view of the late PCP seen in this group with only 1 year of prophylaxis. Given the number of patients who experienced an acute rejection episode or CMV disease preceding PCP, consideration should be given to re-institution of PCP prophylaxis for a period of time after these events in kidney, kidney/pancreas, and liver transplant recipients.

1653

THE RED CELL DISTRIBUTION WIDTH IS ASSOCIATED WITH MORTALITY IN KIDNEY TRANSPLANTED RECIPIENTS

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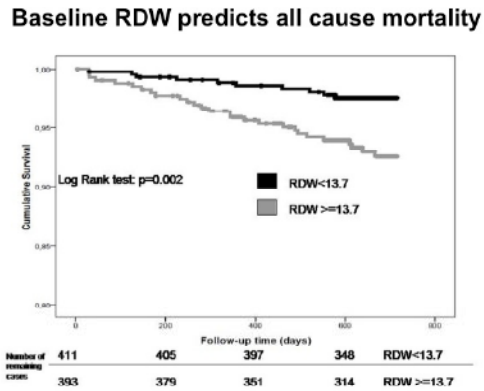
Background: Red Cell Distribution Width (RDW) is associated with inflammation, ineffective erythropoiesis, undernutrition, iron deficiency and impaired renal function in patients with heart failure. It is also associated with increased morbidity and mortality risk. No published data is available about the association between mortality and RDW in kidney transplanted patients.

Methods: We collected socio-demographic parameters, medical and transplant history and laboratory data at baseline from 804 prevalent kidney transplant recipients. RDW was measured as part of a standard complete blood count measurement. To assess if RDW predicts with all-cause mortality risk we used Cox proportional hazards regression.

Results: During the 22 month follow-up, 37 subjects died. Mean age was 51±13 years, 56% of the patients were males, 21% were diabetics. Mortality was significantly higher in patients with high vs low (above vs below median) RDW at baseline (Figure). RDW was a significant predictor of mortality both in the univariate model ($HR_{1\ increase}$ = 1.594; 95% CI: 1.353-1.877) and also in a multivariate model ($HR_{1\ increase}$ = 1.451; 95% CI: 1.139-1.848) adjusting for several co-variables (age, gender, eGFR, Charson Comorbidity Scale, CRP and albumin).

Conclusions: RDW independently predicts mortality in prevalent kidney transplant recipients.



1655

PEDIATRIC PRIORITY IN ORGAN ALLOCATION: ETHICAL PROBLEMS AND PROPOSAL FOR AN ALTERNATIVE MODEL.

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It has been demonstrated in the US that a pediatric deceased kidney allocation priority could be a disincentive for living donation.There is a so called improvement in overall access to transplantation for children based on a shorter waiting time.However,this apparent advantage is lost abruptly at 18y, and is complicated by important ethical issues.In Quebec,our previous allocation rules were essentially based on HLA matching.Since the middle of 2004, we simply give priority for only 1 kidney from each donor between 5 and 45y for a recipient under 18y.The other kidney goes to the adult population. We wanted to see if the phenomenon observed in the US occurred in Quebec,we have examined the ethical problems posed by such a policy, and we propose an alternative, integrated and progressive model,eventually applicable to other organs than the kidney.We compared the living and cadaveric pediatric transplantation activity based on the provincial registry data before and after the implementation of the pediatric priority.Access was measured by multiple parameters(abbreviated data)

	Total	living	cadaveric	Waiting Time cadaveric in days(mo.)	MMDR
2000	7	0	7	428(14)	0.5
2001	6	2	4	718(24)	0.75
2002	13	7	6	428(14)	0.4
2003	22	10	12	700(23)	0.9
2004	16	3 + 2	1 + 10	471(16)	1.3
2005	21	3	18	253(8)	1.2
2006	8	0	8	63(2)	1
2007	13	1	12	95(3)	1.2
2008	13	5	8	191(6)	1.3

The waiting time was unnecessary shortened to 3 to 4 months for the entire cohort,the living donation decreased, the DR Mismatching increased.This new policy,based on seemingly utilitarian principles,could be considered discriminatory(ageism) against the whole adult list, and the brutal transition at 18y seems to be unfair for the children and the young adults.Good kidneys,older than 45y, are inaccessible,even for immunized pediatric patients. Following Veatch's suggestion of justice-over-a-lifetime argument,equity requires that we target organs for the younger persons who are so poorly off that they will not make it to old age without being given special priority:applying a factor Q/Age to the whole waiting list,in addition to the other factors,would produce an entitlement to organs that is inversely proportional to age without any brutal transition at 18y. A safety net of age/10 would secure the kidney system.This continuous factor could be included in the algorithm for other organs than the kidney.

1656

DEBILITATING LOWER URINARY TRACT SYMPTOMS IN THE POST-RENAL TRANSPLANT POPULATION CAN BE PREDICTED PRE-TRANSPLANTATION

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Introduction and Objective: Overactive bladder (OAB) and benign prostatic hyperplasia (BPH) are common entities in the aging population causing lower urinary tract symptoms (LUTS). Renal transplantation (TX) is performed with increasing frequency in older patients who may have occult OAB or BPH masked by the low urine output of end-stage renal disease (ESRD). Sequelae of these disease processes may pose an underlying risk to renal allografts from episodes of retention and infections. While studied extensively in paediatric renal TX patients with ESRD secondary to anomalies of the urinary tract, the effect of LUTS on middle-age and older TX recipients is relatively under-studied. The purpose of our study was to determine the frequency and severity of LUTS in renal TX patients and to determine if validated questionnaires could predict which patients will develop LUTS post-renal TX.

Methods: All adult patients who underwent renal TX at our institution between 2005 and 2010 were invited to participate in this study via mailed questionnaires. The Overactive Bladder Questionnaire (OAB-Q) was completed based on patient symptoms at three time points: pre-TX, and 6 and 12 months post-TX. Male patients also received the International Prostate Symptom Score sheet (IPSS) and were asked to complete the survey for the same three time points. Overall scores were tabulated based on the returned surveys.

Results: Of the 465 patients who underwent renal TX, 105 patients participated in the study (response rate 22.6%). Pre-TX LUTS were common at 15% using the OAB-Q. Post-TX, 31% and 23% had moderate to severe symptoms at 6 and 12 months respectively. Health-related quality of life (HRQL) scores pre-TX were predictive of moderate to severe symptoms post-TX with an odds ratio of 11.2 (95% CI 2.7-45.9, $p = 0.0012$) at 6 months and 9.2 (95% CI 2.0–41.8, $p = 0.0085$) at 12 months. In male patients the IPSS found 40.8% of men had moderate to severe BPH symptoms pre-TX. When their post-TX symptoms were examined these patients were 9.4 times as likely to suffer moderate to severe symptoms as compared to patients with low IPSS scores at 12 months (95% CI 1.7-51.9, $p = 0.0086$). Subgroup analysis examined the quartile of patients with the most severe OAB-Q subscale scores including severity, HRQL, coping, concern, sleep, and social impact. Of these subgroups symptom severity, coping, and sleep components were found to be significantly worse post-TX ($p = 0.001$, 0.003, and 0.014).

Conclusions: The use of validated LUTS questionnaires (OAB-Q and IPSS) prior to renal TX may predict patients who will suffer significant LUTS post renal-TX. Identification of patients at risk for LUTS could allow for screening of inappropriate TX candidates, and treatment of urologic symptoms in the pre-operative or early post-operative period via medical and or surgical interventions avoiding complications which could compromise renal allografts.

1657

FREQUENT EARLY RECURRENCE OF GIANT CELL MYOCARDITIS IN CARDIAC ALLOGRAFT

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Background: Giant cell myocarditis frequently causes irreversible fulminant left ventricular dysfunction. In those patients, cardiac transplantation is the only viable treatment option. The histologic hallmarks of giant cell myocarditis includes a polymorphous inflammatory response with numerous multinucleated giant cells and extensive myocyte necrosis. There were 5 patients who received a cardiac transplant for giant cell myocarditis in our cohort between 2004 and 2011. Among these, 3 patients experienced recurrences of the disease in the allograft including one fatality.
Method/Results: All patients received simulect for induction and a standard protocol including tacrolimus targeting blood concentration > 10ng/ml, cellcept 1.5g bid and prednisone 0.5 mg/kg. Two patients developed asymptomatic systolic dysfunction (ejection fraction < 50%) caused by recurrent giant cell myocarditis demonstrated by endomyocardial biopsy less than 8 weeks after heart transplantation. One patient was treated successfully with methylprednisolone 1g I\V tid for three days and increased of tacrolimus targeting blood concentration > 15ng/ml. Prednisone was increased and sirolimus added in the second patient. After the augmentation of immunosuppression, ejection fraction increased > 60% and repeated cardiac biopsy demonstrated no recurrence of giant cell myocarditis. The third patient had fatal sudden death at home six months after allograft. Focal recurrence of giant cell myocarditis was found at the autopsy. Two days before her death, she complained to her relative of shortness of breath.
Conclusions: Giant cell myocarditis should be expected to recur in the allograft. In most of the cases, the disease in the allograft responds to therapy in a favourable manner. However, in one of our cases, the recurrence was fatal. Surveillance by repeated endomyocardial biopsy and more frequent echocardiography than in the usual transplant population is suggested. Also, in light of the high recurrence rate of giant cell myocarditis in the allograft, higher dosage of calcineurine inhibitor and/or addition of mtor inhibitor may be considered in the early postoperative period.

1658

PREDICTORS OF END-STAGE RENAL DISEASE IN RECIPIENTS OF PANCREAS TRANSPLANT ALONE

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Introduction: Pancreas transplant alone (PTA) has become an accepted therapy for selected non-uremic patients with type 1 diabetes mellitus. The published literature suggests that there is reversibility of diabetic nephropathy after 5-10 years of successful PTA. There is also evidence of development of calcineurin inhibitor nephrotoxicity.
Purpose: To determine predictors of end-stage renal disease (ESRD) after PTA.
Methods: Retrospective cohort study including 43 patients (age 39±8 yrs) with PTA between June 1998 and March 2011. Immunosuppression consisted of ATG-induction, tacrolimus, mycophenolate mofetil, and corticosteroids. The patients were divided into 2 Groups: Group 1: 9 patients (4 women/5 men) with pre-Tx eGFR <60 mL/min/1.73 m^2 and Group 2: 34 pts (14 women/20 men) with pre-Tx eGFR >60 mL/min/1.73m^2. We used multivariate analysis to identify independent predictors of ESRD. The mean pre-Tx eGFR in Group 1 was 44±8 vs. 101±21 mL/min/1.73 m^2 in Group 2 (P<0.0001). Pre-Tx proteinuria (g/d) was 1.6±1.5 in Group 1 vs. 1.2±1.6 and Group 2 (P=0.6). Duration of diabetes was 28±7 yrs in Group 1 vs. 25±7 yrs in Group 2 (P=0.2). Two patients received ACE and/or ARB in Group 1 vs. 10 pts in Group 2 (P=1.0).
Results: The incidence of ESRD requiring chronic dialysis at 1, 3 and 5 years was 0%, 28.57% and 61.9%, respectively in Group 1, and 0%, 8.2% and 12.5%, respectively in Group 2 (P=0.006). Significant predictors of ESRD requiring chronic dialysis included age (HR=0.7, CI: 0.563-0.873, P=0.001, higher risk in younger patients), gender (HR=0.09, CI: 0.012-0.664, P=0.018, higher risk in women), duration of diabetes mellitus pre-PTA (HR=1.24, CI: 1.025-1.510, P=0.02), and pre-PTA eGFR <60/mL/min/1.73 m^2 (HR=0.027, CI: 0.003-0.227, P=0.0009). There was no significant difference in tacrolimus dose and levels between both Groups. Patient survival at 5 years was 89.9% in Group 1 and 85.8% in Group 2 (P=0.73).
Conclusion: Recipients of PTA with eGFR <60 mL/min/1.73 m^2 pre-Tx, younger patients, women, and those with a longer history of diabetes mellitus pre-Tx, were more likely to progress to ESRD requiring chronic dialysis. These factors may highlight patients that could benefit from closer kidney function monitoring and renal protection strategies. Future studies should determine modifiable predictors of ESRD, assess the outcomes of patients on chronic dialysis, and determine the timing and impact of kidney Tx.

1660

AN ASSESSMENT OF THE EFFECTS OF IYENGAR YOGA ON THE HEALTH-RELATED QUALITY OF LIFE OF PATIENTS WITH CHRONIC RESPIRATORY DISEASES: A PILOT STUDY

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Aims: To explore the feasibility of implementing an Iyengar Yoga Program (IYP) for patients with chronic respiratory diseases and to assess its effects on patient health-related quality of life.
Methods: Patients attending the lung transplant clinics in a tertiary institution were invited to participate in a 12-week IYP that included two-hour biweekly classes. Doctors completed a formal physical and clinical assessment on candidates prior to their enrolment. Patients with New York Association Class of III or IV or Dyspnea Grade IV were excluded. At baseline and end of 12-weeks, patients completed the Hospital Anxiety and Depression Scale (HADS), Chronic Respiratory Questionnaire (CRQ), Health Utilities Index (HUI). Medication, six-minute walk test (6MWT) and other clinical parameters were also recorded. Patients recorded in their journals the effects of the yoga practice on their daily living.
Results: Eight patients diagnosed with pulmonary arterial hypertension and chronic obstructive pulmonary disease had a mean age of 60 (range 45 to 67 years old). At the end of the 12 weeks, changes in HADSanxiety and CRQfatigue scores were statistically significant ($p<0.05$) and clinically important. There was an improvement in 6-MWT ($p<0.05$). Two patients on oxygen were able to forgo using it during the class and one decreased her medication. The content of the journals revealed patients improvement in breathing capacity, mobility, energy and sleep. Comments such as “increased tidal volume with slowing expiration”, “breathing improved, feels very good, awesome!” “I have an overall feeling of wellbeing”, “excellent amount of energy”.
Conclusions: Patients overall evaluation suggests potential benefits of the program. The program was well received by healthcare providers.

1661

EVALUATION OF BLOOD GLUCOSE LEVELS AFTER HEPATITIS B IMMUNE GLOBULIN ADMINISTRATION UTILIZING TWO DIFFERENT BLOOD GLUCOSE MONITORING SYSTEMS IN THE ACUTE POST LIVER TRANSPLANT SETTING

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Background: Hepatitis B Immune Globulin (HBIG) is used post liver transplantation (LT) in Hepatitis B surface antigen positive recipients to prevent recurrence of Hepatitis B. One formulation of HBIG, HepaGamB™ (Cangene Corp, Winnipeg, Manitoba) contains 10% maltose, which can potentially falsely elevate glucose readings when glucose nonspecific point of care (GNSPOC) testing is used. This can result in inappropriate administration of antidiabetic agents and resultant episodes of clinically significant hypoglycemia. Glucose specific point of care (GSPOC) testing, however, is not affected by the presence of maltose. The purpose of this study is to determine if there is a significant difference in glucose readings using GSPOC and GNSPOC monitoring devices after HBIG administration.
Methods: This is a non-randomized, prospective study of patients receiving HBIG therapy (HepaGamB™, 20,000 units IV) in the acute post transplant setting (≤7 days post LT). To assess differences in blood glucose levels measured using GSPOC versus GNSPOC meters, capillary blood glucose tests were performed using a GSPOC meter and a GNSPOC meter pre-dose, immediately post-dose, 60 minutes post-dose and 120 minutes post-dose corresponding to the HBIG dosing. Venous blood glucose levels were also assessed in local hospital laboratory pre- and post dose.
Results: Five adult (mean age: 61 years [range, 48-69]; 100% male) LT recipients receiving HBIG therapy, in the acute post operative inpatient setting were enrolled. Prior to HBIG administration, the mean and median difference (GNSPOC minus GSPOC) were -10.0 and -7 g/dL, respectively. Immediately post infusion, the mean and median difference remained consistent (mean, -7.6 and median, -5 g/dL). By 60 minutes and 120 minutes post infusion, the GNSPOC readings were still similar to the GSPOC readings (60 minutes: mean, 10.8 g/dL and median, -2 g/dL; 120 minutes: mean, 11.6 g/dL and median, 1 g/dL). The differences observed between the readings were not considered clinically significant. A random intercept model was used to fit the five subjects’ glucose reading data over time. The fixed effect glucose meters, time effect and their interactions were included in the model. The Meter by Time interaction effect is not significant (P-value=0.72) and the Meter effect is not significant (P-value=0.90) which demonstrates there is no statistical difference between GNS and GS readings following HBIG administration.
Conclusions: Based on these results, there is not a significant difference between GSPOC and GNSPOC readings after administration of this HBIG formulation in the acute post liver transplant setting.

1663

THE INFLUENCE OF IMMEDIATE OUTCOMES ON LONG-TERM RESULTS IN RECIPIENTS OF KIDNEY TRANSPLANTS FROM STANDARD CRITERIA VERSUS EXPANDED CRITERIA DONORS

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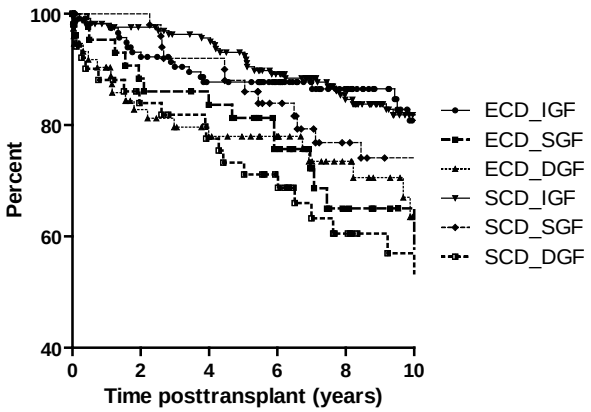
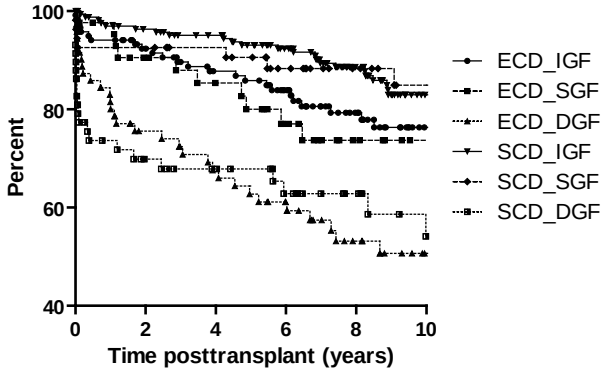
Introduction: The use of kidney transplants (KTx) from expanded criteria donors (ECD) has increased over the past decade. KTx from ECD are associated with an increased risk of graft lost.
Purpose: To determine the impact of immediate outcomes on long-term patient and graft survival in recipients of ECD vs. standard criteria donor kidneys (SCD).
Methods: We studied 471 KTx recipients of deceased donors between 1/1990 and 12/2006. 253 pts (53.7%, 82 women, 171 men, age 49±12 yrs) SCD kidneys, and 218 pts (46.3%, 73 women, 145 men, age 52±13 yrs) received ECD kidneys. Immunosuppression included ATG induction, CNI and an antimetabolite. We performed multivariate analyses using the following variables: gender, age, primary disease, time on dialysis, pre-Tx PRA, HLA-B-DR miss-match, first Tx or redo-KTx, ECD, use of azathioprine or mycophenolate mofetil and cyclosporine or tacrolimus; immediate graft function (IGF, Scr decreased ≥20% within 24 hrs post-KTx), slow graft function (SGF, Scr decreased <20% within 24 hrs post-KTx and no need for dialysis), delayed graft function (DGF, need for dialysis during the first week post-KTx), Tx era (before and after 7/1997), ECD criteria (before and after 2002), Scr and eGFR at 1, 3 and 12 months post-KTx, eGFR drop lower than or greater than 30% between 1-3, 1-12 and 3-12 months, and acute rejection during the first yr post-KTx.
Results: Patient and death censored graft survival are depicted in Figures 1 and 2. Significant predictors of patient survival were: age (HR: 1.058, P<0.0001), Scr at 1 yr (HR: 1.008, P<0.0001), redo-KTx (HR: 2.134, P=0.0003), and use of tacrolimus at 1 month post-KTx (HR: 0.605, P=0.02). Significant predictors of death censored graft survival were: Scr at 1 yr (HR: 1.027, P<0.0001) and eGFR drop >30% (1-12 months) (HR: 2.165, P=0.02).
Conclusion: Recipients of KTx from SCD or ECD with IGF have similar long-term patient and death censored graft survival. In addition to known predictors, eGFR drop >30% (1-12 months post-KTx) is a strong negative predictor of death censored graft survival.

Figure 1. Patient survival*



*Patient survival: ECD_IGF vs. SCD_IGF (P=NS), ECD_SGF vs. SCD_SGF (P=NS), ECD_ DGF vs. SCD_DGF (P=NS).

Figure 2. Death censored graft survival**



**Death censored graft survival: ECD_IGF vs. SCD_IGF (P=NS), ECD_SGF vs. SCD_SGF (P=0.04), ECD_DGF vs. SCD_DGF (P=NS).

1664

SYMBOLISM OF THE HEART AS A TRANSPLANTED ORGAN: "YOU REALIZE YOU HAVE SOMEONE ELSE'S HEART AND IT'S KIND OF WEIRD"

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Purpose: Throughout history, the heart has been viewed as a potent symbol, as well as a vital organ. The mythological and symbolic qualities ascribed to the heart can complicate heart transplant (HTx) patients' acceptance of the new organ. It is posited that any change to the body inevitably transforms the self, hence this study explored possible disturbances to embodiment and personal identity which may be associated with pediatric HTx.
Methods: This qualitative research study explored perceptions of self-identity and bodily integrity in adolescent HTx patients. Participants were recruited from a large pediatric Tx centre and a grounded theory approach guided data collection, data analysis and theory development.
Results: A total of 27/31 HTx patients (18 female, 67%) participated (median age 15.5 yrs; range 12.2 18.4 yrs) with a median age at time of Tx of 12.3 yrs (range 1.7-17.5yrs) and a median time post-Tx of 3.2 yrs (range 0.3 11.1 yrs). Results indicated that many adolescent patients had emotional and psychological concerns regarding accepting a foreign organ as their own and the meaning they associated with the donated organ. This manifested in a range of responses such as sadness or guilt regarding the death of the donor and/or thoughts about potentially acquiring personal qualities or

characteristics of the donor. Many participants speculated extensively about the donor and longed for donor information. A complex relationship with the imagined donor emerged, including a sense of identity assimilation.
Conclusions: Our findings point to the psychological and meaning-making processes adolescent transplant patients encounter as they grapple with the presence of a foreign, life-giving organ within their body. This research highlights the complex process of integrating and adapting to HTx and invites future exploration of the potential impact on adolescents' concept of self and identity which may emerge following HTx.

1665

THE USE OF IMPELLA MICROAXIAL LEFT VENTRICULAR ASSIST DEVICE FOR TREATMENT OF CARDIOGENIC SHOCK: A SINGLE-CENTER EXPERIENCE.

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Background: The aim of this study is to report our experience with the use of the Impella microaxial left ventricular assist device (Abiomed, Danvers, MA, US) in the treatment of intractable cardiogenic shock.
Methods and Results: Charts were reviewed. From October 2010 to September 2011, 10 consecutive patients underwent Impella implantation; one patient had a repeat implantation for device infection. Median patient age was 48, 6 were men, all were in profound cardiogenic shock requiring use of vasopressors and inotropes, 4 had an intra-aortic counterpulsation pump; median EF was 17.5%. Underlying etiologies were: 4 acute ischemiae, 4 myocardites, 1 post cardiotomy and 1 post-transplant. In 3 patients, device implantation was due to inability to wean off bypass (1 CABG, 1 cardiac transplant, 1 MVR/ascending aorta replacement). Three devices were implanted percutaneously in the cath lab via the right femoral artery; one was complicated by cardiac tamponnade, which was drained percutaneously, without further complication. Eight were implanted in the O.R.: 1 percutaneously via the left femoral artery, 7 open technique: 3 via the left femoral artery, 1 via the right femoral artery, 2 via the right subclavian artery, and 1 via a side branch of a aortic Dacron graft. Two implantations failed because of small artery caliber: one was swithed to a smaller 2.5 device, the other ended up with a femoro-femoral ECMO, then was re-transplanted. Median operative time was 138 minutes. One patient had access vessel complication and underwent repair patch angioplasty. 7 patients had massive blood transfusion (defined as 5 or more units of PRBC). One patient was transplanted with concomitant device removal. Another patient was switched to a Heartmate II LVAD and was eventually transplanted, but died of transplant failure. There were 2 other in-hospital mortalities: one was due to intractable haemorrhage, the other was due shock secondary to intestinal occlusion. In 6 patients, the device was successfully weaned off after a median time of 4 days.
Conclusion: Our results support the feasibility and safety of the Impella microaxial LVAD for treatment of intractable cardiogenic shock, as a bridge to recovery, to another more permanent LVAD or to transplant. Prospective randomized trials are needed to ascertain the clinical impact of such an innovative approach.

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AN EXERCISE IN AGE-MATCHING - BALANCING EQUITY AND UTILITY IN ORGAN ALLOCATION

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Background: Maintaining a balance between equity and utility is a key marker of success in policy directing the allocation of deceased donor organs. In recent years, the CST Kidney Working group presented evidence in support of an age-matched allocation policy whereby kidneys from young donors (aged ≤35 years) are preferentially allocated to younger recipients (aged <55 years). In Nov, 2009 our province implemented this policy along with the preferential allocation of kidneys from donors aged ≥ 60 to candidates aged ≥60 years. Donors aged 36-59 are allocated based on wait-time alone and are not age-matched. We report the impact of this policy on allocation practices and on the median wait-times for patients within our program between Nov, 2009 and May, 2011.
Results: Thirty-one percent of deceased donors were young (≤35 years), with 83% them having kidneys allocated to younger recipients (< 55 years) while 10% (n=4) were allocated to recipients aged ≥ 60. Nearly 50% of donors were aged ≥ 60 and 60% of kidneys from these donors were allocated to recipients over 60 years while 22% were allocated to recipients under 55 years.
The table outlines median wait-times for kidney transplant recipients by year, age, and ABO type. Compared to the 2 years preceding the policy change, median wait-times were not prolonged for any age category, regardless of ABO type. Less than 20% of deceased donor transplants were among blood type AB or B recipients, making it difficult to delineate trends in wait-times in these smaller groups. Among elderly blood type A and O recipients there was a decrease in wait-times, with the most dramatic drop in blood type A recipients aged ≥60 from 60 months in 2007 to

under 40 months in 2011. Meanwhile among younger recipients (19-54yrs) in these groups, there was no significant increase in wait-times.

Conclusion: Formal implementation of age-matching resulted in the mismatched allocation of young donor kidneys to elderly recipients in only 4 cases and did not appear to negatively impact wait-times for any age group of recipients, regardless of blood type, with blood type A and O elderly recipients demonstrating a reduction in wait-times. These results suggest that implementation of this age-matching policy has begun to maximize utilization of deceased donor kidneys without evidence of significantly sacrificing equity in access to transplantation.

Table: Median wait-time (months) for first deceased donor kidney transplant, by ABO type, age, and year of transplantation

ABO	Age Group	2007	2008	2009	2010	2011
A	**19-54y (n-66)**	**59.8**	**48.3**	**39.7**	**61.4**	**50.5**
	55-59y (n=16)	**70.0**	**60.3**	**---**	**71.4**	**49.8**
	≥ 60y (n=51)	**60.4**	**52.3**	**28.6**	**43.2**	**36.5**
O	**19-54y (n=57)**	**81.4**	**78.5**	**78.6**	**83.1**	**75.3**
	55-59y (n=17)	**128.5**	**80.6**	**68.3**	**82.5**	**76.3**
	≥ 60y (n=58)	**81.5**	**87.5**	**71.1**	**70.9**	**77.1**
B	19-54y (n=14)	97.9	---	90.8	71.4	84.1
	55-59y (n=4)	---	---	117.5	70.8	69.4
	≥ 60y (n=12)	73.4	---	88.0	77.3	89.5
AB	19-54y (n=12)	40.8	38.8	65.7	43.2	37.2
	55-59y (n=5)	27.9	79.8	59.8	---	---
	≥ 60y (n=4)	---	---	---	51.6	22.2

--- indicates no transplant recipients for the given subgroup and year

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MORBIDITY AND MORTALITY OF INFANTS REQUIRING HEART TRANSPLANTATION IN WESTERN CANADA: WAITING FOR AN ANSWER.

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Introduction: End-stage heart disease in babies remains a challenge with significant mortality and morbidity. Despite recent advances, prolonged time on the waitlist remains a significant concern and also affects post heart transplantation (HTx) outcomes. This study analyzes outcomes of patients listed for HTx under age 3 months in the low population density region of the Western Canada Children's Heart Network.

Methods: Retrospective chart review of all patients listed for HTx under the age of 90 days from January 2007 to October 2011 was performed aiming to determine waitlist mortality and outcome after HTx. Factors that could potentially impact on outcomes before and after Tx were assessed as a secondary objective. Statistical comparison of different groups was performed using Man-Whitney Rank Sum Test.

Results: Throughout the study period, 24 patients were listed for HTx. Median age at listing was 19 days (from 5 days pre-birth to 88 days). The cardiac diagnosis was congenital heart disease in 18 cases and cardiomyopathy, myocarditis and others in the remaining 6. Of the total number of patients, 12 (50%) underwent HTx after a median of 42 days (from 2 to 215 days) on the waitlist. One patient was removed after 112 days due to clinical improvement. Eleven patients (46%) died or were removed from the waitlist in preparation to withdraw life support after a median of 32 days (from 10 to 127 days). Of the patients who died on the waitlist, the main cause of death was sepsis (4). Fourteen patients required some form of extra-corporeal life support pre-Tx. Of these, 9 were ultimately transplanted and 5 died awaiting a suitable donor. In the transplanted group, six patients (50%) received a heart from an ABOi donor and all remain alive. The remaining six received a heart from an ABO-compatible (ABOc) donor, with 3 deaths. Children who received an ABOi heart waited a median of 29 days compared to 43 days for an organ from an ABOc donor (p=0.39). Cumulative survival pre- and post-HTx was 46%.

Conclusion: Waitlist mortality in western Canada exceeds rates reported from higher population density areas. Despite having a nation-wide waitlist for the sickest patients and progressive approaches such as ABOi HTx, the mortality is higher than for any other age group. Since the post-HTx outcomes in early childhood are promising, further strategies to improve organ availability and allocation are required including a possible revision of eligibility criteria for potential organ donors in infant age.

1670

RECIPIENTS OF KIDNEYS FROM EXPANDED CRITERIA DONORS WHOSE EGFR DOES NOT DROP >30% BETWEEN 1-12 MONTHS AFTER TRANSPLANTATION HAVE EXCELLENT LONG-TERM GRAFT SURVIVAL.

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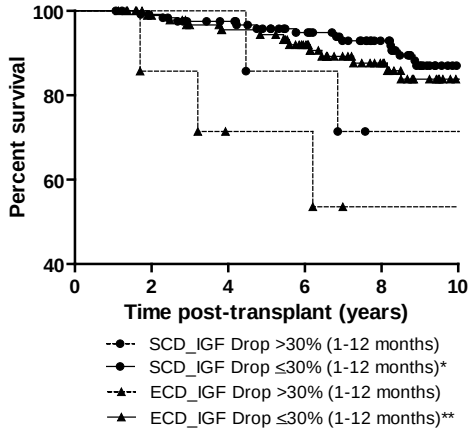
Introduction: The use of kidneys from expanded criteria donors (ECD) is associated with a high risk of graft loss.
Purpose: To determine the impact of eGFR drop lower or greater than 30% during the first year after kidney transplantation (KTx) on long-term graft survival.
Methods: We studied 471 KTx recipients of deceased donor kidneys between 1/1990 and 12/2006. 253 pts (53.7%, 82 women, 171 men, 49±12 years) received standard criteria donor (SCD) kidneys, and 218 pts (46.3%, 73 women, 145 men, 52±13 years) received an ECD kidney. Immunosuppression consisted of ATG induction, CNI and an antimetabolite. CIT was 15.4±8.4 hrs. We analyzed an eGFR drop lower or greater than 30% between 1-3, 1-12 and 3-12 months in recipients of SCD and ECD kidneys, with immediate (IGF, Scr decreased ≥20% within 24 hrs post-KTx), slow (SGF, Scr decreased <20% within 24 hrs post-KTx and no need for dialysis) or delayed graft function (DGF, need for dialysis during the first week post-KTx), on long-term death censored graft survival in pts whose graft survived >1 year post-KTx. 55 recipients of SCD and 34 recipients of ECD were excluded because of graft loss, death or loss to follow-up during the first year.
Results: eGFR (mL/min/1.73m^2) at 1, 5 and 10 years was 71±22, 66±22 and 57±22 respectively, in recipients of SCD and 56±18, 49±23 and 41±20 respectively, in recipients of ECD (P=0.001). An eGFR drop between 1-12 months was associated with lower death censored graft survival (HR 2.16, P=0.02). The impact of eGFR drop on long-term death censored graft survival is depicted in Figures 1, 2 and 3.
Conclusion: Recipients of ECD kidneys without an eGFR drop >30% between 1-12 months post-KTx have excellent long-term death censored graft survival, equivalent to recipients of SCD kidneys.

Figure 1. Actuarial death censored graft survival in pts with IGF stratified by eGFR drop during the first yr post-KTx.



*P=0.002 vs. ECD Drop >30%; **P=0.01 vs. ECD Drop >30%

Figure 2. Actuarial death censored graft survival in pts with SGF stratified by eGFR drop during the first yr post-KTx.

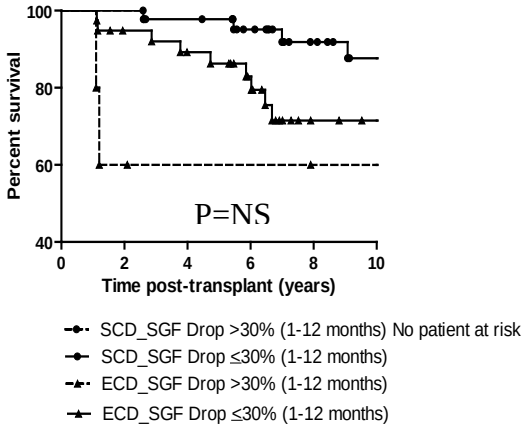
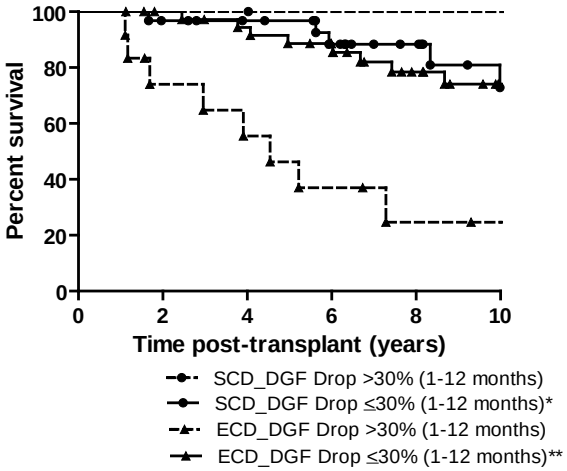


Figure 3. Actuarial death censored graft survival in pts with DGF stratified by eGFR drop during the first yr post-KTx.



*P<0.0001 vs. ECD Drop >30%; **P=0.0003 vs. ECD Drop >30%

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5 YEARS EXPERIENCE WITH LUNG DONATION AFTER CARDIAC DEATH

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Objective: Donation after cardiac death (DCD) was initiated in 2006 in Canada. We reviewed our experience after 5 years of activities of lung transplantation (LTx) using lungs from DCD donors.
Methods: Retrospective study using prospectively collected data. Data were collected from donors and recipients involved

in DCD LTx between September of 2006 and September 2011. Outcomes of these patients were compared with contemporaneous recipients receiving lungs from brain death donors (BDD). Incidence of severe primary graft dysfunction (PGD) after transplantation, ICU and hospital length of stay (LOS), 30 day mortality, and proportional survival were analyzed and compared between the 2 groups. Patients on extra-corporeal life support prior to transplantation and retransplantation were excluded from this study.

Results: The total number of deceased donors, DCD donors, and DCD lung transplants are shown in Table 1. During the study period, 448 LTx were performed; 408 from BDD and 40 from DCD donors. The distribution of recipient diagnosis was similar in both groups (BDD vs. DCD): Emphysema (26% vs. 30%), pulmonary fibrosis (33% vs. 32%), cystic fibrosis (20% vs. 23%), and others (22% vs. 14%). Mean donor gas exchange function (P/F) was 422 mmHg in BDD group and 360 mmHg in DCD group (p=0.004). Mean recipient P/F at ICU arrival was 350 mmHg in BDD vs. 301 mmHg in DCD (p=0.06). Incidence of severe primary graft dysfunction after LTx requiring ECMO support was 3% in BDD and 7.5% in DCD (p=0.14). Median ICU and hospital LOS were 4 and 23 days respectively in both groups (p>0.05). 30 day mortality (4.3% in BDD and 5% in DCD, p= 0.60) and 1 year survival (84% in BDD and 85 % in DCD, p=1.00) were similar in both groups.

Conclusion: DCD LTx activities have significantly increased in Ontario. The use of controlled DCD lungs for transplantation is associated with similar early and intermediate clinical outcomes as LTx using BDD donors.

	2006	2007	2008	2009	2010	2011
Total Ontario Deceased Donors	172	200	175	218	200	183
Ontario DCD Donors	4	17	30	37	35	35
DCD Lung Transplants	0	3	5	9	9	14

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THE MANAGEMENT OF POST-TRANSPLANT LYMPHOCELES: CHANGES IN PRACTICE AT L'HÔTEL-DIEU DE QUÉBEC

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Introduction: Peritransplant lymphocele is a common complication following kidney transplantation. Conservative management is usually recommended unless complication developed as lymphocele can sometimes be the source of significant morbidity in relation to compressive or infectious phenomenons. In our experience, lymphocele management has dramatically evolved overtime toward a less invasive approach. The purpose of this study is to investigate risk factors of symptomatic post-transplant lymphocele, and to review efficacy and complications associated with different therapeutic strategies available in the management of this complication.

Materials and methods: Between January 1990 and December 2010, 962 kidney transplantations have been performed in our institution. 152 recipients (15.8%) developed a lymphocele in the posttransplant period. Relevant demographic characteristics of recipients and donors as well as peritransplant details and long-term fucntion and survivals were retrospectively collected by using our clinical database. Descriptive analysis of patients who developed a posttransplant lymphocele has been performed in order to assess efficacy/ complications of differents management options such as aspiration, drainage, sclerotherapy or marsupialisation of the lymphocele. Moreover, uni- and multivariate comparison between patients with a symptomatic lymphocele and patients without lymphocele will be achieved to evaluate factors that can be associated with this complication.

Results: From the 152 patients with a post-transplant lymphocele, 119 (78% of all patients with a lymphocele, 12% of the total population) required active treatment of their lymphocele. Reasons for treatment were hydronephrosis/ureteral obstruction in 62%. Other reasons included infected lymphocele (8%), scrotum swelling (7%), leg oedema (2%), wound drainage (3%) and vascular compression (3%). The aspiration was the single most practiced procedure (52%) but was associated with a low success rate (15%). When indwelling drainage was performed, success rates improved up to 34% but was associated with higher risk for infectious complications (14%). At the begining of our experience, internal marsupialisation of the lymphocele was the operation of choice for refractory lymphoceles and was necessary for 44% of our patients with symptomatic lymphocele. However, success after one surgery was only 50%. Of the patients who failed, 58% required a second or a third surgical marsupialisation. This surgery was associated with a 17% complication incidence. Finally, sclerotherapy with proviodine was used in 35% of our cohort with a high success rate of 97%. An average of 1.8 (1-11) sclerotherapy session per patient was necessary to achieve lymphocele resolution. It was also used successfully as a salvage treatment in 6 patients that failed surgical marsupialisation. Complication rate was 5% and included mainly infectious events, without negative impact on ureteral stenosis incidence.

Conclusion: In our experience, posttransplant lymphocele management has evolved toward a minimally invasive

approach with proviodine sclerotherapy. This treatment strategy has been associated with a high success rate and low incidence of complications. Consequently, we think this strategy should be favored in the treatment of symptomatic lymphoceles.

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THE EFFECT OF POST-OPERATIVE INTRAVENOUS HEPARIN INFUSION ON SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANT OUTCOMES

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Introduction: Graft thrombosis is the most common cause of technical failure in pancreas transplantation. It can occur in up to 20% of patients and may be due to both donor and recipient factors. There is no current evidence to suggest that the use of anti-coagulation in the peri-operative period has any potential benefit of reducing the rates of portal vein thrombosis in pancreas transplants.
Objective: The aim of the current study was to retrospectively compare the short and long-term clinical outcomes and complication rates in patients who underwent simultaneous kidney-pancreas (SPK) transplants at our institution to determine if the use of post-operative continuous, low-dose intravenous heparin played any effect.
Methods: There were 47 SPK transplants performed at our institution between 2004 and 2011. All patients since July 2009 (n=16) received a post-operative regimen of continuous heparin (Group 1) at 300U/h for 24h, followed by 400U/h until POD#5, whereas patients transplanted prior to that date (n=31) did not (Group 2). There was no bolus given and heparin was started in the recovery unit. All patients were then placed on baby ASA on POD#6 which they continued indefinitely. We assessed the following factors: Donor: age, BMI, DCD/NDD, ischemic time; Recipient: age, previous transplant, previous thromboembolic events, immunosuppression, serum biochemistry (creatinine, glucose, hemoglobin, C-peptide, amylase, lipase, INR/PTT), complications (pancreatitis, DVT, PE, graft thrombosis, hemorrhage, transfusions, re-operation, graft function and loss). Serum parameters were measured pre-transplant and on POD# 1, 3, 7, 14, 30, 180 and 360. Statistical analyses were carried out using a Fisher Exact test and MANOVA.
Results: 18% of Group 2 recipients lost their grafts to portal vein thromboses versus 0% in Group 1 with an overall graft function of 100% in Group 1 and 82% in Group 2 (p=0.11). One patient in Group 2 died from a fatal pulmonary embolus post-operatively whereas no fatalities were observed in the heparinized group. Both groups had equal rates of re-exploration/drain insertion for peri-pancreatic fluid collections. Serum biochemical parameters for renal and pancreatic function were comparable between the groups at the time of last follow-up.
Conclusion: This study provides novel information on the use of peri-operative intravenous anti-coagulative therapy in SPK transplantation. Although not statistically significant, there appears to be a clinically significant trend towards a beneficial effect of the use of post-operative intravenous heparin in this population. A larger cohort will be necessary to confirm these findings.

BASIC SCIENCE

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NANOVESICLES FROM APOPTOTIC ENDOTHELIAL CELLS FAVOR ANTI-LG3 PRODUCTION AND NEOINTIMA FORMATION IN A MURINE MODEL OF VASCULAR REJECTION

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Vascular rejection (VR) is associated with endothelial apoptosis within glomerular and peritubular capillaries of renal allografts. Endothelial apoptosis triggers basement membrane proteolysis and production of a C-terminal fragment of perlecan, LG3. We previously showed that human renal transplant recipients with acute VR show increased LG3 serum levels and elevated anti-LG3 titers. The production of nanovesicles, vesicles distinct from apoptotic bodies and reminiscent of exosomes, is increased during apoptosis. Whether apoptotic nanovesicles contribute to the production of anti-LG3 antibodies and vascular remodelling remains to be established. Here, we aimed at defining the impact of apoptotic endothelial nanovesicles on the production of anti-LG3 antibodies and vascular remodelling in a model of VR in mice.
Nanovesicles were purified by ultracentrifugation from serum-free medium conditioned by apoptotic EC and the presence of LG3 in apoptotic nanovesicles was evaluated by western blotting. Aortas from female BALB/c mice were transplanted in fully MHC-mismatched female C57Bl/6 mice. Syngeneic aortic transplantation served as control. Nanovesicles purified from medium conditioned by apoptotic serum starved murine EC or non-apoptotic serum starved murine EC (pre-treated with the pan-caspase inhibitior ZVAD-fmk) were injected intravenously every other day post-surgery for 3 weeks.

Grafted mice were sacrificed 3 weeks post-surgery and blood and aortas were harvested.
LG3 was recovered within the apoptotic nanovesicle fraction of medium conditioned by apoptotic EC and was not detected in the soluble fraction. Anti-LG3 titers increased significantly in mice transplanted with an allogeneic aortic segment compared with syngeneic controls (p=0.0358; n= 8 for each group). Injection of apoptotic nanovesicles significantly increased anti-LG3 titers in recipients of an allogeneic aortic graft compared to those injected with nanovesicles from non-apoptotic EC (p=0.0422; n= 10 and 9 respectively). At 3 weeks post-transplantation, early neointima formation with accumulation of a-smooth muscle actin positive cells juxtaposed to the allograft endothelium was only observed in recipients injected with apoptotic nanovesicles. Intima/media ratios were higher in aortic allograft recipients injected with nanovesicles from apoptotic EC compared to those injected with nanovesicles from non-apoptotic EC (p=0.0011).

These results suggest that apoptotic nanovesicles are novel conveyors of neoantigens that, like LG3, are of potential importance in vascular remodelling. Apoptotic nanovesicles favour the production of anti-LG3 antibodies and accelerate neointima formation during VR. Strategies aimed at regulating the production of apoptotic nanovesicles could represent future opportunities for controlling maladaptive vascular remodelling of allografts.

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ESTABLISHMENT OF NONMYELOABLATIVE BONE MARROW CHIMERISM BY DOUBLE NEGATIVE TREG CELLS THROUGH INDUCING T CELL CLONAL DELETION AND SUPPRESSING NK CELL FUNCTION

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Keywords: DN-Treg, TCR, clonal deletion, NK cells, chimerism, bone marrow transplantation.

In bone marrow (BM) transplantation, T cells and NK cells play important role for graft rejection. In addition, graft-versus-host-disease, and establishment of stable chimeric states without complete marrow ablation remain as major obstacles post BM transplantation. In this study, we aimed to establish stable mixed chimerism under non-irradiation conditions. The role of 'double negative' (DN) T regulatory cells to control T and NK cell activity has not been tested in this approach. Our data indicate that adoptive transfer of donor-derived $TCR\alpha\beta^{+}CD3^{+}CD4^{-}CD8^{-}NK1.1^{-}$ (double negative, DN) Treg cells prior to C57BL/6 to BALB/c BM transplantation, in combination with cyclophosphamide but not cyclosporine, FK506, or rapamycin, established stable mixed chimerism that led to acceptance of C57BL/6 skin allografts and rejection of 3rd party C3H (H-2k) skin grafts. Adoptive transfer of $CD4^{+}$ and CD8+ T cells, but not DN-Treg cells, induced graft-versus-host diseases in this regimen. Recipient T cell alloreactive responsiveness was reduced in the DN-Treg cell-treated group (4836±2686 cpm vs. 23907±7077 cpm, mean±SD, P<0.01, n=3). The Vβ2, Vβ7, Vβ8.1-2, Vβ8.3 T cell receptor clones were significantly decreased by (71.73±10.89)%, (52.32±19.32)%, (37.32±8.73)%, (46.65±21.09)% respectively in CD4+ T cells, and (77.05±14.39)%, (67.39±14.25)%, (45.93±14.89)%, (52.43±24.76)% respectively in CD8+ T cells (P<0.05, mean±SD, n=3). Furthermore, DN-Treg cell treatment suppressed NK cell-mediated BM rejection （41.22±6.19）%, P<0.05, mean±SD, n=4） in a perforin-dependent manner. Taken together, our results suggest for the first time that adoptive transfer of DN-Treg cells can control both adoptive T cell and innate NK cell immunity and promote a stable mixed chimerism and donor-specific tolerance without an irradiation ablation regimen.

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RECEPTOR INTERACTING PROTEIN 3 (RIP3) REGULATES TUBULAR EPITHELIAL CELL (TEC) INJURY AND INFLAMMATION FOLLOWING RENAL IRI

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Introduction: Renal transplantation is invariably associated with organ injury resulting from ischemia reperfusion injury (IRI) and death of tubular epithelial cells (TEC). TEC death can occur through apoptosis and pro-inflammatory necrosis. Members of the receptor interacting protein family (RIP1, RIP3) have been identified as regulators of a newly described form of cell death termed ‘necroptosis’. Studies have shown that apoptosis and necroptosis are counterbalanced mechanisms of cellular death that allows one pathway to compensate for the other when it becomes blocked. Necroptosis may be enhanced by blocking apoptosis, thus

potentially promoting renal inflammatory injury. Our studies were to determine the role of apoptosis and cellular RIP3 in regulating kidney necroptosis/necrosis with IRI.
Methods/Results: We demonstrate for the first time that murine TEC express RIP3, and both mRNA and protein increased in response to IFNγ and TNFα. TEC underwent necrosis and apoptosis in response to IFNγ and TNFα as determined by Annexin V-PI staining/FACS. Inhibiting apoptosis markedly increased necroptosis mediated by TNFα treatment (15.5% vs. apoptosis inhibition: 37.7%, P<0.05, n=3). Necroptosis in TEC was nearly completely blocked *in vitro* by the RIP1 inhibitor, Nec-1 (37.7% vs. Nec-1: 1.2%, P<0.05, n=3). We then tested the role of RIP3 *in vivo* in a mouse renal IRI model using uni-nephrectomized C57BL/6 mice subjected to clamping of the renal pedicle for 45 min. RIP3 mRNA was detected in kidney tissue after IRI while RIP3 protein was detected in kidney sections by immuno-histochemistry. Necrosis of kidney tissue was quantified using the fluorochrome ethidium homodimer and fluorescent microscopy with automated image analysis. Necrosis was abrogated in $RIP3^{-/-}$ kidneys post-ischemia as compared to the wild type controls (area of necrosis index = $RIP3^{-/-}$ vs. wild type control = 1:18, P<0.0001, n=4/group). Kidney function was preserved in $RIP3^{-/-}$ mice compared to wild type mice using serum creatinine levels 48 hours post-ischemia (61.3±24.9 vs. 137.7±26.3 respectively, P<0.05, n=7/group).
Conclusion: Our data presents for the first time that necrosis in kidney epithelium is regulated by RIP3 expression which permits greater kidney injury and dysfunction following IRI. RIP3 expression is increased with IRI and then may promote further injury by enhancing necroptotic cell death. Blocking apoptosis along with inhibition of renal RIP3 may be both required to reduce renal inflammatory injury following IRI and transplantation.

1680

INFANT CD27+IGM+ B CELLS HAVE AN ELEVATED EXPRESSION OF THE B CELL INHIBITORY MOLECULE CD22

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Background: Immune immaturity allows ABO-incompatible heart transplantation (ABOi HTx) to be performed safely in infants. Generally, a deficiency in antibody (ab) production to donor ABO antigens (ags) is observed following ABOi HTx. ABO abs are normally thought to arise in a T-independent (TI) manner. TI B-cell activation has been shown to be inhibited by the interaction of CD22, an inhibitory B cell co-receptor, with sialic acids on cells and tissues, leading to B cell tolerance. It is unknown whether CD22 plays a role in regulating B cell responses towards ABO ags in the transplant setting. Due to the generally reduced immune response to TI-ags in infants, we hypothesize that CD22 expression and function may be elevated during infancy compared to later in life. In this study, we determined CD22 expression on various B cell subsets from infancy to adulthood.
Methods: We analyzed human splenocytes isolated from organ donors of various age (n=41; ages 4 days - 74 years). Flow cytometric analysis was performed to quantify the expression levels of CD22, CD27, CD38, IgM and IgG on the surface of $CD19^+$ B cells. Six different B cell subsets were investigated including $CD27^-IgM^+IgD^+CD38^{high}$ (previously defined as recent bone marrow emigrants), $CD27^-IgM^+IgD^+$, $CD27^-IgM^-IgD^+$, $CD27^-IgM^-IgD^-$, and the memory-like phenotype $CD27^+IgM^-$ and $CD27^+IgM^+$ B cells.
Results: Significant differences were observed when comparing the Median Fluorescence Intensity (MFI) of CD22 amongst the various B cell subsets (p<0.0001; Kruskal Wallis test). Post testing using the Dunn's multiple comparison test revealed that the $CD27^-IgM^-IgD^-$ B cell subset had lower expression of CD22 (p<0.001) and the $CD27^+IgM^+$ subset had higher expression (p<0.001) compared to all other subsets. Furthermore, the MFI of CD22 on the $CD27^+IgM^+$ B cell subset was inversely correlated with age (p=0.001), with infant samples having the highest level of CD22, and expression decreasing with increasing age.
Conclusion: Based on previous studies showing the inhibitory role of CD22 on B cell activation, our findings suggest that the increased expression of CD22 on the memory B cell subset may cause infant B cells to be more susceptible to down-regulation of B cell signaling leading to subsequent inactivation. CD22 may play a role in the restricted TI immune responses to ABO ags in the ABOi transplant setting. Functional assays are underway to determine its role in B cell signalling during infancy and its role in regulation.

1681

CD133+ STEM CELLS USED FOR CARDIAC REGENERATION MAY BE DESTINED FOR A FIBROBLAST PHENOTYPE RESULTING IN WORSENING MYOCARDIAL FIBROSIS

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Background: Stem cell therapies have been suggested to represent the future of organ regeneration strategies. However, several clinical trials using progenitor cells have failed to result in the expected clinical benefit to patients suffering from heart failure. The present study characterizes how a subset of stem cells, called fibrocytes, may account for some of the discrepancies from clinical trials to date.
Methods: In an established animal model of AngII-mediated heart failure, we administered a potent stem cell mobilizer, AMD3100. We monitored stem cell mobilization using flow cytometry for CD133 in peripheral blood. AngII and saline treated mice served as controls. At day 3, tissues were collected for RNA, protein and histology. Hearts sections were stained with H&E and Sirius Red and analyzed for cellular

infiltrate and collagen, respectively. Fibrocytes were identified by immunofluorescent colocalization of CD45 and α-smooth muscle cell actin (αSMA).
Results: In our heart failure model, we observed significant increases in the transcription of the pro-fibrotic factors TGF-β and CTGF in the myocardium. We administered AMD3100 to increase stem cell mobilization, resulting in an approximate 3-fold increase in the number of circulating $CD133^+$ cells. When increased stem cell mobilization occurred in this pro-fibrotic environment, we observed a significant increase in infiltrating cells in the heart (**78.9±3.1% vs. 30.0 ± 4.0% area of tissue affected)**, which was associated with an almost proportional increase in fibrosis (**29.0±4.8% vs. 11.9±1.9 % area of tissue affected)**. Furthermore, the infiltrating cells were identified as fibrocytes by immunofluorescence ($CD45^+$/ $αSMA^+$).
Conclusion: Despite others showing that stem cell mobilization can have beneficial effects for the treatment of heart disease, we are the first to examine this approach in a pro-fibrotic environment, such as in heart failure. Taken together, our results suggest that stem cells used in the setting of heart failure are destined to become fibrocytes rather than regenerating injured myocardium.

1682

NON-HLA ANTIBODIES IN HEART TRANSPLANT RECIPIENTS WITH AMR: PROFILING WITH ANTIGEN MICORARRAYS

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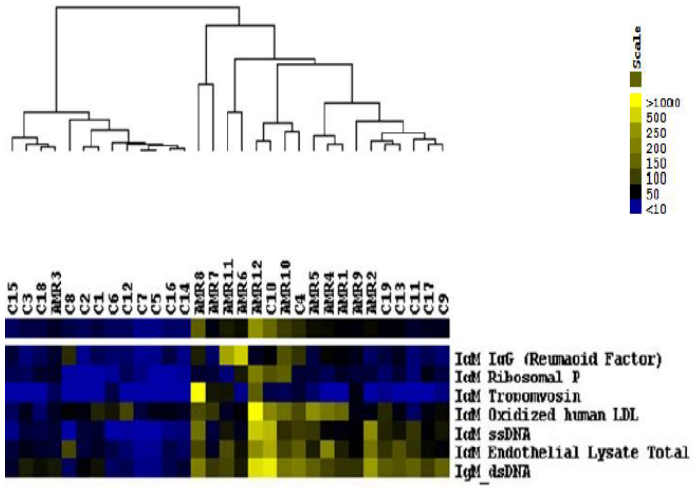
Introduction: While anti-HLA antibodies are the primary component of the humoral response in heart transplant recipients with antibody mediated rejection (AMR), antibodies can also be directed against non-HLA antigens. In order to more fully profile non-HLA antibodies in AMR, we generated custom antigen microarrays comprising 61 antigens and probed the microarrays with serum from recipients with AMR and from controls. Antigen microarrays have been used previously to study antigen reactivities in patients with autoimmune diseases.
Hypothesis: Non-HLA antibodies are elevated in heart transplant recipients who experience AMR as compared to control recipients.
Methods: Recipients were diagnosed with AMR based on parameters of LV dysfunction, new donor specific antibodies, and C4d staining. Control recipients were identified who had not experienced significant rejection, had normal LV function, and did not have cardiac allograft vasculopathy.
Antigen Microarrays were constructed by spotting proteins, peptides, and lysates onto FAST nitrocellulose slides using a microarrayer. After blocking the slides, they were probed with diluted serum (1:150). They were then probed with fluorescently labelled secondary antibodies to detect both IgG and IgM. Fluorescent reactivities were detected with an Axon scanner and quantified with Genepix software. Significance analysis of microarrays (SAM) was used to detect significant changes in antigen reactivities.
Results: Control recipients (n=19) were matched to AMR recipients (n=12) based on gender and pre-transplant diagnosis. Recipients with AMR were younger (40.2 vs. 52.4 years), more likely have had a LVAD (42% vs. 10%), and were more likely to be sensitized prior to transplant as compared to control recipients.
After antigen microarrays were performed, SAM analysis revealed 7 antigen reactivities that were significantly elevated (Q value < 0.05) in the recipients with AMR. The figure shows a heatmap of the significant antigens and clustering of the recipients with AMR. Interestingly, the elevated antibody reactivities were all of the IgM subtype. Given that many of the antigens are intracellular or related to oxidation (e.g., DNA, ribosomal P, tropomyosin, oxidized LDL), the IgM reactivities may reflect a humoral response to tissue damage. Association of these IgM reactivities with AMR has not been described previously.
Conclusions: Antigen microarrays may be a useful tool to identify heart transplant recipients with AMR and may provide insights into pathogenesis of AMR.



1683

REGULATION OF NK CELL-MEDIATED TUBULAR EPITHELIAL CELL DEATH AND KIDNEY ISCHEMIA-REPERFUSION INJURY BY NKR-P1 RECEPTORS AND CLR-B

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Background: Transplantation is invariably associated with organ injury resulting from ischemia-reperfusion injury (IRI), inflammation, drug toxicity and rejection. Tubular epithelial cells (TEC) comprise of more than 75% renal parenchymal cells and their susceptibility to injury directs long-term function of kidney allografts. We have previously shown that natural killer (NK) cells can kill TEC in a perforin-dependent manner and contribute to renal IRI. Recent studies have demonstrated an inhibitory effect of natural killer receptor-protein 1B (NKR-P1B) on NK cell activity. NKR-P1B has been shown to interact with C-type lectin-related protein B (Clr-b), resulting in the suppression of NK cell cytotoxicity. Interestingly, NKR-P1B expression is missing in certain mouse strains, such as C57BL/6, and may explain BALB/c mice's high resistance against IRI relative to C57BL/6 mice. Therefore, up-regulating inhibitory Clr-b – NKR-P1B interaction in kidney transplant patients may protect allografts from NK cell cytotoxicity.
Methods: Cell Characterization: Real-time RT-PCR, flow cytometry, and cytotoxic assays. Cytokine Induction: Cells were cultured with 25 ng/mL of TNF-α and 25 ng/mL of IFN-γ. Hypoxia: Cells were stressed in a hypoxic chamber for 30 min. Kidney IRI: Left renal arteries were clamped for 45 min and right kidney was removed. Anti-Clr-b was kindly donated by Dr. JR Carlyle, University of Toronto.
Results: Real-time analysis of BALB/c Clr-b mRNA expression demonstrated an increase in transcription levels after IRI (4-fold, n = 1). This is supported by flow cytometry data, indicating that Clr-b surface expression in BALB/c (H-2d) mice progressively increased, peaking at 48 h, and persisted after IRI (11.26%, 24.49%, 46.47%, 35.39%, 52.37%, 50.90% for sham, 0 h, 8 h, 24 h, 48 h, 96 h after IRI, respectively, $p < 0.05$, n = 3 per time point). Similar patterns of Clr-b expression was seen in cytokine-induced primary cultured TEC (8.53%, 25.53%, 69.70%, 43.92%, 3.75%, 9.84% for control, 0 h, 24 h, 48 h, 72 h, 96 h after treatment, respectively, $p < 0.05$, n = 4). Furthermore, BALB/c NK cytotoxicity assays demonstrated enhanced necrotic death in TEC after translational interference by Clr-b siRNA. Real-time RT-PCR analysis also indicated that BALB/c NK cells express high levels of NKR-P1B after activation (23-fold, n = 1). Current experiments underway involve the silencing of kidney Clr-b or NK cell NKR-P1B in *in vivo* models, to determine whether NK-mediated TEC death and kidney IRI can be enhanced. In addition, we are studying whether enhancing Clr-b expression can protect the kidney from NK cell-mediated IRI.
Conclusions: Our results indicate that Clr-b expression on TEC and the kidney is up-regulated after injury. The blockade of Clr-b, therefore, may enhance NK cell-mediated TEC death and kidney injury. Furthermore, cytokine-induced Clr-b expression may prevent NK cell-mediated injury in TEC and the kidney. Up-regulating inhibitory Clr-b in transplant patients may protect the kidney from NK cell cytotoxicity.

1684

HUMAN AND MURINE APOPTOTIC ENDOTHELIAL CELL MICROENVIRONMENT REPROGRAMS MACROPHAGES INTO ANTI-INFLAMMATORY CELLS THROUGH MFG-E8 RELEASE.

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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 macrophages into anti-inflammatory, pro-repair phagocytes. We now want to elucidate the role of one candidate molecule present in the apoptotic cell-conditioned medium, *Milk Fat Globule Epidermal Growth Factor-8* (MFG-E8) in this macrophage reprogramming.
To model the CTV environment, EC were serum-starved for 4 hours to create an apoptotic serum-starved conditioned medium. To evaluate the specific role of MFG-E8, we performed immunoprecipitations and siRNA in endothelial cells to obtain conditioned media without MFG-E8. Moreover, we used apoptotic EC from MFG-E8 KO and WT mice and murine recombinant MFG-E8 to highlight the role of this protein in macrophage reprogramming. We also studied the STAT3 pathway in MFG-E8 stimulated macrophages.
We previously demonstrated that MFG-E8 was released by apoptotic endothelial cells in a caspase-3-dependent manner. The high anti-inflammatory, low pro-inflammatory cytokine/chemokine secreting phenotype of macrophages was lost when MFG-E8 was absent from the apoptotic cell-conditioned media (immunoprecipitation, silencing and KO). Treatment of macrophages with recombinant MFG-E8 recapitulated the effect of the conditioned media. Finally, we showed that MFG-E8-mediated reprogramming of macrophages occurs through increased phosphorylation of STAT-3.
Our study demonstrates an important role for the release of MFG-E8 from apoptotic endothelial cells in macrophage reprogramming and the importance of the apoptotic microenvironment in the anti-inflammatory pro-repair

macrophage response. These results suggest an important contribution of macrophage and MFG-E8 in the CTV-associated fibrosis.

1685

EX VIVO ASSESSMENT OF DCD HEARTS WITH STEEN SOLUTION IS ASSOCIATED WITH LESS MYOCARDIAL EDEMA AND IMPROVED CARDIAC FUNCTION

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Background: Previous work has suggested that hearts fromdonors after circulatory death (DCD) can regain sufficient function to supportthe circulation. However the optimal reperfusionstrategy has yet to be determined. Workin rodent hearts has suggested that tepid normothermic, normokalemiccardioplegia (CP) containing adenosine and lidocaine may confer improvedmyocardial protection compared to standard hypothermic, hyperkalemicsolutions. However this has not beenevaluated in DCD hearts. In addition,whether STEEN solution, originally devised as an ex vivo lung perfusate, canalso function as an ex vivo heart perfusate hasn't been studied in thiscontext.
Methods: 60 kg female Yorkshire pigs were subjected tohypoxic cardiac arrest, simulating organ donation following withdrawal of careand subsequent circulatory arrest. Resuscitation of the DCD heart wasapproached in two ways: Group 1, reperfusion with cold blood CP(Plegisol/blood), continuous ex vivo perfusion with crystalloid/blood, coldblood CP for myocardial preservation during transplantation; Group 2: tepidSTEEN solution containing 48 mg/L Adenocard and 100 mg/L lidocaine, continuousSTEEN/blood for ex vivo assessment and continuous warm blood during transplantation. Results: The strategy for Group 2 resulted in a shorter timeto establish continuous ex vivo perfusion (18±6 vs 7±2 min, $p<0.05$), withfewer arrhythmias and the development of less myocardial edema (25±8 vs 14±4g/hr heart weight, $p<0.05$). Cardiac function could be assessed ex vivo withpressure-volume loop analysis equally well in each group. Followingtransplantation, there were fewer arrhythmias and improved function in Group 2.
Conclusion: A strategy employing tepid reperfusion, avoidinghyperkalemia, followed by continuous perfusion of the heart, includingimplantation, results in improved function of DCD hearts. STEEN solution works well as an ex vivoperfusate for hearts. Whether STEENcould be used as an acellular perfusate remains to be determined.

1686

TIMING OF PERFUSATE INFUSION OF CORM TO PROTECT THE RENAL TRANSPLANT: ROLE OF APOPTOSIS

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Carbon monoxide releasing molecules (CORM) have been shown to protect the renal transplant when administered to perfusate solution around the time of cold storage. It is unclear whether protection is from reduction of inflammation at the time of reperfusion or prevention of apoptosis during storage. Isogeneic Lewis rat kidneys were infused with CORM-3 either before cold storage, after cold storage or given combination therapy (double flush). Kidneys were cold preserved for 26 hr and transplanted into isogeneic recipients. CORM-3 supplementation to standard preservation solution was found to be most beneficial prior to cold-storage exposure, however additional re-flushing prior to vascular reperfusion showed an additive benefit to graft survival and function following transplantation (Figure 1). This was confirmed histologically by reduction in glomerular and tubular necrosis and apoptosis (TUNEL) in the double-flushed grafts vs. other groups. In vitro, pretreatment of human umbilical vein endothelial cells in culture with CORM-3 during 4° C storage led to a significant increase in the frequency of live cells ($72.3 \pm 1.9\%$, $p<0.01$), reduced apoptosis ($14.9 \pm 6.1\%$, $p<0.01$) and decreased mitochondrial trans-membrane potential ($40.2 \pm 7.2\%$, $p<0.05$) in HUVEC cells exposed to 20h of cold storage vs. controls ($11.6 \pm 3.5\%$ live cells; $82.2 \pm 2.3\%$ cellular apoptosis; $78.2 \pm 3.2\%$ mitochondrial potential, respectively). In keeping with this anti-apoptotic effect, CORM-3 supplementation led to a 7.4 ± 2.1 fold upregulation in Bcl-2 gene expression. Taken together, CORM-3 protects the renal transplant through upregulation of mitochondrial survival mechanisms, leading to reduced apoptosis from transplant-related stress. In conclusion, we show that CORM-3 supplementation to standard UW solution has a significant impact on reducing cellular and graft injury and improving survival through anti-apoptotic effects, mediated through mitochondrial Bcl-2 associated survival mechanisms in addition to its anti-inflammatory effects during reperfusion.

1687

HYDROGEN SULPHIDE TREATMENT MITIGATES INFLAMMATION AND MODULATES EXPRESSION OF INFLAMMATORY AND ANTI-APOPTOTIC GENES IN SYNGENEIC RENAL GRAFTS SUBJECTED TO PROLONGED COLD STORAGE

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Introduction: Ischemia and reperfusion injury (IRI) is inherent in organ transplantation and leads to increased rates of delayed graft function, acute rejection and early graft loss. Developing novel techniques to protect donor organs against IRI-induced tissue injury is critical in preserving short- and long-term renal graft function and survival. Hydrogen sulphide (H_2S) is a newly discovered, endogenous molecule that has recently been shown to be protective against ischemic tissue injury. We have previously utilized a murine model of renal transplantation (RTx) to demonstrate that addition of H_2S treatment during prolonged cold preservation improves renal graft function and survival compared to UW solution alone and protects renal grafts against IRI-induced injury. In this study, we aimed to characterize specific anti-inflammatory and anti-apoptotic mechanisms by which H_2S protects renal grafts against transplantation-associated renal IRI.
Methods: Bilaterally nephrectomized Lewis rats underwent RTx with left kidneys obtained from syngeneic donors that were flushed, at the time of procurement, with either cold (4°C) UW solution (Control group) or cold UW solution + 150 µM NaHS (H_2S group) and stored for 24 hours at 4°C in the same solution. Sham operated rats were also followed. Control animals did not survive past post-RTx day 5 and H_2S animals were sacrificed between post-RTx day 3 and 5. Post-mortem renal grafts were placed half in formalin and the other stored at -80°C. Formalin specimens were embedded in paraffin before immunohistochemical staining with antibodies against myeloperoxidase (MPO) and
CD68, which are specific markers of neutrophils and macrophages, respectively. Renal grafts stored at -80°C were homogenized and RNA was isolated and subsequently reverse-transcribed into cDNA for quantitative reverse-transcriptase PCR (qRT-PCR) analysis of expression of pro-inflammatory genes Interferon-γ (IFN-γ), Tumour Necrosis Factor-α (TNF-α) and Intracellular Adhesion Molecule-1 (ICAM-1) and anti-apoptotic genes Extracellular Signal Kinase-1 and -2 (ERK-1 and ERK-2).
Results: H_2S treated renal grafts contained significantly fewer MPO-positive and CD68-positive cells compared to Control, whereas Control grafts contained significantly greater numbers of both cell types compared to Sham (Figure 1, $p<0.05$). Relative expression of ERK-2 was significantly increased ($p<0.05$) in the H_2S group compared to Control, while ERK-1 expression was unchanged between groups. As well, expression of pro-inflammatory genes IFN-γ, TNF-α and ICAM-1 was markedly decreased in H_2S treated grafts compared to Control, though these differences did not reach statistical significance (Figure 2).
Conclusions: H_2S treatment of renal grafts reduced the severity of inflammation and expression of pro-inflammatory genes IFN-γ, TNF-α and ICAM-1, as well as mitigated the down-regulation of the anti-apoptotic gene ERK-2 associated with prolonged cold IRI. These phenomena
likely contribute to the mechanisms by which H_2S protects against cold IRI during transplantation and maintains graft function.

1688

INDUCTION OF ALLOIMMUNE TOLERANCE IN HEART TRANSPLANTATION THROUGH GENE SILENCING OF TLR ADAPTORS

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Background: Toll-like receptors (TLRs) can activate two distinct downstream pathways that evoke an innate immune attack against the graft and promote DC maturation, resulting in antigen-specific immune rejection. Using siRNA to knock down the common adaptors of TLR signaling, namely MyD88 and TRIF, may shut down TLR signaling and abolish TLR-induced innate and adaptive immune rejection, thereby protecting cardiac grafts in transplantation.
Methods: Recipients (BALB/c) were treated with MyD88 and TRIF 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. A low dose of rapamycin (2mg/kg) was used after transplantation from day0 to day 13. Control groups that included untreated mice, scrambled siRNA, rapamycin monotherapy groups.
Results: Gene silencing MyD88 and TRIF genes resulted in arresting DC maturation. Treatment with MyD88 and TRIF siRNA significantly prolonged allograft survival (36.7±2.4 days) in heart transplantation. Moreover, the combination of MyD88 and TRIF siRNA along with a low dose of rapamycin

further extended the allograft survival (88.8±7.1 days); Tissue histopathology demonstrated an overall reduction in lymphocyte interstitium infiltration, vascular obstruction, and hemorrhage in mice treated with MyD88 and TRIF siRNA vector. The siRNA treatment promoted $CD4^+CD25^+FoxP3^+$ regulatory T cell generation and Th2 differentiation.
Conclusion: This study is the first demonstration of preventing immune rejection of allogeneic heart grafts through gene silencing of TLR signaling adaptors, highlighting the therapeutic potential of siRNA in clinical transplantation.

1689

ABO-MICROARRAY: A NOVEL TOOL FOR CHARACTERIZING SUBTYPE-SPECIFIC BLOOD GROUP ANTIBODIES FOLLOWING ABO-INCOMPATIBLE TRANSPLANTATION

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Introduction: Histo-blood group ABH(O) antigens are oligosaccharides present as glycolipids or glycoproteins in human cells. The epitope of the A, B or H antigen is carried by at least six different internal carbohydrate backbones (type I–VI), which form antigenically distinct variants of ABH epitopes. Each ABH antigen type is differentially expressed in human tissues. Identifying natural antibody clones specific to these ABH epitopes may be important in the setting of ABO-incompatible transplantation. Our objectives were to 1) develop a diagnostic tool to detect antibodies specific to ABH epitopes on all six carbohydrate backbones and 2) characterize antibodies in A, B, AB and O volunteers and in patients who received ABO-incompatible heart transplants as infants.
Methods: Chemically synthesized A type I–VI, B type I–VI and H type I–VI were printed onto microarray slides in a series of arrays; spot morphology and antigens were confirmed by monoclonal antibodies to ABH antigens. After initial optimization plasma samples from different blood group individuals and patients were hybridized, and bound IgM and IgG detected with fluorescently-labelled secondary antibodies. Slides were scanned with a microarray scanner and analyzed using Imagene software.
Results: Subtype-specific IgM and IgG antibodies varied substantially amongst individuals with different blood groups as well as the same blood group. Antibodies against self-blood group antigens were detected in some individuals (fig 1&2). Antibodies against donor blood group antigens in infants who developed B-cell tolerance following ABO-incompatible transplantation were absent in some (fig 3), yet subtype-specific antibodies were detectable in others, similar to normal individuals.
Conclusions: Current methods to assess ABO antibodies provide limited quantitative information and do not detect fine specificities. The ABO-microarray may be a valuable diagnostic tool for detection of subtype-specific blood group antibodies, although limits of individual variability have yet to be characterized fully. This tool will allow enhanced safety of ABO-incompatible organ transplantation and improved study of mechanisms of tolerance and/or accommodation in the clinical setting. Microarray technology allows for minimal sample volumes to be used (<5 □l plasma), and provides simultaneous characterization of all antibody isotypes. The advantages will be invaluable when testing infants.

1690

INFUSION OF PROGENITOR CELL SECRETORY PRODUCTS RESULTS IN POTENT CARDIO-RENAL PROTECTION IN EXPERIMENTAL CKD

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Background: As in native CKD, chronic renal allograft injury is characterized by progressive cardio-renal fibrosis that leads to dysfunction of both organs, and subsequent morbidity and mortality. Localizing to the liver and spleen post-infusion, bone marrow-derived early outgrowth cells (EOCs) markedly inhibit cardio-renal fibrosis in rats with CKD despite no significant EOC renal or cardiac retention. Recognizing the challenges faced by a cell-based therapy, we tested whether administration of EOC-secreted factors could replicate the tissue protective effects of EOCs.
Methods: Cell free conditioned medium (CFCM) was generated by incubating F344 rat EOCs with serum-free EBM-2 medium to collect their secreted factor(s). Subtotal nephrectomy (SNX) F344 rats, which develop progressive cardio-renal fibrosis that mimics human CKD, were randomized 4 wks post-SNX to receive an iv injection of 1×10^6 EOCs or thrice weekly iv injections of 10x concentrated CFCM or EBM-2 medium for 2 wks. FITC-inulin GFR, urinary protein, left ventricular end-diastolic pressure-volume relationship (LV EDPVR, a marker of diastolic cardiac function), and renal and cardiac fibrosis were measured 4 wks later.
Results: Compared with control medium, infusion of either EOCs or CFCM into SNX rats attenuated further progression of cardio-renal dysfunction and damage, as evidenced by improved GFR, and reduced systolic BP, urinary protein, and renal interstitial collagen IV deposition. Both CFCM and EOC treatment also equally improved diastolic cardiac relaxation and cardiac fibrosis (see Table below).
Conclusions: These data are the first to demonstrate that EOC-derived factor(s) can mimic the effects of cell infusion in attenuating cardio-renal fibrosis and dysfunction in experimental CKD. Identification of the responsible factor(s) and their synthesis may provide a new cell-free therapeutic strategy for the cardio-renal disease seen in patients with chronic renal allograft dysfunction.

	SNX - EBM-2	SNX - CFCM	SNX - EOC
GFR (L/min/g body wt)	1.3 ± 0.2	1.9 ± 0.1 *	2.1 ± 0.3 *
Systolic blood pressure (mm Hg)	201 ± 9	145 ± 14 *	163 ± 9 *
Urinary protein (g/mmol creat)	2.0 ± 0.3	1.0 ± 0.3 *	0.6 ± 0.2 *
Renal interstitial collagen IV deposition (AU)	1.61 ± 0.40	0.47 ± 0.03 *	0.53 ± 0.08 *
LV EDPVR (mm Hg/mL)	56 ± 14	29 ± 4 *	34 ± 4 *
Cardiac fibrosis (AU)	14.8 ± 2.5	7.5 ± 1.1 *	9.2 ± 0.7 *

* $p < 0.05$ vs. SNX - EBM-2 animals

1691

C-TERMINAL FRAGMENT LG3 : A NEW MEDIATOR IN MIGRATION OF VASCULAR SMOOTH MUSCLE CELLS

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Introduction: Sustained apoptosis of endothelial cells (EC) is associated with neointima formation characterized by the migration of vascular smooth muscle cells (VSMC) to the site of insult. In addition, apoptotic EC release cathepsin L, which cleaves perlecan, a proteoglycan of the vascular basement generating a C-terminal fragment LG3. We hypothesize that LG3 acts on VSMC to induce a pro-migratory phenotype. **Methods:** VSMCs are exposed to increasing doses of recombinant LG3. Migration is assessed by 'Wound Assay' and transmigration is evaluated in Boyden Chambers. The involvement of ERK1/2 is verified by the use of biochemical inhibitors (PD98059 and UO126). The inhibition of phosphorylation of ERK1/2 induced by LG3 in the presence of inhibitors was validated by Western blotting. Inhibition of β1 integrin receptor is performed with a neutralizing antibody and its effects are measured by Wound Assay and Western Blot. **Methods:** VSMC migration and transmigration are significantly increased when cells are incubated in the presence of LG3 as compared to vehicle ($p \leq 0.01$et $p \leq 0.001$ respectively). In addition, LG3 induces phosphorylation of ERK1/2 in a dose-dependent manner in VSMC ($p \leq 0.01$). The two specific inhibitors of ERK1/2 (PD98059 and UO126) reduced the migration of VSMC induced by LG3 ($p \leq 0.01$). Incubation of VSMC with a neutralizing antibody against β1 integrin receptor decreases VSMC migration and phosphorylation of ERK1/2 ($p \leq 0.001$ et $p \leq 0.01$ respectively). **Conclusion/Discussion:** These results suggest that LG3 induces ERK1/2-dependent migration of VSMC through interactions with β1 integrin receptor. Collectively, these results demonstrate that the C-terminal fragment of perlecan LG3, released during endothelial apoptosis, is a new mediator contributing to the migration of vascular smooth muscle cells to site of vascular injury.

1692

AUTOCRINE CTGF PRODUCTION AND MYOFIBROBLAST DIFFERENTIATION

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Chronic fibrosis is a major complication of transplantation. Microvascular rarefaction and increased expression of Connective Tissue Growth Factor (CTGF) within renal allografts are important predictors of fibrosis. Microvascular hypoperfusion is linked to chronic oxygen, nutrient and growth factor deprivation. We propose that growth factor (GF) deprivation activates the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway in fibroblasts, leading to autocrine CTGF production. CTGF favors differentiation of fibroblasts into myofibroblasts responsible for extracellular matrix accumulation and fibrosis.

WI-38 human fibroblasts were exposed to serum free medium as a model of GF deprivation. Markers of myofibroblast differentiation (aSMA, collagen I and III and stress fibers) were evaluated by Western blot, qPCR and immunofluorescence confocal microscopy. CTGF mRNA and protein levels were evaluated by qPCR and Western blot. The functional impact of the PI3K/mTOR pathway on myofibroblast differentiation and CTGF production was analysed with biochemical inhibitors (LY294002 for PI3K and rapamycin for mTOR). The importance of autocrine CTGF in GF deprivation-induced myofibroblast differentiation was studied with the use of CTGF siRNA.

GF deprivation for 4 days increases the expression of aSMA and collagen I and III and enhances stress fiber formation. GF deprivation also increases intra and extracellular CTGF levels. LY294002 and rapamycin inhibit myofibroblast differentiation and CTGF synthesis and secretion, suggesting that myofibroblast differentiation and CTGF production are both under the control of the PI3K/mTOR axis in GF-deprived fibroblasts. Silencing CTGF expression in GF-deprived fibroblasts blocks aSMA synthesis, suggesting that autocrine CTGF production is central to myofibroblast differentiation.

Collectively, these results suggest that GF deprivation induces myofibroblast differentiation through PI3K/mTOR-dependent pathways triggering CTGF overexpression and secretion. These results provide novel insights into fibrogenic mechanisms activated in conditions of reduced GF availability such as microvascular rarefaction.

1693

DETECTION OF ABH ANTIGEN-SPECIFIC B CELLS: REFINEMENT AND VALIDATION OF A FLOW CYTOMETRY ASSAY USING CARBOHYDRATE-COATED FLUORESCENT MICROPARTICLES.

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Introduction: In heart transplantation, ABO incompatibility between adult recipients and donors is a seemingly insurmountable immunologic barrier. In infants, however, the ABO barrier can be breached safely and results in spontaneous development of immunologic tolerance to donor ABH antigens. Mechanisms that play a role in the development of tolerance to non-self ABH antigens, or even to self ABH antigens, are still unknown, mainly because of lack of tools to investigate this event. Assays detecting carbohydrate-specific cells are complicated by low frequency and non-specific binding. Previously, we showed reduction of non-specific binding using carbohydrate-coated fluorescent microparticles (MP). This study focuses on refinement and validation of a flow cytometry (FC) assay to detect A antigen-specific B cells.
Methods: MP were produced using multiple conjugation techniques integrating both the anti-biofouling agent polyethylene glycol and A or B antigens onto AlexaFluor (AF)488 or AF647 silica MP (A-MP and B-MP). FC analysis was used to study binding of MP to an EBV-immortalized A antigen-specific B cell line (A-BCL). To determine the assay specificity, IgM^+ A-BCL were isolated, labeled with PKH26 and mixed in defined ratios with blood-group AB peripheral blood mononuclear cells (PBMC) (which should not bind MP) and A-MP.
Results: A subset of A-BCL bound A-MP (mean ± sd: 42.9% ± 3.5%; n=3 experiments); no binding was observed to B-MP (1.0% ± 0.5%). Better separation of cells that bound A-MP and non-binding cells was seen by dual staining with both AF488 and AF647 MP. A-MP-binding cells were IgM^+, whereas non-binding cells were IgM^-. Moreover, the amount of bound A-MP was related to the IgM expression level: IgM^{high} cells had highest MFI for AF488 and AF647 MP (range MFI: 186-877 and 2157-10200, respectively), while IgM^{dim} cells had lower MFI for AF488 and AF647 MP (77-136 and 175-1287). We were able to identify $PKH26^+$ A-BCL when these cells were mixed with group AB PBMC in ratios of 1:1, 1:10 and 1:100; the majority of B cells dual-positive for AF488 and AF647 A-MP were $PKH26^+$ (96.1% ± 0.5%, 76.9% ± 8.3% and 76.5% ± 12.1%, respectively). However, specificity decreased to <20% when A-BCL were mixed with PBMC in ratios of 1:1000 to 1:100000 due to non-specific B-cell binding.
Discussion: The use of carbohydrate-coated fluorescent MP enables a FC assay for specific detection of A antigen-specific IgM^+ B cells. We are currently refining the protocol to increase sensitivity to identify specific B cells present in low frequencies. In addition, similar experiments are underway to detect B antigen-specific B cells.

1694

REGULATORY EFFECTS OF CD1D+CD5+ B CELLS IN THE CONTEXT OF ANTIGEN TOLERANCE FOLLOWING ABO-INCOMPATIBLE HEART TRANSPLANTATION IN CHILDREN

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Introduction: Infants show significantly better graft acceptance and survival after heart transplantation. They are able to tolerate ABO-incompatible hearts; however, the underlying mechanisms are unclear. In mice, a CD1d+CD5+ B cell subset was found to have regulatory capacities and was named 'B10 cells' for their production of IL10 *in vitro*. In humans, this phenotype is more frequent in young children. We aim to determine whether human CD1d+CD5+ B cells are functionally similar to B10 cells in mice.
Methods: Using flow activated cell sorting, CD1d+CD5+ B cells were sorted from pediatric and adult splenocytes and cultured parallel to residual B cells using T-dependent (CD40L+IgM) and independent (CpG, IgM) B cell stimulation. Supernatants were collected to quantify IL10 concentrations using an ELISA. Proliferation was assessed through carboxyfluorescein-succinimidyl-ester stained splenocytes with CD1d+CD5+ B cells at 0%, 100%, 200%, 300% and 500% of their natural proportion using αCD3-αCD28 T cell stimulation along with the B cell stimuli.
Results: We found that CD1d+CD5+ B cells produced IL10 with both CpG and CD40L+IgM stimulation. However, residual B cells also showed IL10 production indicating CD1d+CD5+ is not the only phenotype of regulatory B cells in humans. Proliferation of B cells in absence of CD1d+CD5+ B cells with CpG and CD40+IgM stimulation was markedly increased; it slightly decreased with double and triple proportion of these cells. T cell response was massively increased in absence of CD1d+CD5+ B cells and slightly decreased in presence of the triple proportion of these cells. The results obtained from the adult sample were similar; however, the effects became less pronounced in older donors.
Conclusion: In summary, these results indicate that regulatory B cells include but are not limited to the CD1d+CD5+ subset described in mice. Their high prevalence in early childhood likely contributes to better graft acceptance. Larger data sets

throughout various age groups are required to confirm findings and identify additional regulatory B cell phenotypes.

1695

PREVENTING VEIN GRAFT FAILURE IN VEIN ARTERY TRANSPLANTATION USING STAT-3 SIRNA

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Background: Proliferation and migration of vascular smooth muscle cells (VSMCs) play a key role in neointimal formation which leads to restenosis of vein graft in venous bypass. STAT-3 is a transcription factor associated with cell proliferation. We hypothesized that silencing of STAT-3 by siRNA will inhibit proliferation of VMSCs and attenuate intimal thickening.
Methods: Rat VSMCs were isolated and cultured in vitro by applying tissue piece inoculation methods. In vitro proliferation of VSMC was quantified by the MTT assay, while in vivo assessment was performed in a venous transplantation model. In vivo delivery of STAT-3 siRNA or scramble siRNA was performed by admixing with liposomes 2000 and transfected into the vein graft by bioprotein gel applied onto the adventitia. On day 3 and7 after grafting, the vein grafts were extracted, and analyzed morphologically by HE staining and assessed by immunohistochemistry for expression of Ki-67 and proliferating cell nuclear antigen (PCNA). Western-blot and RT-PCR were used to detect gene expression in vivo and in vitro.
Results: STAT-3 siRNA treatment inhibited the proliferation of VSMCs. On day 7 after operation, a reduced number of Ki-67 and PCNA positive cells were observed in the neointima of the vein graft in the STAT-3 siRNA treated group as compared to the control. The neointima in the experimental group(0.45±0.04μm) was thinner than that in the control group(0.86±0.05μm) ($P<0.05$).Compared with the control group, the protein and mRNA levels in the experimental group in vivo and in vitro decreased significantly. Down regulation of STAT-3 with siRNA resulted in a reduced expression of Bcl-2 and cyclin D-1.
Conclusions: The STAT-3 siRNA can inhibit the proliferation of VSMCs in vivo and in vitro and attenuate neointimal formation.

1696

PRE-OPERATIVE REGULATORY T CELL SUPPRESSIVE FUNCTION CORRELATES WITH EARLY GRAFT FUNCTION AFTER KIDNEY TRANSPLANTATION

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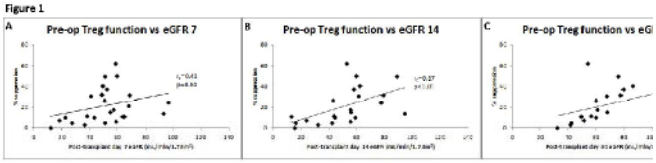
Background: Regulatory T cells (Tregs) are involved in preventing rejection and inducing tolerance after kidney transplantation. A higher Treg frequency or enhanced Treg suppressive function prior to kidney transplantation could limit initial immunologic injury and predict better graft function post-transplant. We investigated whether pre-transplant Treg frequency or function correlated with estimated glomerular filtration rate (eGFR) post-transplant.
Methods: Peripheral blood was collected from 38 kidney transplant recipients (34 brain death donors, 4 live donors) pre-operatively prior to induction immunosuppressive therapy (30 anti-thymocyte globulin, 6 alemtuzumab, 2 basiliximab). All recipients received tacrolimus, mycophenolate mofetil, and corticosteroids for maintenance immunosuppression. Mononuclear cells were isolated by density centrifugation and Treg frequency was measured by flow cytometry using CD4, CD25, and FoxP3 markers. In 25 recipients, sufficient mononuclear cells were available for magnetic isolation of CD4+CD25+ Tregs (purity: 90 ± 1%) and CD4+CD25- effector T cells (Teffs, purity 81 ± 2%). CFSE-labelled Teffs were co-cultured in the presence of anti-CD3/CD28-coated beads with Tregs at a 1:0 and 1:1 ratio for 5 days. Treg function was measured by percentage suppression of CFSE-labelled Teff proliferation. Graft function at 7, 14, and 30 days post-transplant was assessed by eGFR calculated by the MDRD formula. Treg frequency and function were correlated with eGFR using Spearman's rank order correlation.
Results: Frequency of pre-operative CD4+CD25hiFoxP3+ Tregs did not correlate with post-transplant day 7, 14, or 30 eGFR (r_s=0.06 p=0.73, r_s=0.05 p=0.78, and r_s=0.02 p=0.90 respectively). However, significant positive correlations were found between pre-operative Treg suppressive function and eGFR on post-transplant day 7, 14, and 30 (Fig 1A-C).
Conclusion: Pre-operative Treg suppressive function but not frequency correlated with early graft function after kidney transplantation. This suggests that pre-operative Treg function measurement could be a tool to identify patients at risk for early graft dysfunction. Additionally, development of clinical strategies to enhance Treg function pre-emptively could improve outcomes in kidney transplantation.

Figure 1



1697

MINIMIZING DONOR IMMUNOGENEICITY IN HEART TRANSPLANTATION USING SIRNA

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Background: Immune rejection from donorantigen presenting cell (APC) play an important role in graft acceptancein organ transplantation. Wehypothesize that the process of RNA interference (RNAi) may be used to modify a graft so as to effectively suppress genesassociated with maturation and singal ofAPC in order to impair the ability of APC to activate T cells. Such manipulation of immunogeneic gene expression may be performed by treatmentof the organ ex vivo with short interfering RNA (siRNA) as part of thepreservation procedure.

Methods: Hearts were isolated from C57/BL6 mice, and perfused with200 µl of HTK solution that contains 100 µg/ml siRNAs specifically targeting Rel B,CD40 and C3 genes respectively. The donor organs were perfusedand preserved in the siRNA solution at 4 ^{0}C. After preservation insiRNA solution or control solution, hearts were implanted into allogeneic Babl/Crecipient. The protective effect ofsiRNA treatment was determined by heart beating, histopathology and survival.

Results: siRNA was efficiently delivered into heart organthrough perfusion and preservation procedure as shown by thedistribution of the fluorescent labelled siRNA. Gene expression of Rel B, CD40 and C 3 were significantly knocked down by siRNA solution. Perfusion/preservation of donor hearts using siRNAsolution prolong donor heart graft survival (from 6.5 days to 13 days). The protectiveeffect of siRNA was further demonstrated by improved histopathology, lessmonocytes infiltration on day 3 post transplantation.

Conclusion: This is the first demonstration of thatpreservation of donor hearts through RNAi strategy protects cardiac graft fromdirect-pathway initiated immune rejection in heart transplantation.

1698

ENHANCED IMMUNE FUNCTION AND PRO-INFLAMMATORY CYTOKINES IN HIGHLY SENSITIZED PATIENTS AWAITING KIDNEY TRANSPLANTATION

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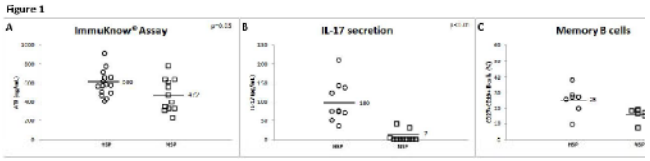
Background: In kidney transplantation, highly sensitized patients (HSP) have inferior outcomes compared to non-sensitized patients (NSP) due to high levels of circulating anti-HLA antibodies. Little is known about the pre-transplant global immune function and cytokine profile of HSP. An altered immune function and cytokine profile could participate in creating or maintaining circulating antibodies. We compared global immune function, leukocyte cytokine secretion, memory B cells, and regulatory T and B cells between HSP and NSP.

Methods: Peripheral blood was collected from 27 patients on the kidney transplant waiting list prior to hemodialysis. HSP (n=15, age=54±3, M/F=6/9) were defined as patients with a panel reactive antibody (PRA) above 70% while NSP (n=12, age=58±4, M/F=11/1) had a PRA below 1%. In 15 HSP and 12 NSP, global immune function was quantified using the ImmuKnow® assay (Cylex Inc., Columbia, MD, USA). In 10 HSP and 11 NSP, enzyme-linked immunosorbent assay was performed on the ImmuKnow® assay supernatant to measure IL-17 secretion. In 7 HSP and 7 NSP, frequency of leukocyte cytokine secretion, memory B cells, and regulatory T and B cells was measured by flow cytometry. Statistical analysis was performed with Student's t-test.

Results: Global immune function and IL-17 secretion were significantly higher in HSP than NSP ($p=0.05$, Fig. 1A and $p<0.01$, Fig. 1B respectively). Frequency of CD14+ monocytes, CD11c+ dendritic, CD56+ NK, CD4+ T, and CD19+ B cells was similar between HSP and NSP. However, frequency of IL-17+ CD14+ monocytes, IL-17+ CD11c+ dendritic cells, IFN-γ+, IL-4+, IL-17+, and IL-6+ CD4+ T cells, and IFN-γ+ CD19+ B cells was significantly higher in HSP ($p\leq0.05$). Frequency of memory CD27+ CD19+ B cells was also higher in HSP ($p=0.04$, Fig. 1C). There was no difference in FoxP3+ regulatory CD4+ T cells or IL-10+ CD19+ regulatory B cells.

Conclusion: HSP awaiting kidney transplantation have a higher global immune function, increased pro-inflammatory cytokine secretion by leukocytes, and increased frequency of memory B cells in comparison to NSP without a compensatory regulatory response. These findings reinforce the use of B and T cells blockade or depletion in pre-emptive desensitization protocols and suggest that the addition of pro-inflammatory cytokines blockade in these protocols could further improve post-transplant outcomes in HSP.



1699

GENERATION OF A RECOMBINANT HEME OXYGENASE TO PROTECT TRANSPLANTED STEATOTIC LIVERS FROM REPERFUSION INJURY

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Objective: To assay the ability of a cell-penetrating heme oxygenase-1 (HO-1) protein to protect organs from ischemia-reperfusion injury in models of steatotic liver transplantation.
Methods: Plasmid constructs expressing cell-penetrating enhanced green fluorescent protein (CPP-EGFP) and HO-1 (CPP-HO-1) were generated by polymerase chain reaction amplification and ligation into a bacterial protein expression system. CPP-HO-1 and CPP-EGFP proteins were isolated from bacterial cultures and purified on nickel agarose columns. HO1-CPP functionality was assayed using spectrophometric quantification of bilirubin production by CPP-HO-1 treated HEK293T cells supplemented with the necessary co-factors. Cell penetration, *in vitro*, was assayed after treatment of various cell lines with CPP-tagged proteins in tissue culture and visualisation of target proteins by immunohistochemistry followed by fluorescence microscopy. Cellular internalisation of protein in rat livers was performed by *ex vivo* hepatic portal vein perfusion of isolated organs with 2mg of CPP-HO-1 or CPP-EGFP in histidine-tryptophan-ketoglutarate solution. Controls included mock treated organs and organs perfused with proteins lacking a CPP. To measure IRI we assayed for damage-associated molecular patterns (DAMPs) associated with oxidative stress, such as mitochondrial DNA (mtDNA), by quantitative PCR.
Results: Purified CPP-HO-1 is functional in a biochemical assay of bilirubin production and is able to penetrate cells in vitro. Histological analysis showed cell penetration of CPP-fused proteins in cells surrounding the hepatic portal vein after *ex vivo* perfusion, but not control proteins. mtDNA was detected in assays for DAMPs in models for IRI and has potential to be used as a marker for protection from IRI.
Conclusions: We have successfully generated a CPP-HO-1 protein which is fully functional and is able to penetrate cells *in vitro* and *ex vivo*. Further work will determine if 1) CPP-HO-1 can provide cellular protection in an in vitro model of IRI 2) whether treatment of steatotic livers with CPP-HO-1 in a rat model of transplantation can protect these organs from IRI.

1700

PHENOTYPIC CHARACTERIZATION OF NATURAL KILLER T CELLS IN HUMAN PERIPHERAL BLOOD, SPLEEN AND LUNGS.

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Introduction: Natural killer T (NKT) cells, a rare heterogeneous group of lymphocytes, express both a limited range of T cell receptors and markers common to natural killer cells (CD56 and CD161). NKT cells recognize lipid antigens, such as Alpha-GalCer which are presented by CD1d-expressing antigen presenting cells. Subsets of human NKT cells have previously been described based on expression of CD4 and CD8. NKT cells are thought to play a regulatory role in alloimmune responses after organ transplantation. However, which NKT subsets play such a role is unclear. We set up and optimized a flow cytometric assay to detect and characterize human NKT cell subsets in human peripheral blood, spleen and lungs.
Methods: Fluorescent human CD1d tetramers loaded with Alpha-GalCer in combination with CD3 expression were used to detect NKT cells in adult blood samples (n=8), spleen samples (n=2) and bronchoalvelar lavage (BAL) samples (n=2) by flow cytometry. Subsequently, NKT cells were characterized by their surface expression of CD4 and CD8, and CD161 expression was determined within the latter subpopulations. To exclude non-specific binding, a viability dye stain and CD19 were used to gate out non-viable cells and B cells.
Results: NKT cells were detected in all peripheral blood samples (median: 0.028%, range: 0.013-0.087%). Within the NKT cell population, cell frequencies positive for either CD4 or CD8 varied among the samples (53%, 18-86% and 8%, 3-40%, respectively). CD4+ cells consistently formed a distinct population, while CD8 was dimly to highly expressed, rendering no clear separation between CD8+ and CD8- populations. A double negative (DN) subpopulation was noted in six samples (18%, range: 0-69%). CD4+ NKT cells were mainly CD161- (82%, 45-90%) while DN NKT cells were mainly CD161+ (96%, 73-100%). The frequency of CD161+ within the CD8+ subset varied among samples (64%, range: 5-97%). NKT cells were also detected in both spleen samples (0.04% and 0.016%). Only one BAL sample contained CD3+ cells; 0.032% of these cells were NTK cells.
Conclusions: In this preliminary study, we demonstrated the detection and characterization of various NKT cell subsets in human peripheral blood, spleen and lungs. These distinct populations may represent different functions. Future experiments will include characterizing the NKT cell populations in samples of healthy individuals and lung and

heart transplant recipients. In addition, functional assays are being set up to investigate the cytokine profile for each subpopulation.

1701

INVESTIGATING DIFFERENCES IN ADULT AND INFANT B-CELL RECEPTOR SIGNALLING

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Introduction: Up to two years of age the immune system has an impaired response to T-independent antigens and does not produce antibodies against the blood-group (ABO) polysaccharides. ABO-incompatible heart transplantation is safe in this period and leads to tolerance of the donor A and B antigens. However mechanisms have yet to be elucidated.

$CD21^{hi}$ splenic marginal zone B-cells are crucial in the immune response to polysaccharides; BCR signalling is enhanced when C3d bound to a foreign polysaccharide engages the CD21 co-receptor. Yet the immature immune system does not suffer from a lack of $CD21^{hi}$ B-cells or from a C3d deficiency. We posit that immaturity in BCR and CD21 co-receptor signalling contributes to the poor polysaccharide immune response in early childhood.

Methods: Adult and infant B-cells were stimulated with anti-human IgM antibodies with and without the addition of soluble C3d and/or CD40L. The progression of the intracellular signalling cascade was monitored at various time points after stimulation using fluorescent antibodies specific for phosphorylated SYK and AKT. Signalling kinetics were characterized using flow cytometry.

Results: In adult B-cells, BCR engagement with anti-IgM antibodies resulted in an increase in pSYK, corresponding to a peak in phosphorylation of the downstream enzyme AKT. The addition of CD40L did not enhance pSYK but was associated with greater pAKT peak. Addition of C3d did not alter phosphorylation kinetics versus anti-IgM stimulation alone.

Stimulation of infant B-cells with anti-IgM with and without C3d or CD40L resulted in dephosphorylation of both SYK and AKT. Dephosphorylation was greatest and most persistent after anti-IgM and C3d simulation.

Stimulation of neonatal B-cells resulted in SYK phosphorylation after anti-IgM stimulation alone and with the addition of CD40L; however only anti-IgM and CD40L stimulation induced AKT phosphorylation. Stimulation with anti-IgM and C3d did not induce SYK phosphorylation and resulted in persisting dephosphorylation of AKT.

Conclusions: Our findings suggest that the poor immune response to T-independent antigens observed in early childhood may be due to a difference in the ability of infant B-cells to transmit intracellular signals after BCR and co-receptor engagement, compared to adult B-cells. The co-signal through SYK could be similarly induced in infant B-cells but lead to dephosphorylation of the downstream enzyme AKT, which is necessary for persistent activation of B-cells. This could explain the unique state of persisting irresponsiveness to the ABO antigens observed following ABO-incompatible transplantation in early childhood. The signaling pathway of the B-cell co-receptor may be a possible target for therapeutic intervention to translate the benefits of the immature immune system into older patients.

1702

BK POLYOMA VIRUS INDUCES EPIGENETIC REPROGRAMMING OF RENAL EPITHELIAL CELLS TO PROMOTE CELL PROLIFERATION AND FIBROSIS

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BK Polyoma virus (BKV) is acquired during early childhood with peak seroprevalence ranging from 60 to 100%. While usually persistent in a latent state throughout life, it is reactivated in some renal transplant patients, and may lead to BKV Nephropathy (BKVN). BKVN is currently the leading cause of graft loss in the first two years after transplant.

BKV causes renal graft destruction through a process of inflammation and fibrosis. Identifying the mechanisms and markers of BK activation and BK induced fibrosis may help in better understanding and management of BKVN. Since BKV does not integrate into the host genome, it cannot cause genetic changes in mammalian cells. However, human gene expression can be controlled by epigenetic modifications of the human genome, including DNA methylation which can inactivate key genes. We hypothesized that BKV infection would change epithelial cell phenotype epigenetically. We specifically aimed to identify BKV induced promoter CpG methylation changes in key host genes which are important for maintaining cellular integrity and may have a role in Epithelial-Mesenchymal Transition (EMT), which leads to fibrosis.

We used primary human renal proximal tubular epithelial cells (HPTC) isolated from donor kidneys. Real time gene expression profiling and DNA CpG methylation analysis of BK infected HPTC was done at different time points. For DNA CpG methylation, we did Bisulfite sequencing and Methylation specific PCR (MSP) of different promoter CpG islands in the genes which were modulated by BKV.

Our study showed that BKV induces the expression of proinflammatory genes such as Cox-2 and Interleukin 1 Beta, and Vimentin which is an important marker of EMT. Importantly, BKV inhibits the genes for E-cadherin (CDH1) and retinoblastoma protein 1 (RB1) during infection of renal tubular cells. We are the first to identify that BKV inhibits CDH1 and RB1 by causing hypermethylation of specific CpG islands in their upstream regulatory regions. This epigenetic inhibition of CDH1 and RB1 by BKV may lead to loss of cell adhesion and cell cycle progression, respectively. These changes in gene expression, both induction and inhibition, are expected to contribute to the pathogenesis of BK nephropathy

by inducing inflammation, cell proliferation and the initiation of EMT. Specific CpG methylation changes identified in this study may also serve as biomarkers for early prediction of BKV reactivation in renal transplant patients.

1703

WHITE BLOOD CELL DIFFERENTIALS ENRICH WHOLE BLOOD EXPRESSION DATA IN THE CONTEXT OF ACUTE CARDIAC ALLOGRAFT REJECTION

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Background: Traditional microarray analysis does not account for sample heterogeneity. It cannot distinguish between variations in RNA transcript abundance that result from actual transcriptional changes and those consequent to differences in sample composition. It is also unable to determine the cell-specific contribution of a given cell type within a mixed sample to the overall gene expression of the whole sample. Isolating subpopulations of cells from tissues for microarray analysis is prohibitively expensive, however, and may affect gene expression. Cell-specific Significance Analysis of Microarrays (csSAM) is a statistical alternative that integrates whole blood microarray data and sample composition information to yield cell-specific expression. This approach is particularly interesting in the context of whole blood microarray studies, which are often used to assess global patho-physiological perturbations. Conveniently, sample composition information for whole blood is readily available in the form of white blood cell count differentials.
Hypothesis: Cell-specific expression can be statistically inferred from whole blood expression data and sample composition information (white blood cell differential counts) using csSAM.
Methods: csSAM was applied to data obtained from heart transplant recipients that were either not rejecting the transplant [ISHLT grade=0R; NRs; n=16] or undergoing treatable acute rejection [ISHLT grade>=2R; ARs; n=10]. RNA was isolated from whole blood samples using PAXgene Blood RNA kits and run on Affymetrix HG-U133 plus 2 microarrays. Blood was further submitted to an automated hematology analyzer to obtain white blood cell differential counts. CEL files were RMA-normalized before submission to the csSAM algorithm.
Results: csSAM identified 8 differentially expressed probesets in monocytes (mapping to 6 genes, down-regulated in ARs as compared to NRs) at a false discovery rate (FDR) of 15%. Interestingly, none of these had been detected in whole blood. Gene set enrichment analysis (GSEA) identified >30 canonical KEGG pathways significantly down-regulated in monocytes in ARs at an FDR of 1%.
Conclusion: Three of 6 genes have been previously reported to be highly expressed in monocytic lineage cells. The down-regulation of 2 of these genes suggests a disruption in metabolic homeostasis of monocytes in AR subjects, resulting in an overall pro-inflammatory state. GSEA suggests that monocytes may disrupt tissue remodeling, provide pro-apoptotic feedback, and inhibit development of immune tolerance in AR subjects.

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A NOVEL ORGANIC DYE ENHANCES THE SURIVIVAL OF ISLET ALLOGRAFTS WHEN COMBINED WITH ANTI-LFA-1 MAB THERAPY

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Background: The major obstacle to the clinical application of islet transplantation for the treatment of Type I Diabetes Mellitus is the cell mediated immune rejection. Monoclonal antibody (mAb) therapy directed against several leukocyte cell surface antigens has been utilized in preventing the rejection of islet transplants. Transient therapy with anti-LFA-1 mAb has been shown to be effective in preventing the rejection of islet allografts, however the percentage of long-term islet allograft survival (>100 days) varied from 89% in CBA mouse recipients transplanted with BALB/c mouse islets to only 38% of B6 mouse recipients bearing BALB/c mouse islet grafts. When anti-LFA-1 mAb was combined with anti-CD154 mAb, significant prolongation of BALB/c mouse islet allografts was observed. Direct Red 80 (DR-80) is a novel organic dye that has been recently shown to inhibit the interaction of CD154 to CD40. In this study, we determined the effect of Direct Red 80 alone or in combination with anti-LFA-1 mAb in prolonging the survival of BALB/c mouse islet allografts in B6 mice.
Methods: Islets were isolated from adult BALB/c mouse pancreata by collagenase digestion and Ficoll purification, and then 500 islets were handpicked and transplanted in the subcapsular space of the left kidney of diabetic B6 mice. B6 mice were rendered diabetic by a single intraperitoneal

injection of 200 mg/kg body weight of streptozotocin. Once deemed diabetic, they were then transplanted with islets and received one of the following treatments intraperitoneally: 1) Rat IgG2α as isotype control (200µg on days 0, 1, 7, 14); 2) anti-LFA-1 mAb (200µg on days 0, 1, 7, 14); 3) DR-80 (250µg on days -1, 1, and then twice a week for additional 4 weeks); 4) Combination therapy of anti-LFA-1 mAb and DR-80. Blood glucose levels of B6 mouse recipients were monitored twice weekly for more than 100 days after transplantation.

Results: Our preliminary results show that all B6 mouse recipients with blood glucose levels higher than 25 mmol/ml on the day of transplantation restored their normal blood glucose levels within 24 hours after transplantation. Mice treated with isotype and DR-80 alone became diabetic at 12 days post-transplantation and were euthanized. While, mice treated with anti-LFA-1 mAb alone or in combination with DR-80 maintained their normal blood glucose levels for 100 days post-transplantation.

Conclusion: DR-80 can prevent the rejection of mouse islet allografts however; the combination of DR-80 with anti-LFA-1 mAb is more efficacious in prolonging islet allograft survival in B6 mice.