BRITISH TRANSPLANTATION SOCIETY

11th ANNUAL CONGRESS

16-18 April 2008

The Scottish Exhibition & Conference Centre (SECC)

Glasgow

BTS 11th ANNUAL CONGRESS: 16-18 April 2008

The SECC, Glasgow

Welcome to Glasgow!

Welcome to the 11th Annual Congress of the BTS.

The Local Organising Committee is delighted to welcome you to Glasgow for what we hope will be both an educational and enjoyable three days in the company of your transplantation colleagues from the UK and our visitors from overseas.

Our overriding theme is “Better Organs for More Patients”, focusing, clinically, on maximising donation, improving initial and long term graft function; and, scientifically, on exploring new concepts of organ support and replacement as well as advancing ways of manipulating the immune system. There is an impressive faculty of speakers addressing these different aspects and the anticipation of many good quality presentations from our members, as well as the favourites of the UK Transplant report session, other named sessions and rounding off with “breaking news” in What’s hot, what’s new. There are also poster presentations of members’ work which will be well worth studying.

The social programme gives the opportunity to move out with the Conference Centre to partake in sustenance while enjoying chat, “bopping” and the odd bit of “Ceildh”.

The Glasgow transplant community and our patients are very appreciative of all the people who have donated organs and so please spend a few minutes sitting on “The Love Seat”, commemorating all our West of Scotland donors from the beginning of this century, when you explore the delights of Kelvingrove Art Gallery and Museum at the pre Gala Dinner reception.

As in previous years our corporate partners are well represented and we thank them for their ongoing support of our Annual Congress. Please take time during the meeting to visit the trade exhibition and talk to the company representatives about their products.

Deirdre Walsh **Laura Buist**

Co-chairmen BTS Congress Glasgow 2008

ACKNOWLEGEMENTS

The British Transplantation Society would like to give special thanks to their Corporate Partners for their support throughout the year and during the Congress:

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The abstracts for this meeting were kindly reviewed by:

Laura Buist, Lisa Burnapp, Deirdre Cunningham, Chris Dudley, Phil Dyer, Sue Fuggle, Jane Griffiths, Nicholas Jones, John Kirby, Jen Lumsdaine, Lorna Marson, James Neuberger, Marlene Rose, Paul Shiels, Colin Short, Jacqui Spencer, Deirdre Walsh, Steve Wigmore

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Please note that we have also provided 15 travel bursaries with sponsorship money provided by Astellas and Novartis

ABSTRACTS

Parallel Session

Post Transplant Complications

Wednesday 16 April

11:30 – 12.30

O000

OKT3 Effectively Treats Rejection in Antibody Incompatible Transplantation.

Rob Higgins[1], Nithya Krishnan[1], Rizwan Hamer[1], Habib Kashi[1], For Lam[1], Lam Chin Tan[1], Peter Roberts[1], Chris Imray[1], Beth Harrison[1], Sarah Clarke[1], David Briggs[2], Daniel Zehnder[3]

[1]University Hospital, Coventry, United Kingdom, [2]NHS Blood and Transplant, Birmingham, United Kingdom, [3]Warwick Medical School, Coventry, United Kingdom

Introduction: The optimal treatment regimen for acute renal transplant rejection associated with donor-specific antibodies (DSA) has not been established. Since DSA cannot be completely removed by plasmapheresis (PP) during rejection, and rituximab seems ineffective during acute rejection, timely diagnosis and optimal choice of drug treatment are critical. We report the outcomes of treatment with the monoclonal T cell antibody muromonomab-CD3 (OKT3).

Methods: Fifty one patients were transplanted, 45 with HLA antibody incompatibility (HLAi), 3 with blood group incompatiblity (ABOi), and 3 with HLAi and ABOi. 24 patients had rejection or suspicious biopsy changes in the first 3 months. 12 were treated with 5-7 day courses of OKT3; 11 of these cases rejection was antibody-associated, 1 case had mixed cellular/vascular rejection with low antibody levels.

Results: OKT3-treated rejection had onset at mean day 12 (range 0-16) post transplant. Mean creatinine was 230 (range 87-dialysis) umol/l before rejection, peaked at 367 (range 126 – dialysis) umol/l, and fell post-treatment to 157 (range 70-271) umol/l. 5 patients were treated with dialysis for severe rejection. One graft failed from thrombotic microangiopathy at 3 months, the others are currently functioning with mean creatinine 147 (range 78-245) umol/l. Complications were more frequent in patients who also had PP, and included a probable fungal pneumonia and encephalopathy with fits, but no patient has died. One patient developed a testicular tumour. In order to reduce adverse effects, PP has been minimised or not used during rejection. We are now using lymphocyte subset monitoring by flow cytometry to optimise dosing.

Discussion: The use of OKT3 for early rejection was associated with 92% graft survival and 100% patient survival. It is interesting that T lymphocyte-specific therapy was effective, since plasma cells, B cells, monocytes and macrophages are also important in antibody mediated rejection. However, the effects of OKT3 on CD3- lymphocyte lineages are not well described. Preliminary results showed that some patients experienced falls in the numbers of CD19+ and CD56+/CD3- cells, as well as CD3+ cells, during OKT3 treatment.

O001

Peritonectomy Is A Successful Treatment For Patients With Encapsulating Peritoneal Sclerosis (EPS) Following Renal Transplantation

Declan G de Freitas, Angela M Summers, Helen Hurst, Paul Taylor, Alastair J Hutchison, Louese Dunn, Paul EC Brenchley, Titus Augustine

Manchester Royal Infirmary, Manchester, United Kingdom

Encapsulating peritoneal sclerosis (EPS) is an increasingly recognised complication of long term peritoneal dialysis (PD), associated with deposition of fibrous sheets which constrict and restrict the bowel. Surgical intervention has in several cases been associated in the past with high mortality. EPS following renal transplantation is a new phenomenon, occurring relatively soon post transplant despite immunosuppression. Peritonectomy has been used to treat PD patients with EPS. We report the first experience of peritonectomy in the management of EPS in the transplant population.

We collected outcome data on renal allograft recipients who developed EPS following transplantation in a single centre during the period of 3yrs from 2004 to 2006. Diagnosis was based on both clinical and radiological findings, with surgical confirmation. Patients with a clinical picture which included ascites, deteriorating nutritional status, raised inflammatory markers and bowel obstruction underwent adhesiolysis and complete peritonectomy.

11 patients developed EPS following renal transplantation in this period. Clinical findings included ascites and symptoms of bowel obstruction. CT findings included ascites, peritoneal thickening and calcification, abdominal cocoon, bowel thickening and dilatation. 10 patients underwent surgery while one patient was treated conservatively and has achieved a normal nutritional state. 9 patients underwent peritonectomy and adhesiolysis, of whom 7 are now eating a normal diet. 5 of these patients have residual symptoms including nausea, abdominal pain and constipation. One patient underwent a repeat peritonectomy and is now almost symptom free. In this group of patients there has been one death.

This is the first report of peritonectomy in the management of EPS following renal transplantation with achievement of normal diet in the majority of patients. Unlike previous reports with high surgical mortality up to 33%, there has only been a 10% mortality in this group. This is a condition which should be considered in patients with post transplant chronic abdominal symptoms and ascites. The key to successful surgery is early semi-elective peritonectomy after adequate preparation.

O002

Renal allograft rejection after Campath induction and medium dose

Tacrolimus monotherapy without steroids

Jack Galliford[1], Kakit Chan[1], Adam McLean[1], Terry Cook[2], Rawya Charif[1], Tony Dorling[3], Anthony Warrens[3], David Taube[1]

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Although Campath is now commonly used as an induction agent in renal transplantation, there are few reports detailing the incidence, nature and outcome of subsequent rejection following its use with Tacrolimus [Tac] monotherapy.

In this study, we report our experience of renal allograft rejection in 233 patients [141 M, 92 F; mean age 46.1±13.0 yrs, 135 live donors, 98 deceased donors] receiving Campath

induction [30mg iv immediately post operatively] medium dose Tac [0.1mg/kg/day, target level 5-8 ng/l] and 7 days of oral steroids. Mean follow up was 15.8±10.8 months. Allograft rejection [AR] was diagnosed by biopsy and designated to be cellular [ACR] or antibody mediated [AMR]. ACR was treated with iv methyl prednisone, 0.5g x 3, the introduction of oral steroids and Mycophenolate Mofetil. AMR was similarly managed with the addition of plasma exchange and ivIg [2g/kg] if severe or steroid unresponsive.

Allograft survival, censored for death with functioning graft, at 6, 12 and 24 months was

96.9%, 96.3% and 96.3%. 2 patients [<1.0%] with AR lost their allografts; 1 from treatment resistant AMR and the other from non compliance after ACR. 27 episodes of AR occurred in 26/233 [11.2%; 17M, 9F] patients, at mean time of 165.8 days [range 14-546]. Rejection free survival [Kaplan Meier] at 6, 12 and 24 months was 92.1%, 87.9% and 84.9%. There was no statistically significant difference between AR and non-AR patients with regard to recipient sex, age [46.6+13.0 vs 42.9+12.4 years], cold ischaemia time [20.5+11.0 vs 20.6+8.2], mismatches to HLA A, B and DR [2.9±1.5 vs 3.3±1.3], ethnicity or pretransplant presence of Class I and II HLA antibodies.

22/27 [81.5%] AR episodes were designated ACR; 13/27 [48.1%] were C4d positive. 5/27 [18.5%] were designated AMR; 5/5 were C4d positive. ACR occurred significantly later than AMR [mean time 196.3 vs 31.8days; p=0.007 (Mann-Whitney U test)]. Allograft function in the AR group was significantly impaired at 6, 12 and 24 months after transplantation when compared with the non AR group.

This study shows that in patients receiving Campath and Tacrolimus monotherapy, AR is relatively uncommon and although is associated with impaired function, it is rarely associated with allograft loss.

O003

Withdrawal of all Immunosuppressive Therapy Except Corticosteroid in Renal Transplant Recipients Who Experience Life-threatening Malignancy.

Meetali Kolhatkar, Stuart Rodger, Colin Geddes

Western Infirmary, Glasgow, United Kingdom

Introduction. Patients with functioning kidney transplants are at increased risk of malignancy mainly as a direct consequence of immunosuppressive therapy. It is widely accepted that immunosuppression (I/S) should be reduced in transplant patients who have life-threatening malignancy. There are no randomised controlled studies to guide how I/S should be reduced in these patients to balance the optimal management of the malignancy with prevention of kidney transplant failure. There are no published studies of corticosteroid-only immunosuppression in this setting. We present our single centre experience of withdrawing all I/S except prednisolone in patients with life-threatening malignancy.

Methods. All kidney transplant patients who had a period of corticosteroid-only immunosuppression due to life-threatening malignancy since 2000 were identified from the electronic patient record. Ages, sex, malignancy type, duration of prednisolone only I/S acute rejection date of graft failure (return to dialysis) and patient death were identified.

Results. 15 patients were identified who had I/S except prednisolone withdrawn because of life-threatening malignancy. 11 patients were male and mean age was 52.8 years (range 34.7-68.2). 5 patients had lymphoma, 1 had metastatic melanoma and the remainder had a range of solid organ malignancies. Median time since transplant was 18… years (range 7 years… - 27 …years.). Median duration of follow-up on prednisolone monotherapy was 18.7 months (range 0.3 – 95.0). 7 patients have died and none have been lost to follow-up. No patients experienced clinical acute rejection although 1 patient was found to have histological acute rejection at post mortem 0.3 months after withdrawing other I/S. No patients experienced graft failure requiring return to dialysis. No patients restarted other I/S. Median serum creatinine at time of withdrawing other I/S 153µmol/L and at 3 months, 6 months and 12 months was 121.5µmol/L, 137µmol/L and 116µmol/L respectively.

Discussion. These data suggest that patients with life-threatening malignancy can reduce immunosuppression to prednisolone monotherapy without an adverse effect on transplant function. Further studies are required to determine if this applies to all patients with life-threatening malignancy and to explore the mechanisms for this apparent immunological tolerance.

O004

Transplant Renal Artery Stenosis; Early Detection and Management are Mandatory for Short and long Term Graft Functions

Abbas Ghazanfar, Afshin Tavakoli, Neil Parrott, Heny Riad, Titus Augustine

Manchester Royal Infirmary, Manchester, United Kingdom

Introduction: Transplant renal artery stenosis (TRAS) is a recognized complication resulting in post-transplant hypertension with associated allograft dysfunction. It is potentially treatable complications that can occur from months to years after transplantation. Current prevalence widely ranges from 1% to 23% in different series.

In this retrospective study we analyzed diagnosis, management and outcome of TRAS in recipients from 1990 to 2005.

Methods: The clinical diagnosis of TRAS was based on uncontrolled refractory/new onset hypertension and/or unexplained graft dysfunction in absence of rejection, ureteric obstruction, or infection. One hundred and ninety-six renal transplant angiograms were performed between 1990 and 2005 to confirm the diagnosis of TRAS. 67 angiograms confirmed TRAS were included in study.

Results: Mean age of the patients with diagnosis of TRAS was 55.5 years (range 21yrs to 73yrs). Mean time interval from transplant to diagnosis was 11.5 months (range < 1mo to 13yrs). Cadaver transplant recipients had a higher incidence of TRAS than living donor recipients (59 *vs* 8). 14 patients needed surgical intervention, 9 as primary procedure for tight stenosis and 5 after failure of Primary Percutaneous Transluminal Renal Angioplasty (PTRA). Primary PTRA was undertaken in 39 patients with 13 needed stenting. 14 patients were primarily managed conservatively.

Rise of 187mmol/L (range: 47 to 389) above the baseline mean serum creatinine (MSCr) of 173mmol/L (range: 68 to 351) was noted with the mean drop of 163mmol/L (range: 58 to 227) after the management. There was no significant difference in the MSCr level of the patients either treated with surgery (147mmol/L) or PTRA (123mmol/L) however patients managed conservatively had a static raise in MSCr from base line of 153 to 185. The mean time taken for MSCr to come back to normal was 4.5 months post procedure. Good blood pressure was obtained in the 61 patients. 39 patients needed mono-antihypertensive therapy while 18 needed more than one antihypertensive to control BP in addition to the procedures. Mean systolic BP dropped from 195 to 135 with mean diastolic BP dropped from 110 to 85. Nine patients lost their grafts, 5 following surgery and 4 following angioplasty.

Discussion: The treatment of TRAS includes PTRA or surgical intervention. As it is associated with higher incidence of graft loss, TRAS should be suspected in any renal transplant patient with severe or worsening hypertension and/or unexplained renal function deterioration. Awareness of the problem, high clinical suspicion, and liberal use of non-invasive procedures like ultrasonography helps in early diagnosis. The importance of early diagnosis is self-evident because unrecognized cases invariably result in graft loss and potentially fatal systemic complications, whereas cases promptly recognized and treated before irreversible structural changes of the graft develop may achieve full recovery of kidney perfusion and function with minimal risks.

Parallel Session

Liver

Wednesday 16 April

11:30 – 12.30

O005

Combined Liver Kidney Transplants (CLKT) in small children <15 kg: Technical Aspects and Outcome

Thamara Perera, Khalid Sharif, Carla Lloyd, Patrick McKiernan, David Milford, David Mayer, Dierdre Kelly, Darius F. Mirza

Liver Unit, Birmingham Children's Hospital, Birmingham, United Kingdom

Background: Multi-organ transplantation is a surgical challenge in small children owing to technical aspects and body size. Herein we report the outcome following CKLT in children below 15kg.

Method: Prospective analysis of data of all children undergoing CLKT at a UK transplant centre during 1994-2007. Analysis of peri-operative data in children <15kg were done with figures expressed as median (range), and graft and overall survival was compared with that of children over 15kg.

Results: Twenty (20) children underwent CLKT during study period [11 male (55%), age 9.5 (1.6-16.7) years]. Six (6/20; 30%) patients were <15kg [Recipient vs. Donor; age - 2.2 (1.6-5.3) vs. 13 (3-40) years, weight 12.2 (9.5-15) vs. 60 (15-65) kg and height 77 (67-98) vs. 156 (83-168) cm] and transplanted for Primary hyperoxalosis (5/6) and congenital hepatic fibrosis (1/6) with 12 (0.7-104) months follow up. Pre-operative renal support was received by 4/6 (66%) compared to 10/14 (71%) children over 15kg. All, except one patient received a split liver graft (segments II/III) weighing median 281 (267-307)g along with a solitary kidney (2 intra-peritoneal) from the same donor. Warm ischaemia times for liver and kidney were 37 (32-70) and 35 (33-45) minutes respectively, whilst liver arterialization time was 63 (57-112) minutes. Total intensive care unit and in-patient stay was 2(1-22) and 25(15-93) days respectively. One patient died due to multi-organ failure following severe renal rejection at 21 days. When compared to children over 15kg with CKLK, survival figures at 3, 6 and 12 months were; liver graft [83%, 80% and 75% vs. 92%, 85% and 78% respectively], kidney graft [83%, 80% and 75% vs. 92%, 92% and 85% respectively] and overall survival [83%, 80% and 75% vs. 92%, 92% and 85% respectively] (p=n.s).

Conclusion: CLKT in small children results in comparable outcome despite obtaining suitable donors and technical difficulties; consequently, body size/stature should not be a limiting factor for multi-organ transplant.

O006

An Artificial Neural Network predicting survival following liver transplantation

Glenn Bonney[1], Lee Lancashire[2], Stephen Pollard[1], Giles Toogood[1], Peter Lodge[1], Raj Prasad[1]

[1]St James' University Hospital, Leeds, United Kingdom, [2]Paterson Institute for Cancer Research, Manchester, United Kingdom

Background:

The United Kingdom has seen a fall in number of donors and rise in number of patients on the liver transplant waiting list. There remains a need for a model to predict survival that may improve allocation of an increasingly scarce resource. The Donor Risk Index (DRI) and UKELD/MELD scores, based on donor and recipient factors respectively have been validated to predict outcome of transplantation. We aimed to develop a sensitive and specific model to predict post-transplant survival using an Artificial Neural Network.

Method:

A prospectively collected database of donor and recipient variables from transplants performed from January 1995 to December 2005 (n=1090) inclusive was analysed. The DRI, MELD and UKELD were calculated using formula. 18 donor and recipient variables were entered into a Bayesian Artificial Neural Network (ANN18). This enabled four datasets to be generated, allowing for the modelling of patient status up to and including 3 month, 12 month, 3 year and 5 years. Cases were randomly split with 50 % assigned to training, 25 % testing, and 25 % independent validation (blind). The neural network was trained using the training set and tested with the test set. Once trained, the model was then validated using the independent test set of cases.

Results:

The accuracy, sensitivity, specificity and c-statistic of the ANN18 at predicting survival for the 4 time intervals are as shown in Table 1. The model accuracy for ANN18 for the prediction of survival were statistically more significant than random for the 3 month data ($p = 5*10^{-5}$), the 12 month data ($p = 9*10^{-6}$), the 3 year data ($p = 4*10^{-7}$) and 5 year data ($p = 0.03$). The predictive power of the model was significantly better then MELD and UKELD.

Conclusion:

Survival from liver transplantation consists of a dynamic range of donor and recipient influences. Artificial Neural Networks may better lend itself to correlating these complex factors in predicting outcomes. Here we have developed an ANN based on donor and recipient factors that can accurately predict short- and long-term post-transplant survival which may better inform organ allocation. We are currently undertaking further validation of these findings on a national dataset.

Table 1: Accuracy, sensitivity, specificity and c-statistic of ANN18 at predicting 3-month, 1-year, 3-year and 5-year survival

Model	Accuracy (%)	Sensitivity (%)	Specificity (%)	C-statistic
3-month	91.6 (±1.8)	99.8	50.0	0.916
1 year	85.2 (±2.2)	100.0	41.3	0.836
3 year	81.7 (±2.5)	98.9	52.5	0.863
5 year	84.5 (±1.9)	96.7	72.9	0.956

O007

Assessment of Donor Liver Steatosis using Electromagnetic Probes

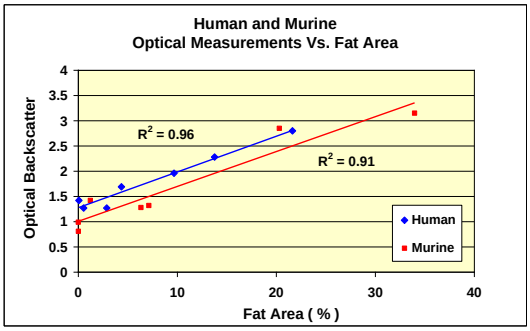
Bryan McLaughlin[1], Antonia Wells[2], Rebecca Roberts[2], Antonio Vidal-Puig[4], Susan Davies[3], Sam Virtue[4], Christopher Watson[2], Paul Robertson[1]

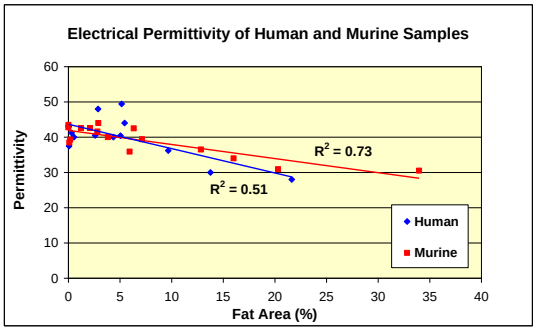
[1]Department of Engineering, University of Cambridge, Cambridge, United Kingdom, [2]Department of Surgery, Addenbrooke;s Hospital, Cambridge, United Kingdom, [3]Department of Pathology, Addenbrooke's Hospital, Cambridge, United Kingdom, [4]Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom

Introduction: Moderate or severe steatosis in donor livers is a major cause of mortality and morbidity following liver transplantation but surgical assessment of steatosis is subjective and unreliable. Electromagnetic techniques can instantly quantify lipid and water content of tissues. Two minimally invasive liver probes were developed to measure hepatic water and fat content.

Methods: Liver samples from 17 hepatectomy patients were subjected to electromagnetic measurements. The results were compared to histopathology grading, computerized pathology grading (percent fat area) and biochemical analyses. 12 murine samples (OBOB, Wildtype, POCO, and PPAR γ_2 genotypes) with steatosis induced by known pathways were also analysed.

Results: The electromagnetic properties of human and murine liver tissue correlated with the fat area measured using microscopy. The optical backscatter results were more quantitative than pathological grading (R^2 = 0.96 for humans). Electrical permittivity measurements were sensitive to tissue temperature and micro anatomies in comparison with optical backscatter. 'Gold standard' pathology methods have significant error margins compared to biochemical





techniques which have about 2-5% error in total lipid estimation.

Conclusion: The liver probe allows immediate and quantitative assessment of liver steatosis and may be a useful tool for assessing steatosis at the time of organ donation.

Parallel Session

Basic Science 1

Wednesday 16 April

11:30 – 12.30

O008

CD8$^+$ Memory T Cells Elicit Rapid Skin Allograft Rejection Through Enhanced Recruitment Of GR-1$^+$ Cells

Nick Jones, Matthew Brook, Shiqiao Luo, Manuela Carvalho-Gaspar, Kathryn Wood

University of Oxford, Oxford, Oxfordshire, United Kingdom

Introduction

Increasing evidence suggests that memory T cells (Tm) play an important role in allograft rejection and may form a barrier to the induction of tolerance. We have used TCR-transgenic mice (BM3; H2K^b-reactive CD8$^+$ T cells) to compare the response of naïve and memory BM3 T cells to alloantigen where the number, specificity and affinity of alloreactive naïve and memory T cells was identical.

Methods

Naïve BM3 T cells were harvested from BM3 TCR-transgenic mice; BM3 Tm were isolated from mice that had rejected a skin allograft. In all experiments 1x10^5 memory or naïve BM3 T cells were transferred to syngeneic RAG$^{-/-}$ mice the day before H2K^{b+} skin transplantation. The response of the memory and naïve T cells was visualised using FACS (lymphoid tissues) and immunohistochemistry and real-time PCR for pro-inflammatory genes (skin graft).

Results

A comparison of the response of memory and naïve BM3 T cells to skin allografts revealed that Tm rejected grafts with an enhanced kinetic (17 versus 27 days, respectively). Surprisingly, when the response of BM3 Tm to the skin allograft was determined, we found that memory and naïve BM3 T cells proliferated to a comparable extent following transplantation and that the resulting effector cells infiltrated skin grafts with a similar kinetic. Furthermore, infiltration of effector cells derived from both memory and naïve BM3 T cells induced a similar gene expression profile. However, BM3 Tm were able to recruit large numbers of GR-1$^+$ cells (neutrophils) to the graft by 10 days post transplant. Furthermore, administration of a depleting anti-GR-1 mAb attenuated graft rejection mediated by Tm which now rejected skin grafts at an identical rate to naïve BM3 T cells.

Discussion

Tm are able to rapidly reject allografts through the enhanced recruitment of GR-1$^+$ cells to the graft. These data extend our knowledge of the function of alloreactive Tm in transplantation. Furthermore, we have identified an under-appreciated role of GR-1$^+$ cells in transplant rejection and suggest that the transient depletion/manipulation of GR-1$^+$ cells may be of therapeutic benefit in the control of rejection responses elicited by Tm.

O009

Donor Brain Death: Measurement of the Activation and Modulation of Cardiac Poly-ADP Ribose Polymerase in a Rat Model

John Brain[1], Anthony Rostron[1], John Dark[2], John Kirby[1]

[1]Instiitute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom, [2]Department of Cardiopulmonary Transplantation, Freeman Hospital, Newcastle upon Tyne, United Kingdom

Introduction

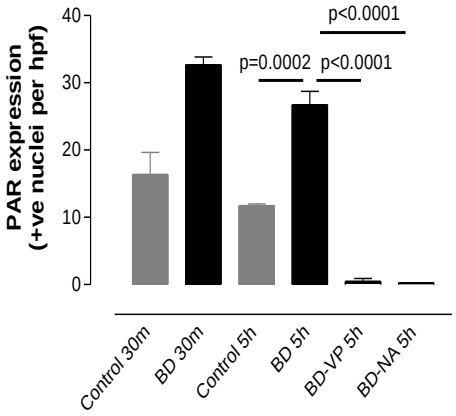
Transplantation can damage DNA resulting in the activation of poly-ADP ribose polymerase (PARP). This enzyme generates polymers of ADP-ribose (PAR) which are linked to nuclear proteins resulting in cell death and allograft dysfunction. Up-regulation and subsequent pharmacological blockade of PARP improves cardiac morphology and function after both heart transplantation and episodes of normothermic cardiac ischaemia. This series of experiments was designed to determine how brain death and resuscitation affect PARP activation in donor myocardial tissues.

Materials and Methods

Brain death was induced in Wistar rats by intracranial balloon inflation. Animals were monitored for either 30 minutes or 5 hours after brain death. Rats monitored for 5 hours had either no resuscitation (5 rats; BD) or were resuscitated by infusion of vasopressin (5 rats; BD-VP) or noradrenaline (5 rats; BD-NA) to normalise the mean arterial pressure to pre-brain death levels. Control animals underwent craniotomy and placement of intracranial balloon which was not inflated (10 rats). Histological sections of myocardial tissue were stained by immunocytochemistry to detect the presence of PAR.

Results

The expression of PAR-linked nuclear proteins by cardiac myocytes was greatly increased after the induction of donor brain death (versus control p<0.0002), the expression of PAR did not alter between the 0 minute and 5 hour groups. Importantly, infusion of noradrenaline or vasopressin to normalise the chronic hypotension produced by brain death reduced the expression of PAR to a level below baseline (both p<0.0001). These data are shown in the **Figure**.



Discussion

These data suggest that chronic hypotension produced by donor brain death has the potential to limit cardiac allograft function through the activation of PARP and subsequent myocardial cell death. Importantly, this early cause of graft damage can be mitigated by appropriate donor resuscitation.

O010

In-vitro Generated Human $CD4^+CD25^{high}$ Regulatory T Cells with Indirect Allospecificity as Potential Patient-specific Reagents to Promote Donor-specific Transplantation Tolerance

Shuiping Jiang, Julia Tsang, Yakup Tanriver, Eva Leung, Giovanna Lombardi, Robert Lechler

Department of Nephrology and Transplantation, King's College London, London, United Kingdom

Introduction: The holy grail in transplantation medicine is the induction of donor-specific tolerance with minimum use of non-specific immunosuppressive drugs. It has been established that $CD4^+CD25^+$ regulatory T cells (Tregs) play a crucial role in maintaining transplantation tolerance. Thus, harnessing regulatory T cells for potential adoptive cell therapy is very promising strategy for the induction of donor-specific transplantation tolerance.

Methods: Human $CD4^+CD25^+$ regulatory T cells with indirect allospecificity for an HLA-A2 (103-120) peptide were generated from purified peripheral blood $CD4^+CD25^{high}$ and $CD4^+CD25^{dim}$ cells by priming with HLA-$DR0101^+A2^-$ autologous dendritic cells pulsed with the A2 peptide.

Results: The antigen-specificity for the A2 peptide was determined by flow cytometry analysis using a fluorescent tetramer composed of HLA-DR0101 and the A2 peptide. The $CD4^+CD25^+$ Tregs with indirect allospecificity for the A2 peptide showed more potent antigen-specific suppression of proliferation and cytokine secretion by effector $CD4^+CD25^-$ T helper cells with indirect allospecificity for the same allopeptide than polyclonal $CD4^+CD25^+$ Tregs. Importantly, pure HLA-DR1:A2 (103-120) tetramer positive (more than 90%) $CD4^+CD25^{high}$ and $CD4^+CD25^{dim}$ Tregs were achieved by flow cytometry cell sorting. Furthermore, the selected $CD4^+CD25^+$ Tregs can be expanded substantially, i.e. a 40-fold increase over a week after T-cell receptor stimulation by CD3/CD28 Dynal beads in the presence of 1000 U/ml of interleukin-2. The expanded $CD4^+CD25^+$ Tregs highly expressed Foxp3, and retained their suppressive properties and maintained expression of lympoid homing receptor CD62L. Finally, we generated and expanded murine $CD4^+CD25^+$ Tregs from C57BL (B6) mice with indirect allospecificity for an K^d allopeptide. In cardiac transplantation, wild-type B6 mice were treated with anti-CD8 antibody to deplete $CD8^+$ T cells one-day before heart transplantation from BALB/c mice. The B6 mice carrying a full mismatch BALB/c heart were subsequently treated with Rapamycin and the K^d-specific $CD4^+CD25^+$ Tregs for two weeks, followed by another two-week treatment with the K^d-specific $CD4^+CD25^+$ Tregs only. After 150 days, the full mismatch BALB/c cardiac transplants were still fully functional and had normal histology.

Discussion: Taken together, these data provide a platform for clinical studies using in-vitro generated and expanded human $CD4^+CD25^+$ Tregs with indirect allospecificity as individualized medicine to promote donor-specific transplantation tolerance.

Parallel Session

Post Transplant Follow up

Wednesday 16 April

13:30 – 15:00

O011

Randomised Controlled Trial of Campath and Tacrolimus-Monotherapy With Daclizumab, Tacrolimus and Mycophenolate Mofetil In Renal transplantation; Interim Results.

Kakit Chan, David Taube, Jack Galliford, Rawya Charif, Antony Dorling, Dawn Goodall, Nadey Hakim, Vassilios Papalois, Anthony Warrens, Adam McLean

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Although Campath induction is widely used in transplantation, there have been no formal controlled trials using this agent with Tacrolimus [Tac] monotherapy. In this study, we report the interim results of our CamTac trial which compares Campath induction and Tac monotherapy with Daclizumab, Tac and Mycophenolate Mofetil [MMF]. This study is stratified for live vs deceased donors, and randomization is 2:1 to the Campath and Daclizumab arms. The target enrolment of 120 patients is powered to detect a 10% difference in rejection rates in the first year. Over a 2 year period 113 patients have been enrolled with mean follow-up 12.3 months. (Campath arm 77 patients LD:DD 50:27, Daclizumab arm 36 patients LD:DD 22:14):

Immunosuppression:

	Daclizumab	Campath
Induction	2 x 1mg/kg IV	30mg IV
Steroids	Day 0 – 6	Day 0 - 6
Tacrolimus	Trough target 8-12ng/ml	Trough target 5-8ng/ml
MMF	Trough target 1.5-3.0mg/L	Nil

Results:

	Daclizumab	Campath
Patient survival	96.6%	100%
Graft survival	91%	98.6%
Graft function (eGFR ml/min)	45.1 [38.4-51.9]	52.8 [48.1-57.4]
Tacrolimus level (LC-MSMS) ng/ml	9.4 [8.2-10.7]	6.8 [6.1-7.6]

Patient and graft survival is similar in both arms. Allograft function is better in the Campath arm but does not reach statistical significance at 12 months [p=0.07] Crude rejection rate in the Campath arm is 5.4% with a median time to rejection of 3.2 months. The rejection rate in the Daclizumab arm is 17% with a median time to rejection of 4.4 months.

Conclusions: In this ongoing randomized controlled trial, Campath induction with

Tacrolimus monotherapy provides a steroid sparing regime with excellent patient and graft survival with low rejection rates and improved graft function compared to a similar regime based on Daclizumab, Tacrolimus, and MMF.

O012

Renal Function Recovery is Delayed in Children With Primary Hyperoxaluria Type 1 [PH1] Undergoing Combined Liver Kidney Transplants [CLKT]

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Background: CLKT is a recognised treatment option for primary hyperoxaluria type 1 [PH1] and renal cystic diseases. However, there is paucity of data on post operative renal function of children undergoing CLKT.

Method: Analysis of prospectively collected data of all consecutive children undergoing CLKT at a tertiary care setting in the UK. Patients were grouped [Oxalosis and non-Oxalosis group] and assessed for post operative renal function with creatinineGFR [cGFR] calculated by Schwartz formula.

Results: Twenty (20) patients underwent CLKT between 1994 and 2007 [Oxalosis gp: 9 patients (5 male); median (range) age 8.6 (1.6-16.7) years: non-Oxalosis gp: 11 patients (6 male); median (range) age 11.5 (5.4-13.8) years]. Median (range) follow up was 71 (1-101) and 17 (2-109) months respectively. Both groups were transplanted with comparable organs. Eight (8/9) and six (6/11) patients received pre-operative renal support in each group respectively, whilst more patients in the oxalosis group [4/8; 50% vs. 2/6; 33%] needed continued post operative renal support [p=>0.05]. Glomerular function (CreatinineGFR) was significantly different between groups until first year post-transplant [median cGFR : Oxalosis vs. non-Oxalosis at Pre-transplant, 3m, 6m and 12m post transplant respectively: 11.06 vs. 12.61 (p=0.4), 40.78 vs. 71.78 (p=0.02), 43.78 vs. 82.74 (p=0.01) and 53.40 vs. 77.43 (p=0.005)]. Overall one year survival is 89% vs. 90% and 5 year survival is 89% vs. 62% respectively.

Conclusion: Children with PH 1 receiving CLKT appear to have delayed recovery of renal function possibly due to mobilization of systemic oxalate.

O013

Antibody Incompatible Renal Transplantation: Early to Medium Graft Survival is Equivalent to Standard Transplantation

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[1]University Hospital, Coventry, United Kingdom, [2]NHS Blood and Transplant, Birmingham, United Kingdom, [3]Warwick Medical School, Coventry, United Kingdom

Introduction: Although antibody incompatible transplantation (AIT) appears reasonably successful in clinical practice, the outcomes for HLA antibody incompatibility (HLAi), are not well defined outside the USA.

Methods: From 2003-2007, 54 patients entered our programme, 45 with HLAi, 3 with blood group incompatibility (ABOi), and 3 with HLAi and ABOi. The early (06/2003 – 08/2006) and later (09/2006 – 12/2007) cohorts of 27 patients each were compared. The initial protocol was similar to that used at the Johns Hopkins University. From case 16, OKT3 was used for established rejection, and IVIg and rituximab were not used. From case 29, ATG was used pre-emptively in selected patients, and post-transplant plasmapheresis (PP) was phased out.

Results: The two cohorts were similar in respect of mean age (39.7 vs 42.6 years), proportions of regrafts (59% vs 56%), cytotoxic crossmatches +ve (33% vs 22%), ABO incompatible or flow crossmatch +ve (41 vs 52%); >10yr treatment of established renal failure (56% vs 56%) and serious comorbidity (53% vs 41%), but differed in the proportions who had developed renal failure as children (37% vs7%). One case in each cohort did not proceed to transplant, and one case was excluded as +ve crossmatch was later found not to be due to donor-specific antibodies.

At latest follow up in the early and late cohorts respectively, patient survival was 92% vs 92%, graft survival was 92% vs 100%. All losses so far have been in the first 4 months. The mean number of pre-transplant PP sessions was 4.3 vs 3.1 and post transplant was 3.1 vs 0.28. In the early and late cohorts respectively, the rates of delayed graft function were 8% and 32% (increase due to immunological and non-immunological risks); acute rejection in the first 3 months 42% vs 40%; mean eGFR at 3 months 49.7 vs 74.6 ml/min/1.73m^2.

Discussion: The results of AIT improved with experience and empirical protocol changes, with graft function and graft survival in the later cohort the same as in standard transplants performed in our unit, despite a high rate of delayed graft function. Patient survival was less than that of standard transplantation. This reflected increased comorbidity, and compared with a survival of only 65% in another 23 patients not accepted for AIT because the recipient was unfit.

O014

Forty Five Consecutive Pancreas Transplants Without Steroids; A Successful Alternative

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BACKGROUND: The widely known adverse effects of long-term therapy with steroids have motivated interest in steroid-free immunosuppression. It is especially attractive in pancreas recipients because it avoids the increased insulin resistance and eye complications caused by steroids. This should be counterbalanced with the increased risk and detrimental effect of rejection in Pancreas Transplantation.

METHODS: We evaluated the outcome of a steroid-free immunosuppressive protocol in pancreas transplant recipients. Between December 2004 and December 2007, a total of 45 pancreas transplant recipients received steroid-free immunosuppression, consisting of induction with thymoglobulin (1.5 mg/kg for five days) and maintenance on tacrolimus adjusted to a target trough level between 5-10 ng/l for the first month (and 5-8 subsequently) and Mycophenolate Mofetil up to 2g daily (median dose 1g).

RESULTS: 30 patients received simultaneous pancreas and kidney transplant (SPK) and 15 pancreas after kidney transplant (PAK). Median recipient age was 38 years, median donor age was 40 and mean HLA mismatch was 4.2. Patient and pancreas survival rates were 93.3% and 86.7%, respectively. Pancreas censored for death survival was 88%.

Among the SPK there were 3 grade IA and IB biopsy proven rejections as well as 2 biopsy proven borderline rejections, which were treated giving an overall rejection rate of 16% (including borderline). Two of those patients had severe rejection 3 months post-transplant. The first one due to severe gastroparesis and malabsorption lost both the kidney and the pancreas despite aggressive treatment. The second one had steroid resistant rejection and was successfully rescued with ATG, repeated steroid pulses and Rituximab. Only this patient required long term steroids following her treatment. There were also two presumed pancreas only rejection in the PAK group diagnosed on the basis of elevated amylase, CRP and absence of clinical symptoms of pancreatitis who were treated successfully. None of them required long-term steroids.

Reasons for patient loss included one death from aspiration pneumonia, and one early cardiovascular death.

All patients who have been treated for rejection have been off steroids for the duration of follow-up. Patients with functioning pancreata had a mean HB1Ac of 5% and normal insulin and peptide C levels.

CONCLUSION:

Excellent graft survival with a low incidence of acute rejection can be achieved using a fully steroid-free immunosuppressive regimen using appropriate induction treatment.

O015

Does Mycophenolate Mofetil (MMF) Confer Clinical Benefit Over Azathioprine (AZA) In Renal Transplantation? A Systematic Review and Meta-Analysis

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Introduction: MMF has increasingly become the anti-metabolite of choice in immunosuppressive protocols since its introduction in the early 90's, replacing AZA. Initial large multi-centre RCTs showed a clinical benefit in reducing the incidence of acute rejections, but without significantly improving graft survival at 1 year. More recently it has been questioned whether this benefit is significant when using newer formulations of Cyclosporine (Neoral) and Tacrolimus.

Methods: A detailed literature search was performed using the Transplant Library from 1995-June 2007. The library, produced by the Centre for Evidence in Transplantation, includes all randomised controlled trials in organ transplantation from 1995 to the present day. For trials before 1995 we searched Medline and Embase combining all search terms for MMF and AZA. All RCTs which directly compared MMF to AZA from time of transplantation were included. All trials were assessed for quality using the Jadad scoring system. The results were analysed using the meta-analysis software Review Manager. All groups were analysed with a fixed effects model and confidence intervals (CI) were set at 95%.

Results: 20 studies were identified which met the inclusion criteria (mean Jadad score = 1.4); these included a total of 3218 transplant recipients. MMF is found to significantly reduce the risk of acute rejection and importantly its action is independent of type or formula of Calcineurin Inhibitor (CNI) used (18 studies, 3043 patients, RR 0.63, 0.56-0.71 $p<0.00001$). The risk of graft loss is also significantly reduced in patients treated with MMF (14 studies, 2800 patients, RR 0.76, 0.60-0.97, $p< 0.03$). There is no significant difference in patient survival between the two groups, nor in renal transplant function as determined by serum creatinine. There is no significant difference in rate of CMV infection, anaemia, leukopenia or rates of malignancy. There is however a significant increase in GI side effects with MMF. Use of MMF results in a greater risk of vomiting (4 studies, 1487 patients, RR 1.27, 1.01-1.61, $p<0.04$) and a greater risk of diarrhoea (6 studies, 1903 patients, RR 1.56, 1.32-1.84, $p<0.00001$).

Conclusion: We have shown that MMF used with a CNI does indeed confer a clinical benefit over AZA by reducing the risk of acute rejection and also in reducing graft loss. We have also shown that this beneficial effect is independent of whether MMF is used in combination with Sandimmune, Neoral or Tacrolimus.

O016

Outcomes of Kidney and Kidney/Pancreas Allograft Recipients After Cardiac Surgery

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The Ohio State University, Columbus, Ohio, United States

Objective: Cardiovascular disease is a common and often debilitating disease in renal allograft recipients. Surgical intervention in renal transplant patients is associated with the perception that these patients have more difficult post-operative courses with higher morbidity and mortality. We sought to retrospectively review the outcomes of the kidney and kidney/pancreas transplant recipients who underwent cardiac surgery.

Methods: A retrospective review of the comprehensive transplant center database was performed. All patients that underwent kidney or kidney/pancreas transplantation between January 1st 1996 and December 31st 2006 were queried for having undergone either coronary artery bypass grafting, valvular repair/replacement or other open cardiac surgery after having received their allograft(s). The mortality rate, complication rates, including infections, stroke, renal failure and allograft loss were determined and compared to non-transplant patients undergoing cardiac surgery.

Results: Of the 8182 open cardiac surgery cases reviewed over the ten year period, 135 appropriate kidney and kidney/pancreas transplant cases were found. The overall mortality rate for these transplant recipients after cardiac surgery was 4.4 % as compared to 6.2% in the non-transplant patients. (p= ns) The outcomes and complication rates are outlined below.

	Kidney Transplant Patients	**Kidney/ Pancreas Transplant Patients**	**Transplant Patient Mortality Rate**	**Non-Transplant Patient Mortality Rate**
Coronary Artery Bypass (CABG)	52	17	2.9%(2)	3.8% (19)
CABG + other	4		0	8.0% (40)
CABG + valve	20	1	4.8%(1)	8.2% (41)
CABG + Valve + Other	5		0	8.3% (41)
Valve	26	6	9.4%(3)	7.1% (35)
Valve + other	4		0	7.4% (37)
Total Cases/Deaths	111	24	6	497
Mortality Rate	4.2% (5)	4.5% (1)	4.4%	6.2%

Complications (<30 days post Cardiac Surgery)	**Transplant patients**	**Non-transplant patients**
Deep Sternal Wound Infection	0.7%(1)	0.5%(38)
Pneumonia	2.2%(3)	3.2%(257)
Stroke	0.7%(1)	1.8%(142)
Renal Failure	2.2%(3)	4.0%(322)

Dialysis	1.5%(2)	1.8%(141)
Allograft Loss	0	

Conclusion: Although kidney and kidney/ pancreas patients are immunosuppressed and have a number of associated co-morbidities, they can undergo cardiac surgery with morbidity and mortality rates similar to or better than those currently reported for non-transplant cardiac surgery patients. Despite their renal transplant status, post operative renal failure and dialysis dependence was not higher in the transplant group. Prior kidney and kidney/ pancreas transplantation should therefore not dissuade us when considering cardiac surgical intervention in this patient population.

O017

SIMULTANEOUS PANCREAS KIDNEY TRANSPLANTATION DOES NOT WASTE KIDNEYS

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Introduction

UK guidelines dictate allocation of kidneys from optimal donors for SPK transplantation over all kidney transplants other than paediatric 0:0:0 mismatched grafts. This is based on an assumed survival/quality of life benefit for diabetic patients which has not been validated in the UK population. Associated mortality and major surgical morbidity means some kidneys used in SPK's inevitably fail. These grafts come from selected, high quality donors and may have provided excellent long-term renal function for less high risk recipients. This study investigates the outcome of kidney transplants as part of SPK's compared with standard deceased donor kidney transplantation.

Methods

From June 2001 to November 2006, we performed 72 SPK transplants. Demographic, clinical and biochemical data were prospectively collected in an electronic database . Analogous data for recipients of kidneys alone from the same donors (paired kidneys) was obtained from the UK Transplant database. Recipient demographics and graft/patient survival were compared at 1 year (χ^2) for each group. Serum creatinine was compared at 3 months (t-test).

Results

Other than primary disease, there were no significant demographic differences between recipients. 15 patients had a follow-up period of between 3 months and 1 year. 51 had a follow-up period of greater than 1 year. 1 year patient survival was similar: 44/51(86.3%) for SPK and 49/51(96.1%) for paired kidney recipients (p=0.16). 1 year graft survival was identical 44/51(86%) in both groups. 3 month serum creatinine values were also not significantly different: 122.2+/-42µmol/L for SPK and 119.7+/-46µmol/L for paired (p=0.76). The incidence of delayed kidney graft function was similar, 24% for SPK's and 16% for kidneys alone (p=0.37).

Discussion

Despite SPK's associated mortality and morbidity, there is no evidence of reduced kidney graft survival at 1 year or renal function after 3 months. The preferential allocation of high quality, deceased donor kidneys for SPK's over most kidney alone transplants cannot be considered unjustified on grounds of kidney utility.

O018

Pancreas Transplantation: The Real Cost Of The Service On Critical Care

Otilia-Maria Mitu-Pretorian, James Barnard, Elijah Ablorsu, Nile Alaf, Abbas Ghazanfar, Bence Forgacs, Sanjay Mehra, Hany Riad, Ravi Pararajasingam, Tunde Campbell, Titus Augustine, Neil Parrott, Afshin Tavakoli

CENTRAL MANCHESTER AND MANCHESTER CHILDREN'S UNIVERSITY HOSPITALS TRANSPLANT UNIT, Manchester, United Kingdom

Introduction: Organ transplantation is among the most expensive surgical treatments performed today; estimates of the cost of various organ transplants vary widely between different settings. The real costs of transplantation are ward costs, pharmacy acquisition costs, laboratory investigations, investigative procedures, and blood products. This audit aimed to analyse the costs of bed stay for pancreatic transplantation in our unit.

Method: Resource use data were collected for 129 patients transplanted between June 2001 and December 2007. We analysed length of stay in the Intensive Care Unit (ICU), High Dependency Unit (HDU) and the Transplant Ward according to the organ transplanted and the procedure performed. We also analysed subsequent re-admissions to hospital. Costs of one night bed-stay in HDU/ICU/ Surgical Ward used in our analysis were obtained from the Department of Health which estimated cost of bed occupancy at £1400 for ICU, £900 for HDU and £350 for the Transplant Ward.

Results: 100% of pancreas transplant recipients were admitted to ICU or HDU. 93 (72%) patients were admitted to HDU, 36 (28%) were admitted to ICU with subsequent step down to HDU or the ward. Median ICU and HDU stay were 3 days. Median ward stay was 17 days. Median length of the first, second and third readmissions were 6, 8 and 6 days respectively. 64(50%) patients were re-admitted only once, 38(30%) were readmitted twice and 27 (21%) were-admitted three times. Total ward care costs for ICU, HDU and the transplant ward were £371,000, £330,300, and £1,337,000 with total cost of re-admissions being £526,050. The mean cost of care per patient was £19,878.68. Prolonged hospital stay was directly associated with factors such as age, donor BMI and Cold Ischemic Time

Median Inpatient stay (days)		HDU	ITU	Inpatient	Readmission
Recipient age	<50	3	1	17	1
	>50	2.5	3	16.5	2
Donor age	<45	3	1	17	1
	>45	3	3.5	19	3
Donor BMI	<26	2.5	1	17	1
	>26	3	3	23	0
Cold Ischemic Time (CIT)	<12	2	1	15	2
	>12	3	2	17	1

Discussion: This study represents the first detailed evaluation of the cost of inpatient care for pancreas transplant patients in our unit. This study may prove useful in a number of ways. It provides a framework for accurately estimating the burden of pancreas transplantation and related complications within surgical and its related specialities. Hospital healthcare managers can utilise cost data to more precisely project future expenditure and resource consumption by this cohort. Optimal donor selection and minimising CIT should be primary target cost reduction.

Parallel Session

Best Abstract

Wednesday 16 April

15:30 – 17.00

O19

Machine Preservation Of Kidneys Donated After Cardiac Death Does Not Reduce Delayed Graft Function: The PPART study

Antonia Wells[1], Rebecca Roberts[1], Joanne Blackwell[2], Jacob Akoh[3], Peter Friend[4], Murat Akyol[5], Francis Calder[6], Andrew Bradley[1], David Collett[2], Chris Watson[1]

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Background. Cold machine preservation has been shown to improve outcome following transplantation of deceased heart-beating donor kidneys. To determine whether cold machine preservation also improves outcome of kidneys donated after cardiac death (DCD) we undertook a multicentre randomized controlled trial in the UK comparing machine perfusion with simple cold storage.

Methods. One kidney from each DCD donor was randomized to machine perfusion (Organ Recovery Systems LifePort device with KPS-1 solution), the other to perfusion with UW (ViaSpan) solution followed by simple cold storage. The primary outcome measure was the need for dialysis in the first 7 days (delayed graft function, DGF); secondary outcome measures included GFR at 1 week (MDRD technique); 3 and 12 month graft and patient survival. At the time of writing, one week outcome data are available for all patients.

A sequential design was used with the data inspected after 60 transplants and then every 20. All recipients received basiliximab, mycophenolate sodium, tacrolimus and prednisolone. The significance of the initial results has been tested using paired T tests and McNemar's exact tests.

Results. Recruitment was stopped after 46 donors when the unadjusted sequential analysis concluded that there was no difference in the incidence of DGF. 91 of the 92 kidneys were transplanted; one was not used for anatomical reasons. One kidney suffered primary non function; it had been machine preserved.

The results shown below represent intention to treat.

	Machine perfusion (n=45)	Simple cold storage (n = 45)	Significance (p-value)
Recipient Age: Mean (SD)	50 (14)	49 (14)	0.55
Cold ischaemic time: Mean (SD)	14h44m (4h36m)	14h53m (4h41m)	0.98
DGF incidence	25 (56%)	24 (53%)	0.99
GFR on day 7: Mean (SD)	17.0 (14.7)	13.4 (11.1)	0.09

Conclusion. Cold machine perfusion of kidneys from DCD donors does not reduce the incidence of delayed graft function in the context of the short ischaemic times seen in the study.

O20

Location and Time Dependent Control of Rejection by Regulatory T cells Culminates in a Failure to Generate Memory T cells

Manuela Carvalho Gaspar, Nick D. Jones, Luo Shiqiao, Martin Laurent, Matthew O. Brook, Kathryn J. Wood

University of Oxford, Oxford, United Kingdom

Introduction

We have shown previously that adaptive $CD25^{+}CD4^{+}$ regulatory T cells (Treg) suppress skin allograft rejection. However, the location of Treg as they function *in vivo* and the mechanisms used to control donor-reactive T cells remain ill-defined.

Methods

Treg were purified from tolerant CBA mice that had accepted a cardiac allograft (C57BL/10; B10; $H2^{b}$) following administration of anti-CD154 mAb. $1x10^{5}$ BM3 T cells (TCR-transgenic, $H2K^{b}$-reactive $CD8^{+}$ T cells) were CFSE labelled and transferred alone or together with $5x10^{5}$ Treg into syngeneic $RAG^{-/-}$ mice one day before transplantation of a B10 skin allograft. The response of the BM3 T cells to skin allografts was visualised using FACS (lymphoid tissues), immunohistochemistry and real-time PCR for pro-inflammatory genes (skin graft).

Results

We found that initially Treg prevented the priming of BM3 T cells to the skin allograft. The suppression of T cell priming by Treg also resulted in the absence of T cell infiltration into skin grafts and in a marked reduction in the intragraft mRNA expression of IFNγ (84 fold), Perforin (19 fold), CCL5 (11 fold) and XCL1 (19 fold) by day 10 post-transplant compared to grafts undergoing rejection (BM3 T cells without Treg). However, with time, peripheral suppression was overcome resulting in the activation and infiltration of BM3 T cells that induced a 'Tc1-like' intragraft gene expression profile. In spite of this, such effector T cells were deleted prior to eliciting rejection by Treg that had also infiltrated skin allografts resulting in a marked deficit in the peripheral $CD44^{+}$ BM3 memory T cell pool.

Discussion

These data demonstrate for the first time that donor-reactive Treg can suppress allograft rejection using distinct mechanisms at different sites *in vivo* with the overall outcome of preventing the generation of donor-reactive memory T cells. Extrapolation of these data to clinical transplantation suggests that the identification of patients that have become "operationally" tolerant to their foreign organ grafts will require a multi-faceted approach involving both peripheral and perhaps more importantly analyses that focus on the transplanted organ itself

O21

Indirect pathway CD4 T cells provide help for generating cytotoxic effector responses through recognition of MHC class II on CD8 T cells.

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Background: CD4 T cells that recognise processed alloantigen on *recipient* APC can provide help to alloreactive cytotoxic CD8 T cells that recognise intact MHC I alloantigen on *donor* APC, but how such 'un-linked' help is provided is not clear.

Methods: The ability of direct and indirect pathway CD4 T cells to provide help for CD8 alloimmunity was compared in a mouse heart transplant model in which recipients contain only monoclonal helper CD4 T cells, specific for self-restricted H-Y Ag (♀ B6 Mar/RAG1$^{-/-}$ mice). Recipients were reconstituted with 10^6 B6 CD8 T cells, and then challenged with ♀ BALB/c (no CD4 T cell help), or ♂ BALB/c (indirect pathway help), or ♂ B6xBALB/c F1 hearts (direct pathway help).

Results: Un-reconstituted Mar/RAG1$^{-/-}$ mice lack effector B and CD8 T cells, and consequently all heart grafts survived indefinitely. In contrast, reconstituted Mar/RAG1$^{-/-}$ mice rejected ♂ F1 grafts rapidly (MST 8d), whereas ♀ BALB/c grafts survived indefinitely, confirming a CD4-dependent effector role for the transferred CD8 T cells. CD4 T cell help through the indirect pathway, although sufficient to elicit rejection, was less efficient than direct pathway help, because ♂ BALB/c grafts were rejected more slowly than the F1 grafts (MST 12d, $p<0.001$). We next considered whether indirect pathway CD4 T cells provide help through recognition of MHC II on the surface of alloreactive CD8 T cells, analogous to the cognate interaction between B and T lymphocytes. In support, flow cytometric analysis of mitogen-stimulated CD8 T cells revealed surface MHC II expression. Moreover, Mar/RAG1$^{-/-}$ recipients that were instead reconstituted with MHC II-deficient CD8 T cells rejected ♂ BALB/c hearts more slowly (MST 21d, $p<0.01$), whereas ♂ F1 grafts, that still permit provision of linked help, were rejected at the same tempo (MST 8d). Most tellingly, Mar/RAG1$^{-/-}$ mice that received simultaneously a ♀ BALB/c heart and ♂ B6 APC (to activate Mar CD4 T cells) rejected their grafts rapidly when reconstituted with ♂ CD8 T cells (MST 9d). In contrast, grafts survived indefinitely when ♀ CD8 T cells were transferred.

Conclusions: Indirect recognition can provide help for cytotoxic alloimmunity, albeit not as effectively as via the direct pathway. Indirect pathway help is potentiated by linkage through recognition of MHC class II on the CD8 T cell.

O22

Causes of early graft failure and outcome of SPK and PAK.

UK experience 2001-2005

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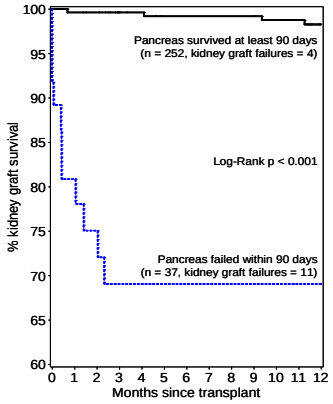
Background: The number of pancreas transplant has been constantly increasing in the UK. The aim of this study is to analyze the causes of early graft failure in SPK and PAK. Also, to compare graft and patient survival following SPK and PAK. **Methods:** All SPK and PAK performed in the UK were retrospectively reviewed from data prospectively collected by UK Transplant between January 2001 and December 2005. The follow-up was 12 months. Early failure is defined as failure within 90 days of transplantation. Data are death censored. **Results:** In the study period 340 pancreas transplant were performed in the UK (306 SPK and 34 PAK). Pancreas graft loss occurred early in 85% of total graft loss (51 out of 60); and the main cause of early pancreas graft loss was thrombosis. Kidney graft loss occurred early in 15 cases. Causes of failure were equally distributed. Kidney graft loss was more commonly associated with early pancreas graft loss (log rank; p<0.001) Tab I. Pancreas graft survival showed to be inferior in PAK compared to SPK (log rank; p=0.04).

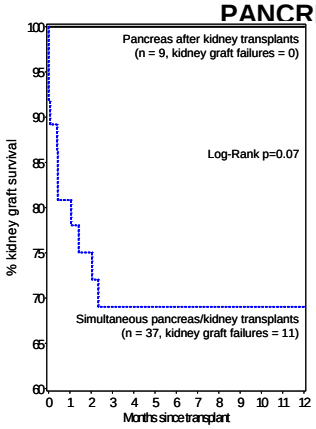
The comparison of 1 year kidney graft survival following SPK and PAK where the pancreas graft failed showed that early pancreas graft loss has a greater detrimental effect on SPK when compared with PAK (log rank; p=0.07) Tab II.

Conclusion. The most commonly observed early cause of pancreas graft loss was thrombosis and no specific cause of early kidney graft failure was identified. The data obtained show that SPK carry better pancreas graft survival when compared to PAK. Kidney graft survival is linked with pancreas graft loss; and PAK host a better kidney graft survival even if the pancreas graft fails. Although there is a limited number of PAK performed there are potential advantages arising from this procedure.

Table I. ONE-YEAR KIDNEY SURVIVAL FOLLOWING PANCREAS TRANSPLANT BY 90 DAY PANCREAS OUTCOME



TableII. ONE-YEAR KIDNEY GRAFT SURVIVAL FOLLOWING SPK AND PAK WHERE THE PANCREAS FAILED WHITHIN 90 DAYS



O23

Ischaemic reperfusion injury and its influence on the epigenetic modification of the donor kidney genome.

Marie D Parker[1], Philip Chambers[2], J. Peter. A Lodge[3], Julian R Pratt[1]

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In clinical transplantation, there is a link between organ ischaemia and damage that occurs upon reperfusion. This is caused by O_2 and OH free radicals that are formed by the degradation of ATP to Hypoxanthine during ischaemia, and by the further conversion to Xanthine upon organ reperfusion. Oxidative damage of 5-methyl-Cytosines (5mC) in genomic DNA is known to be capable of converting such 5mC to unmethylated Cytosines for which no known DNA repair mechanism exists. We hypothesise that such oxidative damage leads to aberrant demethylation of CpG sites in regulatory sites within gene promoters of donor tissue. Normally, such methylated CpG sites control the accessibility of DNA binding proteins to regulate transcription factor binding, and therefore gene expression. Conversion of methylated Cytosines to non-methylated Cytosines by oxidative damage in post-ischaemic organs may modulate gene expression in donor organs and affect transplant outcomes in the short and long term.

Kidneys were exposed to 4 hours cold ischaemia and 2 hours of reperfusion. Ischaemia / reperfusion injury was assessed by histopathology and DNA methylation by bisulphite modified PCR. Primers were designed to assess methylation in the promoter of the Complement C3 gene – known to be important in the donor inflammatory response to I/RI. Our data produced an epigenetic map of the rat renal C3 promoter and identified methylated CpG sites coincident to cytokine response elements and NF-κB binding sites. A similar map has been produced for the rat renal TGFβ promoter. Analysis of the samples showed a significant ($p<0.05$) decrease in methylation in the C3 promoter in response to ischaemia, and a further decrease in response to reperfusion. In order to assess site specific demethylation at the IL-1/IL-6 response element (RE), and the NF-κB binding site, we have undertaken pyrosequencing of the genomic DNA taken from our tissue samples. Preliminary results from pyrosequencing show that compared to normal rat kidney the CpG sites within the IL-2/IL-6 RE and the NF-κB binding site are less methylated following I/RI. Since such epigenetic modification is believed to be stable and heritable to daughter cells upon mitosis, we speculate that aberrant demethylation in genes linked to chronic allograft nephropathy (CAN) such as TGFβ may play a role in chronic fibrotic changes after transplantation. To assess this we are repeating this study in a model of CAN.

In conclusion, the findings of aberrant demethylation of DNA resulting from ischaemia and reperfusion injury describe an unrecognised phenomenon in transplantation. Aberrant demethylation has been linked to the development of tumours and our data suggest a similar mechanism of gene dysregulation may be initiated by the ischaemic insult inherent in organ transplantation. These data may contribute to a further understanding of the how the short lived and transient ischaemic insult influences chronic pathological changes that occur even in syngeneic models of transplantation in the long term.

O24

By How Much does Transplantation Improve Survival For Established Renal Failure Patients In The UK? Data from 7,088 wait listed patients

David Ansell[1], John O'Neil[2], Rommel Ravanan[1], Rachel Johnson[2], Chris Dudley[1]

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Background: The effect of transplantation on patient survival from time of listing for kidney transplant was investigated for renal failure patients in the UK. The analyses have been extended to examine the effect of differing periods of suspension on survival benefit and also to calculate life years gained for the different patient subgroups.

Data: The cohort analysed consisted of 7088 adult patients who were first registered between 1995 and 2000 for a kidney transplant in the UK and did not experience any continuous period of suspension from the transplant list exceeding 60 days. These patients were followed-up from date of listing to date of death or 10th October 2005, the end-point of the study.

Results: 5905 (83%) of the 7088 patients received a transplant in the study period. A Cox regression analysis was used to model the effect of transplantation after adjusting for patient-specific factors. The Cox model incorporated a time-dependent variable which allowed for an immediate increase in the risk of death after transplant and a decrease with time post-transplant. The number of days following transplantation at which the risk of death for transplanted and non-transplanted patients is the same was then calculated, as was the time at which the survival proportions in both groups became equal. Time to equal risk is independent of waiting time and was found to be 145 days. Time to equal survival depends on waiting time and was estimated as 428 days for patients who receive an immediate transplant, rising to 513 days for patients with a 5 year waiting period. Further analysis showed that the decrease in risk associated with transplanted patients varied according to the time spent on dialysis prior to listing (pre-emptively listed, < 1yr, 1 or more years; $p<0.001$) together with the age at listing (18-29, 30-39, 40-49, 50-59, 60+ years; $p<0.001$), ethnicity (white/non-white; $p<0.001$) and renal disease (diabetic/non-diabetic; $p=0.01$) of the patient. Patients with longer histories of dialysis gain from transplantation more quickly in terms of time to equal risk and time to equal survival. However, both these times increase with age at listing, with non-white patients being the most disadvantaged. Within each ethnic group, diabetic patients experience the benefit of transplantation in a shorter period of time than non-diabetics. A preliminary analysis of lifeyears gained from transplantation suggests a relative benefit for non-diabetic patients which decreases with age.

Parallel Session

Basic Science 2

Wednesday 16 April

15:30 – 17.00

O025

Accelerated Rejection of Allografts Following Homeostatic Proliferation of T Cells Despite Disproportionate Expansion of Regulatory T Cells

Kathryn Brown, Thomas Tapmeier, Victoria Moxham, Julieta Karegli, Steven Sacks, Wilson Wong

King's College London School of Medicine at Guy's King's and St Thomas' Hospitals, London, United Kingdom

Homeostatic proliferation of peripheral lymphocytes becomes the main source of new T cells in adults following thymic regression. This process is accelerated in transplant recipients that are lymphopaenic as a result of immunosuppressive therapy. Importantly, T cells that have undergone homeostatic proliferation acquire the phenotype and functions of memory T cells. However, the fate of the regulatory T cell component of the T cell population following homeostatic proliferation has so far revealed contradictory results.

To determine the effect of homeostatic proliferation on $Foxp3^+$ regulatory T cells, we reconstituted $RAG^{-/-}$ mice with wild-type C57BL/6 splenocytes. The proportions of $Foxp3^+$ cells in wild-type spleens, and $RAG^{-/-}$ spleens 14 days following reconstitution, were analysed using flow cytometry. Surprisingly, while only 0.73% $\pm$ 0.16% $CD4^+$ T cells from spleens of naïve animals also stained positive for intracellular Foxp3 (n =10), 20.21% $\pm$ 4.9% $CD4^+$ T cells from spleens of reconstituted $RAG^{-/-}$ mice were double positive, representing a 28 fold increase (n =6, p = 0.0002). To ascertain whether the increased numbers of $Foxp3^+$ T cells in the spleens of reconstituted $RAG^{-/-}$ mice compared with wild type would infiltrate subsequent renal allografts and have a bearing on the outcome of the alloresponse, immunohistochemistry was performed on BM12 allografts transplanted into WT or reconstituted $RAG^{-/-}$ recipients. Although the level of $CD4^+$ infiltration was similar in BM12 kidney grafts transplanted into reconstituted $RAG^{-/-}$ or WT recipients, the level of $Foxp3^+$ T cells was more than 2 times higher in BM12 allografts transplanted into reconstituted $RAG^{-/-}$ recipients (27.7$\pm$6.7 and 13.5$\pm$3.5 cells per HPF respectively, n = 4). This is despite the fact that BM12 kidneys are rejected more rapidly by $RAG^{-/-}$ reconstituted compared with WT mice.

$Foxp3^+$ regulatory T cells have a greater ability to undergo homeostatic proliferation compared with non-regulatory T cells. Despite this, rejection of BM12 kidney grafts are accelerated, further supporting the notion that effector T cells that have undergone homeostatic proliferation have enhanced “memory” like functional capabilities and are therefore more difficult to regulate.

O026

Thrombin Fine-tunes the Allogeneic Stimulatory Capacity of Human Monocyte-derived Dendritic Cells

David Game, Seema Shrivastava, Anthony Dorling

Imperial College London, London, United Kingdom

Introduction: There is increasing evidence of the interplay between the immune and coagulation systems. Thrombin is a key coagulation molecule, acting to catalyse the conversion of fibrinogen to fibrin, amongst other effects to promote clotting. Thrombin can also mediate effects via the protease activating receptors cell surface receptors (PAR), subtypes 1, 3 and 4. The presence of these receptors on the surface of immune cells, including monocytes, has been reported, but the functional consequences of receptor ligation are not defined. The aim of this study is to test the influence of thrombin and PAR peptide agonists and antagonists on the transition from peripheral blood monocytes to derived dendritic cells.

Methods: Monocytes were isolated from human buffy coats by CD14 bead selection. These were then stained for PAR 1-4 using flow cytometry and immunocytochemistry. After this monocytes were cultured in serum supplemented medium with GM-CSF for 5 days. The effects of thrombin and PAR agonists and antagonists were tested as well as the influence of IL4, LPS and dexamethasone on the monocyte culture. Phenotypic changes were detected by flow cytometry for HLA-DR, CD80 and CD86. Function was tested by the stimulatory capacity of the DCs to allogeneic CD4+ cells.

Results: (1) Freshly isolated monocytes expressed PAR1-4 although this was variable.

(2) PAR expression on monocyte derived DCs was reduced, but not abolished by the addition of IL4. (3) Low dose thrombin increased the expression of HLA-DR and CD86 whereas high dose thrombin reduced expression. Consistent with this, allogeneic T cells proliferated more when stimulated by DCs cultured with low dose thrombin and less with high dose thrombin. (4) PAR1 and PAR4 agonists could reproduce the increased stimulatory phenotype and function, as could thrombin with either PAR1 or PAR4 antagonists. (5) This effect was seen whether, or not, IL4 was added to the monocyte culture. (6) This effect was also seen if low dose dexamethasone was added to the DC culture. At high dose dexamethasone, neither thrombin nor PAR agonists could increase costimulatory phenotype or function. (7) When DCs were matured with LPS, thrombin and PAR agonists had no additional effect.

Discussion: Low dose thrombin, acting via either PAR1 or PAR4 can increase the stimulatory capacity of monocyte derived DCs, in part by the increased expression of HLA and costimulatory molecules. This appears to be a “fine tuning” effect, as it is not additive to the effect of LPS nor can it overcome the effect of high dose dexamethasone. It is not clear why high dose thrombin caused a decrease in stimulatory capacity. These effects provide further evidence of the interaction between the coagulation and immune systems, which may have therapeutic implications given the development of clinical PAR reagents.

O027

Intranasal administration of HY peptides induces FoxP3 and alters the migratory properties of antigen specific T cells

Katy Derbyshire, David Coe, Eduardo Fernandes, Jian-Guo Chai, Diane Scott, Julian Dyson

Imperial College London, London, United Kingdom

The male-specific minor transplantation antigens are the best characterised of all the minor H alloantigens. It has previously been shown that intranasal (i.n.) administration of the MHC class II restricted peptide, HY-A^{b}*Dby* can induce tolerance to male skin grafts expressing up to 5 additional HY epitopes. The experiments described here aim to identify the mechanisms whereby tolerance is induced and how this leads to linked suppression, by examining changes in the gene expression of antigen specific T cells during tolerance induction and migration of antigen specific CD8 T cells following male skin allograft.

Gene expression in antigen specific T cells was analysed by real time quantitative PCR using Immunoquant array analysis. 5 days after i.n. administration of HY-A^{b}*Dby* peptide the expression of 11 genes was significantly changed in antigen specific CD4 T cells. When peptide was given with LPS, 79 genes involved in TCR signalling, cytokine signalling and toll-like receptor signalling were significantly altered. 6 genes: FoxP3, Lag3, Ifng, IgG2b, Cd80 and Mmp1a were specifically up regulated by peptide alone.

Under rejecting conditions, 22 days after application of a male skin graft, class I restricted HY-D^{b}*Uty* specific (tetramer positive) T cells could be found in the draining and non draining lymph nodes, spleen and a small number in the skin graft itself. However, when i.n. HY-A^{b}*Dby* peptide was given before the male skin graft, ie tolerising conditions, HY-D^{b}*Uty* specific T cells could only be found in the graft draining lymph nodes.

Taken together these results suggest that i.n. peptide induces regulatory T cells that can prevent allograft rejection and may also alter the migratory properties of antigen specific CD8 T cells. Further work will look at the gene expression in both the antigen specific CD4 and CD8 T cells after HY mismatched skin grafts.

Parallel Session

Medawar Medal

Thursday 17 April

10:30 – 12.30

O028

Graft-protective Regulatory T cells Develop From FOXP3-negative Precursors In Vivo

Ross Francis, Andrew Bushell, Kathryn Wood

University of Oxford, Oxford, United Kingdom

Naturally-occurring $FOXP3^+CD4^+$ regulatory T cells (Treg) play a crucial role in the prevention of autoimmunity. The ability to generate similar populations of Treg against alloantigens offers the possibility of preventing transplant rejection without the requirement for indefinite global immunosuppression.

Pre-treatment of mice with a donor-specific blood transfusion (DST) combined with anti-CD4 antibody generates alloreactive Treg with the capacity to prevent rejection of cardiac, skin and islet allografts. The aim of this project was to investigate whether these graft-protective Treg are derived from naturally-occurring Treg or are generated by the peripheral conversion of non-Treg precursors.

A novel cell sorting strategy was developed, based on the expression of the cell surface markers CD25 and GITR, which yields a population of $CD4^+$ cells that consistently contain ≤0.5% $FOXP3^+$ cells by FACS. The purity of sorted $GITR^-CD25^-$ cells was confirmed using qRT-PCR for foxp3 mRNA.

T cell deficient CBA.$RAG^{-/-}$ mice were reconstituted with this sorted population, devoid of naturally occurring Treg but containing potential effector cells prior to tolerance induction with anti-CD4/DST. Control mice did not receive anti-CD4 or DST. 28 days later, full thickness donor-type skin grafts were performed. Spleen cells were isolated from additional cohorts of mice given the same adoptive transfer but without skin grafting for analysis of FOXP3 expression. In the absence of a skin graft, $FOXP3^+CD25^+$ T cells were readily detected in both control and tolerised mice almost certainly reflecting Foxp3 induction resulting from homeostatic proliferation. However, whilst control mice rejected their skin grafts acutely (n=6, MST 19 days), mice given the tolerance induction protocol accepted their grafts long-term (n=9, MST>100 days).

These data demonstrate that naïve $CD4^+$ cells with the potential to reject an allograft can undergo peripheral conversion into graft-protective Treg following an alloantigen-dependent tolerising protocol. The fact that skin grafts were not protected by $FOXP3^+$ cells that arose in the absence of alloantigen indicates a clear qualitative difference in the two $FOXP3^+$ populations and emphasises the benefit of driving Treg selection using alloantigen pre-treatment. Naturally-occurring Treg are scarce (≈5% peripheral $CD4^+$ cells) while the precursor frequency of alloreactive T cells can be as high as 10%. Tolerance induction strategies that do not rely on expansion of naturally-occurring Treg and are able to convert potential effector cells into graft-protective Treg are very attractive for the establishment of transplantation tolerance.

O029

Intracellular Retention of CD80/86 by Dendritic Cells, Unlike Tryptophan Catabolism, as a Mechanism to Induce Treg-Mediated Donor-Specific Tolerance

Adnan Khan[1], Jennifer Harper[1], Sven Beutelspacher[2], Giovanna Lombardi[3], Myra McClure[4], Andrew George[1]

[1]Department of Immunology, Imperial College London, London, United Kingdom, [2]University Eye Hospital, University of Heidelberg, Heidelberg, Germany, [3]Department of Nephrology and Transplantation, King's College London, London, United Kingdom, [4]Jefferiss Research Trust Laboratories, Imperial College London, London, United Kingdom

Introduction: Allogeneic T cell stimulation requires not only antigen-specific signals but also costimulatory signals, most importantly between CD80/86 on the antigen presenting cell (APC) and CD28 and CTLA4 on the T cell. Engagement of the T cell receptor without costimulation can lead to anergy and the induction of regulatory T cells (Tregs). T cell activation is also controlled by expression of the tryptophan-catabolising enzyme indoleamine 2,3-dioxygenase (IDO). Depletion of this essential amino acid, and/or the production of tryptophan metabolites inhibits T cell proliferation.
Methods: A genetic approach to confer tolerogenic properties on murine dendritic cells (DCs) has been explored using lentiviral vectors, based on the Equine Infectious Anaemia Virus. Firstly, an intracellular method that prevents costimulation has been developed: A fusion protein consisting of CTLA4 and KDEL [an endoplasmic reticulum (ER) retention signal] is expressed in DCs. The CTLA4-KDEL binds to CD80/86 in the ER and prevents expression of these proteins on the DC surface. A second approach uses an elevated expression of the IDO enzyme by transduced DCs.
Results: CTLA4-KDEL- or IDO-transduced DCs were unable to induce allogeneic T cell proliferation. However, using two-stage DC:T cell co-culture assays, it was shown that CTLA4-KDEL-, but not IDO-transduced DCs, can induce donor-specific T cell anergy *in vitro* and *in vivo*. Tolerance to both the direct and indirect pathways was shown using CTLA4-KDEL-transduced DCs, and linked suppression was mediated by the generation of donor-specific Tregs. IDO-transduced DCs did not generate Tregs. Furthermore, it was shown separately that DCs expressing IDO whilst lacking CD80/86 expression for potential ligation by CTLA4 (although CTLA4-CD80/86 ligation upregulates IDO, it downregulates T cell activation) failed to generate or even sustain FoxP3+ Treg populations. The ability of the transduced DCs to induce tolerance to allografts was assessed in a corneal graft model, in which rejection results from the indirect pathway, the predominant pathway of recognition during the chronic phase of allograft rejection.

Discussion: These results support a clinical strategy to induce Treg-mediated, donor-specific tolerance using CTLA4-KDEL-, rather than IDO-expressing DCs.

O030

Is there Equity of Access to the Renal Transplant Waiting List in the United Kingdom?

Deepika Akolekar, Gabriel C Oniscu, John LR Forsythe

Royal Infirmary of Edinburgh, Edinburgh, United Kingdom

INTRODUCTION
This study evaluated the variations in the assessment practices among transplant centres and the impact of comorbidity on access to the kidney transplant waiting list in the United Kingdom.

METHODS
908 patients assessed for transplantation in 10 transplant centres across the UK were included in this multi-centre study. Sociodemographic and comorbidity data were prospectively collected over a nine month period from October 2006 to June 2007. Centre variations and the effect of comorbidity on the likelihood of listing were ascertained using Chi square and Cox Proportional analyses.

RESULTS
720 patients (80%) have at least one comorbid condition at the time of assessment with wide centre variations. These include significant differences in the incidence of ischaemic heart disease ($p<0.0001$) and diabetes ($p<0.0001$) among the assessed patients. Overall 41% patients were assessed pre-emptively but the proportion varies significantly between centres ($p<0.001$, Chi square test). There are significant differences in the time from assessment to listing among transplant centres even after adjusting for comorbidity and other sociodemographic covariates ($p<0.0001$, Cox Regression analysis). On multivariate analysis the other independent predictors of access to waiting list are ischaemic heart disease ($p=0.027$) and smoking ($p=0.003$). Furthermore, at a given time comparable patients are managed differently in different transplant centres.

CONCLUSION
This is the first prospective study demonstrating significant variations in the assessment of patients for renal transplantation across the United Kingdom leading to inequity in access to the waiting list. Comorbidity alone does not explain centre differences. Uniform assessment guidelines are required to ensure an equitable access to the renal transplant waiting list.

O031

Donor CD4 T cells Within Solid Organ Transplants Contribute To Graft Rejection

Thet Su Win[1], Sylvia Rehakova[1], Margaret Negus[1], Kourosh Saeb-Parsy[1], Martin Goddard[2], Eleanor Bolton[1], J. Andrew Bradley[1], Gavin Pettigrew[1]

[1]Department of Surgery, Addenbrooke's Hospital, Cambridge, United Kingdom, [2]Department of Pathology, Papworth Hospital, Papworth, United Kingdom

Background: This study examines how donor CD4 T cells within heart allografts contribute to the development of chronic allograft vasculopathy (CAV), by providing help to recipient B cells for generating pathogenic autoantibody.
Methods: B6 mice were transplanted with MHC II-disparate bm12 hearts. The *allo*- and *auto*-antibody responses were quantified and their contribution to CAV assessed by serum transfer to B cell deficient recipients. The role of donor CD4 T cells in providing help for autoantibody was tested by removing CD4 T cells from donor hearts before transplantation by three approaches: treating with anti-CD4 depleting mAb, administering 1300Gy lethal irradiation, and using $RAG^{-/-}$ bm12 donors.

Results: Bm12 heart grafts developed progressive vasculopathy and were rejected slowly (MST 95 days, n=17). The contribution of antibody-mediated effector mechanisms was confirmed by histopathological examination (fibrinoid necrosis and vascular proliferation), by complement C4d staining, and by long-term graft survival and reduced vasculopathy in B cell deficient recipients. Surprisingly, no alloantibody was detected, but recipients instead developed autoantibody. The autoantibody response was completely dependent upon the provision of help from donor CD4 T cells; it was abrogated by transplanting CD4 T cell deficient hearts. To confirm an effector role for autoantibody in CAV, B6 mice were primed for humoral autoimmunity, but not for alloimmunity, by injecting highly purified bm12 CD4 T cells prior to transplantation. Heart grafts developed severe vasculopathy and were rejected more rapidly (MST 29 days, n=4, $p<0.01$). Furthermore, the serum transfer of autoantibody to B cell deficient recipients induced rejection (MST=30 days, n=4) and restored C4d deposition. Finally, bone marrow chimeric recipients that lacked MHC class II expression specifically on B cells did not develop autoantibody, demonstrating that transplantation-induced autoantibody results from cognate interaction between donor CD4 T cells and MHC class II of recipient B cells.

Conclusions: Our results demonstrate the novel finding that help for autoantibody production is provided by graft-versus-host cognate recognition of recipient B cell MHC class II by donor CD4 T cells. This autoantibody contributes to the development of vasculopathy independently of alloantibody.

O032

The Role of Mannosidase I (Man1a) and N-Glycosylation in the Function of $CD25^+CD4^+$ Regulatory T Cells

Elaine Long, Stephanie Chapman, Kathryn Wood

University of Oxford, Oxford, United Kingdom

Introduction: Pre-treatment of mice with a donor-specific blood transfusion (DST) and a non-depleting anti-CD4 antibody (anti-CD4/DST) results in specific tolerance to heart allografts. This tolerance induction protocol is thought to enrich for alloantigen-specific regulatory T cells (Treg). Mannosidase I (Man1a) is an enzyme involved in the synthesis and processing of N-linked glycoproteins. Man1a is highly expressed in both graft and peripheral blood lymphocytes of tolerant mice. Moreover, Man1a expression increases in Treg when they encounter alloantigen *in vivo*, indicating that N-glycosylation may influence Treg behaviour. Our aim was to determine the importance of Man1a for the function of Treg.

Methods: $CD25^+CD4^+$ Treg purified from CBA mice following anti-CD4/DST pre-treatment were incubated with either a specific Man1a inhibitor Kifunensine ($Treg^{KIF}$), or control PBS ($Treg^{PBS}$). The adherence and regulatory function of $Treg^{KIF}$ and $Treg^{PBS}$ was compared *in vitro*. $Treg^{KIF}$ and $Treg^{PBS}$ function also was analysed *in vivo* after co-transfer with effector T cells into T and B cell deficient mice that received a donor allograft.

Results: $Treg^{KIF}$ retained their capacity to suppress effector T cells *in vitro* but were unable to prevent B10 allograft rejection *in vivo* ($Treg^{KIF}$ 33% survival, $Treg^{PBS}$ 100% survival; each n=9). Further analysis demonstrated that $Treg^{KIF}$ numbers were significantly lower in the draining axillary lymph nodes (LN), ($Treg^{KIF}$ 6592±1092, $Treg^{PBS}$ 653±322; $p<0.01$) at day10 post-transplant, where they were less able to inhibit CFSE labelled effector T cell proliferation. $Treg^{KIF}$ expressed equivalent levels of the homing molecule CD62L, but adherence to its ligand MAdCAM-1 was decreased *in vitro* ($Treg^{KIF}$ 15.7%±4, $Treg^{PBS}$ 3.7%±0.6; $p<0.05$).

Discussion: Taken together, our results suggest that activation of Treg results in increased Man1a expression and altered N-glycosylation of cell surface proteins. In our experimental system, altered N-glycosylation is not essential for intrinsic Treg suppressive capacity *in vitro*, but is essential *in vivo* where it facilitates Treg migration to sites where they can regulate immune priming. Treg migration is central to its role in regulating *in vivo* immune responses and may require specific changes in N-glycosylation upon alloantigen encounter.

O033

Rate of change of GFR in 2,927 Kidney Transplant Recipients – Influence of donor and recipient factors.

Udaya Prabhakar Udayaraj[1], Anna Maria Casula[1], David Ansell[1], Christopher Dudley[2], Rommel Ravanan[3]

[1]UK Renal Registry, Bristol, United Kingdom, [2]Southmead Hospital, Bristol, United Kingdom, [3]University Hospital of Wales, Cardiff, United Kingdom

Introduction: There has been only modest improvement in long term graft outcome despite significant improvement in 1 year graft survival in the last decade. In this study, we investigate factors associated with the rate of decline in graft function after the first year of transplantation. **Methods:** 2927 kidney only transplant recipients (2000-2004) with a functioning graft at one year and eGFR (4v MDRD equation) of 10-59 ml/min/1.73m^2 were included. Annualised change in eGFR (Δ eGFR, ml/min/1.73m^2/year) after the 1st year was calculated using linear least square regression. Simple and multivariate linear regression was used to study donor and recipient factors that were associated with Δ eGFR. Weighted analysis using inverse of Δ eGFR slope variance was used to account for varying precision in Δ eGFR slope estimate. **Results:** Overall, the mean Δ eGFR ± SD was -1.3 ± 6.3 ml/min/1.73 m^2/year. 25% of patients had stable eGFR (Δ eGFR -1 to +1 ml/min/1.73 m^2/year); 29.3 % had improvement in eGFR (Δ eGFR ≥ +1 ml/min/1.73 m^2/year) and 45.7% had decline in eGFR (Δ eGFR ≥ -1 ml/min/1.73 m^2/year). In multivariate regression, recipient factors associated with more rapid decline in eGFR were younger recipient age, Caucasian race, diabetes, and longer duration of renal replacement therapy prior to transplantation. There was no association between baseline graft function and Δ eGFR. Donor factors associated with a faster decline in GFR were older age and male sex. Male to female transplants were associated with more rapid decline in eGFR than any other gender combinations. The decline was faster in patients with DR mismatch compared to A/B mismatch or no mismatch. There was no association between Δ eGFR and donor type (live/cadaveric). Conclusions: Rate of change of eGFR post renal transplantation is very different to native kidney disease .In most patients the GFR tends to be stable or actually improve. Both donor and recipient characteristics may influence the rate of decline in eGFR. Surprisingly, male to female transplants have more rapid decline in eGFR compared to female to male transplants and donor type [live vs cadaveric] does not influence rate of decline after the 1st year of transplantation.

O034

Inferior long term graft and patient survival in Asian patients after adult liver transplantation compared to a matched Caucasian cohort

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Background: Outcomes in liver transplantation are dependant on donor, operative and recipient factors. Liver disease is common in patients of Asian origin and almost all UK cadaveric donors are Caucasian. These factors may impact on graft availability and outcome.We compared outcomes in matched groups of adult Asian and Caucasian recipients of cadaveric liver grafts at a single UK centre. Methods: Demographics, risk factors, graft availability, survival and mortality data on all adult Asian patients transplanted between 1986 and October 2006 were analysed from a prospective database. Results: For each Asian recipient (n = 111), one Caucasian transplant recipient (n = 111), matched for age, gender, race, diagnosis, and year of transplantation, was selected from the transplant database. Blood group distribution, MELD, Creatinine, BMI and time on waiting list were also recorded. (table1)

	Asian (n=111)	Caucasian (n=111)	P value
Graft failure	50	36	0.017
Total deaths	48	30	0.005
Diabetes	30	15	0.019
Cardiac co morbidity	9	2	0.05
Time on waiting list	73 days	43 days	0.01
Graft failure in blood group (B+AB)	20/41	9/33	0.041
Death in blood group B+AB	19/41	7/33	0.016

Patient and graft survival by Kaplan-Meier analysis was better in Caucasian patients (Patient: 5 and 10 year survival 60% and 50% in Asian versus 77% and 70% in Caucasians; Graft survival 5 and 10 years survival was 57% and 46% in Asian versus 71% and 63 % in Caucasians).Conclusion: Liver transplant recipients of Asian origin have worse long term outcomes having waited longer for their grafts. The higher incidence of diabetes, cardiac comorbidity and incidence of blood group B may contribute to this.

O035

Campath and Tacrolimus monotherapy without steroids or Mycophenolate Mofetil: 3 year results.

Kakit Chan, Jack Galliford, Rawya Charif, Rania Betmouni, Antony Dorling, Nadey Hakim, Adam McLean, Vassilios Papalois, Anthony Warrens, David Taube

West London Renal and Transplant Centre, Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom

Complex immunosuppressive regimes which include steroids, Tacrolimus [Tac] and Mycophenolate Mofetil [MMF] are effective, although expensive. We have developed a simple and cheap regime using Campath induction and medium dose Tac monotherapy avoiding steroids and MMF.

In this study we report our 3 year experience comparing our results with a similar group of patients receiving immunosuppression with Daclizumab [Dac] induction, conventional dose Tac and MMF. Neither group of patient received steroids more than 7 days. In the Campath group, 160 patients [94m, 66f,mean age 45.8 yrs + 12.7 (Mean+1SD)] received 30mg Campath 1H iv, day 0, prednisolone [60mg daily for 4 days, 30mg daily for 3 days and then stopped] and Tac, 0.1 mg/kg/day [12 hr trough level 5 -8 ng/L (LCMS)]. 87 patients [45m, 42f, mean age 45.6 yrs + 12.6] acting as historical controls received 2 iv doses of Dac [2mg/kg, day 0 and day 14], prednisolone [60mg daily for 4 days, 30mg daily for 3 days and then stopped], Tac, 0.15 mg/kg/day, [12 hr trough level 8-11 ng/L (LCMS)] and Mycophenolate Mofetil 1.5g/day [12 hr target trough level 1.5-3.0 mg/L]. Mean follow up in the Campath group was 17.1 months and 45.8 months in the Daclizumab group.

At 3 years, patient survival in the Campath group was 98.8% and 95.3% in the Daclizumab group [p=ns]; allograft survival was 91.9% in the Campath group and 94.0% in the Daclizumab group [p=ns]. The Campath group showed significantly better allograft function at 3, 6 and 12 months. The incidence of rejection, infection and malignancy were similar in both groups.

The mean annual drug cost of the Campath regime [NHS list price] was £3900 and £8000 with Daclizumab [$p<0.001$].

This study shows that in the medium term, this simple and cheaper regime which also avoids steroids, produces similar outcomes to a conventional immunosuppressive regime with better allograft function during the first year after transplantation.

Parallel Session

Transplant Co-ordinators Session

Thursday 17 April

10:30 – 12.30

O036

A Novel Solution To An Old Problem -The impact On Donation Rates Using The Liverpool Integrated Care Pathway As A Trigger For Referral- The Results From One Acute General Hospital Trust.

Fiona Murphy

Bolton HOSPITAL NHS TRUST, Bolton. Manchester, United Kingdom

The Liverpool integrated care pathway for the dying has been successfully used to direct end of life care and decision making throughout this Acute Hospital NHS Trust since April 2003. Use of the pathway ensures that bereavement care is delivered in a consistent way across the trust to nationally agreed standards of practice.

It is recognised that the critical care area and its staff are pivotal to the required increases in the identification and referral of potential solid organ donors. Working in collaboration with the critical care end of life group and the Trust Clinical Effectiveness Group to build on the success already achieved in the use of the current pathway we registered as a pilot site for the Liverpool Care Pathway for the care of the dying in critical care for the last days of life. This pilot commenced June2007.

A simple addendum has been added to this pathway for ventilated patients, which requires all patients to be considered for Non Heart Beating Organ Donation (NHBOD). The pathway requires staff to refer all patients with suitable renal and liver function to the Donor Transplant Coordinators to assess their suitability for solid organ donation before the critical care staff approach family to ascertain the patents wishes.

The pilot is in the early stages, however the first six months data is very encouraging showing a referral rate of 100% of all potentially suitable NHBOD or tissue donors. This represents a significant increase in donation compared to the previous 6 months figures.

Although still in its infancy the pilot is showing that a combination of excellent support for bereaved families and collaborative working with the critical care staff and transplant services can generate an increase in the number of deceased donors. This simple and easy to follow addendum to a recognised pathway enhances its value made in life and increasing the choice available at the end of life. It is proposed that this pilot could be rolled out to other critical care units as a trigger for donation. There is also an expectation that because the requirement to consider the suitability for donation is documented it will be carried out by staff. Thus we have ensured that donation is considered part of normal practice when delivering high quality end of life care that fully respects the wishes of the dying patient.

O037

NHS staff and the organ donor register (ODR)

Aftab Fazal[1], Natasha Ahmad[1], Kasim Ramzan[1], Haroon Waheed[1], Susan Richards[2], Simon Bramhall[2]

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Organ transplantation is the only treatment available for those in end stage organ failure but the waiting list continues to grow and is limited by the supply of donor organs. The Potential Donor Audit has revealed that there is a large pool of potential donors that are never converted into actual donors. There is little published evidence of what influences staff and what impact this has on discussing organ donation with the families of potential donors.

A descriptive cross sectional study was conducted using 257 questionnaires from Physicians and ICU Nurses in 6 hospitals in the West Midlands. Statistics were calculated using simple proportions, Fischer's exact and Kendall's tau-b test.

There was a higher rate of staff on the ODR in the study population compared with the general population. Factors that significantly increased the likelihood of the respondents being on the ODR included female, age 21 to 30 years and being Caucasian. In the Caucasian group 54.6% of people were on the ODR, which was significantly greater than for other ethnicities ($p=0.00$). There was also a significantly greater proportion of people on the ODR in the Christian group (51.2%) compared with other religious groups ($p=0.00$). Involvement with more than 11 organ donations increased the likelihood of respondents being on the ODR ($p=0.052$).

These data suggest that targeted education to specific groups of healthcare professionals might improve organ donation rates.

O038

Has the Human Tissue Act Altered the Work-up Time For Living Kidney Donors?

Kay Hamilton, Andy Weale, Christopher Dudley, Justin Morgan, Najib Kadi, David Mitchell, Paul Lear

Southmead Hospital, Bristol, United Kingdom

Introduction

The Human Tissue Act (HTA) came into force across the United Kingdom on September 1st 2006. Concern was raised by the British Transplantation Society that the new legislation may result in delays in the time taken to process live donors, as every patient must now be seen by an independent third party assessor. Prior to the Act only non-genetically related donor-recipient pairs underwent such assessments. The aim of this study was to examine the impact of the Act on the work up time to donation for living kidney donors, in a single centre.

Method

We compared the time from tissue typing and CT angiogram to transplantation for all living kidney donors within our centre for the year prior to the Act (1st September 2005-31st August 2006) with the year following the Act (1st September 2006 – 31st August 2007).

Results

There were 40 living kidney donor transplants performed in the year preceding the Act and 36 in the year following the Act. The median time from tissue typing to donation was 255 days (IQR 166-534) prior to the act and 318 days (IQR 219-436) following the act, whilst the median time from CT angiogram to donation was 68 days (IQR 27-137) prior to the act and 68 days (IQR 40-151) following the act. Univariate analysis of variance using log transformed data revealed there was no significant effect on time to donation whether the donor or recipient was related or unrelated ($p=0.464$ time from tissue typing; $p=0.986$ time from CT), prior to or following the act ($p=0.973$ time from tissue typing; $p=0.668$ time from CT), and there was no interaction between these factors ($p=0.301$ time from tissue typing and $p=0.673$ time from CT).

Conclusions

The HTA provides a robust framework for assessment of all living donors and within our centre has not increased the work-up time to transplantation.

O039

Exploration of key stakeholders valuation of priority criteria for the allocation of deceased donor kidney transplants

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Aims: Those designing systems for the allocation of deceased donor kidney transplants need to be informed by the views of professionals and patients in order to produce equitable allocation algorithms. We studied a large sample of patients and professionals, including carers and for the first time donor families.

Methods: Extensive piloting was used to establish criteria (attributes) that may be valued when prioritising patients. Discrete choice experiments (DCE) (using questionnaires) were used to explore the different weightings respondents placed on these criteria. Final analysis was undertaken using appropriate regression techniques, and according to respondents' ethnicity, gender, transplant status, quality of life, age, dependents (adults or children), and stated altruism.

Results: Final priority criteria included waiting time; level of HLA matching; differences in the patients' number of dependents; age of recipients; and illnesses affecting both length of life and quality of life. Responses came from 908 patients; 113 healthcare workers; 41 carers and 48 donors. All the attributes were statistically significant. Significant differences in preferences were observed in some categories of patients. Patients of non-white ethnicity had less strong preference for HLA matching. There were also differences by transplant status, age, respondent quality of life, altruism of responders, and whether patients had dependent children. Respondents' gender and having adult dependents did not influence preferences. Compared to patients, healthcare workers placed more value on prioritizing young patients and on prioritizing those with diseases having a moderate effect on life expectancy.

Summary: This large study indicated that respondents valued multiple factors affecting the allocation of deceased donor kidneys. DCE methodology had not previously been used in this area, but proved very powerful. The results are broadly supportive of the changes made in the UK 2006 transplant allocation policy, in which more priority was given to long waiters and young adults. Despite HLA matching now only producing a small numerical benefit in transplant survival, it remained an attribute valued by all groups of patients and professionals, though was valued less by patients of non-white ethnicity, who may be disadvantaged by allocation systems using HLA matching.

O040

Consent to enter the transplant waiting list and consider kidney offers from extended criteria donors

Diane Evans, Janet Fenning, Christopher Dudley

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Introduction: The use of kidneys for transplantation from extended criteria donors (ECD) is increasing and may be associated with less favourable outcomes. Because of the stress from an unpredictable admission for deceased donor renal transplantation, it is difficult to discuss the risks associated with the use of ECD organs with recipients and to obtain informed consent when called in. A refusal by the recipient to proceed with transplantation may result in an increase in the cold ischaemic time (CIT) for the next recipient. Therefore, we have developed a process where patients consent to entry onto the transplant list and decide in advance whether they wish to consider a transplant from an ECD. ECDs in our centre are identified as donors with:↑creatinine, diabetes, CVA,↑CIT, hypertension, age >60 yrs or risk of communicable disease. We present the process we have developed and our experience with using it for 6 months.

Methods: Patients have both verbal and written information (in house Transplant Information Booklet) including details regarding the definition of ECD and the risks and benefits of using kidneys from these donors are discussed at a transplant assessment clinic or transplant education clinic. A consent form for entry onto the transplant waiting list is provided and patients are encouraged to take this form away to discuss with family members before signing. Patients are required to sign this consent form stating that they have read and understood the Transplant Booklet and had the opportunity of asking questions regarding renal transplantation. They are also asked to state whether they wish to consider offers of kidneys from ECDs.

Results: Between June and September 2007 58 consecutive adult patients completed the process. The decision of these patients regarding NHBD/ECDs was:

	No. of patients		**No. of patients**
No to NHBD	**4**	**No to ECD**	**4**
No to transplant	**1**	**Transplanted**	**1**

Conclusion: The process lets patients consider the risks and benefits associated with the use of kidneys from NHBD and ECD in advance of a transplant. Those who will decline such offers are identified beforehand and not called in. Obtaining informed consent from patients at the time of transplantation has been enhanced.

O041

How People From Three Ethnic Backgrounds Developed A Positive View of Organ Donation

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Imperial College London, London, United Kingdom

Introduction: To explore how people from a white British/Irish, African Caribbean and South Asian/Indo-Asian ethnic background developed a positive view of organ donation. **Methods:** A United Kingdom based qualitative study using oral history interviews currently with 55 participants. **Results:** The ability to achieve a positive view of organ donation is hindered or encouraged by an interplay of family background, cultural and religious values, feelings about death, personal relationships, age, personality, and experiences of donation and identity over time. These dynamic relationships are influenced by their historical and social contexts. Despite being positive about organ donation many people failed to register this view and some peoples' views towards organ donation changed during their lifetime. The white British/Irish saw organ donation and death in practical or secular terms and some older participants recalled the media coverage of the development of organ donation as a scientific “cultural” advancement. Developing a positive view of organ donation was more complex for the two ethnic minorities. Organ donation generated stronger and more emotional conflicts concerning individual and social identities that were often already in a state of cultural tension. Many participants overcame these issues through emphasising religious values over religious rituals, discussing and thinking about organ donation over time, rationally separating out good publicity from bad publicity about organ donation, believing in religious fate, self interest, real life exposure to the benefits of organ donation and seeing organ donation as another struggle against “oppression.” Some of these participants hinted that they would like to see a positive shift in opinion about organ donation from people of the same ethnic background before fully embracing the idea of organ donation. **Discussion:** Future campaigns should be grounded in peoples' realities and reflect the tensions that they face when deciding to be an organ donor. Previous campaigns that have highlighted the lack of donors from ethnic minorities may have reinforced the idea that organ donation goes against cultural “norms,” while campaigns based on the idea of “ethnic communities” have failed to reflect or understand the complexity and politics of ethnic identity. Exploring effective strategies that reflect this politics and enable people to trust the NHS and embrace the idea of organ donation is crucial.

Parallel Session

Cardiovascular & Immunological Disease in Transplant Patients

Thursday 17 April

13:30 – 15.30

O042

Long-term safety and efficacy of Calcineurin (CNI) Free Immunosuppression for the Failing Kidney Transplant

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Chronic allograft nephropathy (CAN) remains the most common cause of graft loss following transplantation. CNI toxicity has been implicated in the pathogenesis of CAN. Evidence is increasing for the use of CNI free regimes in the early and late post transplant period as well as for the patient with a failing kidney transplant.

We initially reported the benefits of CNI free/ MMF based therapy in these patients, in 2000. Following this unit policy changed and all patients with CAN had their immunosuppression changed, with the CNI withdrawn and maintenance being achieved with MMF and prednisolone only. We report the long-term follow up of patients with CAN maintained on MMF and prednisolone from 2002.

53 patients were included in this analysis. Mean (±SD) follow-up is 64±28 months (range 1-105). Immunosuppression was changed to Tacrolimus, Rapamycin or Cyclosporine based therapy for acute rejection (n=1), progression of CAN (n=4) and diarrhoea (n=2) respectively. 6 patients died with a functioning graft. 3 grafts failed as a consequence of CAN and one patient was lost to follow up. 36/53 grafts are still functioning on MMF based immunosuppression.

Analysis of serum creatinine and calculated GFR (Cockroft-Gault and MDRD GFR) over time showed significant improvements compared to pre-conversion values.

Months from conversion	**-6**	**0**	**12**	**24**	**36**	**48**	**60**	**72**	**84**
N	53	53	51	48	43	39	37	24	17
Mean Creatinine (μmol/L)	203	223	175	168	175	135	147	144	150
Stand dev	71	73	70	77	115	38	43	45	67

$p \leq 0.01$ T Test paired sample 2 tail

Improvements in systolic and diastolic blood pressure control were achieved with an associated reduction in the number of antihypertensive medications taken by the patients. Absolute cholesterol levels were also reduced.

There is unlikely to ever be a “golden bullet” that will enable us to treat the variety of pathological entities that encompass CAN. It nevertheless remains the goal. We have shown that graft survival can be maintained without the use of CNIs and that this is safe. This protocol results in sustained improvement in kidney function over time with other secondary benefits also being seen .

O043

Lisinopril And Chronic Allograft Nephropathy (CAN): Graft Function, Proteinuria And Proximal Renal Tubular (PTC) Peptide (Trasylol) Catabolism: A Novel RCT

Asheesh Sharma[1], Alieu Amara[1], John Alexander[3], Liliana Shalamanova[1], Ana Alfirevic[1], Munir Pirmohammed[1], Malcolm Jackson[1], Steve Grime[4], Alan Shenkin[1], Sally Heyworth[2], Graeme Close[1], Howeda Shawki[5], Linda Smith[4], Francis McArdle[1], Ajay Sharma[2], Abdel Hammad[2], Ali Bakran[2], Rana Rustom[1]

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Background: ACE inhibitors reduce proteinuria and delay progression to renal failure in diseased native kidneys. Proteinuria is a common feature in CAN. We previously proposed, in pilot studies, a pathogenic role for proteinuria in CAN and demonstrated in patients increased PTC catabolism of a radiolabelled peptide marker, aprotinin Apr*. Proteinuria and Apr* catabolism were reduced after two months treatment with lisinopril without compromising graft function but there was significantly *increased metabolic acidosis* and graft progression was not addressed.

Methods: 47 patients with biopsy proven CAN and ≥1g/24h proteinuria were recruited (2001-6). Patients were randomised to either lisinopril (2.5-40 mg/d titrated to reduce proteinuria to <1 g/day without a fall in BP) (Group A, ^{51}CrEDTA clearance 25.2±1.7 ml/min/1.73m^2) or to other hypotensive therapy (Group B, 29.8±3.4) for one year. Sodium bicarbonate was given to all patients to maintain normal plasma K^+ and HCO^-_3. Serial ^{51}CrEDTA clearances (primary outcome) and 24 h proteinuria (secondary outcome) measurements were made and genotyping studies carried out. In Group A patients Apr* (injected i.v. 0.5 mg, 80 MBq) catabolism was measured from renal imaging and urinary metabolic studies).

Results: At baseline, the two groups were comparable except for a higher level of proteinuria in Group A (2.8±0.3 vs 1.7±0.2g/24h; p<0.01). The rate of decline of renal graft function was comparable after one year (-7.7 in Group A vs -8.4 ml/min/y/1.73m^2 in Group B). Proteinuria decreased significantly after 1y in Group A only (to 1.8±0.2 g/24h; p<0.03 vs 2.0±0.3 g/24h). Additionally, after one year lisinopril significantly reduced PTC uptake and metabolism of Apr* (from 21.2±1.6 at baseline to 14.3±2.5 % of dose and 0.94±0.06 to 0.70±0.08 %/h respectively; p<0.03). MAP was comparable in both groups (96.3± 2.4 mmHg), as was plasma K^+ and HCO_3. In all patients with ≥30% reduction in proteinuria, there was a significant association with rs699 polymorphism in angiotensinogen (OR 3.1, p<0.02, 95% CI 1.12-8.8). **Conclusion:** The rate of decline of renal graft function in patients with CAN and severe renal impairment was unaffected by lisinopril therapy for one year. However, Lisinopril led to a significant fall in proteinuria, PTC Apr* uptake and catabolism suggesting relative preservation of graft function despite increased baseline proteinuria in these patients compared with Group B.

O044

Increased expression of the protein cross-linking enzyme, transglutaminase 2 (TG2), in the Fisher-to-Lewis rat model of chronic allograft nephropathy

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INTRODUCTION: Chronic allograft nephropathy (CAN) is the leading cause of renal allograft loss. It is associated with tubular atrophy, interstitial fibrosis, glomerulosclerosis and obliterative arteriolopathy. The development of CAN involves expansion of the extracellular matrix (ECM). TG2 is believed to be central to this expansion by crosslinking proteins through the formation of ε(γ-glutamyl)-lysine dipeptide bonds that causes accelerated ECM deposition and resistance to proteolytic clearance. The aim of this study was to investigate the activity and distribution of TG2 and ε(γ-glutamyl)-lysine in the Fisher-to-Lewis (F-L) rat model of CAN.

METHODS: Under isoflurane anaesthesia, donor kidneys obtained from Fisher rats (allograft) (n=7) or Lewis rats (isograft) (n=5) were transplanted into Lewis rats using end-to-side anastomosis employing aortic and inferior venacaval conduits. Total renal Tg activity was measured using the [^{14}C] putresceine incorporation assay. TG2 protein and its cross-linked product ε(γ-glutamyl)-lysine were detected by immunofluoresence and quantified by computerised image analysis.

RESULTS: F-L allografts were hypertensive (systolic BP 124 ± 11 vs. 159 ± 11 mmHg, $p<0.05$) and proteinuric (37 ± 17 vs. 303 ± 80 mg/24h, $p<0.05$) with a lower creatinine clearance (1.57 ± 0.19 vs. 0.62 ± 0.18 ml/min, $p<0.05$) than Lewis-to-Lewis (L-L) isografts. Masson's trichrome staining showed an increased expansion of the ECM in the F-L allografts together with tubular atrophy and tubular dilatation. Electron microscopy confirmed the changes of CAN in the allografts. Kidneys from the F-L allografts had higher transglutaminase activity (0.41 ± 0.03 vs. 1.09 ± 0.13 nmol/h/mg protein, $p<0.05$) than the L-L isografts. Immunofluoresence showed a marked increase in the area of both Tg protein (glomeruli: 40 ± 8.7 vs. 60 ± 14.9 %, $p<0.001$; interstitium: 11.4 ± 3.8 vs. 44.9 ± 10.9 %, $p<0.001$) and ε(γ-glutamyl)-lysine (glomeruli: 20 ± 9.6 vs. 56.9 ± 15.5 %, $p<0.001$; interstitium: 1.87 ± 2.2 vs. 35.4 ± 25 %, $p<0.001$) in the F-L allografts.

CONCLUSIONS: An increased expression of renal TG2 and ε(γ-glutamyl)-lysine was observed in both glomeruli and the renal interstitium in the F-L rat model of CAN. This therefore provides a suitable model for interventional studies using TG2 inhibitors to treat CAN based on the successful use of these compounds in slowing the development of renal scarring in other animal models of chronic renal failure.

O045

Sildenafil Citrate Augments Current Myocardial Protective Strategies in Cardiac Transplantation.

Phil Botha, John Dark, Guy MacGowan

Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction. Sildenafil citrate has recently been demonstrated to have profound myocardial protective effects in both in-vivo animal and isolated cellular models of myocardial ischaemia-reperfusion injury. We aimed to investigate whether these effects can be used to augment cardioplegic, hypothermic and anaesthetic techniques aimed at protecting the heart during transplantation.

Methods. We employed a rat model of heterotopic cardiac transplantation, including pre-and postconditioning by volatile anaesthetic, in-situ cardioplegic arrest, cold storage (3 or 6 hours) and blood reperfusion (3 hours). An intra-ventricular balloon was used to assess functional recovery and myo-cellular injury and apoptosis quantified by serum cardiac troponin-I and activated Caspase-3 immunohistochemistry respectively. The protective effect of Isoflurane anaesthesia, with and without the addition of Sildenafil preconditioning was quantified. The role of the mitochondrial ATP-sensitive potassium channel (mK_{ATP}) commonly implicated in preconditioning was investigated using a selective pharmacological inhibitor (5-Hydroxydecanoate).

Results. Sildenafil preconditioning (0.7mg/kg IV) 30 minutes prior to cardioplegic arrest significantly improved post-reperfusion recovery of systolic and diastolic function after a clinically relevant ischaemic time of 3 hours. Maximum ventricular generated pressure over a range of end-diastolic pressures form 0-35mmHg was significantly greater at 0.5, 1, 2 and 3 hours post reperfusion in the Sildenafil group (116.3 ± 11.6mmHg vs.87.5 ± 15.3mmHg for Control at 1 hour of reperfusion, $P<0.01$ ANOVA). Similarly, maximum dP/dt, minimum dP/dt and ventricular compliance were significantly improved by Sildenafil pre-conditioning. Both systolic and diastolic function showed a decline over the three hour reperfusion period but remained significantly better in the Sildenafil group at three hours ($p<0.01$). Neither cellular apoptosis (1.4 ± 0.6 Sildenafil, 1.5 ± 0.3 cells/hpf control, $p=0.54$) nor cardiac troponin-I release (299.4 ± 78.5 vs. 290.3 ± 62.4n/ml control, $p=0.41$) were significantly reduced by Sildenafil preconditioning. 5-Hydroxydecanoate, administered 5 minutes prior to preconditioning, abolished the additional protective effects of Sildenafil over Isoflurane anaesthesia. In the setting of a prolonged ischaemic time of 6 hours, resulting in a significantly greater degree of cellular injury and apoptosis, Sildenafil preconditioning had no statistically significant effect on the recovery of function, nor cellular necrosis and apoptosis.

Conclusion. Our findings suggest that Sildenafil citrate may represent a valuable adjunct in the improvement of myocardial function after ischaemia-reperfusion injury in the setting of cardiac transplantation. The effect appears to be mediated by the mK_{ATP} channel and independent of the known protection Sildenafil induces against myo-cellular necrosis and apoptosis.

O046

Immune Tolerance Fingerprint in Renal Transplants

Maria Hernandez-Fuentes[1], Birgit Sawitzki[2], Esperanza Perucha[1], Pervinder Sagoo[1], Patrick Miqueu[3], Stephanie Chapman[4], David Stevens[5], Ligia Craciun[6], Ian Roberts[4], Rachel Hilton[1], Ruhena Sergeant[7], Stefan Tomiuk[8], ' http://www.transplant-tolerance.org.uk/Participant%20Physicians.aspx[1], Uwe Janssen[8], Michel Goldman[6], Jean-Paul Soulillou[3], Anthony Warrens[7], Kathryn Wood[4], Hans-Dieter Volk[2], Robert Lechler[1]

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Identification of immunological tolerance is an important prerequisite in order to establish an individually-tailored approach to the post-transplant management of allograft recipients. It will also provide new insight into the mechanism underlying the balance between tolerance and rejection. Here we present data from a multi-centre study aimed at identifying tolerance to renal allografts.

Methods*:* We have collected samples from five selected groups of renal transplant recipients: drug-free tolerant patients that were functionally stable despite remaining immunosuppression-free for more than one year; functionally stable patients on minimal immunosuppression (<10 mg/day prednisone); stable patients maintained with calcineurin inhibitors (CNI); stable patients maintained on CNI-free immunosuppression regimen; and patients showing signs of chronic rejection. A group of age and sex matched healthy volunteers was also included as control. Several biomarkers and bioassays, were combined to provide an immunological 'fingerprint' of the tolerant state.
Results*:* Drug-free tolerant patients displayed an expansion of peripheral blood B and NK lymphocytes in the absence of donor-specific antibodies. The differential expression of several immune relevant genes and a high ratio of foxp3/ α-1,2 mannosidase expression in these patients was observed. TCR analysis highlighted differences between the Vβ repertoires of drug-free tolerant recipients and chronic rejection patients. Additionally, direct pathway donor-specific hyporesponsiveness by IFNγ ELISpot and lack of indirect pathway anti-donor responses assessed by *trans-vivo* DTH were detected in drug-free patients.
Discussion**:** The diagnostic capabilities of a signature consisting of: i) the fold expression over reference of the 9 most significant genes, ii) the ratio of percentages of B/T lymphocyte subsets, iii) the percentage of $CD25^{mid}CD4^{+}$ and iv) the ratio of anti-donor to anti-3^{rd} party ELISpot frequencies is as follows: specificity 0.964, sensitivity of 0.933, a positive predictive value of 82.4% and a negative predictive value of 98.7%. These biomarkers, after being validated in a prospective study, could be used to inform drug weaning protocols in kidney transplant recipients.

O047

Are We Storing Up Vascular Catastrophes For The Future In Long Term Allograft Survivors?

A.O. Mahendran, P.S. Veitch, Y. Aggarwal, A. Emin, E Alnaeb, O. Fernando, D. Yu, P. Sweny

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INTRODUCTION: Following two index cases involving undiagnosed arterial aneurysm formation in long standing renal allograft recipients (one fatal, one repaired), we designed and instituted a vascular screening programme. Our initial survey detected a 33% prevalence of aneurysm in recipients with grafts surviving 20 years or more. If our experience is widely applicable then this finding will require surveillance scans in all long term renal transplant recipients.

METHOD: We have established a retrospective study of recipients with grafts functioning 20 years or more. Exclusion criteria included; patient refusal, a return to regular dialysis and those recipients unfit for surgery. The abdominal and pelvic vascular tree was imaged using one of three modalities; MRA or CT angiography and IA DSA in those patients with hip replacements or pacemakers. The patient cohort was stratified according to risk using; current age, original disease, MACE (major adverse cardiovascular events), creeping creatinine and immunosuppressive regime. The high risk recipients have been the first to be screened.

RESULTS: Our analysis identified a total of 182 patients with a minimum allograft age of 20 years, of which 54 patients met our inclusion criteria. The median patient age and graft age were 50 and 25 respectively. Twelve high-risk transplant recipients were imaged initially and 4 patients were identified with 1 aneurysm or more of their vascular tree. These included; external iliac artery (1 patient), internal iliac artery (2 patients), abdominal aorta distal to anastomosis site (1 patient), gastroduodenal artery (1 patient). This represents a 33% prevalence of aneurysm in renal allograft recipients of > 20 years. One patient died following aneurysm rupture. One patient has undergone successful excision and repair of aneurysm with preservation of graft function. Two recipients are planned for surgery.

CONCLUSION: The data from this single unit study demonstrates a significantly high prevalence of aneurysm formation in asymptomatic long term graft recipients which hitherto has been unreported. This screening programme is unique. Very long term patient and graft survival is an emerging phenomenon, posing new medical problems. Aetiological factors for late aneurysm formation include; hypertension, long term steroids and altered haemodynamics. It is possible that patients with adult polycystic kidney disease will be at particular risk.

O048

Pentosan Polysulphate: Potential as an anti-inflammatory anti-coagulant

Aimee Kibondo, Vijay Anand, Lisa Clarke, Andrew Nelson, David Talbot, Anne Cunningham, Noel Carter

University of Sunderland, Tyne and Wear, United Kingdom

Introduction: Heparinoid compounds have a long history of use in transplantation; primarily as anti-coagulants, but the literature demonstrates that these compounds are also effective anti-inflammatory compounds. The mechanism of their anti-inflammatory activity is the inhibition of key heparin binding cytokines and chemokines that are sequestered at the cell surface by the glycosaminoglycan moieties of proteoglycans. This process appears to be critical in defining localised signalling cues, presentation to receptors and to facilitate the sustained signalling required for a biological response.

Hypothesis: The heparinoid pentosan polysulphate (PPS) will inhibit the chemotactic activity of chemokines and the pro-inflammatory effects of cytokines.

Methods: Transmigration assay: Endothelial cell lines HMEC-1 or EAHY-926 are seeded into 3μM pore transwells (Falcon) and placed in 12 well culture plates. The lower chamber contained Gey;s Balanced Salt solution with or without 10 μg ml^{-1} of CCL-5. Into the upper chamber are seeded peripheral blood mononuclear cells (PBMCs) with or without PPS at various concentrations. Cells migrating across the membrane stained using Diff Quick and enumerated by counting 10 high power fields in a microscope.

Cytokine stimulation of endothelial cells: Confluent mono-layers of the endothelial cell line HMEC-1 are grown in the presence or absence of the cytokines TNF-α and IFN-γ at a concentration of 10 ng ml^{-1} with or without PPS at a concentration of 10 μg ml^{-1}. Cells were analysed using both *taqman* gene expression assays and by flow cytometry for the expression of ICAM-1 as a marker of cytokine stimulation.

Results: In the transmigration assay there was a significant increase in the number of cells migrating in response to CCL-5 ($P<0.05$) and the addition of 10 μg $ml^{-1}$of PPS reduced the migration to the level of the no CCL-5 control ($P<0.05$) in both cell lines. The use of 5 μg ml^{-1} of PPS showed a reduction in migration but this was not significant. In the second assay the addition of cytokines saw a 26 fold increase in ICAM-1 gene expression and a 4 fold increase in cell surface expression of the ICAM-1 protein. In both cases the addition of PPS reduced the levels back to that of the control ($P<0.01$).

Discussion: The assays used in this work are common *in vitro* assays to measure the activity of heparinoid compounds in the field of immunology. What is surprising in this case is the relatively low doses of PPS required to elicit an inhibitory effect, we typically require about 10X more heparin weight for weight. PPS is also approximately 4 times less anti-coagulant. We are currently repeating these and other assays in comparison with heparin to quantify the relative activity of this molecule.

Significance: It would appear that PPS is ~10 times as effective as heparin weight for weight and less anti-coagulant. If PPS were to replace heparin as the anticoagulant of choice it could be used at a higher dose providing a stronger anti-inflammatory effect.

Parallel Session

Preservation & Machine Perfusion

Thursday 17 April

16:00 – 18.00

O049

Outcomes Following Pancreas Transplantation From Expanded Criteria Donors

Anand Sivaprakash Rathnasamy Muthusamy[1], Mano Navarathnarajah[1], Shrikanth Reddy[1], Doruk Elker[1], Steve Garnett[1], Susanna Fernandez-Diaz[1], Isabel Quiroga[1], Sanjay Sinha[1], Anil Vaidya[1], Peter Friend[2]

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Introduction: The disparity between the availability and demand for pancreases has increased rapidly in the recent years. In an effort to expand the donor pool, we liberalized our criteria for acceptance of pancreas allografts. The donor profiles and graft outcomes are compared with the standard donor grafts. **Methods:** From April 2004 to December 2007, 174 pancreas grafts were procured either from 123 standard criteria (SCD, 12-45 years) or 51 expanded criteria donors (ECD, <12 or >45 years)and 9 grafts from non-heart beating donors. Pancreases were accepted based on gross appearance and quality of perfusion during retrieval. All grafts were implanted intraperitoneally with enteric exocrine and caval venous drainage. PTx outcome was evaluated as incidence of delayed graft function (DGF) of pancreas & kidney, graft & patient survival. **Results:** There were 102 SPK, 17 PAK and 4 PTA from SCD; 38 SPK, 9 PAK and 4 PTA from ECD group. Median follow up was 16 months for SCD and 8 months for ECD. Mean SCD recipient age (41.07±6.9) was significantly less (P<0.0001) than mean ECD age (47±7.56), due to an effort to match the donor age in SCD (30.7±9.2) & ECD (53.1±5.38). ECD had a significantly lower (P<0.0001) body-mass index than SCD (28.5±3.37 Vs 23.7±3.8), and were predominantly female in comparison with SCD. The average hospital stay for SCD recipients was similar to ECD recipients (18.9±12.9 vs. 18.9±11.2 days), as were the re-admissions rates (16% vs. 19.6%), rejection episodes (24% vs. 16%, P=NS) and re-operations (20% vs. 16%). ECD grafts had a higher incidence of DGF of kidney (29% vs. 15%, P<0.05) and of pancreas (4% vs. 0%, P=ns) despite similar mean cold ischemia time (11h30min vs. 11h24min). However, this early difference was lost by 3 months post transplant with equivalent mean serum creatinine values (120±49 SCD vs 129±36mmol/L, P=ns), mean fasting glucose (5.48±0.64 SCD vs. 5.45±0.63 mmol/L) and fasting c-peptide production (0.76±0.38 vs.0.94±0.57 pmol/L, P=ns).Overall pancreas graft survival (91% vs. 94%), kidney graft survival (99% vs. 100%) and patient survival (96% vs. 100%) was similar. **Conclusions:** ECD offers a potentially large donor pool of pancreas grafts with similar early patient and graft related outcomes compared to SCD, despite a higher incidence of DGF. These donors will provide a significant increase in the number of available pancreases for transplantation.

O050

Long-term outcome of non-heart-beating-donor kidney transplants is influenced by baseline donor renal disease, but not warm ischaemic insult.

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Introduction: Although long-term results for non-heart-beating-donor (NHBD) kidney transplantation are reportedly similar to heart-beating-donor (HBD) transplants, NHBD are generally younger, with less co-morbid disease. Here, we analyse how warm ischaemic damage (WID) and baseline disease within the transplant impacts on short and long term outcome of NHBD kidneys.

Methods: The results of 104 'controlled' NHBD kidney transplants, performed at our centre from 1996 to 2006, were compared to the HBD kidneys transplanted immediately before and after each NHBD kidney. Baseline renal disease was scored by analysing implantation biopsies for the presence of glomerular, tubular, parenchymal and vascular disease, as described previously (N Engl J Med. 2006 Jan 26; 354(4): 343-52).

Results: Delayed graft function (DGF) occurred more frequently in NHBD kidneys than HBD kidneys (64% vs. 29%, $p<0.001$) but graft function at one year and long term graft and patient survival were equivalent in both groups (mean follow up 25 months). NHBD were younger (mean 41 vs. 46 years, $p=0.027$), with a higher pre-donation eGFR (101 vs. 81 ml/min, $p=0.001$) and were associated with shorter cold ischaemic times (14.6 vs. 17.3 hours, $p <0.001$), suggesting that NHBD kidneys are better 'quality' but receive additional WID at time of retrieval. Histological scoring of underlying donor renal disease was, however, the same for both groups. Overall, the presence of baseline disease was associated with earlier graft failure (relative risk 1.55, $p=0.001$); but the risk of failure was no higher for NHBD than HBD kidneys. On multivariate analysis, no other donor variable impacted significantly on graft survival. Finally, if WID influences long-term graft outcome, one would expect paired kidneys from the same NHBD to have similar long-term function. This was not the case. WID does impact upon initial function because there was an effect of pairing on the incidence of delayed graft function (test of independence of pairs $p=0.025$).

Conclusion: Long term outcomes of HBD and NHBD kidneys are similar. Although WID from NHBD influences immediate function, the only identifiable donor factor that impacts on long-term survival is the presence of baseline disease in the transplant kidney. The combination of baseline donor disease and NHB donation does not confer an additional risk of graft failure.

O051

Modulation of ischaemia reperfusion injury by the nanoparticle delivery of C3 specific small interfering RNA (siRNA)

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RNA interference blocks gene expression using small unique segments of gene sequence. This natural process can be exploited to reduce transcription of specific genes and offers a new strategy to block gene expression. In transplantation, it has been established that donor derived Complement C3 is rapidly upregulated at the time of transplantation, and that such C3 contributes to ischemia/reperfusion injury and inflammation during immune rejection of the organ. This study sought to exploit a novel nanoparticle strategy to treat donor organs with siRNA to knockdown C3 gene expression in the donor kidney.

10µg of siRNA was packaged into synthetic polycationic histidine:lysine nanoparticles to facilitate *in vivo* siRNA transfection. The nanoparticles were added to Hyper Osmolar Citrate perfusion fluid and administered to donor rat kidneys at the time of organ harvest. After 4hours of cold ischaemia, the kidneys were transplanted into syngeneic hosts, and 2 days later were harvested. C3, IFNγ, IFNα and IL-1β gene expression were then determined by Real-Time PCR to assess the degree of gene knockdown and the effect of C3 gene knockdown on other pro-inflammatory genes that could be influence by 'off-target' effects of siRNA. Histopathology of the tissues was performed to assess the effect of C3 gene knockdown on post-ischaemic tissues, and immunohistochemistry for C3 assessed the affect of C3 siRNA on local C3 deposition.

Delivery of naked C3 siRNA did not produce significant C3 gene knockdown, however, using the siRNA packaged in the nanoparticle co-polypeptide at ratios of 4.5:1, 3:1 and 1.5:1 w/w with C3 siRNA produced significant C3 gene knockdown (50% / 61% / 46% respectively, compared to untreated control transplanted kidneys: $P<0.05$, n=3/group). The use of a scrambled control sequence of siRNA did not reduce C3 expression, suggesting the effect was target specific. Assessment of off-target effects so far indicates the siRNA sequence used did not itself induce an inflammatory response and analysis to date does not indicate any toxicity associated with the *in vivo* use of the co-polypeptide packaged siRNA. By histology, the reduced local expression of C3 in the donor organ was associated with diminished acute tubular necrosis characteristic of severe ischaemia / reperfusion injury, and immunohistochemistry revealed a marked reduction in the deposition of C3 in the transplanted tissues.

In conclusion, packaging siRNAs into the nanoparticles provides a novel strategy to effectively reduce pro-inflammatory gene expression in the transplanted kidney. The strategy offers clear clinical potential to reduce the local expression of genes contributing to post-transplant inflammation and in the future we will develop arrays of specific siRNA to diminish pro-inflammatory gene expression in donor organs as adjunct therapies to conventional immunosupression or tolerance induction.

O052

Macrophages and renal ischaemia reperfusion injury: from disease initiation to novel cell therapy.

David Ferenbach, Vasudev Ramdas, David Kluth, Jeremy Hughes

University of Edinburgh, Edinburgh, United Kingdom

INTRODUCTION: Ischaemia Reperfusion Injury (IRI) may result in delayed graft function in renal transplants and is a risk factor for acute rejection and reduced graft survival. We examined the role of macrophages (MΦ) in disease initiation and also used genetically modified Mφ as novel therapeutic agents in renal IRI. METHODS & RESULTS: Circulating monocyte and renal MΦ ablation was achieved by administering either IP diptheria toxin (DT) to CD11b-DTR transgenic mice or IV liposomal clodronate (LC) 24hr prior to IRI (20 min left renal pedicle clamp and right nephrectomy with mice sacrificed at 24hr). LC-mediated depletion of monocytes and renal MΦ was significantly protective at 24hrs (serum Cr and ATN score reduced by 46% and 43% respectively vs CON). The more potent DT-mediated monocyte ablation (5.8±1 vs 3.2±0.6 vs 0.8±0.5 $x10^5$/ml: CON vs LC vs DT) and MΦ depletion (23.7±1.3 vs 11.2±0.9 vs 5.1±0.8 $F4/80^+$ MΦ/hpf: CON vs LC vs DT) had no protective effect (Cr 110±17 vs 99±18 μmol/l; DT vs CON: %ATN 52±6 vs 57±5; DT vs CON). Unlike DT, LC induces dramatic systemic apoptosis of MΦ in spleen, liver etc. We tested the hypothesis that the widespread generation of apoptotic cells (AC) by LC was key to its protective effect by injecting $20x10^6$ AC IV 24 hrs prior to renal IRI. AC administration *per se* was protective (44% reduction in serum Cr vs CON, $p<0.05$) in the absence of monocyte/MΦ depletion. Hemeoxygenase-1 (HO-1) upregulation is protective in IRI and we examined whether exogenous HO-1 overexpressing MΦ could 'deliver' therapeutic HO-1 to the injured kidney. MΦ were transduced with recombinant adenovirus expressing HO-1 (Ad-HO-1) or control virus expressing β-galactosidase (Ad-βgal). *In vitro*, Ad-HO-1 MΦ generated significantly less TNF-α and increased IL-10 following LPS compared to Ad-βgal MΦ. $10x10^6$ MΦ were injected IV following IRI with Ad-HO-1 MΦ administration being significantly protective (Cr 133±30 vs 102±18 vs 68±4μmol/l; non-transduced MΦ vs Ad-βgal MΦ vs Ad-HO-1 MΦ, $p<0.05$). CONCLUSION. Modulation of monocyte/renal MΦ numbers as well as ACs *per se* can ameliorate IRI and ACs may exert immunomodulatory effects in this context. These data are also 'proof of principle' that 'cell therapy' with modified HO-1 overexpressing MΦ is renoprotective in IRI.

O053

Significance of Day Zero Donor Renal Allograft Biopsy in Predicting Long Term Outcome of Recipient's Renal Functions

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Introduction: Long term outcome of renal transplant is unpredictable. Many recipient factors can affect the survival of renal allograft. Because of the selective selection criteria of the donors, invariably the donor factors are less important in predicting renal allograft outcome. The problem usually arises with asymptomatic healthy donors of upper age limit and with cadaver donors of young age with undiagnosed underlying medical co-morbidity. Day zero renal allograft biopsy is not a common practice because of the invasive nature of the procedure.

In this retrospective study we analyzed the safety and significance of day zero renal allograft biopsy in predicting long term renal functions of recipients.

Methods: From January 1999 to December 2005 two hundred and seven day zero cadaver donor renal allograft biopsies (CRABx) were performed. All biopsies were taken in cold phase during bench dissection, including 171 wedge and 36 trucut biopsies. 183 patients where up-to-date records were available were included in this retrospective study. In pair kidney donation only the recipient with biopsy kidney was included. Day zero CRABx were classified into normal, mild, moderate or severe nephropathy depending upon degree of arteriosclerosis (AS), arteriolar hyalinosis (AH), interstitial fibrosis (IF), tubular atrophy (TA), glomerular mesangial sclerosis (GS) and mononuclear interstitial infiltration (MI). Serum creatinine levels (s/Crt in mmol/L) and GFR were recorded at year one and two to access the renal functions.

Results: The overall donor mean age (DMA) was 43 years (range: 14yrs to 68yrs) with M:F of 4:1. Heart beating and non-heart beating donors were 149 and 34 respectively. 141, 28, 1 and 3 biopsies were put into normal, mild moderate and severe nephropathy group. In normal group (n=141), DMA was 38yrs with recipients mean age (RMA) 44yrs. Mean s/Crt at 1, 6, 12 and 24 months was 103, 109, 107 and 117 respectively. In mild group (n=28), DMA was 41yrs compared with RMA of 41yrs. Mean s/Crt at 1, 6, 12 and 24 month was 117, 123, 113 and 103 respectively. In moderate group (n=11), DMA was 53 with RMA of 39 years. Mean s/Crt at 1, 6, 12 and 24 months was 139, 143, 168 and 166 respectively. In severe nephropathy group (n=3), DMA was 66yrs (63+68+67) with RMA 61yrs (51+65+68). Mean s/Crt at 1, 6 and 12 months was 211, 253 and 389. Two patients from this group lost their graft and went back on haemodiaysis within 24 months.

Discussion: Although many factors are responsible for the overall renal allograft outcome after transplant, day zero biopsy can be used as a guide to predict these functions at an early stage. In a long run it can help to improve graft survival by more vigilant follow-up in patient with moderate to severe group.

O054

Renal Vascular Function During Hypothermic Pulsatile Perfusion

Ian Winburn, Jonathan Hill, Robert Walker, Zoltan Endre, Ivan Sammut

University of Otago, Dunedin, New Zealand

Introduction- Hypothermic pulsatile perfusion (HPP) of the donor kidney has been reported to improve graft outcome. However the mechanism by which protection is offered remains unclear. It has been hypothesised that preservation of endothelial function may be responsible for the improved outcomes observed following HPP. This study details both vascular smooth muscle and endothelial function during HPP which have not been previously investigated.

Methods- Kidneys were retrieved from Sprague-Dawley rats following renal artery cannulation and perfused *ex vivo* with a modified Krebs Henseleit solution at either 37 or 8ºC. Following equilibration, perfusion parameters were recorded in response to angiotensin II (AngII; $1x10^{-11}$-$1x10^{-7}$ M) or methacholine (MCh; $1x10^{-9}$-$1x10^{-4}$ M). Cumulative pressure, flow and vascular resistance dose response curves were generated. Endothelial denuded kidneys (with 0.1% CHAPS) were utilised to examine the vasodilatory effects of adenosine diphosphate (ADP). The kinetics of vascular constriction/relaxation were also investigated in both 37 or 8ºC kidneys following AngII administration in the presence or absence of losartan.

Results- Kidneys perfused at both 37 and 8ºC demonstrated dose dependent vasoconstriction in response to AngII (EC_{50} 37ºC= 0.32±0.028nM vs EC_{50} 8ºC = 9.0±1.39 nM; p<0.001). The rate of vasoconstriction was also significantly slower (p<0.001) in 8ºC kidneys as compared to warm. Interestingly 8ºC kidneys remained vasoconstricted following cessation of AngII delivery unlike the 37ºC controls. This persistent pressor response was reversed in a dose dependent manner by losartan. Only 37ºC kidneys vasodilated (EC_{50}= 187±35nM) in response to MCh. However, ADP (another endothelial dependent vasodilator) induced vasodilation in 8ºC kidneys (EC_{50}=1330±295nM) and these effects were significantly reduced following endothelial disruption with 0.1% CHAPS.

Discussion- This is the first time that endothelial function has been described during HPP, and our data supports the notion that the endothelium remains active. Although MCh responses were lost at 8ºC, ADP mediated vasodilation was observed implying altered MCh pharmacodynamics at 8ºC. This has strong clinical implications and supports the hypothesis that pharmaco-modulation of HPP kidneys is a therapeutic strategy that can improve post transplant function.

Parallel Session

BSHI

Thursday 17 April

16:00 – 18.00

O55

Is there a place for beneficial matching of complement C3 allo types in the improvement of long term renal transplant outcome?

Magdi Yaqoob[1], Mira Varagunum[1], Bernd Dohler[2], Gerhard Opelz[2]

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Background: Activation of complement appears to play an important role in the development of chronic allograft nephropathy (CAN), a common cause of delayed graft loss. The recently reported prospect of improving long term renal graft outcomes by using complement C3 F/S allele as a putative target for matching donors and recipients in kidney transplantation is exciting (Brown et al 2006). It is very important to confirm this finding in a larger patient series before the recommendation of this approach in clinical practice.

Aim: A random sample of 1147 pairs of donor and recipients from the Collaborative Transplant Study (CTS) DNA bank (mean follow up = 7.4 years) was genotyped for the C3 F/S allele and association with graft outcome was analysed. Polymerase chain reactions with validated primers were used for genotyping. Transplants were divided into four groups according to the genotype of recipients (R) and donors (D) (R=SS D= FS/FF, R=SS D=SS, R=FS/FF D=SS, R=FS/FF D=FS/FF).

Results: Baseline characteristics of the four transplant groups were similar. Genotype distribution was in agreement with Hardy Weinberg equilibrium. The graft outcome of selected cohort was identical with the remaining patients in the CTS database excluding selection bias. There was no statistically significant difference in graft survival between the main comparison group of interest R =SS, D= FS/FF, and of the other three groups (R=SS D=SS, R=FS/FF D=SS, R=FS/FF D=FS/FF), with associated hazard ratios (HR) of 0.90, 0.87, and 0.89, respectively ($p>0.05$). Moreover, no difference in patient survival rates, cumulative rates of acute rejection and graft function as assessed by serum creatinine levels was seen.

Conclusion: In a large patient cohort, we were unable to replicate the previously reported positive association between C3 polymorphism and graft outcome in renal transplantation. In the light of this data, we cannot recommend matching for C3 polymorphisms in renal transplantation. We suggest that the quest for improving long term renal allograft outcome should continue and must include multi-targeted genetic and non-genetic approaches in future.

Brown KM et al. Influence of donor C3 allotype on late renal transplantation outcome. N Engl J Med 2006; 354:2014-23

O056

Predicting the Immunogenicity of HLA Class I Alloantigens Using Structural Epitope Analysis

Vasilis Kosmoliaptsis[1], J. Andrew Bradley[2], Linda D. Sharples[3], Afzal N Chaudhry[4], Eleanor M. Bolton[2], Timothy Key[1], Reyna S. Goodman[1], Craig J. Taylor[1]

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Introduction: The full benefit of HLA matching strategies in kidney transplantation is not realised because all donor mismatches within a given HLA locus are currently assigned equal weighting despite wide variation in immunogenicity according to the recipient HLA type. We therefore examined the extent to which the additional structural information provided by inter- and intra-locus analysis of amino acid polymorphisms at continuous (triplet) and discontinuous positions (eplet) defined by the HLAMatchmaker algorithm enables prediction of immunogenic (unacceptable) HLA mismatches.

Methods: Sequential sera obtained from 34 highly sensitised patients (HSP) awaiting renal transplantation were screened using single antigen HLA antibody detection beads covering 64 HLA-A and –B specificities to determine immunogenic HLA alloantigens and the antibody levels. The 34 HSP HLA class I types and each mismatched HLA specificity represented on the single antigen beads (giving 2088 mismatched combinations) were entered into the HLAMatchmaker program to perform inter- and intra-locus amino acid sequence comparisons and determine the number of triplet and eplet mismatches.

Results: There was a strong positive correlation between the number of triplet/eplet mismatches and the presence of antibody to the mismatched HLA specificity ($p<0.0001$). For mismatched HLA specificities with no triplet/eplet mismatches, 19 of 66 (29%) and 15 of 54 (27%) combinations respectively were antibody positive, increasing to >85% with eight or more triplet/eplet mismatches. The magnitude of antibody response also increased as the number of triplet/eplet mismatches increased from zero to five, at which point maximal antibody reactivity was reached. In four of the 34 HSP, high antibody levels were observed for several mismatched HLA combinations with zero triplet/eplet mismatches, indicating that self triplets/eplets expressed in different conformations on donor alloantigens do not always predict non-immunogenic epitopes.

Conclusion: Structural analysis of the recipient HLA type and mismatched HLA alloantigens using the HLAMatchmaker algorithm to identify triplet/eplet mismatches allows prediction of immunogenic (unacceptable) donor HLA types.

O057

Pretransplant Crossmatch Using Peripheral Blood Derived Lymphocytes (PBL) is Reliable and Reduces CIT

Mohamed Ilham[1], Sandra Lloyd[2], Robert Walters[2], Timothy Rees[2], Argiris Asderakis[1]

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Introduction: Pretransplant crossmatch (CXM), along with identification of HLA specific antibodies is prerequisite for a successful kidney and pancreas transplant. Organs from donors after cardiac death (NHB) represents one of the attractive solutions to organ shortage, and pancreas transplantation (PTX) simultaneously or following kidney transplant is a successful treatment for selected diabetic patients. Cold Ischemic Time (CIT) is an important predictor of long term kidney survival and is known to be detrimental in early and late pancreas survival, thus it might be even more important in those situations. Therefore every effort that can effectively minimise CIT should be used.

Aim: Evaluate crossmatch using donor peripheral blood derived lymphocytes (PBL) rather than spleen or lymph nodes once a donor has been identified (in some cases even before the donor procedure takes place) in NHB and PTX.

Methods: In a 2-year period all NHB and PTX donors were CMX against recipient's most rent sample available. All recipients had HLA specific antibodies previously identified and actively avoided. There were 28 CMX for NHB and 10 for PTX performed using donor PBL. Both complement dependent (CDCXM) and flow cytometry cross match (FCXM) were performed. In 12 patients the CMX was performed using spleen cells (as PBL were not available prior to spleen becoming available from the donor), and were excluded from further analysis. All 26 remaining CMX were repeated the following day using spleen cells and the results compared.

Results: All CDCXM using donor PBL were negative, and concurred with CDCXM results against spleen-derived lymphocytes (SDL). Two FCXM were not performed with PBL due to lack of enough cell yield while six were not performed with SDL for the same reason.

One case was FCXM positive with both materials. Only one case had negative B-cell FCXM on PBL and was positive on SDL (linear channel shift 21 vs 51, cut off 41), whereas it was CDC and Flow T-cell negative on both. There were no immunologic Pancreas or kidney losses. The median CIT for the kidney for those cases was 13 hours and compared to our unit overall CIT of 18 hours. There were 3 cases of NHB donors where the CIT of the first kidney was less than 6 h.

Conclusion: Pretransplant final crossmatch using donor PBL is as reliable and precise as crossmatch using cells derived form donor spleen or lymph nodes.

This method might significantly reduce CIT in NHB and Pancreas transplantation and may also have wider implications for classic brain stem kidney donors

Parallel Session

B Cell Immunology

Friday 18 April

10:30 – 12.30

O058

Do donor specific anti-HLA antibodies in renal transplant recipients with negative cross matches matter?

Amany Ballow[1], Jack Galliford[2], Kakit Chan[2], Adam McLean[2], David Taube[2], Anthony Warrens[1]

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Introduction: Flow Cytometric Crossmatching [FCXM] using single antigen beads and Luminex xMAP technology has improved the sensitivity of screening for anti-HLA donor specific antibodies [DSAbs]. Whilst there are good data to suggest that the presence of DSAbs with a negative CDC but positive FCXM crossmatch at the time of transplantation are associated with a higher incidence of rejection and graft loss, the significance of DSAbs in the presence of negative CDC and FCXM crossmatches remains uncertain.

In this study we have assessed the effect of DSAbs with a negative CDC and FXCM crossmatch at the time of transplantation on graft outcome [survival, rejection, function].

Methods: We have retrospectively analysed 461 consecutive transplants [283m, 178f, mean age 45.7±12.7 years, mean follow up 34.0±24.2 months]. All were CDC and FCXM at the time of transplantation and received Tacrolimus based immunosuppression. Rejection was diagnosed by allograft biopsy and treated with iv Methyl Prednisolone, oral prednisolone, ivIg and plasma exchange if necessary.

63 [13.7%] patients were found to have anti-HLA antibodies by Luminex screening. 38/63 [60.3%] patients were DSAb+; class I, class II and class I + II DSAbs were detected in 15, 5 and 18 patients respectively. 398 patients at the time of transplant were found to be DSAb– and acted as controls.

Results: Table 1 [below] shows that although graft survival and rejection free survival was poorer in DSAb+ patients this did not achieve statistical significance at any time point. Similarly DSAb IgG subtyping showed that patients with complement fixing antibodies had a higher incidence of rejection but this was not statistically significant.

		DSAb-	DSAb+	P value
Allograft	1 year	96.1%	91.9%	
survival	3 years	94.6%	87.7%	
	5 years	92.5%	87.7%	0.71
Rejection free	1 year	85.4%	78.0%	
survival	3 years	80.9%	69.3%	
	5 years	79.5%	69.3%	0.28
Creatinine	1 year	135.0 ± 39.41	134.7 ± 68.24	0.97
[umol/l]	3 years	150.2 ± 64.76	134.9 ± 34.49	0.46
	5 years	142.7 ± 46.48	126.5 ± 25.41	0.49

Discussion: This study shows in the medium term [5 years] that allograft survival, rejection and allograft function was similar in patients with and without DSAb. Long term data will be important as this group of patients may be at risk of developing transplant glomerulopathy, particularly those with complement fixing antibodies.

O059

Double Filtration Plasmapheresis in Antibody Incompatible Transplantation; Effective but with Shortcomings.

Robert Higgins[1], David Briggs[2], Rizwan Hamer[1], Nithya Krishnan[1], Dave Lowe[2], Mark Hathaway[2], Kath McSorley[1], Andrew Short[1], Simon Fletcher[1], Daniel Zehnder[3]

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Introduction: We used double filtration plasmapheresis (DFPP) in antibody incompatible transplantation (AIT). The use of DFPP has not been reported extensively in HLA antibody incompatibility, and we review its efficacy.

Methods: 54 patients entered the AIT programme. 41 had 267 sessions of DFPP, 1 had 10 sessions of plasma exchange, and 3 had 13 sessions of ABO immunoadsorption. Some patients had more than one modality of treatment, 11 patients had no antibody removal.

Results: In patients treated with DFPP alone, the mean number of sessions pre-transplant was 4.8, mean of 4.6 litres per session, total treatment 325 ml/kg. Post-transplant, 18 received DFPP, for a mean of 3.5 sessions, mean 6.2 litres per session, total treatment 309 ml/kg. Mean reductions in IgA, IgG and IgM per treatment session were consistent with the plasma volume treated, 63.9%, 57.8%, 70.2% pre transplant, and 59.3%, 48.4% and 65.4% post-transplant. 70% of patients were transplanted with a flow cytometric (FC) relative median fluorescence (RMF) >2.5, and 65% were transplanted with microbead DSA mean fluorescence intensity (MFI) of >2000u. Failure to achieve negative crossmatch was strongly related to the starting level of donor specific antibody (DSA).

Patients who had a course of DFPP and who had complete data and no IVIg administered were studied in more detail. Pre-transplant, in 21 patients, the mean fall in IgG over the course of treatment was 70.0%, with a fall in FC RMF 25.7%, and fall in microbead MFI 52.6%. There was variation with levels in some patients responding well, but others resistant to DFPP. Post transplant, in 8 courses of treatment administered during DSA resynthesis, IgG fell by a mean of 45.3%, but the microbead MFI level rose by 41.2%, and no patient achieved a reduction in MFI to <2000 by DFPP alone. Combined DFPP and rejection treatment were associated with more adverse effects, including infections and fluid shifts.

Discussion: Larger volumes of plasma were treated with DFPP than were feasible with plasma exchange. Although graft survival was excellent, most patients were transplanted with potentially damaging DSA levels, and DFPP post transplant was unable to control rising antibody levels; we have phased out the use of DFPP post-transplant. There is a need for improved methods of DSA removal.

Parallel Session

General Transplant Topics

Friday 18 April

13:30 – 15.00

O060

Two Year Results of a BK Viraemia Surveillance Program in all New Kidney Transplants.

Manolis Mazonakis[1], Rory Gunson[2], Lynne Thompson[1], William Carman[2], Colin Geddes[1]

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Introduction. Published literature suggests that BK virus nephropathy (BKVN) is an increasing problem in kidney transplantation and guidelines recommend regular surveillance to detect BK viraemia to prompt transplant biopsy or reduction in immunosuppression. We introduced a surveillance program in our centre in 2006 and report our experience in the first two years.

Methods All new kidney transplants since 01/01/2006 have had blood sent for BK virus surveillance using a sensitive, quantitative, PCR-based assay each time they attend transplant follow up clinic. Clinicians are alerted to positive results and take action in the context of the transplant function and immunosuppression. Our primary interest was the associations between BK viraemia, biopsy proven BKVN and transplant function during this period. In addition baseline characteristics and transplant function in patients who developed BK viraemia were compared with those who did not. Transplant function was assessed by estimated glomerular filtration rate (eGFR). Transplant biopsy with immuno-staining for evidence of BKVN was performed at the discretion of the attending clinicians and histopathologist.

Results. 114 consecutive transplants with > 3 months follow up were analysed (maximum follow up 23 months). 35 (26.3%) patients developed BK viraemia with median time to onset of 81 days (range 19-473 days), and median duration of first viraemia 38 days (range <14 to 493 days). 6 of these patients developed a recurrence of viraemia. During this period no patients had biopsy proven BKVN. Mean eGFR 1 month before, at the time of, and 6 months after onset of BK viraemia was 42.4, 47.8 and 43.8ml/min/1.73m^2 respectively. There was no significant difference in recipient age, sex, proportion of live donor transplants, incidence of acute rejection and immunosuppression at baseline, 1 month or 3 months comparing the patients who experienced BK viraemia and those who did not. However comparing the 30 patients who experienced onset of BK viraemia within the first 6 months with those who did not, mean eGFR at 1 month after transplant was similar (48.2 v 47.5ml/min/1.73m^2), but lower at 3 months (45.4 v 49.4ml/min/1.73m^2) and 6 months (42.5 v 51.7ml/min/1.73m^2; p=0.03).

Discussion. This is the first UK centre that we are aware of reporting the results of BK virus surveillance. In contrast to reports from North America we found no cases of biopsy proven BKVN. However BK viraemia occurred in 26.3% of patients within the first 6 months after transplant with highly variable duration. We found no definite risk factors for BK viraemia, no change in transplant function around the time of onset of viraemia, but BK viraemia in the first 6 months was associated with significantly worse transplant function at 6 months compared to patients who had no BK viraemia. Further study is required to determine if early BK viraemia is an early independent risk factor for poor kidney transplant function.

O061

Effect of 1 year of senior critical care support to an organ retrieval team.

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Methods

For a one year period Sep 04-Aug 05 a consultant intensivist/anaesthetist attended all potential multiorgan donors in Scotland. They travelled to the donor hospital as soon as possible after confirmation of donor status and provided intensive care and anaesthesia support pre retrieval in ICU and during the donation procedure in theatre.

Intensive pre-retrieval critical care aimed to improve donor physiology with institution of triple therapy hormonal resuscitation, goal directed cardiovascular and respiratory therapy and correction of temperature and electrolyte abnormalities.

A retrospective analysis of core donor data forms for the East of Scotland was performed for the periods Sep 03 – Aug 04 and Sep 05 –Aug 06 and was compared to the data collected during the period of support.

Results

	Sep03-Aug04	Sep04-Aug05	Sep05-Aug06
Median Age	42	46	42
Median time BSD to coordinator contact	57.5 mins	28.5 mins	10 mins
No. of Retrievals	14	23	24
Organs Retrieved	44	80	92
Organs/donor	3.14	3.48	3.83
Hormone Resus	nil	13/14 (93%)	23/24 (96%)
Reduction inotropes from ICU to theatre	nil	50%	30%

Discussion

Senior critical care support ensures donor stability both in ICU and during the theatre retrieval process. A self sufficient team causes minimal disruption to the donor hospital and allows independent scheduling of donor and recipient operations. The number of organs retrieved and the number of organs retrieved per donor increased during the study period and this increase has been sustained. Reasons for this may include the introduction of triple hormone resuscitation, and continuation of more active protocol-directed donor management by referring ICUs.

O062

Allograft Quality And Recipient Risk Status In Liver Transplantation

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Background: The relationship between allograft quality and recipient risk status was investigated in relation to patient survival 1 year after liver transplantation.
Data: The classification for deceased donor livers and patients on the liver transplant list was derived from a cohort of 723 first adult elective liver only transplants performed in the UK 2000-2001, using livers from deceased heartbeating donors. A similar cohort of 1,615 liver transplants performed between 2002-2006 was then analysed to determine whether the survival of liver recipients depends on the matching of allograft quality to recipient risk status.
Results: An iterative technique using logistic regression was applied to the 2000-2001 cohort to investigate several donor and recipient factors which allowed us to define poor vs good quality allografts and high vs low risk patients. Kaplan-Meier (K-M) survivor functions obtained from analysing the 2002-2006 cohort revealed differences between the 4 groups (p=0.08). The worst outcome was observed when poor quality allografts were transplanted to high risk recipients (86% survival). Low risk recipients who received poor quality allografts had poorer survival (89%) than either high risk or low risk recipients of good quality allografts (91% survival in both groups). A Cox regression analysis was then performed to assess the combined effect of liver quality and cold ischaemia time (CIT) on patient death within 1 year post-transplant. The analysis showed that although poor quality livers were associated with significantly worse patient survival than good quality livers, the difference could be explained by the inferior survival associated with the poor quality livers with long CITs (>12hrs). Patients who receive poor quality livers with CITs $\leq$12 hours have comparable survival to those who receive good quality livers. A comparison of the K-M survivor functions for the poor quality long CIT group (85% survival) and the group containing all other livers (90% survival) revealed a statistically significant difference (p=0.02).
Conclusion: Allograft quality allied with CIT is of prime importance in determining the outcome of patients following liver transplantation. Poor quality allografts with CIT >12hrs are associated with the lowest patient survival one year after transplantation. Current policy of matching poor quality allografts to low risk patients is justified if the CITs can be kept below 12hrs.

O063

Access to cadaveric renal transplantation – Is it equitable across the UK?

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Introduction: For suitable patients with ESRD, renal transplantation is recognised as the optimum treatment. In the context of cadaveric renal transplantation, patients are able to access this treatment option only if they are included on the national transplant waiting list [WL]. Further, current allocation rules favour patients who have been on the list longer. Therefore, both access to the WL and time taken to activate on the WL are key measures of administered clinical care as recommended in the recently published Renal Association [RA] kidney transplantation practise guidelines. Analyses were undertaken to see if patients across the UK have equitable and timely access to the WL.

Methods: Patients aged <65 years were assigned according to the renal centre responsible for their dialysis treatment. Start date of dialysis was obtained from the UK Renal Registry and date of activation on the WL provided by UK Transplant. The data were analysed according to [1] Point prevalence analysis – proportion of patients on dialysis in a given centre on 31/12/06 [n = 11,554] who were active on the WL on the same day [n = 4333] and [2] Incident patient analysis – proportion of incident patients commencing dialysis in a given centre between 01/01/03 and 31/12/04 [n = 4816] who were activated on the WL within 2 years [n = 2161]. The average time taken to activate a patient on the WL was also calculated for each centre by the Kaplan-Meier method.

Results: In both the prevalent and incident patient analyses, several centres [both transplanting and non-transplanting renal centres] differ significantly from the national average. The analysis of prevalent patients showed nationally 37.6% of patients were active on the WL on 31/12/06, ranging from 16.7% to 51.6% across the UK centres. The incident patient analysis revealed 45% of under 65 year olds were active on the WL within 2 yrs of starting dialysis, but this varied from 23% to 66% across UK renal centres. Analysis with appropriate risk adjustment was also carried out. Variation in the average time taken to activate patients onto the WL was also noted.

Conclusions: The data indicate there are significant differences in the UK, between centres, in both access to the transplant waiting list and the time taken to list such patients. Further work to understand the reasons behind these findings is necessary. Access to renal transplantation needs to be regularly included in the core clinical audit for centre performance in the care of patients with ESRD as recommended in the RA transplantation guideline document.

POSTERS

Basic Science

P01

Differential gene expression and anoxic survival in PHD1,2, and 3 knock-out mouse embryonic fibroblasts.

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Introduction: Hypoxia Inducible Factor (HIF) upregulates genes important in ischaemic protection such as HO-1, erythropoietin, iNOS and VEGF as well as pro-death genes that may be detrimental in context of ischaemia. HIF is primarily controlled by 3 oxygen dependent prolyl hydroxylases (PHD1, PHD2, PHD3), and inhibition of these prolyl hydroxylases leads to HIF activation. We hypothesised that differential inhibition of PHD1,2 or 3 may result in selective gene regulation and may confer protection against ischaemia reperfusion injury. To investigate this hypothesis we isolated mouse embryonic fibroblasts (MEFs) from PHD1,2,&3 KO embryos and compared them to MEFs derived from WT litter mate controls.

Methods: Primary MEFs were incubated in either hypoxia (1% O2) or normoxia (21% O2) for 16 hours and mRNA extracted for analysis with Illumina gene expression arrays. Selected genes were confirmed with real-time rtPCR and by immunoblotting. Further studies measured glucose, lactate and pH in media of cultured MEFs as well as oxygen consumption in PHD2 KO and WT MEFs. Finally MEFs were incubated for 12-48 hours in anoxia followed by 24 hours reoxygenation to simulate ischaemia reperfusion injury. Cell survival was measured by flow cytometry with propidium iodide and annexin V staining.

Results: HIF was constituitively upregulated in normoxia in PHD2 KO MEFs but not in PHD1 and PHD3 KO MEFs. Greater than 60% of genes upregulated in the PHD2 KO MEFs are hypoxia regulated genes. In contrast there was very little concordance between genes upregulated in PHD1 and PHD3 KO MEFs and known HIF regulated genes. PHD2 MEFs showed marked differences in cell metabolism with greater glucose consumption and lactate production as well as lower oxygen consumption compared to WT controls. There were no differences, however, in cell survival between PHD1,2 and 3 MEFs compared to controls following anoxia reoxygenation injury.

Discussion: PHD2 is the dominant prolyl hydroxylase controlling HIF in normoxia. PHD2 KO MEFs have anaerobic metabolism in normoxia and lower oxygen consumption. This did not , however, confer greater protection against anoxia reoxygenation injury in our model. Genes upregulated in PHD1 and PHD3 KO MEFs may represent HIF independent genes and are the focus of further study.

P02

Alternative-Splicing of the 5' Untranslated Region of foxp3 mRNA Differs Between Natural and Induced $CD25^+$ $CD4^+$ Regulatory T cells in Mice

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Introduction: Regulatory T cells (Treg) play a critical role in tolerance induction in several experimental transplant models. *foxp3* is an essential transcription factor for Treg differentiation and function, and $CD25^+CD4^+$ Treg express high levels of FOXP3 protein. However, in our mouse models of tolerance induction we have not observed changes in total foxp3 mRNA or protein levels which correlate with graft outcome. Patterns of alternative splicing in the 5' UTR of mRNAs determine mRNA stability and protein translation, and commonly depend on the differentiation and activation status of cells. The aim of our study was to compare distinct populations of Treg and correlate their function with their foxp3 mRNA 5' untranslated region (UTR) expression profile.

Methods: Initially, we determined expression of alternative-splice variants within the 5' UTR of foxp3 mRNA using rapid amplification of cDNA ends (RACE) PCR. Using real-time PCR we analysed expression of these splice-variants and assessed their stability following treatment of cell groups with Actinomycin D.

Results: RACE analysis of $CD25^+CD4^+$ T cells confirmed the expression of multiple foxp3 mRNA transcripts encoding alternative-spliced 5' UTRs. Real-time PCR analysis of *foxp3* splicing patterns showed that whilst there is a similar level of total mRNA, significant variation exists in the pattern of 5' UTR expression in distinct Treg subsets.

Discussion: Our findings suggest that there is a significant level of regulation through alternative splicing of *foxp3* at the 5' UTR in different groups of Treg. This may provide us with a "molecular footprint" in Treg which is not offered by analysis of total foxp3 mRNA or protein. Understanding this regulation may allow us to optimise techniques to generate and harness the populations of Treg with greatest therapeutic value.

P03

A Novel Approach for Predicting Immunogenic B Cell Epitopes Based on the Degree of HLA Alloantigen Disparity Using Structural Modelling and Digital Image Analysis

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Introduction: The ability of an HLA alloantigen to induce alloimmunity is dependent on the number, position and physiochemical properties of the amino acid polymorphisms. We report a novel approach aimed at predicting the relative immunogenicity of individual HLA alleles using three-dimensional structure data to generate models of the tertiary conformation of common HLA-A, B and -C alleles. Computer images of the HLA molecule surface were then used to compare the stereochemical structure and electrostatic properties and calculate potentially immunogenic differences between individual HLA alloantigens.

Methods: Homology modelling and structure prediction was used to generate tertiary models of common HLA class I alleles using the Modeller program. The structural quality of the models was validated using several bioinformatics methods. Parameters including the position of atoms in space, inter-atomic interactions, folding interactions, surface accessibility of amino acid side chains and amino acid protonation states were used to calculate the electrostatic potential on the surface of each HLA molecule using the Deep View program and the results were represented as digital images of the alpha helices and peptide binding groove. Pixel values in the digital images reflected the stereochemical and electrostatic properties of individual HLA molecules; a computer program was written to calculate differences between HLA molecules by pixel subtraction.

Results: Structural models of 95 HLA alleles were generated and verified by bioinformatics to be >95% accurate. Comparison of common HLA class I alleles using the digital image subtraction method gave a numerical range of 8.786 to 31.041. In general, the pixel subtraction values correlated with known serological HLA cross-reactivity. For example the serologically cross-reactive specificities HLA-A2 and A68 and A2 and A69 had low pixel subtraction values (12.080 and 11.122 respectively) whereas the serologically disparate HLA alleles, HLA-A2 and A30, had a high pixel subtraction value (22.599). Interestingly, this approach also highlighted unexpected variability among serologically similar HLA specificities.

Conclusion: Modelling the structure of HLA molecules and calculation of their surface electrostatic charge enables comparison of individual alleles, providing a novel index for predicting the relative immunogenicity of HLA alloantigens.

P04

Therapeutic Strategy For Counteracting Complement In T Cell Activation

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Introduction: Our previous studies in murine acute rejection and in the analysis of human long term graft outcome have shown that local production of C3 in donor organs appears to be a major determinant of graft survival. In addition, we have shown that APCs prepared from $C3^{-/-}$ mice have reduced ability to stimulate alloreactive T cells *in vitro* and *in vivo*. We envisage that complement activation at the surface of APCs will lead to enhanced allo-stimulatory capacity of those cells and/or provide essential help for T cell stimulation at the immunological synapse. Conversely, we propose that therapeutic overexpression of complement regulator will lower the alloreactive T cell stimulatory capacity of APCs in favour of immune suppression/tolerance.

Methods and Results: We first assessed if APT070, a membrane–localising complement regulator can inhibit murine complement activation and bind to murine bone marrow dendritic cells (DCs). Using a haemolytic assay, we show that APT070 (20□g/mL) inhibits >75% of complement mediated cell lysis. Using immunochemical staining and flow cytometry with anti-human CD35 mAb, we show that APT070 efficiently binds to DCs.

We next determined if APT070 treatment modulates DC activation and their function in alloreactive T cell stimulation. APT070 (20□g/mL) or control molecule (APT544) was added to the DC culture medium, from the beginning of BM cell culture, with repeated addition every two days. We show that compared to control molecule treated DCs, APT070 treated DCs exhibit a tolerogenic cytokine profile with a lower IL-12 and higher IL-10 production; when co-cultured with naïve allogeneic CD4 T cells, they elicit significantly lower T cell responses measured by IFN-γ production and thymidine uptake.

Discussion: Our findings showing that APT070 treatment reduces the alloreactive T cell stimulatory capacity of donor DCs may have therapeutic potential for targeting the alloreactive T cell response in allograft rejection.

P05

Complete reversal of STZ induced diabetes using adult PDPC cells

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Introduction:

Cellular therapies hold potential for the treatment of diabetes. Evidence that candidate cells which might fulfill this role, reside in the pancreatic ducts, has been provided by rodent models of pancreatic regeneration. We have sought to isolate such cells and demonstrate that they can mitigate the effects of diabetes in vivo.

Methods:

Novel candidate cells were isolated from adult rat and human pancreatic ducts and characterized by ICC, FACs and RT-PCR. The ability of these cells to mitigate the effects of diabetes was assessed in a double-blinded trials in a xenogeneic mouse model of streptozotocin-induced diabetes mellitus. Female C57Bl/6 mice (n=4 per treatment group) were made diabetic by injection of streptotozocin (STZ) to give a dose of 250 mg/kg on day 0 of the experiment. PDPCs were injected into the tail vein on day 3 after STZ injection. Control animals were given no cells. Blood glucose was monitored every 3 days. All procedures were conducted under authority of the UK Home Office.

Results:

We have isolated a novel cell type from adult pancreatic ducts, in both rat and man, which we have termed Pancreatic Derived Pathfinder Cells (PDPCs), on the basis that they appear to navigate a path towards sites of damage in vivo. Direct intravenous injection of either rat, or human, PDPCs into STZ diabetic mice, completely normalizes blood glucose levels for over 100 days. Body weight and pancreatic histology in treated animals also appears normal. Crucially, the insulin produced in these treated animals is principally mouse in origin and is of both type I (embryonic) and II (adult). These data indicate that the primary mechanism of PDPC action is by stimulation of host tissue to regenerate.

Discussion:

These results described for the first time the use of an adult human cell type to achieve complete correction of diabetes in a rodent model for an extended period of time. The results reproducibly demonstrate the feasibility of using adult cells to regenerate damaged tissue and enhance our understanding of the mechanisms relating to such repair. Furthermore, they suggest a means for novel therapeutic intervention in diabetes.

P06

Regulation of allospecific T cell responses by donor antigen presenting cells depends on local generation of complement C5a

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The innate system of immunity plays an important role in ischemia-reperfusion injury and allograft rejection. The early stages of inflammatory processes are accompanied by complement activation. One biological consequence of this activation is the release of potent inflammatory anaphylatoxins, C3a and C5a, which have been reported to regulate a range of inflammatory responses. We previously reported that DCs express C3aR and C5aR, and C3a-C3aR interaction has a positive impact on murine BM DCs, in terms of activation phenotype and capacity for Ag uptake and allostimulation. However, the role of C5a in modulating DC function remains unclear. The aim of this study is to investigate the role of local C5aR signalling in modulating murine BM DC function and subsequent regulation of the allospecific T cell response. We first evaluated if C5a-C5aR interaction could result from local expression of factors. Our results showed that C5aR mRNA was detected in WT DCs at different stage of DC culture by RT-PCR, and C5a was detected by ELISA in the culture supernatants from different stages of DC culture. We next determined if C5a-C5aR interaction modulates DC function in allospecific T cell stimulation *in vitro* and *in vivo*. We found that BM DCs cultured from C5aR-/- mice or treated with C5aR antagonist (C5aRa, W54011) exhibited a less activated phenotype (producing significantly less IL-12 and more IL-10, in response to LPS stimulation); both C5aR-/- and antagonist-treated DCs (LPS stimulated) showed reduced capacity to stimulate naïve alloreactive T cells, as measured by IFN-γ production and thymidine uptake. As regards interaction *in vivo*, following i.p. administration of the C5aRa-treated DCs into allogeneic mice for 10 days, *ex vivo* mixed lymphocyte reaction showed that CD4+ T cells from those recipients have reduced thymidine uptake, but increased IL-4 production compared to that with untreated DCs. Conversely, DCs treated with C5aR agonist (C5a) exhibited a more activated phenotype (producing more IL-12 and less IL-10) and were more potent in allospecific T cell stimulation.

Our findings demonstrate that murine BM DCs can express C5aR and C5a can be generated locally; C5a-C5aR interaction up-regulates murine BMDC activation and their allostimulatory capacity. Thus, targeting C5a-mediated signal may be able to prevent allograft injury.

P07

HY-specific T cells constitutively producing IL-10 are unable to mediate tolerance to murine minor antigen mismatched skin graft transplants

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Interleukin 10 is a potent anti-inflammatory cytokine. Evidence suggests that IL-10 can have an inhibitory effect in the adaptive immune response. Previous reports have shown that IL-10 producing, antigen specific T cells are able to dampen the inflammatory response induced by pathogenic self-antigens causing rheumatoid arthritis. Our aim here was to determine whether constitutive production of IL-10 by HY specific T cells would confer a regulatory function in the context of a syngeneic male to female skin transplant.

TCR transgenic CD4+ T cells specific for the HY-A^b *Dby* minor H antigen were transduced with an active IL-10 gene and EGFP. A MIGR-1 empty vector carrying only the EGFP protein was used as a control. Upon transduction, EGFP positive cells were sorted and adoptively transferred into C57BL/6 females which were then grafted with male syngeneic skin. The effect of the IL-10 producing CD4+ T cells was assessed by comparing graft survival between the two groups.

The results of the experiment suggest that IL-10 transduced T cells did not have significant regulatory activity and did not prevent graft rejection. By day 22, all skin grafts from the IL-10 and control group had been rejected. Expansion of CD8 T cells specific for the class I restricted peptide HY-D^b*Uty* did not differ significantly between the two groups.

These data show that HY-specific CD4+ T cells constitutively producing IL-10 are unable to control the immune response of female mice to transplanted syngeneic male skin. The overproduction of IL-10 is not sufficient by itself to confer a regulatory function to these cells. Unlike other models where the inflammatory response towards certain antigens could be modulated by the presence of IL-10, in the minor mismatch transplantation setting, this cannot be achieved. Further experiments will be done transducing antigen specific CD4+ T cells with other immunosuppressive cytokines such as TGF-β, or by transducing naturally occurring 'professional' $CD4^+CD25^+$ T cells with such cytokines to enhance their regulatory function.

P08

Erythropoeitin Modulates Apoptosis Related Gene Expression in Human Microvascular Endothelial and Renal Tubular Epithelial Cells

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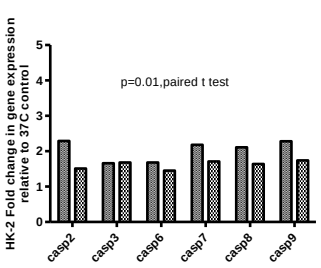
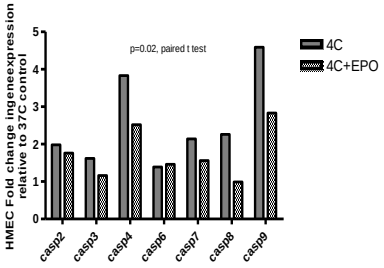
Ischaemia-reperfusion injury (IRI) is a major contributor to both short and long term renal allograft failure. Evidence from the literature supports the hypothesis that erythropoietin (EPO) is tissue protective, reducing both inflammation and apoptosis related to IRI. Key cell types targeted by IRI within the kidney include endothelial and tubular epithelial cells.The aim of the study was to examine an in vitro model of the conditions experienced by a deceased donor kidney following retrieval, and to define the effects of EPO on the expression of apoptosis related genes. Human microvascular endothelial cells (HMEC-1) and human proximal renal tubular epithelial cells (HK-2) were incubated at 4ºC for 24 hrs. with and without the addition of 10iu/ml of rHuEPO. Control cultures were incubated at 37ºC. Gene expression was quantified using low density arrays. Statistical analysis used paired students t test.

There was significant upregulation of the caspase pathway in both cell types at 4ºC compared to 37ºC (figures 1 and 2), (p=0.02, p=0.01 respec). The TNF and Fas pathways were up-regulated at 4ºC in both cell lines. The relative ratio of pro- to anti-apoptotic Bcl2 family members, including BAD, BAK1 and BID, favoured apoptosis. EPO significantly down regulated the caspase pathway in both cell lines, with a resulting downregulation of the Fas and TNF pathways in HK-2 cells, and the Fas pathway in HMEC's. In addition the expression of several pivotal genes was modulated by EPO, including RELA and RELB, data not shown. In conclusion, downregulation of pro-apoptotic gene expression is one of the mechanisms through which EPO exerts its tissue protective effects. This model promises to be a useful tool with which to identify candidate genes and their potential modulation by EPO in clinical trials.





P09

A Novel Model To Study Remote Ischaemic Preconditioning In The Mouse Liver

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Introduction: Remote ischaemic preconditioning (RPC) of the liver protects against liver ischaemia-reperfusion (IR) injury and may be beneficial in liver transplantation. We have developed a novel remote preconditioning of the liver model in transgenic and wild type mice.

Methods: In these preliminary experiments, we developed 2 models : (1) an external tourniquet on the mouse hindlimb (n=2) to produce the remote ischaemic preconditioning effect prior to the liver ischaemic insult; (2) microscopic clamps on the femoral artery and vein to induce the ischaemic stimulus of the hind limb (n=4) following microsurgical isolation of the femoral triangle contents. RPC was induced by 6 cycles of 4 minutes (min) of ischaemia to the hind limb followed by 4 min of reperfusion in both groups. Interruption of the limb blood supply was confirmed with Laser Doppler Flowmetry. The liver IR consisted of standard laparotomy with left and middle lobe isolation (70% of liver mass) and micro-clamping. This was followed by 40 min of liver ischaemia and 2 hours reperfusion. Plasma ALT and AST levels of preconditioned (Group (1)n=2; Group (2) n=4) and non-preconditioned (liver IR alone n=3) mice were measured at 2 hr of reperfusion.

Results: RPC in group (1) - tourniquet technique - resulted in paralysis of the hind limb on recovery from anaesthesia that persisted for the 2 hour recovery period and was discontinued. However, group (2) - the open clamping - resulted in no neuro-muscular deficit and good postoperative recovery. In RPC animals following liver IR, mean plasma ALT was 3434 +/- 929 and mean plasma AST was 2144 +/- 356 as compared to the non-preconditioned animals which had undergone liver IR alone, ALT was 6180 +/- 1161 and AST was 7467 +/- 200

Discussion: We have developed a new standardised model of hindlimb RPC of the liver in wild type and transgenic mice. The use of an external tourniquet resulted in crush injury to the neuro-muscular structures in the thigh which was not detected in our previous studies in the rat or rabbit as these were non-recovery models. However, the microscopic clamping of the femoral vascular bundle resulted in no such injury. This model could prove useful for future research on the role of remote ischaemic preconditioning in liver transplants using the wide diversity of transgenic mice strains to study molecular pathways involved in RPC.

P10

The Effect of HTK and Marshall's Solutions on Renal Tubular Preservation in a Rat Model for Non Heart Beating Donor Allografts

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Introduction

Marshall's hypertonic citrate and Histidine Tryptophan Ketoglutatrate (HTK) are commonly used for in situ cooling in NHBD kidney procurement. We aimed to assess the efficacy of the two solutions for renal tubular preservation using a simple animal model for NHBD allografts.

Methods

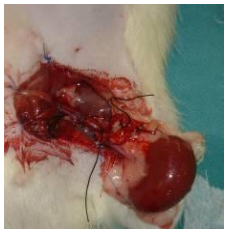
Donor male, Wistar rats were killed and after 30 minutes warm ischaemia the left kidney was cooled in situ using a streptokinase flush and either solution. The donor kidney was then static cold stored in the same solution for 20 hours. For each kidney, a recipient, male, Wistar rat was anaesthetised and heparinised (100 IU). The retrieved kidney was reperfused via cannulae in the recipient's femoral vessels. After 3 hours the donor kidney and the recipient's native left kidney (negative control) were immediately fixed and prepared for electron microscopy. Tubular epithelial cells were viewed under 40, high-powered fields selected at random by a single, blinded observer. Tubular viability was assessed using the semi-quantitative, Trump scale for mitochondrial injury (stages 1-4, viable, stages 5-7 non viable).

Results

Kidneys preserved in HTK had a significantly lower ($p<0.0001$, MWU) median injury stage (n=6, stage3) than those preserved in Marshall's (n=6, stage4).

Discussion

HTK appears to preserve renal tubules better than Marshall's solution and this in turn may lead to a reduced incidence or length of delayed graft function. HTK may be the optimal solution for in situ cooling of NHBD as well as marginal HBD kidneys.



P11

Reperfusion Tolerance Changed by Different Preservations with or without Erythropoietin in Isolated Ischemic Porcine Kidneys

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Background: Ischemia/reperfusion tolerance is important in kidney transplantation. EPO inhibits apoptosis and inflammation, and provides renoprotection. This study investigated effects of different preservations with or without EPO on reperfusion tolerance of kidneys.

Methods: Porcine kidneys subjected to 10-minute ischemia were preserved by four methods: 16-hour cold storage (16hCS) followed by 2-hour warm preservation (2hWP)±5000 units/L EPO; or 18hCS±EPO; then assessed by 3-hour haemoreperfusion (3hR) using an isolated organ perfusion system.

Results: Caspase-3 activity was increased by 2hWP±EPO and 3hR compared with post-CS kidneys; elevated further by EPO in 2hWP that was fallen post-3hR; but no significant difference between 16hCS+(2hWP±EPO) or 18hCS±EPO in post-3hR kidneys. Tubular apoptosis was greatly augmented by 3hR in kidneys preserved by 16hCS+(2hWP±EPO) and 18hCS+EPO compared with pre-3hR or 18hCS kidneys, but reduced by EPO in 2hWP. In tubular lumens, apoptosis was increased by 2hWP±EPO and 3hR compared with post-CS kidneys, decreased or maintained by 3hR in the kidneys preserved by 16hCS+(2hWP±EPO). Interstitial apoptosis was raised by 2hWP±EPO and 3hR in all groups. HSP70 was enhanced by 2hWP±EPO and 3hR compared to post-CS kidneys, maintained or decreased by 3hR in 16hCS+(2hWP±EPO) kidneys. 16hCS+(2hWP±EPO) appeared to be better preservations indicated by oxygen consumption and renal blood flow (RBF). EPO in 2hWP and 18hCS increased urine output; the later also improved RBF. The change of caspase-3 matched apoptosis in tubular lumens and urine output, while HSP70 is consistent with oxygen consumption and RBF.

Conclusion: WP and EPO, in contrast to CS, improved reperfusion tolerance and early renal function, which might be partially through caspase-3 activation, HSP70 induction and inflammation clearance.

POSTERS

Clinical Science

P12

Activation of hypoxia inducible factor (HIF) for protection of the liver from ischaemia reperfusion injury

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Introduction: Hypoxia inducible factor (HIF) upregulates a number of genes that have been shown to protect organs from ischaemia reperfusion injury including HO-1, iNOS, VEGF, and erythropoietin. HIF is activated by prolyl hydroxlase inhibition and we hypothesised that dimethyloxalyglycerine (DMOG), a 2-oxoglutatarate analogue that inhibits prolyl hydroylases, would activate HIF in the rat liver and protect it from subsequent ischaemia reperfusion injury.

Methods: Male Wistar rats were used for all experiments. HIF target gene induction was measured by real time rtPCR using RNA extracts from DMOG treated and control livers. Immunohistochemistry was performed on formalin fixed paraffin-embedded sections with antibpdies against HIF1alpha and HIF2alpha. Rats were subjected to 45 minutes partial liver ischaemia (left and middle lobes) and liver transaminases were measured 24 hours following reperfusion. Experimental groups were DMOG treated rats (3 intraperitoneal injections of 40mg/kg DMOG over 24 hours pre-op, n=6), normal saline treated CONTROL group (3 intraperitoneal injections of normal saline), and a positive control group of ischaemic preconditioned (IP) rats (3 intraperitoneal injections of normal saline, 10 minutes ischaemia and 10 minutes reperfusion prior to 45 minutes liver ischaemia, n=7).

Results: Maximal upregulation of the HIF target genes Glut-1, VEGF and HO-1 was achieved using 3 doses of 40mg/kg over 24 hours. Immunohistochemistry demonstrated strong HIF1alpha and HIF2alpha nuclear staining in DMOG treated livers and no staining in control livers. Following 45 minutes partial liver ischaemia, liver transaminases were markedly reduced in the DMOG and IP treated rats compared to CONTROLS both at 24 hrs post ischaemic injury (AST: CONTROL 989+/- 400, DMOG 460+/-127, IP 438+/- 182; ALT: CONTROL 1094+/-467, DMOG 445+/-172, IP 365+/-161; all values U/L +/- SEM).

Discussion DMOG effectively upregulates HIF and HIF target genes in the rat liver. Preliminary studies suggest that DMOG may protect the liver from ischaemia reperfusion injury and that this protection parallels that conferred by surgically induced ischaemic preconditioning.

P13

The Rise and Fall of Donor Specific HLA Antibody Levels in the first 30 Days after Antibody Incompatible Transplantation.

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Introduction: Successful antibody incompatible transplantation and microbead methods to measure donor specific HLA antibody (DSA) levels mean that it is now possible to study DSA levels post-transplant in some detail.

Methods: 42 patients who had HLA antibody incompatible transplantation were studied, another 6 who also had ABO incompatibility, or died post transplant were excluded. DSA were measured by microbead method daily for the first 2 weeks, then at least twice a week for the first month. No grafts failed in the first 30 days. 23 patients had rejection or delayed graft function (in the presence of DSA).

Results: Each patient's peak DSA level in the first 30 days was identified, and compared with the levels pre-treatment (PRE) and at day 30 (D30). PRE, peak and D30 median fluorescence intensity (MFI) levels were 4833 (range 113 - 14846)u, 6602 (range 171-15914)u and 3137 (range 105-10164)u respectively. Peak was higher than PRE in 25/42 (60%) cases, and higher than D30 in 42/42 (100%). Mean time to peak was 12.5 (range 1-25) days.

We looked at patients with low PRE levels; 14 had PRE MFI <2000u, of these 9/14 (64%) had peak MFI <2000u, and none had rejection. However, the other 5/14 (36%) had peak MFI >2000u, 3/5 of whom had rejection.

We also looked at patients with high peak levels; 19 had peak MFI >7000u, 14/19 (74%) of whom had a D30 MFI >2000u, and 7/14 (50%) had rejection. However, 5/19 (26%) of the patients with peak MFI >7000 then had a low D30 MFI<2000u, 5/5 (100%) of whom had rejection.

25 patients had a total of 50 additional DSA identified. In 18/25 (72%) patients the relative levels of DSA paralleled both each other and third party HLA antibodies. In 7/25 (28%) patients antibody patterns diverged. In 2 cases the levels were low initially and stayed low. The other 5 cases all experienced rejection.

Discussion: Mean DSA levels post transplant rose to a peak at a mean of 12.5 days, and then fell in all cases. Patterns of HLA antibody levels were heterogeneous, but there were some consistent observations. No patient who had DSA with MFI <2000 throughout had rejection. Rejection was associated with divergent DSA patterns in patients with multiple DSA. There were extremely rapid falls in DSA levels after resolution of rejection in some patients.

P14

Mitochondrial uncoupling as a protective strategy during hypothermic liver preservation

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Introduction. Mitochondrial damage during hypothermic organ preservation is well-recognised, and has been linked to oxidative stress and apoptosis in early reperfusion. We have investigated mild reversible uncoupling of hepatic mitochondria during cold preservation, followed by measurement of organ recovery and oxidative stress during reperfusion using a rat liver model.

Methods. A standard model of rat liver cold flush and isolated reperfusion were used, with / without addition of low dose dinitrophenol (DNP) in the flush solution, and cold storage for 18h in 3 study groups (n=7 in each) : control fresh livers (CON); livers stored for 18h (STOR); livers stored for 18h + 100 uM DNP (STOR+DNP) in the flush. Reperfusion was carried out with Krebs Ringer Solution at 37^{o}C for 60 min. Liver tissues were used to measure mitochondrial substrate-linked respiration, ATP content, lipid peroxides, and antioxidant enzymes (catalase, gluthathione peroxidase, glutathione reductase, glucose 6 phosphate dehydrogenase) by standard biochemical assays. Liver enzymes AST and ALT were measured in the perfusate. Comparisons were made by ANOVA and Fisher test.

Results. Mitochondrial injury as shown by respiratory control index was reduced by >70% in STOR compared to CON, but there was a significant protection noted in STOR+DNP (RCI = 1.62± 0.07) versus STOR (1.00±0.06); $P<0.05$. Lipid peroxideswere higher in STOR (520±46 pmol/g) than in STOR+DNP (298±61); $P<0.05$. Activities of antioxidant enzymes in STOR were lower than in STOR+DNP for catalase - 78±8 vs. 108±12 umol H_2O_2 ; $P<0.05$; glutathione peroxidase – 0.18±0.03 vs. 0.26±0.08 umol GSSG; $P<0.05$; glucose 6PDH – 12.3±1.9 vs.20.3±2.3 nmol NADH; $P<0.05$, whilst there were no statistical differences in glutathione reductase. ATP was lower in STOR than in STOR+DNP (1.24±0.24 vs. 1.77±0.5 uMol/g); $P<0.05$. Enzyme release did not differ between STOR and STOR+DNP.

Discussion. Mild uncoupling protected liver mitochondrial function after cold preservation / reperfusion, and reduced oxidative stress. Uncoupling agents other than DNP should be investigated for organ protection during storage, since DNP has known pharmaco-toxicity, but its' use has allowed an initial proof of concept.

P15

The induction of pan-familial tolerance by bi-parental stem cell transplantation and mixed tri-chimerism in a murine model.

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Introduction: The paucity of organs for solid organ transplantation (SOT) and the delay in their achievement, increased the use of a living family member as donor. Still there is a constant need for post-transplant immune suppression.

We have established a murine model of combined bi-parental bone marrow transplantation without T cell depletion for the induction of pan-familial tolerance.

Methods: (BALB/c x C57BL/6)F_1 (F_1) served as recipients while C57BL/6 and BALB/c were the donors. Animals were conditioned using total body irradiation in a myeloablative dose (9Gy) and non myeloablative (7 or 5 Gy) in a single fraction on day -1 and then received T cell repleted bone marrow from either F_1, C57BL/6, BALB/c or both C57BL/6 and BALB/c simultaneously (MDT – multi donor transplantation). Animals were followed for the hematological reconstitution, development of clinical signs of GVHD including hunched back, diarrhea and weight loss, GVHD related mortality and donor(s)-recipient chimerism.

Results: results were similar regardless of conditioning used. For example: despite bi-parental chimerism, in F1 animals conditioned with 9 Gy, the time to the clinical appearance of GVHD was not different in MDT as compared to the control groups (C57BL/6 or BALB/c; p= 0.29, figure 1A). GVHD associated weight loss was again indifferent in the MDT animals (figure 1B). GVHD related mortality in the MDT group was similar to the allogeneic single donor control groups and was also similar to the syngeneic group mortality (2/10, 1/12, 1/9 and 1/12 respectively in 2 separate experiments, figure 2, p=0.68).

Conclusion: we conclude that combined simultaneous bi-parental bone marrow transplantation without T cell depletion induces bi-parental tolerance (and therefore pan-familial) and does not increase GVHD. This may serve as an immunological platform for SOT from father, mother or other siblings.

P16

Haemodynamic resuscitation of brain dead organ donors: comparison of the effect of vasopressin and noradrenaline on potential viability of liver and kidney grafts.

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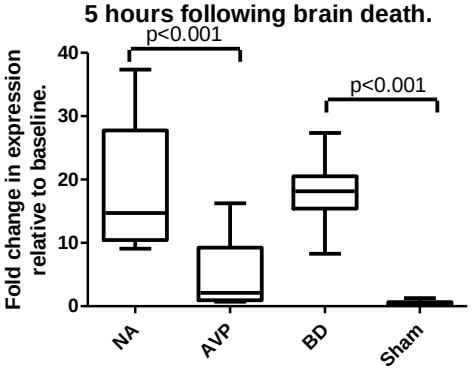
Background Hypotension is the most common clinical problem following brain death and causes a deterioration in organ function. Resuscitation with noradrenaline can increase the number of organs suitable for retrieval but also causes cardiac dysfunction in the donor and predisposes to failure of cardiac allografts. Vasopressin is a less cardiotoxic alternative but the effect on intra-abdominal organs is unknown. We sought to identify the optimal drug by comparing the relative effects of resuscitation with noradrenaline and vasopressin upon regulation of renal and hepatic inflammatory markers.

Methods Brain death was induced by intracranial balloon inflation in 30 rats: in 10 (BD) a hypertensive crisis and subsequent hypotension were observed. 20 received haemodynamic resuscitation to restore perfusion pressure: In 10 (NA) the hypotensive phase was corrected with noradrenaline; in 10 (AVP) hypotension was corrected with arginine vasopressin. In 10 rats (Sham), a balloon was inserted but not inflated. Organs were recovered at 2 or 5 hours following balloon insertion. Real time reverse transcription polymerase chain reaction was used to determine the changes that occurred in these organs in response to BD and resuscitation.

Results Significant upregulation of CXCL1, IL-1β and haem oxygenase-1 (HO-1)($p \leq 0.01$) occurred in both organs following BD. Neither NA nor AVP increased renal inflammatory activation, but both caused significant upregulation of hepatic CXCL1 and IL-1β ($p<0.05$) compared to Sham. This effect was significantly greater in AVP compared to NA ($p<0.05$). Hepatic HO-1 expression was significantly reduced in vasopressin treated groups (see graph).

Conclusions The liver, but not the kidney, is particularly susceptible to vasoconstriction by exogenous vasopressors. The decrease in HO-1 expression, and increase in hepatic inflammatory activation that occurred in response to vasopressin should be balanced against the potential cardiac benefits of haemodynamic resuscitation with this drug.

Hepatic haem oxygenase-1 expression 5 hours following brain death.



P17

Pre-Transplant IgM non-HLA Antibodies Are An Independent Risk Factor For Graft Survival.

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Introduction: It is recognised that pre-existing IgG antibodies to donor HLA antigens may result in rapid rejection of a transplanted organ, and this situation is avoided whenever possible. However, non-HLA antibodies, especially of the IgM class, also called autoreactive antibodies, are not thought to affect graft survival. The purpose of this study was to perform a large retrospective single centre investigation of the effects of pre-formed IgM non-HLA antibodies on cardiac allograft survival. **Methods:** The survival of cardiac allografts in 616 adult transplant recipients have been investigated. Antibodies were initially defined using complement-dependent cytotoxicity (CDC) assays, and subsequently re-analyzed for HLA specificities using Luminex technology. **Results:** HLA-specific antibodies were present in 69/616 heart recipients (58 IgG, 11 IgM); in 22 of these patients, the antibodies were donor-specific. Non-HLA IgM antibodies were detected in 59/616 recipients who did not have HLA-specific antibodies; these patients had a 1yr, 2, 5 and 10 year survival of 55.9, 54.2, 49.9, and 43.3% compared to 75.8, 73.7, 66.6 and 52.8% for those without antibodies (p=0.006 logrank). Multivariate analysis using Cox proportional hazards model was carried out on a subset of recipients (n=483), the presence of pre-transplant non-HLA IgM antibodies remained a risk factor for mortality independent of the other variables (p=0.0001). 12/16 post-mortem grafts from non-HLA IgM positive patients showed capillary deposition of C4d. **Conclusions**: The presence of cytotoxic IgM antibodies to non-HLA antigens prior to heart transplantation is a risk factor for graft failure.

P18

Surprises in Pediatric Sirolimus Metabolism

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Introduction: In a clinical study of pediatric solid organ transplant recipients converted to sirolimus immunosuppression, the metabolite profile in children was markedly different than the typical adult profile. In order to better understand these observations, we utilized human liver microsomes (HLM) to investigate the differential metabolism of sirolimus in children and adults.

Methods: At steady-state, 13 pediatric solid organ recipients (age 9.4 ± 4.3 years) underwent a sirolimus PK profile, with samples drawn 0-12 hours. AUC and metabolite patterns were determined after analysis and quantification with a fully validated LC-MS assay. HLM from cadaveric adult and pediatric donors were incubated with sirolimus at 37^{o}C for 20 minutes, and the resultant metabolites were analyzed and quantified with the same LC-MS assay.

Results: In the clinical samples and the HLM incubations, pediatric metabolism of sirolimus was primarily accomplished via hydroxylation (86%), unlike in adults where demethylation is preferred. CYP2C8 is responsible for the formation of a piperidine-hydroxy metabolite rarely seen in adults but commonly seen in children (~30% of metabolites). Its formation was correlated to age in the pediatric patient samples ($p=0.0097$) and in pediatric HLM incubations, decreasing with age as does the activity of CYP2C8 (411 pmol/mg/min in children to 200 pmol/mg/min in adults). Chemical and immunological inhibition of HLM metabolism demonstrate that CYP3A4 and CYP2C8 are equally responsible for the metabolism of sirolimus in children, and that from 30 days to 1 year of age a third enzyme may contribute. Contrary to our expectations based on this evidence and that sirolimus is a substrate of CYP3A4/5, sirolimus is not metabolized by CYP3A7 at all. Drug interactions with sirolimus seem to be age-dependent. Furthermore, the primary metabolite in adults, 39-O-desmethyl-sirolimus, has only 10% biological activity and cross-reactivity >100% with the immune assay used for therapeutic drug monitoring of sirolimus. The biological activity, potential toxicity, and cross-reactivity of the hydroxylated metabolites seen in children have not previously been investigated.

Conclusions: Our results have clinical significance. Since metabolism of sirolimus in children is different than that of adults, caution should be used when translating sirolimus PK and therapeutic drug monitoring results from adults into pediatrics.

P19

Metabolic And Biochemical Analysis Of Hearts Preserved In Different Preservation Solutions

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Introduction: A solution has been under development at our institute, based on Phosphate-Buffered Sucrose, which has shown good preservation for kidneys and livers. This work used a refined version of this solution to be used in heart preservation and compared it to solutions, which are already widely used.

Method: Hearts were mounted initially mounted in a Langendorff configuration for a washout and equilibrium period, during which cannulation of the left atrium took place. Hearts were then flushed with 15ml of the preservation solution and left at 4˚C to be reperfused 6 hours later in a Langendorff model for 15 minutes followed by working perfusion for 30 minutes.

For the measurement of the enzymes the initial 1.5ml of the coronary effluent was collected immediately after the storage period and tested for Creatine Phosphokinase (CPK) and Lactate Dehydrogenase (LDH). The hearts were then dropped immediately into liquid nitrogen in order to measure the Adenosine Triphosphate (ATP), Lactate and Creatine Phosphate (CP). Spectrophotometric analysis was used to assess both the releases of CPK and LDH into the coronary effluent, and the level of ATP, Lactate and CP in the frozen heart tissue.

Results: Table 1:

Solutions	CPK(IU/L)	LDH(IU/L)	Lactate(µM)	ATP(µM)	CP(µM)
PBSH (n=6)	18.9 ± 2.8	26.4 ± 3.6	5.7 ± 0.4	1.7 ± 0.4	2.2 ± 0.4
STH (n=8)	19.9 ± 4.0	31.0 ± 3.3	7.5 ± 0.5	0.8 ± 0.2	0.7 ± 0.2
UW (n=7)	41.0 ± 4.9	42.1 ± 3.2	7.8 ± 0.9	0.3 ± 0.1	1.3 ± 0.5
CS (n=8)	22.9 ± 2.4	20.4 ± 7.8	6.5 ± 0.5	0.9 ±0.2	2.2 ± 0.7

Discussion: High-energy phosphate compounds, including ATP and CP, are markers of energy preservation within the cardiac muscle after preservation. The higher the level of these compounds the better the quality of the organ preservation, as the more energy remaining in the heart the higher the possibility that it will start working again. ATP was found to be higher in heart preserved in PBSH than in any other solution, and this reached significance over the UW preserved hearts. Overall these results, coupled with better haemodynamic recovery (not shown), show that PBSH is at least as effective in cardiac preservation in the rat model of 6 hours cold ischaemia, as the other widely used solutions tested.

P20

Receptor For Complement C5a Mediates Renal Allograft Rejection

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Recent studies have suggested that local production and activation of complement participates in modulating antigen presenting cell (APC) function in allostimulation and exogenous Ag presentation. One of the proposed mechanisms is up-regulating APC activation and Ag uptake through engagement of C3aR and C5aR. In addition, C3aR expression on donor tissues accelerates skin allograft rejection (refs). However, the role of C5aR in organ transplantation is unknown. To explore this role, we employed a kidney allograft model (C57BL6 to BALB/c) and (C5aR-/-) mice. We performed four groups of kidney transplantation with different combinations of donor and recipient, in terms of their C5aR status: 1) C5aR-/- to C5aR-/- (n=15), 2) C5aR-/- to wild type (WT) (n=12), WT to C5aR-/- (n=5), 4) WT to WT (control group) (n=7). Native kidney was removed at day 7 post-transplantation. The end-point of graft survival was taken as the time to blood urea nitrogen >50mmol/l or death, depending on which was first. We found that kidney grafts in the group 1, when both donor and recipient do not express C5aR, survived significantly longer that the control group (40% survived more than 29 days vs 100% rejection less than 10 days). However, donor or recipient lacking expression of C5aR alone did not prolong the graft survival compared with the control group. Thus, our data demonstrate a contributory role for C5aR in renal allograft acute rejection. It also indicates that rejection is dependent on expression of C5aR on both donor and recipient tissues, suggesting that C5aR signal may up-regulate both donor and recipient APC function, which contributes to allospecific T cell responses elicited through the direct and indirect presentation pathways. Therefore, targeting C5a-mediated signal in prevention of graft injury may require inhibition of both donor organ and recipient factors.

P21

Donor Specific HLA Antibody Levels at the Time of Renal Transplantation are Associated with Early Graft Function.

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Introduction: Donor specific HLA antibody (DSA) testing by microbead analysis and successful antibody incompatible transplantation make it possible to dissect out relationships between DSA levels and graft function. The aim of this study was to examine DSA present at transplantation and function over the first 4 days, thus excluding post-transplant DSA resynthesis.

Methods: Of 48 patients who received HLA antibody incompatible transplants; 6 had either HLA and ABO antibody incompatible transplants or died early post transplant, and were excluded, leaving 42 in this study.

Results: Early rejection (starting up to day 4 post-transplant) or delayed function (ER/DF) was associated with pre-transplant crossmatch (XM) status (after any plasmapheresis) – cytotoxic XM +ve, 2/4 (50%), flow cytometric (FC) XM +ve 7/21 (33%), microbead +ve 1/19 (5%) ($p<0.01$). High, intermediate and low risk strata were separated in the FC XM by relative median fluorescence (RMF) values of 10 and 2.5, and in microbead testing, by median fluorescence intensity (MFI) levels of 7000u and 2000u. The two methods had similar receiver operator characteristic curves. Patients in either low risk group with ER/DF all had either higher risk level measured by the other technique, or high pre-treatment DSA.

For high/intermediate/low levels of risk defined by the microbead MFI, respectively, the rates of ER/DF were 5/ 10 (50%), 3/14 (21%) and 2/18 (11%)($p<0.01$); mean urine output for days 2-4 was 42, 113, 139 ml/hr ($p<0.01$ high vs low); mean day 4 creatinine was 309, 218, 134 umol/l ($p<0.05$ high vs low). When patients with ER/DF were excluded, there was still a trend to association with risk level. Mean urine output days 2-4 in high/intermediate/low risk groups was 85.8, 134, 148 ml/hr (p = ns, high+intermediate vs low), and mean day 4 creatinine was 204, 169, 119 umol/l ($p<0.04$, high +intermediate vs low).

Discussion: Levels of DSA at the time of transplantation were associated with early graft function. It was possible to identify patients at low risk of early graft dysfunction (though some of these had later rejection after DSA resynthesis). However, at higher levels of DSA, only about 50% of patients had graft dysfunction, even when the transplant was performed across a low level cytotoxic +ve crossmatch.

P22

Peripheral Blood Monocyte IMPDH Activity Is Lower In Female Patients Of Asian And Black Ethnicity

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Introduction: Mycophenolate mofetil (MMF) prevents acute rejection following solid organ transplantation by inhibition of inosine monophosphate dehydrogenase (IMPDH) to limit lymphocyte activation. IMPDH activity displays a wide variability between individuals and therefore patients may require personalized dosing regimes of MMF to minimize adverse effects without compromising efficacy. The aim of this study was to assess the effect of demographic and clinical variables on IMPDH activity in patients awaiting renal transplantation.

Methods: IMPDH activity was measured in peripheral blood monocytes isolated from 54 patients by quantification of xanthosine monophosphate (XMP) by HPLC. Results: IMPDH activity ranged from 46-331 nmol XMP / h/ µg total protein with a mean of 106 and standard deviation of 42 nmol XMP/ h/ µg total protein. Males (n=35) exhibited higher IMPDH activity compared to females (n=19) (115 v 90 nmol XMP/ h/ µg, P=0.036). There was no overall difference in IMPDH activity between ethnic groups (P=0.60), however females of asian and black ethnicity had significantly lower IMPDH activity compared to all males (P=0.047). Within each ethnic group, females tended to have lower IMPDH activity compared to males with statistically significant lower IMPDH activity in females compared to males of black ethnicity (P=0.01). There was no difference in IMPDH activity relating to age (P=0.51), primary renal disease (P=0.84) or dialysis modality (P=0.86).

Discussion: This is the first study to demonstrate lower IMPDH activity in females particularly those of asian and black ethnicity. It suggests that these patients may require lower doses of MMF to limit drug toxicity.

Ethnicity / Gender	Frequency (%)	Mean IMPDH activity (SD)
White Male	10 (18)	99 (27)
White Female	7 (13)	96 (42)
Asian Male	16 (30)	123 (58)
Asian Female	5 (9)	88 (27)
Black Male	5 (9)	115 (15)
Black Female	5 (9)	84 (13)
Other Male	4 (7)	123 (45)
Other Female	1 (2)	93 (-)

P23

Characterisation of Intra-Islet Collagenase within Different Anatomical Regions of the Human Pancreas

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Introduction: Whilst the composition of collagenase batches is clearly important, for optimal islet isolation targeted delivery of collagenase to the islet-exocrine interface is also paramount. We have shown previously that collagenase enters human and porcine islets during standard infusion techniques. The aim of this study was to further characterise the distribution of intra-islet collagenase within different anatomical regions of the pancreas following both syringe loading and recirculating perfusion techniques.

Methods: With appropriate consent and ethical approval, human pancreases (n=18) were retrieved from multiorgan donors (age range 19-61 years). Following collagenase infusion by either manual syringe loading (n=10) or recirculating perfusion (n=8), specimens were taken from the neck, body and tail of the pancreas and snap frozen in liquid nitrogen. Frozen sections (8µm thick) were immuno-labelled for collagenase, insulin, CK19 (ductal marker), collagen VI and CD31 (endothelial marker). Intra- and peri-islet collagenase was assessed by confocal microscopy in double-labelled sections, with 47±5 islets assessed per section.

Results: Collagenase labelling was widespread throughout the pancreas, associated with collagen VI, adjacent to CK19-labeled ducts. Collagenase was found within 67±2% of islets, localised within capillaries (CD31 positive). Intra-islet collagenase labelling was observed in 70±3% of islets in the tail of the pancreas, compared to only 58±2% and 53±2% of islets in the pancreatic body and neck, respectively ($p < 0.05$ tail vs neck). Intra-islet collagenase labeling was more prevalent in islets with diameters >150µm (98±1% of islets >150µm vs. 52±2% of islets <150µm, $p < 0.05$). There was no difference in the extent of intra-islet collagenase labeling between perfused and syringe-loaded pancreases (69±3% vs 65±3%, $p >0.05$).

Discussion: Using current infusion techniques, collagenase enters the majority of islets and is most frequently observed in islets in the pancreatic tail. Intra-islet collagenase digestion can lead to islet fragmentation, which may result in low yields and islet dysfunction. Localisation within islet capillaries suggests that following delivery into the ductal system, collagenase is able to enter the vasculature of the pancreas, with large islets at particular risk. This study again underlies the need to optimise collagenase delivery.

P24

Resuscitation Of Non-Heart-Beating-Donor (NHBD) Pancreas By Hypothermic Machine Perfusion (HMP) And Venous Oxygen Persufflation (VOP) Before Pancreatic Islet Isolation

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Introduction: Pancreases from NHBD with prolonged warm ischaemia (WI) are not routinely used for islet transplantation due to the low yield and poor quality of the isolated islets. Improved cold preservation techniques that can resuscitate the NHBD pancreases during their transport to an islet isolation centre can increase their utilisation. We investigated the role of a short period of Hypothermic Machine Perfusion (HMP) or retrograde Venous Oxygen Persufflation (VOP) in increasing the yield, viability and in-vitro function of islets isolated from pancreas in an experimental NHBD rat model.

Methods: Pancreases along with adjoining duodenum, spleen and a segment of abdominal aorta was retrieved from male Wistar rats (350-450g) after 35 minutes of warm ischaemia. The pancreas was preserved at 4°C for 5 hours by either simple cold storage in UW solution (SCS group) or HMP with UW (flow:0.3 ml/min; perfusion pressure:10-15mmHg) through the aorta (HMP group) or underwent VOP with 100% oxygen (10-15mmHg) through the portal vein (VOP group). Other pancreases were retrieved with minimal warm ischaemia (<5 minutes) and underwent immediate islet isolation to serve as Heart-beating-donor controls (CONT group).All pancreases underwent collagenase digestion and discontinuous gradient separation with Histopaque (1.083 gm/ml). Islet yield in terms of crude and purified islet counts and islet equivalents (IEQ), and islet viability using propidium iodide and trypan blue stains were assessed. In-vitro function was assessed using static glucose stimulated insulin secretion test.

Results: 25 successful isolations (Control-9, SCS-5, HMP-3, VOP-4) were carried out. The purified islet count, purified IEQ yield and %viability in the control group (428±146,1360±558,86±8%) was significantly higher than the SCS group (218±94,364±128,75.5±4.6%) and the HMP group (253±64.3,500±181,67±16%). Purified islet count, IEQ, % viability of VOP group (337±93,994±477,80±1.6%) was not significantly different from the CONT group.

Conclusions: Exposing the rat pancreas to 35 minutes of warm ischaemia produced a significant decrease in the purified yield and viability of islets as compared to control pancreases. Preservation with VOP, but not HMP, appears to partially ameliorate the deleterious effect of warm ischaemia on purified islet yield.

P25

Investigation Of HLA Specific IgG Subclass Antibodies In Renal Patients

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It is established that the presence of preformed donor specific HLA antibodies (HLA Abs) in a potential recipient presents a risk to successful transplantation. However, the role of IgG subclasses in graft survival is still unclear. In a study to determine the distribution of IgG subclass HLA Abs in patients with varying sensitisation sources, a serum sample known to contain IgG HLA class II specific antibodies was tested from each of 100 renal patients. The 100 sera were tested with FlowPRA® II screening (FL2-30) and LABScreen® Mixed (LSM12) (both One Lambda) adapted to identify IgG1, IgG2, IgG3 and IgG4.

42% and 36% of sera were negative when tested by FlowPRA® II screening and LABScreen® Mixed respectively. This was possibly due to the lower sensitivity of the assays for the detection of subclasses as compared with total IgG.

Table 1 shows the number of sera positive for IgG1, IgG2, IgG3, IgG4 when tested with each method.

Table 1

Subclass	IgG1	IgG2	IgG3	IgG4
FlowPRA	49	21	23	23
LABScreen	63	18	30	16

More than one subclass was detected in 50% of positive sera by FL2-30 and in 58% of positive sera by LSM12. Table 2 shows the number of sera positive for more than one subclass.

Table 2

No of Subclasses	None	1	2	3	4
FlowPRA	42	29	9	13	7
LABScreen	36	27	17	14	6

IgG1 was the predominant IgG subclass regardless of sensitisation source. IgG2 was more prevalent in patients who had had a previous transplant (31% FL2-30, 39% LSM12) or transfusions (17% FL2-30, 50% LSM12) rather than pregnancy (18% FL2-30, 5% LSM12).

Further work will concentrate on testing patients with failed transplants to determine if HLA specific antibodies produced at the time of transplant failure are of a particular subclass.

P26

Post transplant renal allograft function is reflected in peri-transplant expression of telomere associated genes

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Introduction: Accelerated biological ageing predisposes to disease. This is reflected in telomere biology and changes in cellular stress responses. Recently, telomere attrition has been demonstrated to be strongly associated with the risk of mortality in chronic kidney disease patients (1). Consequently, we have investigated whether cold ishaemia, as part of peri-transplant stresses on renal allografts, accelerates biological ageing and whether the affect of this on graft function is reflected in the expression of telomere associated genes and cell cycle control.

Methods: RT-PCR for p16, p21, POT1, SIRT 2 and XRCC5 was performed on human renal peri-transplant biopsies (n=61). Expression, relative to HPRT, was then associated with cold ischaemic time (CIT), age, sex and creatinine clearance (CC) at 6 months post transplant

Results: XRCC5 expression was found to be associated significantly with sex (p=0.019) and showed a trend to association with age (p=0.068), CIT (0.059) and CC (0.099).

P16 expression showed a trend to association with age (p=0.092). When analysed in time zero biopsies, p16 expression showed a significant association with age (p=0.008). Other genes showed no significant associations.

When analysed by sex XRCC5 expression in males was significantly associated with CIT (p=0.044, n=31)

Discussion: These data suggest that DNA damage and cellular senescence may be associated with increasing CIT and poorer graft function at 6 months post transplant. XRCC5 is a key mediator of DNA double strand break repair. P16 is a regulator of cellular senescence. The data indicate that peri-transplant analysis of renal allografts for signs of DNA damage and senescence may be beneficial for prognosis of post transplant graft function. They also suggest higher levels of damage in males, which again may influence subsequent graft performance and half life. These data are in keeping with published reports of telomere attrition associated with mortality risk in haemodialysis patients.

Reference

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Telomere attrition is associated with inflammation, low fetuin-A levels and high mortality in prevalent haemodialysis patients.

J Intern Med. 2007

P27

The Effect Of Acidosis On Innate Immunity In Patients With Renal Dysfunction

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Introduction: Systemic metabolic acidosis, often observed in patients with renal failure, is a life-threatening condition that has detrimental effects on a range of tissues, organs and cellular processes. Localised acidosis is commonly observed at inflammatory loci and within incidences of ischemia. Bacterial infection is a significant clinical problem in patients with renal dysfunction suggesting that innate immunity in these individuals is compromised. This study aimed to investigate how acidosis affects neutrophil apoptosis, a key mechanism that regulates the ability of these cells to function during immune challenge.

Methods: Neutrophils were isolated from venous blood of healthy controls by a minimally-perturbing one step isolation method using Polymorphprep. Cells were incubated in (control) RPMI 1640 medium (+HEPES, +10% pooled human Ab serum) at pH 7.3 or in medium adjusted to pH 6.5 by the addition of isotonic HCl (pH 6.5). Apoptosis was measured by morphology changes and positive Annexin V staining measured by flow cytometry. Mcl-1 protein levels were quantified by Western blot.

Results: Cells incubated at pH 6.5 exhibited enhanced survival after 6h compared to cells incubated at control pH values ($p<0.05$). Levels of the anti-apoptotic protein Mcl-1 in neutrophils incubated at pH 6.5 were significantly increased compared to controls over 6h ($p<0.05$). When de novo synthesis of Mcl-1 was blocked with the translational inhibitor cycloheximide (CHX), Mcl-1 levels were decreased in control samples compared to pH 6.5 suggesting that the stability of the protein is enhanced under acidic conditions.

Conclusions: Previous work has shown that Mcl-1 plays a central role in regulating neutrophil apoptosis and that its levels in cells are acutely controlled. An acidic environment commonly observed at inflammatory loci has been found to enhance neutrophil survival, and concomitantly increase levels of Mcl-1. This may involve changes in the rate of turnover of the protein that is usually rapidly degraded by the proteasome. This is the first observation that acidosis can mediate changes in the cellular levels of this protein, likely to occur via post-translational modification (phosphorylation) of the protein. Understanding how neutrophil function is affected by a reduced pH could help to reduce risk of bacterial infection risk in patients with renal dysfunction.

P28

The Effect Of Immuno-suppressant Drugs On Innate Immunity In Renal Transplant Patients

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Introduction: Following renal transplantation, patients receive a cocktail of immuno-suppressant drugs that prevent organ rejection, but which can adversely suppress immunity to microbial and viral infections. Many of these immuno-suppressants are designed to down-regulate T-cell function, as these cells are the major effectors of tissue rejection. However, bacterial infections are common in renal transplant patients, indicating that innate immunity is impaired. The purpose of this study was to determine if commonly used immuno-suppressant drugs, administered following renal transplantation, had any *in vitro* effects on neutrophil function, an important component of the innate immune response.

Methods: Neutrophils were isolated from the venous blood of healthy controls by a minimally-perturbing one step isolation method on Polymorphprep. Cells were incubated in RPMI 1640 medium (+HEPES, +10% pooled human Ab serum) in the absence (control) or presence of a variety of immuno-suppressants (or their solvent vehicle) at concentrations representing “peak” and “trough” serum levels after administration. After 30 min pre-incubation, activation of the neutrophil respiratory burst was determined by luminol-dependent chemiluminescence following stimulation by the phorbol ester, PMA.

Results: Under these experimental conditions, prednisolone (100-1000 ng/mL), 6-mercaptopurine (50-150ng/mL, active ingredient of azathioprine), rapamycin (0.1-1000 ng/mL), ciclosporin (200-1000 ng/mL) had little or no effect on neither magnitude nor kinetics of the respiratory burst. However, mycophenolic acid (MPA, active ingredient of mycophenolate mofetil, 1 µg- 100 µg/mL) resulted in a significant ($p < 0.05$), dose-dependent inhibition of activity. Also, while tacrolimus at 10 ng/mL -1000 ng/mL had no effect on activity, at lower concentrations (0.1 ng/mL) the response appeared “primed” in that it was triggered more rapidly than controls.

Conclusions: Most of the immuno-suppressant drugs had little or no effect on activation of the neutrophil respiratory burst under these experimental conditions. However, MPA, at clinically relevant concentrations, markedly impaired neutrophil function. As bacterial infections are a common cause of morbidity and mortality in renal transplant patients, these studies show that they may arise, at least in part, from direct effects on innate immune processes. Indirect effects on innate immunity may also be important as; *in vivo*, T-cell derived cytokines are important regulators of neutrophil function.

P29

Peri-Insular Collagen V and VI within Pancreases of Young Human Donors are not Resistant to Collagenase Digestion

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Introduction: Efficient islet isolation is dependent on collagenase to digest the extra-cellular matrix (ECM) in the islet-exocrine interface of the human pancreas. Islet yields from young donors (<30 years) are usually insufficient for transplantation, although these islets potentially provide better physiological function than those from older donors. In this study, we used a novel assay to compare collagenase digestion of two principle components of the islet-exocrine interface in young and old donors. **Methods**: With appropriate consent and ethical approval, human pancreata were retrieved from 5 young (19-28 years) and 6 old (45-60 years) donors. Cold ischaemia time was <10 hours. Tissue blocks (~0.5 cm^3) were snap-frozen in liquid N_2. Specimens were cryo-sectioned onto slides at 10-15-μM thickness and stored at -25^oC. Specimens were incubated ±Liberase at 1.4mg/mL in HBSS for 5 min. at 37^oC. Digestion of the ECM within the islet-exocrine interface was analysed by double immuno-labelling for insulin and Collagen V or VI and semi-quantified by morphometry (Zeiss KS-400 image analyser). A mean of 13±1 or 15±1 islets were assessed in control or Liberase-treated sections respectively. Data were expressed as area of collagen/ islet area. Statistical analysis was by Mann-Whitney U-test. **Results**: After 5 min. incubation, Liberase had begun to digest the ECM although the mean islet area was unchanged in specimens from young (14572±2400 vs 12698±1604 μm^2 in untreated) and old (11881±1095 vs 12541±806 μm^2 in untreated) donors. Peri-islet Collagen V content in young and old donors was significantly reduced by 34% (from 0.210±0.022 to0.138±0.014) and by 35% (from 0.235±0.024 to 0.153±0.007) respectively. Similarly peri-islet Collagen VI content in young and old donors was significantly reduced by 29% (from 0.298±0.017 to 0.213±0.022) and by 34% (from 0.322±0.015 to 0.212±0.011) respectively. Collagen VI remained significantly higher ($p<0.05$) than Collagen V in specimens from both young and old donors. **Discussion**: Collagenase digestion of peri-insular ECM Collagen V and VI within the pancreas of young donors is not impaired which suggests that inadequate digestion of these components is not the reason for poor islet yields from young donors. Digestion of other ECM components in young donors is currently under investigation.

P30

Successful Transplantation in the Presence of Luminex Defined Donor Specific HLA Antibodies

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Introduction:. The presence of luminex-detected donor specific HLA antibodies (DSA) pre-transplant should not always be considered a contraindication to transplantation. We present our experience of transplanting seven patients (median follow up 229 days) with luminex defined DSA in the presence or absence of a positive B cell flow cytometry crossmatch (FCXM). Pre-transplant antibody removal was not undertaken although post-transplant monitoring of HLA antibody levels and graft function were used to manage each transplant.

Methods: HLA antibodies were defined pre and post transplant by luminex using One Lambda LABScreen® PRA or single antigen bead testing. Complement dependent cytotoxicity (CDC) and FCXM were performed for all patients pre-transplant. Post-transplant antibody monitoring was undertaken for 6/7 patients.

Results: Six of 7 recipients had class II DSA. Three cases involved DP antibodies, two DR (DR7 & DR8), one DQ5 and one class I B55 DSA. CDC crossmatches were negative in all cases. In the two cases with DR antibodies, DSAs were present at levels detectable by luminex but did not cause a positive B cell FCXM (i.e. luminex bead positive). DSA in the remaining five recipients (HLA-B, DQ & DP specific) were present at levels resulting in a positive B cell FCXM. Successful transplantation with fully functioning grafts and a reduction in serum creatinine was achieved in all cases. No change in DSA levels post transplant was observed for transplants involving B, DR & DQ antibodies. Antibody monitoring was undertaken in 2 of the 3 patients with DP specific DSA. A reduction in DSA levels was observed by day 10 post-transplant in both cases however, there was no evidence of antibody mediated rejection or elevation of serum creatinine in these patients. Five patients are currently well with functioning grafts. One patient died with a funtioning graft and one returned to dialysis 17 months post transplant due to graft failure attributable to BK virus.

Discussion: An assessment of risk, taking into account the levels of DSA is required before the decision to transplant can be made. In this study, successful transplantation was achieved without pre-transplant antibody removal, in the presence of a positive B cell FCXM due to HLA-B, DQ or DP DSA and in the presence of FCXM negative -luminex bead positive HLA-DR DSA.

P31

An Investigation Into The Incidence Of Major Histocompatibility Complex Class I-Related Chain A Antibodies In Sensitised Renal Patients

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Antibodies directed against Major Histocompatibility Complex Class I-Related Chain A (MICA) antigens have previously been associated with renal allograft rejection. The aim of this study was to investigate the incidence of MICA-directed antibodies in sensitised renal patients.

Sera from 124 renal transplant waiting list patients, with >75% antibody reactivity to Human Leukocyte Antigens (HLA), including 38 un-transplanted patients and 86 with failed transplants, 22 of whom had received more than one graft, were simultaneously assayed using Luminex® LABScreen® mixed bead technology for the presence of MICA and HLA specific antibodies. MICA antibody-positive samples were assayed by Luminex® LABScreen® MICA Single Antigen bead technology for antibody definition. MICA antibody-positive patients, their kidney donors and/or the partners of previously parous females were Luminex® LABType® MICA genotyped to determine the source of MICA sensitisation.

MICA-directed antibodies were detected in 27% of the sensitised renal patients. Chi-Squared analysis revealed an association between MICA antibodies and kidney transplantation ($P=0.054$). This was statistically significant in patients who had received more than one graft ($P=0.037$). A further significant association was observed between the production of MICA and HLA antibodies in previously transplanted patients ($P=0.009$). Of the MICA positive patients, 76% were found to have antibodies directed against multiple MICA antigens, amongst which, antibodies directed against donor and/or partner antigens were identified. MICA*019 antigen-directed antibodies were detected most frequently, found in 67% of MICA antibody positive patients.

Further investigation of MICA antibodies in a larger cohort of transplanted and also non-HLA sensitised renal patients is required to fully assess the incidence of MICA antibodies in renal patients. The detection of donor-specific MICA antibodies in such patients may indicate a beneficial role for MICA matching in renal transplantation.

P32

Application of flow cytometry to monitor antibody levels in ABO incompatible kidney transplantation.

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Introduction: Current methods of measuring ABO antibody levels based on the haemagglutination (HA) titres has the disadvantage of being both non-reproducible and having inter-observer variability.

Methods: We have therefore developed and validated flow cytometry (FC) as a semi quantitative method to monitor the amount of IgG and IgM ABO antibodies in individuals undergoing ABOi transplantation.

Results: To validate our method, we analysed plasma samples from 79 healthy donors using the FC method. We have shown that in healthy donors, the relative levels of IgG and IgM antibodies against A and B in subjects of blood groups O, A and B were same as previously reported using the HA technique. We also analysed 40 consecutive samples over 32 days from group O patient who received group A2 kidney. On comparison, there is a significant positive relationship between FC RMF (relative median fluorescence) and HA for both IgM ($p<,0.01$) and IgG ($p<0.01$).

Discussion: Our data demonstrates that FC monitoring of ABO-specific antibodies in incompatible transplants is effective in terms of detecting quantitative changes and reproducibility. We have simplified the FC method, by overcoming the tendency of sensitised RBCs to agglutinate by using dilute cells rather than use fixation buffers. Thus, FC may be a practical method of measuring antibodies and monitoring changes in ABO antibody level.

P33

Soluble CD30 in Antibody Incompatible Renal Transplantation

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Introduction

CD30 is expressed by CD4+ and CD8+ T cells that secrete Th2-type cytokines. CD30+ T cell activation leads to release of the soluble form of CD30 (sCD30).In diseases where Th2-type immunity dominates high levels of sCD30 is associated with increased disease activity. Some studies in kidney transplant recipients have suggested that elevated pre- and post-transplant sCD30 levels may be related to acute rejection episodes.

Methods

We retrospectively analysed serum samples from 22 patients who had received antibody incompatible transplantation (AIT). sCD30 was measured on samples that had been stored at various time points including: Prior to starting treatment with plasmapheresis (PP), pre-transplantation, post-surgery, at onset of rise and at peak anti-HLA antibody titres post-transplant, at onset and resolution of rejection and several weeks post-transplant

Results

19 patients received HLA-incompatible and 3 ABO-incompatible renal transplants. There was a fall in sCD30 levels post-PP ($p<0.001$) and post-surgery (<0.001). 9/19 patients developed an episode of rejection. Mean pre-treatment sCD30 level was 106.52U/ml (±59.4) in rejectors and 100.6 U/ml (±58.0) in non-rejectors (p=NS). Post surgery mean Scd30 levels in rejectors was 52.9 U/ml (±33.9) and in non-rejectors 48.3 U/ml (±25.6) (p=NS). At peak anti-HLA antibody levels, mean sCD30 was 55.59 U/ml (±26.0) in rejectors and 55.98 U/ml (±65.1) in non-rejectors (p=NS). No significant difference was seen between the two groups at other time points. None of the 3 ABO incompatible transplant recipients developed rejection.

Discussion

We report for the first time on sCD30 levels in patients undergoing AIT**.** Previous studies have shown a drop in sCD30 levels post-transplantation, an effect seen in our cohort of patients. However, we did not detect any correlation between sCD30 levels measured at different time points and early rejection. Studies with larger cohorts of patients need to be conducted.

P34

Serum B Cell Cytokine Levels In Renal Transplant Recipients: A Pilot Study

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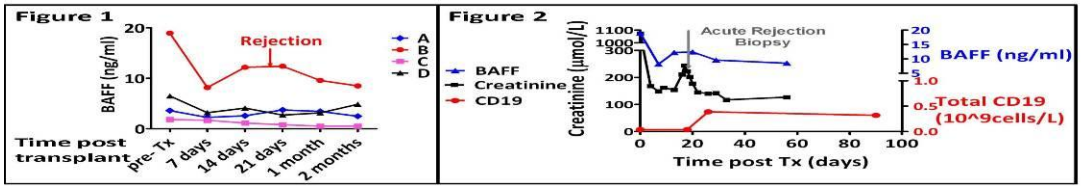
Introduction: Acute cellular rejection (ACR) has traditionally been viewed as a T cell-mediated process. More recent studies have identified CD20+ve cells (B cells) in grafts with ACR and have shown that their presence is associated with steroid-resistant rejection[1] suggesting that B cells may play an important role in ACR. BAFF and APRIL are soluble factors which drive B cell survival and maturation and have been implicated in a number of autoimmune diseases[2]. BAFF can also promote T cell activation[3]. It may therefore be a useful target in the prevention of ACR, however no studies to date have assessed BAFF and APRIL levels in the context of renal transplantation. **Aims:** We wished to perform a pilot study to determine serum BAFF and APRIL levels in renal transplant recipients.

Methods: BAFF and APRIL levels were assessed by ELISA in serum samples taken from 7 renal transplant recipients prior to transplantation and at days 7, 14, 21, 28, and 56 post-transplant. Patient demographics, B and T cell counts, and ACR episodes were also documented. All patients received daclizumab at day 0 and 7 and tacrolimus (0.075mg/kg) and mycophenolate mofetil (1g BD) maintenance.

Results: 4/7 patients had detectable levels of BAFF in serum in the pre and post-transplant period (patients A-D, Figure 1). Patient B had particularly high levels of BAFF and low B cell numbers pre-transplant. He subsequently developed an episode of ACR (Figure 2) characterised by both T and B cell infiltrates on biopsy. No other patients experienced a rejection episode. 2/7 patients (patients A,C) had detectable APRIL levels which did not greatly change in the post-transplant period. Neither patient developed ACR.



Conclusions: We have demonstrated for the first time that BAFF and APRIL levels are detectable in some patients post-renal transplant. In this small pilot study, high BAFF levels were associated with the development of acute rejection, an interesting observation requiring validation in a larger cohort.

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P35

Successful Kidney Transplantation In A Patient With Donor-Directed HLA-DP Specific Antibodies

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Characterisation of HLA-DP specific antibodies (Abs) has been facilitated by advances in technology and an understanding of amino acid sequence epitopes but the relationship between recipient sensitisation to donor HLA-DP mismatches and transplant outcome remains poorly defined. The relevance of a B cell positive (pos) crossmatch (Xm) is also sometimes questioned and it is clear that interpretation of such a result requires full knowledge of the patient's HLA antibody profile.

We report a successful second kidney transplant for a sensitised patient with a current pos B cell flow cytometry (FC) Xm due to donor directed HLA-DP Abs.

The 36 year old female patient with lupus erythematosus had a history of pregnancy, blood transfusion and a failed kidney transplant. At the time of listing for re-transplant, her HLA Abs reaction frequency was 45% with class I but no class II specificities defined. Kidneys were offered from two donors with no mismatches at HLA-A, B, Cw, DR, DQ. For both donors, the cytotoxic (CDC) and FC B cell Xm were pos due to IgG Abs. The patient had no IgG autoantibodies.

Patient sera were tested using LABScreen Single Antigen Class II (One Lambda) and Luminex technology and HLA-DP Abs identified. The patient, her son, the first transplant donor and both potential donors were typed for HLA-DP by LABType (One Lambda). The son, first donor and potential donors all shared HLA-DP epitopes absent from the patient, accounting for her HLA-DP Abs profile.

In May 2007 she was offered a kidney from a heart-beating deceased donor who was only mismatched for HLA-DPB1*0201, to which she was known to be sensitised. The Xm was CDC negative but current FC B cell pos. She was transplanted and given simulect as induction immunosuppression and maintained on tacrolimus, prednisolone and mycophenolate mofetil. She was discharged on day 10 with a serum creatinine of 262 mmol/l. This fell to 148 and 153 at 3 and 6 months respectively.

In conclusion, a comprehensive knowledge of this patient's HLA Abs profile and the donor's HLA-DP mismatch enabled the FC B cell pos Xm to be interpreted with confidence and the patient to be successfully transplanted in the presence of donor-directed HLA-DP Abs. Collation of comprehensive data on such cases will facilitate risk assessment for patients with donor-directed HLA-DP Abs.

P36

Zinc Supplementation Promotes the Prevention of Allograft Rejection by Decreasing IFNγ and IL-6 Levels

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PURPOSE

Zinc plays an important role in cell-mediated immune function and has been reported to be an effective anti-inflammatory agent by altering cytokine production. Its potential role in regulating allograft rejection is unknown. The current study is designed to measure the effect of zinc supplementation on the production of IFNγ and IL-6, cytokines associated with allograft rejection, in human lymphocytes.

METHODS

Human lymphocytes were separated from fresh human blood obtained from the American Red Cross. The lymphocytes were treated with either zinc chloride ($ZnCl_2$: 5, 50 and 100 μM), Concanavalin-A (ConA) (10 mg/ml) ,or both, for 2, 6, 24 or 48 hours. The $ZnCl_2$ concentrations were representative of dietary supplementation doses. Con-A stimulation mimicked lymphocyte activation as seen in acute rejection. The cell supernatants were collected and the cells were lysed.. Cytokine levels were measured using ELISA assays. The difference between the means at different time points of treatments was calculated. Results were interpreted using T-test data testing.and $p<0.05$ was considered significant.

RESULTS

Both IFN-γ and IL-6 were secreted by unstimulated human lymphocytes (25.2 +/- 0.35 pg/ml and 10.4 +/- 1.23 pg/ml, respectively) at time zero. At 6, 24 and 48 hours, Con-A stimulated cells had significantly increased levels of IFNγ compared to non-stimulated cells (38.5 +/-0.24 vs 23.0 +/- 1.69 , 55.7 +/- 1.12 vs 23.0 +/- 0.17 and 56.1 +/- 3.20 vs 21.3 +/- 0.18 pg/ml, respectively)($p<0.05$). At 6, 24 and 48 hours, Con-A stimulated cells had significantly increased levels of IL-6 compared to non-stimulated cells (19.0 +/- 1.0 vs 12.1 +/- 0.33, 375.3 +/- 27.1 vs 14.8 +/- 1.61 and 1021.7 +/- 193.9 vs 17.1 +/- 1.61 pg/ml, respectively) ($p<0.05$). The addition of $ZnCl_2$ to Con-A stimulated cells significantly decreased the levels of IFNγ at 6 and 24 hours, at all $ZnCl_2$ concentrations, when compared to Con-A alone stimulated cells (6 hours: 5 μM $ZnCl_2$: 17.2 +/- 0.33 vs. 38.5 +/- 0.24 pg/ml, 50 μM: $ZnCl_2$ 15.9 +/- 3.59 vs 38.5 +/- 0.24 pg/ml, 100 μM $ZnCl_2$: 16.3 +/- 1.33 vs 38.5 +/- 0.24 pg/ml. 24 hours**:** 5μM $ZnCl_2$: 23.5 +/- 2.33 vs 55.7 +/-1.12 pg/ml, 50 μM $ZnCl_2$: 30.5 +/-0.36 vs 55.7 +/- 1.12 pg/ml; 100μM $ZnCl_2$: 28.4 +/- 0.64 vs 55.7 +/- 1.12 pg/ml. ($p<0.05$)). The addition of $ZnCl_2$ to Con-A stimulated cells, significantly decreased the level of IL-6 at 6 hours, at all $ZnCl_2$ concentrations when compared to Con-A alone stimulated cells (5 μM $ZnCl_2$: 10.8 +/- 0.20 vs. 19.0 +/-1.0 pg/ml, 50 μM: $ZnCl_2$ 11.4 +/- 0.47 vs 19.0 +/- 1.0 pg/ml, 100 μM $ZnCl_2$: 12.3 +/- 0.61 vs 19.0 +/- 1.0 pg/ml ($p<0.05$))

CONCLUSIONS

Early in the human lymphocyte inflammatory response, levels of INFγ and IL6, cytokines associate with allograft rejection, are significantly decreased by zinc supplementation. These results suggest that dietary zinc supplementation may be useful post transplant to prevent allograft rejection.

P126

Using Donor Specific Antibodies and diffuse C4d staining to help stratifying for risk of graft failure in patients with kidney transplants.

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Purpose: This retrospective study looked at the impact of the detection of diffuse (>50%) C4d staining on Transplant Kidney biopsy on outcome. The impact of associated Donor Specific HLA Antibody (DSA) detection was also looked at to see if this influenced transplant graft function.

Methods: This was a retrospective single centre study reviewing all the adult patients with kidney transplants who had had kidney biopsies between April 2003 and October 2006 at Guy's Hospital.

Results: 45 patients had diffuse (>50%) C4d staining out of 228 who had kidney transplant biopsies in this time and had been followed up within the centre. Of these 13 out of 45 and 16 out of 45 had this finding on biopsy within 3 months and 6 months (respectively) of transplantation. The further 29 cases occurred between 6 and 240 months from engraftment without any obvious clustering of cases.

Of the 45 patients with diffuse C4d staining on biopsy, 20 were also found to have DSA prior to or at the time of biopsy. The fall in eGFR in this group a year post biopsy was greater than those with diffuse staining for C4d but no DSA. The mean change in eGFR from the day of biopsy at a year was -3.1ml/min/1.73m2 (+/-12.1) in those with DSA compared with +17.7 ml/min/1.73m2 (+/-23.2) for those with C4d but without DSA. The changes in eGFR from the pre-biopsy baseline at one year showed a fall in eGFR in both groups but this was greater in those with DSA (- 21.5 compared with -9.6 ml/min/1.73m2).

Of 179 patients who never had diffuse or focal C4d staining on biopsy, only 81 had their DSA tested. Of these only 8 (10%) had a positive DSA result. From these eight, four had features of rejection and four did not. One person in each of these groups is dialysis dependant and one person in the rejection group has eGFR <15 ml/min/1.73m2. Although small numbers, this outcome appears to be worse than that of C4d negative patients with no DSA but features of rejection who in fact showed an improvement in eGFR from the day of biopsy by 14.4 m l/min/1.73m2 or an improvement from their pre-biopsy baseline of 1.3 m l/min/1.73m2 at one year.

Conclusion: DSA is of additional value in evaluating risk of graft failure. This appears to be of value in those with and without diffuse C4d staining on biopsy.

POSTERS

Infection

P37

Efficacy of leflunomide in treating CMV disease in renal transplant recipients

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Introduction: CMV disease is a major problem post transplantation. Treatment consists of ganciclovir and reducing immunosuppression (IS). Ganciclovir is efficacious but development of viral resistance is well recognized. Reducing IS in the setting of CMV disease may be complicated by acute rejection. Leflunomide (LF) has both immunosuppressive and anti-viral properties. We report a single centre experience of using LF in the management of CMV disease in renal transplantation. **Methods**: IS consists of tacrolimus, MMF and prednisone. CMV DNA PCR is performed weekly post transplantation. Preemptive treatment with valganciclovir (VGC) is initiated when the CMV DNA rises above 3.6 (log) copies/ml. Baseline IS is lowered. VGC is continued until serum CMV DNA is undetectable. LF is initiated if 1) the CMV DNA does not reduce after 10 days of treatment with VGC 2) if ganciclovir resistance gene mutation is identified or 3) there is a coexisting biopsy proven rejection. LF dose is 200mg/d for 5 days followed by 50mg/d (MMF is discontinued). VGC is stopped if resistance is proven. **Results:** We have treated 5 transplant patients with LF to date. In one patient with proven ganciclovir resistance (UL97 gene mutation), and very high CMV DNA titres, LF alone was effective at inducing a negative CMV DNA within 3 weeks. In 2 patients CMV DNA remained static or increased despite full dose VGC and reduction in IS. Both patients responded rapidly when LF was added to VGC. No resistance mutation was identified in these patients. In 2 patients with recurrent CMV disease responsive to VGC, lowering of IS was associated with type 1 ACR. Addition of LF treatment in these cases resulted in resolution of both the rejection and recurrence of CMV. LF has been well tolerated. Side effects were mild diarrhoea and a mild transaminitis in one patient. Both resolved with a reduction in dosage. **Conclusion**: This is the first report of successful management of ganciclovir resistant CMV infection with LF in a renal transplant patient in the UK. Addition of LF to patients who do not initially respond to VGC treatment results in rapid reduction in viral replication. In patients who have coexisting acute cellular rejection and active CMV disease, the combined immunosuppressive and anti-viral properties appear to be particularly useful. Leflunomide is well tolerated and does not exhibit the nephrotoxicity of foscarnet or cidofovir.

POSTERS

Kidney Clinical

P38

Validity Of The MDRD Estimated GFR At 1 year v Creatinine As A Marker Of Transplant Outcome In 4,628 Kidney Transplant Recipients

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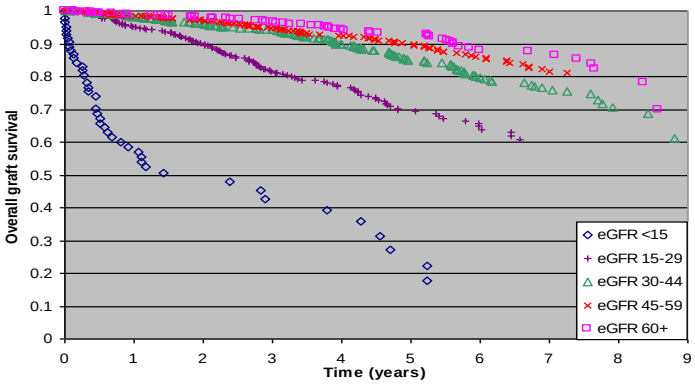
Introduction The 4 variable Modification of Diet in Renal Disease (MDRD) formula was developed to estimate GFR in the native kidney disease population. There have been some studies validating its use as a measure of function in tranplant recipients, although none have compared using the 1 year eGFR as a predictor of longer term kidney transplant outcome compared with the accepted use of serum creatinine (sCr) at 1 year post-transplant (Hariharan).

Methods All adult recipients on the UK Renal Registry database with a first, kidney-only transplant between 1997 and 2004 were included. Data on HLA matching were provided by UK Transplant. There were 4,628 patients who were alive at 12 months post transplant and also had complete data for serum creatinine at 6 and 12 months, age, gender, ethnicity, primary renal disease, donor type (live or deceased) and HLA matching score. Overall graft survival at six years post transplant was analysed by eGFR (CKDt stage) and sCr at 6 and 12 months post-transplant using a Cox regression analysis. In addition, we also tested change in both eGFR and sCr between 6 and 12 months. These surrogate markers of outcome were compared using likelihood ratios.

Results Cox regression analysis showed that eGFR at 12 months had a significant effect on subsequent graft loss or death (Figure 1). This was similar to outcomes stratified according to sCr. The calculated likelihood ratio found that eGFR at 12 months is at least as good a marker or subsequent transplant outcome as sCr at 12 months.

Discussion Using the same methods as employed by Hariharan we found that the MDRD eGFR at 1 year is at least as good a marker of subsequent kidney transplant outcome as serum creatinine at 1 year. It should be noted that this methodology of a single creatinine result at 1 year does not make allowance for the fact that within the high creatinine group a proportion of patients will be having an acute rejection episode which will recover.

Figure 1. Long-term graft survival by eGFR at 1 year from transplant



P39

Significance of 'mild arteritis' in early renal transplant biopsies

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Introduction: Banff Type 2a acute cellular rejection (ACR) is generally steroid resistant, requires ATG treatment and is associated with a poor prognosis. Correct histological diagnosis of Type 2a ACR therefore is essential. It is defined as the presence of mild to moderate intimal arteritis and characterized by intimal thickening with inflammation of the arterial subendothelial space. Banff criteria state that arteritis may be diagnosed by the presence of a single intimal lymphocyte in a single artery on light microscopy. However there is no information in the literature regarding the prognostic implications of very mild arteritis. **Methods**: We examined biopsies from patients transplanted between 2005 and 2006. Biopsies were performed either 1) weekly post transplant in the setting of DGF or 2) with a significant increase in serum creatinine. 2 cores are routinely taken. Anti-CD3 immunoperoxidase was performed to confirm the presence of a T cell infiltrate. 'Mild arteritis' was defined as the presence of less than 3 CD3+ T cells in 1 or 2 arteries. Case records were examined to determine donor and recipient details, treatment given and clinical outcome. **Results:** During this period 101 transplants were performed (38 controlled non heart beating donor(CNHB)- and 27 live donor transplants(LDT)). 56 patients had a total of 84 biopsies. Anti-CD3 staining was performed on all these biopsies. 12 patients had a diagnosis of definite type 2 ACR and were treated with a course of ATG. 14 patients had 'mild arteritis' diagnosed by the above criteria. None of these patients were treated with ATG. 2 of these patients had coexisting type 1 ACR and were treated with methyprednisolone. Repeat biopsies were performed in 9 of 14 patients and showed either resolution or persistence of 'mild arteritis' but none showed worsening. Overall graft function of these kidneys is good (mean creatinine 147 with 6-24 months f/up). The majority of biopsies with 'mild arteritis' were taken early post transplant (1 to 5 weeks) in the setting of delayed graft function (DGF) and were from CNHB kidneys. Mild to moderate donor arteriosclerosis coexisted in 7/14 biopsies. **Conclusion:** This report suggests that the finding of 1-2 lymphocytes in the intima of 1-2 arteries (confirmed by anti-CD3 staining) is not uncommon post transplant, is associated with DGF and donor arteriosclerosis and is associated with good graft function in the short term despite no treatment with ATG.

P40

Open Source Software Analysis Of Blood Pool Contrast Enhanced MRI Accurately Predicts Kidney Volume As Measured By A Peri-operative Fluid Displacement Technique In Live Renal Donors

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Introduction: Enhanced renal parenchymal volume is a surrogate of nephron number and decreases with declining renal function when kidney length may not. The volume of perfused functional renal tissue is required to calculate split renal function. Contrast-enhanced MRI has been shown to be accurate for assessing porcine(1) renal volume in an experimental model, however, there has been no validation in humans or with blood-pool contrast agents which have theoretical benefits for MRI renal perfusion analysis over conventional gadolinium agents.

Methods: Ethics approval was obtained and 10 live renal donors undergoing pre-operative MRA to exclude underlying disease and evaluate renovascular anatomy were studied. Post contrast enhanced (10ml Vasovist) coronally acquired breath-hold T1w THRIVE 3D datasets were analysed using OsirisX(2) - an open source image processing software. Using the semi-automated region growing tool renal parenchymal volume was calculated. During transplant surgery the volume of the transplant kidney was measured using a fluid displacement technique. Data was analysed using Pearson product-moment correlation coefficient.

Results: Excellent agreement was found between MRI measurement of total renal enhanced parenchymal volume and peri-operatively assessed volume, with a Pearson product-moment correlation coefficient of 0.96.

Discussion: MRI measurements of enhanced renal volume with blood pool contrast agent are accurate. This knowledge will aid in developing an accurate MRI assessment of differential renal function.

1 Measurement of renal volumes with contrast-enhanced MRI

Journal of Magnetic Resonance Imaging. 15(2):174-9, 2002 Feb.

2 http://www.osirix-viewer.com

P41

Post transplant anaemia is associated with reduced renal allograft survival but not patient survival

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There is conflicting evidence for the association of post transplantation anaemia with patient and renal allograft outcomes. This study interrogated the LOTESS (Long Term Efficacy and Safety Surveillance) database for clarification.

The LOTESS database is a pharmacovigilance project with detailed laboratory, demographic and clinical data collected from a UK cohort of >6,500 patients from 64 centres between 1995 and 1998. Using Cox regression analysis we assessed patient and graft survivals for adult renal transplant recipients who had a complete dataset for the following variables: recipient and donor age, sex and race; dialysis modality and duration; prior transplantation; time post transplantation; HLA mismatch; body mass index (BMI); immunosuppression regimen; antihypertensive use; Charlston comorbidity index (CCI); blood pressure (BP); donor type; delayed graft function; acute rejection; creatinine; eGFR; albumin; ciclosporin level and dose; haemoglobin (Hb); and Hb variability.

3839 patients (mean age: 43yr; 63% male; 90% Caucasian; 7% live donor) had complete data and were 45±42 months post transplantation at study onset with follow-up of 4±1.6 years. All were treated with ciclosporin microemulsion. Hb levels were positively associated with patient survival on univariate but not multivariate analysis. Recipient age, CCI and urea were negatively associated, and albumin positively associated with patient survival ($p<0.001$ for all). Live donor transplants had a weaker positive association ($p=0.016$). Lower Hb levels were associated with reduced graft survival when death censored. A 1g/dl rise in Hb was associated with a hazard ratio for graft loss of 0.91 ($p=0.003$). Recipient age, albumin, BMI and eGFR were positively associated with death censored graft survival; male recipient and systolic BP were both negatively associated ($p<0.001$ for all). Urea had a borderline negative effect ($p=0.03$). Similar results were generated with the uncensored graft survival model. Hb variability was not associated with mortality or graft loss.

No association between lower Hb and mortality was found in this study which is the largest body of evidence, to date, describing the relationship. This supports current recommendations that full correction of Hb in transplant recipients is unjustified and its role in delaying functional decline requires further investigation.

P42

Donor quality scores and parameters of early graft function are associated with renal allograft outcomes

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Recent analyses identify the utility of donor quality scores and definitions of early graft function in predicting late renal allograft outcomes. This study aimed to validate these parameters and identify the optimal predictors of late graft function.

Details on 217 consecutive deceased donor renal transplants performed between 2003 and 2006 were collected. All recipients received cyclosporine-based immunosuppression. Multivariate analysis was used to test the following donor quality scoring systems: i) The "Nyberg et al" Deceased Donor Score (DDS), ii) The "Schold et al" Donor Risk Score, iii) Expanded Criteria Donor (ECD) status, and iv) The "Irish et al" Delayed Graft Function (DGF) Nomogram. Similarly, markers of early graft function were assessed: a) Dialysis requiring DGF and duration, b) The expanded definition of DGF [Boom et al, 2000], c) Creatinine at day 5 [Humar et al, 2002], and d) Creatinine reduction ratios at day 2 and day 7 [Rodrigo et al, 2004 and Johnston et al, 2006]. We investigated the impact of using different combinations of predictor variables on transplant outcomes.

When comparing pre-transplantation scores in the patient population as a whole, the "Schold Donor Risk Score" was most significantly associated transplant outcome. A 0.1 unit rise in the score resulted in a 5% increase in creatinine at 12 months, an increased likelihood of CKD stage 4 at 12 months (OR: 1.28; 95% CI 1.08,1.51; p=0.005) and increased CKD stage 4 over a mean follow up of 40 months (HR: 1.22; 95%CI: 1.10,1.34; p<0.001; Cox model). Post-operatively, the "Boom" definition of DGF was similarly associated with poorer renal allograft function (p<0.01 for all); dialysis requiring DGF had no independent impact on outcome. In non-dialysis requiring patients, creatinine at day 5 outperformed all other postoperative markers in predicting suboptimal allograft function. "Boom DGF" and creatinine at day 5 held their associations with later function when "Schold DRS" was included in the multiple regression model, but their level of significance was reduced. None of the pre or post operative variables showed an association with change in creatinine between 6 and 12 months post transplant.

Donor quality scores, in particular the "Schold Donor Risk Score", and markers of early graft function are associated with late renal transplant function. This has implications for organ allocation policy, clinical management and future research.

P43

Steroid Avoidance - Good for most but is there a price to pay?

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Introduction: Since 2004 we have been using a steroid avoidance regime and we have previously reported good short term results. However we were concerned over the fate of those patients who suffered an episode of rejection and compared their outcome with historic controls with clinical rejection episodes.

Methods: All low to medium risk adult patients who were transplanted between Jan-2004 and Oct-2006 (n=258) formed the study group while those transplanted between Jan-2001 and Dec-2003 formed the historical controls (n=216). Baseline demographics were analysed in both groups and the results including DGF rate, acute CMV rate acute rejection rate, e-GFR and survival with a functioning graft at 1 year were compared. Subsequently the outcomes of those patients with at least one rejection episode were compared for both groups.

Results: Baseline demographics were similar apart from increasing proportions of DCD and living donors in the later cohort. Both groups had similar outcomes apart from a significantly higher acute rejection rate in the study group (19.8% v/s 12.5%, p=0.045). However in patients who underwent a rejection episode the one year graft survival was signicantly lower in the steroid avoidance group versus the control arm, 80.3% vs.100% respectively (p=0.045). All the patients who remained on steroids after the rejection episode had a preserved graft at one year compared to 27.7% graft loss (10/36) in patients who did not start steroids after the rejection episode.

Discussion: This data suggests that the extremely popular steroid avoidance regime may benefit the majority of patients to the detriment of a small minority who suffer rejection episodes. We advocate introduction of maintenance steroids in all patients who suffer an episode of rejection.

	Rejection-SA	Rejection-Control	P value
Acute Rejection	19.8% (51/258)	12.9% (27/217)	0.05
BANFF 1	80.4% (41/51)	85.2% (23/27)	NS
BANFF 2/3	19.6% (10/51)	14.8% (4/27)	NS
DGF	47% (24/51)	44.4% (12/27)	NS
eGFR at 1 year	43mls/min	42mls/min	NS
Graft survival 1 yr	80.3%	96.4%	0.04
% On steroids at 1yr	29.4% (15/51)	100%	

P44

Does complex or unexpected donor vascular anatomy influence outcomes in living renal transplantation?

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Introduction: Mapping of renal anatomy assists planning of donor nephrectomy for live donor transplantation. Our unit moved to CT-angiographic (CT-A) assessment in 2005. Here, we present data on 100 sequential live donor transplants to explore the incidence of complex renal anatomy, discordance of CT-A and operative findings, and subsequent outcome in the recipient.

Method: CT-A reports and operative anatomical findings were collected for all live donor transplants performed between Jan 2005 and Dec 2007. Recipient serum creatinine (SCr) at day 0, 3 and 180 was recorded.

Results: 92 cases were hand assisted retroperitoneoscopic live donor nephrectomies (HARLDN). The remainder were open. Complex anatomy was present in 40, including 5 donors with retro-aortic left renal vein and 1 with a double IVC. All donor arteries were always anastomosed. In 29 cases there was discordance between CT-A and operative findings. 16 discrepancies involved the venous anatomy. CT-A underestimated the number of renal veins in 12 cases.

CT-A 'Errors'	Vein	Artery	Artery + Vein	Ureter
Overestimate	5	2	0	0
Underestimate	12	5	4	1

There was a small difference in graft outcomes at 6 month between cases with simple and complex transplant anatomy.

	Simple	Complex	p*	CT-A=Op	CT-A≠Op	p*
Median day 3 SCr	157	178	0.285	159	192	0.162
Median % fall in SCr day3	72.1	69.1	0.077	71.2	66.7	0.084
Median day 180 SCV	126	134	0.031	128	130	0.348
Median day 180 eGFR	53	46	0.021	53	48	0.097

* Mann Whitney U

Conclusions: There is often discrepancy between CT-A and operative anatomy. HARLDN, with anastomosis of all donor arteries, can offer similar results in simple and complex anatomy live donor transplants. Complex anatomy need not preclude patients from transplantation. In our centre, we have not declined a patient for donor nephrectomy on the basis of anatomy since introducing CT-A in Jan 05.

P45

Regional audit of cardiovascular drug prescribing in renal transplant recipients following introduction of a nurse-led review clinic

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Introduction: Vascular disease is the leading cause of mortality in renal transplant recipients. Although multi-factorial in nature, there is some evidence that conventional prophylaxis with blood-pressure control, aspirin, statins and inhibitors of the renin-angiotensin system can improve outcomes. We aimed to establish current prescribing patterns, to identify deficiencies and to see whether the implementation of a nurse-led vascular intervention clinic at one centre had improved practice.

Methods: Electronic drug records were analysed for all regional patients with a 2006 drug entry (n=794), and for all patients from one centre with a nurse led vascular intervention clinic were analysed between 2005 and 2007 (n=627 and 682). Approximately 75% of patients have attended this clinic.

Results: Prescribing patterns were similar between the three units. Statins and anti-hypertensives were extensively used. Aspirin (the predominant anti-platelet agent) was prescribed in less than ⅓, and in less than ½ of over 45s. Aspirin was not less likely to be used in those on prednisolone. In the unit with a nurse-led clinic prescribing rates in most classes improved over a 2 year period (Table 1).

Table 1 - Percentage of patients on drugs in unit with nurse-led clinic

	2005 (n=627)	2007 (n=682)
ARB	13.9	18.0
ACEI	35.9	44.6
Calcium CBs	40.7	46.3
Beta Blockers	40.4	38.4
Statins	56.1	66.6
Aspirin	22.7	34.0

Discussion points: 1.Aspirin is underused, even in those at highest risk of cardiovascular events (based on observational studies we estimate that aspirin is likely to be effective primary prophylaxis in the over 45s)

2. A nurse-led clinic has led to improved prescribing rates in one centre. It is essential to follow these patients to see if this translates into better cholesterol and blood pressure control and, more importantly, better long-term outcomes.

P46

Successful Transplantation Without Desensitisation Despite the Presence of HLA DSA

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Introduction

Transplanting sensitised patients is a complicated issue involving finding a reasonably matched organ, increased level of immunosuppression and long term implications to both patient and graft survival. In the last few years desensitisation protocols have found widespread application in the setting of live donation. Retransplanted (RTX) patients who have developed donor specific antibodies (DSA) pose a significant risk for graft survival although their outcome is not entirely clear.

Aim

To review the outcome of all retransplants that were performed with negative cytotoxic crossmatch despite possessing DSA.

Method and results

From 1998 to 2003 there were 55 RTX from cadaver donors. As all these patients were transplanted with the knowledge of a negative Cytotoxic crossmatch (CDCXM), no specific changes were made in their immunosuppressive management. On retrospective analysis using solid phase assay and then flow cytometry-based techniques we identified 10 patients with DSA. On flow cytometry (FC) cross match out of those 10 patients, there were three patients T-cell positive and B-cell positive, three were T-cell positive and B-cell negative and four were T and B-cell negative.

Median follow up was 6 years. Two kidneys were lost early postoperatively, due to renal vein thrombosis (on biopsy no sign of rejection) and recurrent FSGS respectively. No graft was lost due to acute rejection and no humoral rejection occurred in the 1st year post transplant.

One patient died at 6 years with a functioning graft. Two grafts failed at 6 and 8 years, and 4 grafts are still functioning at 6 to 8 years post transplant giving a 5-year graft survival of 80%. Functioning grafts have a mean creatinine of 175 and median of 155 μmol/l.

Conclusion

DSA detection in the presence of negative CDCXM does not always predict an adverse outcome or need for desensitisation even in the presence of positive FC crossmatch. We present 10 patients with DSA and surprisingly excellent long term outcome. In the current era the level of DSA antibody as assessed by more sensitive/ sophisticated methods as Luminex technique might be more predictive than the actual FC crossmatch results.

P47

Change in Practice from Heparin to Aspirin Prophylaxis Significantly Reduced the Thrombosis Rate in Renal Paediatric Recipients in a Single Centre

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Objectives of study: Graft loss due to thrombosis is a major problem in paediatric transplantation. A prior retrospective study in our unit from 1987-2000 revealed the same thrombosis rate in patients with no antithrombolytic therapy (11.1%), as in those receiving heparin (9.3%). This study evaluates the impact of changing to aspirin prophylaxis at 1mg/kg (maximum dose 75mg) for one month. Aspirin was not discontinued at the time of renal biopsies. **Methods**: 156 consecutive transplants on aspirin (Oct 2000-Dec 2006) were analysed retrospectively using the same variables as in the previous study: live/deceased donor, donor and recipient age and gender, cold ischaemia time, single v multiple vessels, side of graft, aortic anastomosis. The patients were divided into 3 groups: group 1: no prophylaxis, group 2: heparin and group 3: aspirin. **Results:** Groups 1 (N=126), 2 (N=128) and 3(N=.156.): Live Donor:23%, 27% and 50.6 %, recipient age 0-5 yrs:31%, 27% and 17% , male recipient:66%, 68% and 58%, donor age 0-5yrs:15%, 7% and 0%, male donor:54%, 54% and 54.4%, multiple donor vessels:23%, 16% and 30%, onto aorta:48%, 65% and 21.7%. Graft loss from thrombosis occurred in 2/156 pts (1.2%) in group 3, compared to 11.1% and 9.3% in groups 1 and 2. There were no graft losses from haemorrhage and none in the 0-5yr (n=17) old in group 3. **Conclusions:** Over the past 7 years there has been an increase in live donors and hence more left kidneys were transplanted. Fewer recipients in group 3 were under 5, but no graft losses were observed in the 0-5yr children on aspirin.The fall in thrombosis rate from 10% to 1.2. % is greater than could be expected by the change in the group characteristics. We would therefore advocate the use of aspirin prophylaxis in paediatric renal transplantation.

P48

Quality of life following live kidney donation- a single centre experience

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INTRODUCTION**:** The aim of this study was to assess the quality of life (QoL) pre- and post-kidney donation using standardised assessment tool and to compare this with a control group of potential donors who did not proceed with donation.

METHODS: 78/82 live donors, who proceeded with kidney donation between January 1978 and May 2006, were included in the study. Short Form-36 (SF-36) Health Survey questionnaire was used as the assessment tool.

RESULTS: 66/78 (84.6%) donors responded. The median post-donation period was 4.57 years (range, 3 months to 27 years). 38 individuals were included in the control group. The SF-36 scores are shown in the table below.

SF-36	Pre-donation (a)	Post-donation (b)	Control (c)	P value (a vs. b)	P value (b vs. c)
PF	93.11 ± 14.02	84.54 ± 21.45	83.68 ± 23.00	0.0071	0.851
PR	95.08 ± 16.53	70.83 ± 43.53	92.10 ± 21.82	<0.0005	0.001
BP	94.68 ± 14.44	78.03 ± 24.55	84.47 ± 25.60	<0.0005	0.214
GH	80.44 ± 16.06	72.9 ± 23.73	73.34 ± 28.08	0.0347	0.936
Vit	79.3 ± 14.54	66.59 ± 22.38	69.21 ± 22.04	0.00169	0.563
SF	90.9 ± 17.88	78.78 ± 26.49	80.92 ± 35.68	0.0025	0.749
ER	90.9 ± 31.24	79.79 ± 41.29	90.35 ± 36.27	0.0836	0.178
MH	82.90 ± 13.86	80.67 ± 17.10	76.74 ± 22.78	0.409	0.359
PCS	88.52 ± 9.65	74.58 ± 22.41	80.56 ± 16.77	<0.0005	0.125
MCS	84.89 ± 11.7	75.75 ± 20.28	78.11 ± 24.73	0.0018	0.619
Total	88.42 ± 9.94	76.52 ± 21.09	81.35 ± 19.09	<0.0005	0.235

(PF-Physical Function, PR-Physical Role, BP-Body Pain, GH- General Health, Vit- Vitality, SF-Social Functioning, ER-Emotional Role, MH-Mental Health, MCS-Mental Component Summary, PCS- Physical Component Summary)

DISCUSSION: There was no significant difference in the post-donation scores between the donors and the controls. The post-donation SF-36 scores were increased in 16/66(24%), unchanged in 6/66 (9%) and reduced in 44/66 (66%) donors. The reduced scores resulted from musculoskeletal pain, migraine, myocardial infarction, diabetes, and peptic ulcer. 83% of the donors wished to donate again if possible and 90.9% wished to encourage live kidney donation.

Conclusions: Assessment of the QoL following LKD using SF-36 showed no difference of QOL score post-donation compared to the control group. Overall, donors were positive about live kidney donation and wanted to encourage the programme.

P49

Creatinine reduction ratio of less than 30% between days one and two (CRR2): an early independent predictor of poor long term renal graft survival.

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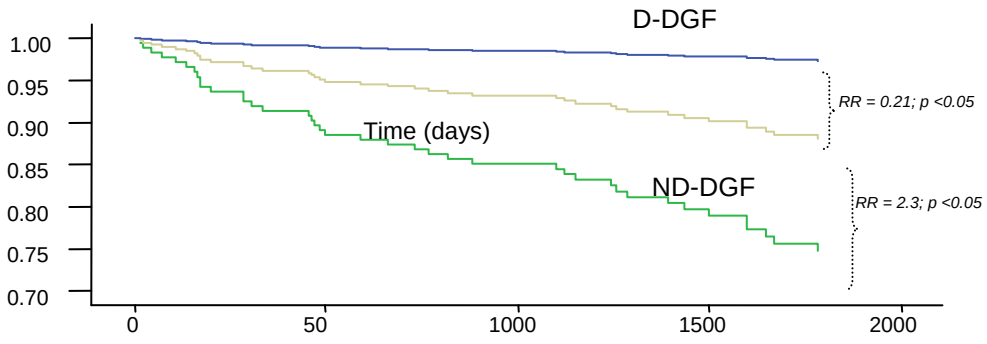
Introduction: Dialysis requiring delayed graft function (D-DGF) is independently associated with inferior long term renal transplant survival. More recently non dialysis requiring delayed graft function (ND-DGF) as defined by a creatinine reduction ratio between days 1 and 2 (CRR2) of $\leq$ 30% has been shown to have similar outcomes to D-DGF. We applied this definition in our cohort of patients to examine outcomes.

Methods: All transplants between 1996 and 2004 at our centre were assessed and 361 included (mean age of 44 (range 17-73), 66% male). Patients were divided into 3 groups: IGF (immediate graft function; CRR2 >30%), D-DGF and ND-DGF. Follow up was for a mean of 4.2 years (SE $\pm$ 0.06).

Results: IGF accounted for 36% of patients, D-DGF 22% and ND-DGF 42%. CRR2 was inversely correlated with serum creatinine on day 7 (r =-0.673 p <0.001), day 30 (r=-0.377 p <0.001), day 90 (r=-0.256) and day 365 (r=-0.186 p=0.008). Risk of graft loss was significantly different between the groups (shown below) having adjusted for recipient age and sex, donor age and sex, HLA mismatches and immunosuppression.

Conclusion: Our study shows that CRR2 influences long-term graft outcomes. Unlike the original description however, patients with ND-DGF carried an intermediate risk and hence could be considered as early as Day 2 for calcineurin inhibitor sparing treatment regimens.



P50

The Effects Of Rosuvastatin On ^{51}Cr-EDTA Measured Glomerular Filtration Rate And Urinary Albumin Excretion In Non-diabetic Renal Transplant Recipients

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Background. Hyperlipidaemia contributes to chronic allograft injury, leading to eventual graft loss. Emerging literature from the general population suggests statin treatment has pleiotropic and renoprotective properties. This raises the possibility of using statin therapy to attenuate chronic allograft injury in renal transplant patients. This study aimed to assess the change in ^{51}Cr-EDTA measured glomerular filtration rate (GFR) and urinary albumin excretion after short-term rosuvastatin treatment in renal transplant recipients.

Methods. Twenty patients were recruited into a randomised, double blind, placebo controlled, crossover study. ^{51}Cr-EDTA GFR and urinary albumin excretion was assessed after 12 weeks of either rosuvastatin or placebo before patients crossed over to the opposite table and the investigations repeated after 12 weeks. Clinical and biochemical investigations were conducted at the end of each 12-week period.

Results. Rosuvastatin was a safe and effective lipid-lowering agent, lowering total cholesterol by 31% ($p<0.001$), LDL cholesterol by 48% ($p<0.001$) and triglycerides by 19% ($p=0.018$) when compared to placebo. It also had a significant anti-inflammatory effect, lowering C-reactive protein by 43% ($p=0.042$). However there was no difference in creatinine, estimated GFR, ^{51}Cr-EDTA derived GFR or urinary albumin excretion between rosuvastatin and placebo (Table 1).

Table 1 – Change in renal indices between placebo and rosuvastatin (mean ± standard error of mean)

	Placebo	Rosuvastatin	Change	*p* value
Creatinine (mmol/l)	112 ± 5	109 ± 5	- 3%	0.100
Est. GFR (ml/min)	61 ± 3	63 ± 3	+ 3%	0.102
^{51}Cr EDTA GFR (ml/min)	61 ± 3	62 ± 4	+ 2%	0.612
Albumin/Creatinine Ratio	2.3 ± 0.8	3.3 ± 1.0	+ 43%	0.274

Conclusions. Short-term rosuvastatin treatment does not affect renal allograft function, despite a significant lipid-lowering and anti-inflammatory effect. Whether long-term treatment slows the decline of GFR remains unanswered. The renoprotective properties of statins remain speculative and we should hesitate from advocating benefits without evidence from large, clinical studies specifically designed to answer this question.

P51

Single centre experience of the natural history of haemodialysis vascular access in kidney transplant recipients.

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Introduction: Maintaining the functionality of vascular access is central to the well-being of the haemodialysis patient. After a successful renal transplant, patency of vascular access becomes of secondary concern. In this group of patients, there is a paucity of data guiding clinicians as to whether the policy of intervening only for vascular access complications resulting in patient morbidity is best. With this in mind, our objective was to evaluate the fate of haemodialysis vascular access in our renal transplant (RT) recipients.

Methods: A retrospective study of 562 RT recipients between January 1993 and October 2007 was conducted.

Results: A total of 359 patients were on haemodialysis prior to their transplant date. Of these, 56 patients died during the period of study. Out of the 303 living patients, 259 patients have a functioning renal transplant whereas 44 have returned to renal replacement therapy (RRT). Of the 259 with a currently functioning transplant the fistula in use prior to the transplant has stopped functioning in 60 patients (51 spontaneous thromboses, 9 operative ligations). Ligations were performed for steal syndrome, Raynaud's syndrome, venous hypertension, sepsis and aneurysmal dilatation. Of the 44 patients returned to RRT, 36 demonstrated a patent, useable fistula and were all able to restart haemodialysis without disruption; one patient however elected to commence peritoneal dialysis. The remaining 8 of 44 all suffered from spontaneous thrombosis of their fistula during the functional life of their transplant. All 8 elected to return to haemodialysis thus requiring a further vascular access procedure. The distribution by anatomical site of fistulae in use prior to transplantation was as follows: autogenous (wrist: 154; forearm: 90; and cubital fossa) and PTFE grafts (groin:11 and upper limb: 6). Of these 18, 27, 32, 2,1 accesses respectively stopped functioning during the life of the renal allograft. The median patency was 730 days (range 0-4378 days) and 9 patient's access sites thrombosed within 30 days of transplantation.

Discussion: The majority of transplant patients do not suffer significant vascular access related morbidity. Most patients maintain a patent fistula throughout their transplant life. Based on this data even a rudimentary programme of active surveillance that may be used as a pretext to pre-emptive radiological or surgical intervention is not required.

P52

Pre-implantation activity of mitochondrial complexes in cadaveric renal grafts, cold ischaemia time and subsequent recovery of graft function

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Background

It is known that Cold ischemia time is associated with increased risk of delayed graft function (DGF). One explanation for DGF could be that anoxia adversely affected mitochondrial function in proportion to its duration. The purpose of this study was to examine this possibility.

Methods

Subject to informed consent obtained from potential recipients, needle biopsies were obtained from cadaveric kidneys at the end of the period of cold ischaemia and stored at -80º Celsius until subsequent analysis of mitochondrial complexes I, II/III and IV activity (indexed according to citrate synthase activity) using established methods. The relationship between these activities, CIT and the speed of recovery of renal function (time taken for serum creatinine concentration to fall to half that at the time of surgery, $Cr_{t½}$) were examined using statistical methods. Recovery of renal function was also classified according to the need for dialysis in the week following transplantation (delayed graft function, DGF) and mitochondrial function was compared between the two groups. Grafts from heart-beating donors (HBD) and NHBD and were also compared.

Results

Frequency of DGF increased with increasing CIT (p=0.01). CIT was significantly different between those without and with DGF (p=0.02) but there was no significant difference in any of the indexed mitochondrial complex activities (MCA_i). There was a significant correlation between $Cr_{t½}$ and CIT (r=0.43, p=0.003) but no significant correlation between MCA_i and CIT. Frequency of DGF, which was greater in NHBD, was significantly different from that of HBD (p=0.04). While there was no significant difference in CIT or MCA_i between HBD and NHBD, $Cr_{t½}$ was significantly different (p=0.01), being longer in NHBD.

Conclusion: The risk of DGF and duration of $Cr_{t½}$ both increase with duration of CIT. In no case were any differences in MCA_i at the end of cold ischaemia found to account for these observations. Mitochondrial function remains stable during an inevitable period of anoxia related to cold storage of kidneys. Impairment of cellular functions other than mitochondrial activity or events subsequent to cold storage such as quality of allograft perfusion and reperfusion injury could be responsible for the differences observed and need to be examined.

P53

Factors Associated With Hospitalisation Post-Transplantation In Renal Allograft Recipients

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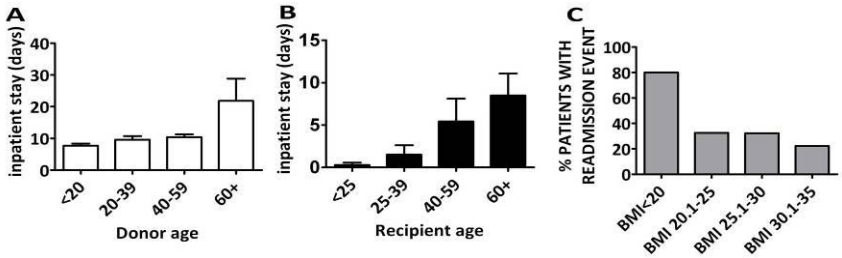
Introduction: Hospitalisation post-transplantation has significant implications both economically and in terms of patient quality of life. **Aims**: (1)To ascertain the duration of hospital stay peri-transplant (PT) and in the first 3 months post-transplantation (3Mo) in renal allograft recipients. (2)To analyse factors associated with duration of hospitalisation. **Methods**: The number of hospitalisation days in the first 3 months post-transplant and reason for re-admission were retrospectively documented in all renal transplant recipients (n=108) from an annual period (Jan 1st 2006-Dec 31st 2006) in a single UK centre. Additional data was collected on type of donor, donor age, recipient age, body mass index (BMI), cause of end stage renal failure (ESRF), ethnicity, and CMV status. **Results**: Overall duration of hospital stay was shorter in patients receiving an allograft from a living donor (mean=11.43 days) compared to a deceased donor (mean=18.31 days. Table 1).

Table 1

Admission days (mean)	Living (n=21)	Heart-beating (n=47)	Asystolic (n=40)	Deceased comb.(n=87)
Total	11.43	21.51	14.55	18.31
PT	7.67	14.33	11.13	12.75
3 Mo	3.76	6.23	3.40	4.93

A longer PT admission was associated with an older donor age (Fig. 1A) and 3Mo admission with an increasing recipient age (Fig.1B). Patients with a low BMI (<20) had a significantly increased frequency of re-admissions (Fig.1C). Diabetics did not have an increased duration of hospital stay. There were no significant differences in hospitalisation in different ethnic groups.

Figure 1



Conclusions: 1) The use of asystolic donors does not appear to be associated with an increased duration of hospital stay. 2) The use of older deceased donors and the transplantation of older recipients increases hospitalisation. 3) Low BMI significantly increases the risk of readmission.

P54

Lack of antibody rebound after ABO incompatible transplantation with anti-CD 20 induction and specific immunoabsorption

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Introduction

Blood group incompatible renal transplantation is becoming increasingly common, yet little is known about how long and how frequently blood group titres should be measured after surgery. Recent work from Freiburg has suggested that postoperative immunoabsorption (IA) may only be necessary on an 'on-demand' basis. We reviewed our experience of ABO incompatible transplants and the postoperative ABO titre response.

Methods

Six patients underwent ABO incompatible renal transplantation following pre-operative rituximab and 3 to 6 sessions of blood group specific IA (Glycorex). One patient with HLA antibodies also underwent plasma exchange. Postoperatively, 1 to 3 IA were performed within the first week. No further IA was carried out and splenectomy was not used. Immunosuppression consisted of Tacrolimus, MMF and prednisolone. ABO titres were checked daily in hospital, then weekly for a month, monthly for 3 months and then every 3 months.

Results

At follow-up ranging from 6 to 30 months (mean 16 months), all grafts are working well. 3 patients sustained mild biopsy proven acute cellular rejection, which were C4D negative and responded to steroid therapy. There was no antibody mediated rejection.

Postoperative ABO titres remained below the limits set pre-transplantation (1 in 8 for A1 or B donors, 1 in 16 for A2) in all cases throughout follow-up. Cellular rejection episodes were not accompanied by a rise in antibody titres. Patients with minimal post-operative IA showed no rise in titres.

Discussion

Our initial experience in ABO incompatible transplantation suggests that antibody rebound is uncommon, as is antibody mediated rejection. We suggest that titres should only be measured for the first month postoperatively and if rejection occurs, and that routine postoperative IA may be unnecessary.

P55

Genetically Predicted CYP3A5 Expression Influences Changes With Time In Tacrolimus Pharmacokinetics But Not Long-term Outcomes

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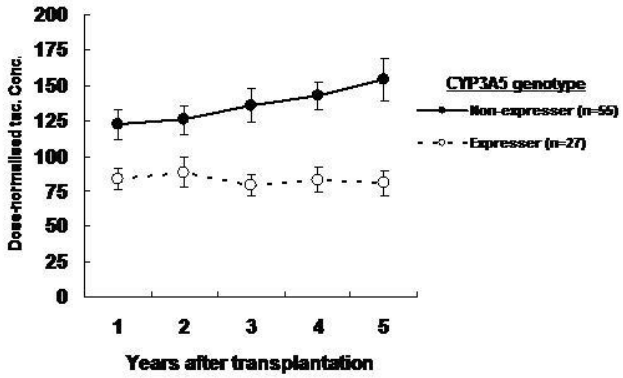
Introduction. CYP3A5 expressers achieve lower blood concentrations of tacrolimus for a given dose than CYP3A5 non-expressers. Here we confirm the previously reported observation of a time-related increase in dose-normalised tacrolimus blood concentrations over five years in CYP3A5 non-expressers, but not in CYP3A5 expressers in a cohort of patients with complete follow-up for 5 years.
Methods. Out of our 219 patients genotyped at the *CYP3A5*1/*3* SNP, we identified 82 (27 expressers; 55 non-expressers) with complete 5-year follow-up data for tacrolimus dose, blood concentration and patient weight. Dose-normalised tacrolimus concentration (ng/mL/mg/kg) was calculated annually. The study received ethical approval and subjects gave written informed consent.
Results. There was a progressive increase in the dose-normalised tacrolimus concentration at each follow up year in the CYP3A5 non-expresser group which was not seen in the CYP3A5 expressers (Fig 1, repeated measures ANOVA for change with time, p = 0.001). Exclusion of Black patients from the analysis did not alter these findings. In our full cohort of genotyped patients, there were no significant differences between the CYP3A5 expresser (n = 66) and non-expresser (n = 153) groups in graft failure and mortality rates (mean follow-up 6.3 years).
Discussion. We have confirmed that dose-normalised tacrolimus concentrations increase over time in CYP3A5 non-expressers, but not in CYP3A5 expressers in a cohort of patients with complete follow-up for 5 years.



P56

Black Renal Transplant Recipients Have Poorer Long-term Graft Survival Than CYP3A5 Expressers From Other Ethnic Groups

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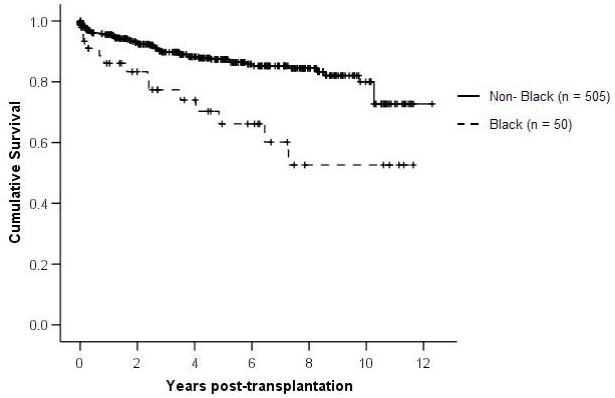
Introduction. African American renal transplant recipients have poorer long-term outcomes than Caucasian Americans, but this was not found in a French cohort.

Methods. Records of 555 (50 Black; 505 non-Black) sequential renal transplant recipients from a single UK centre from September 1995 to April 2006 (mean follow-up 3.9 years) were analysed. Our Black population was also compared to a sub-population of non-Black CYP3A5 expressers (n=42).

Results. Outcomes were significantly worse for Black patients: 5 year graft survival (Kaplan-Meier) censored for death (Fig 1, 66% vs 87%, p=0.001), and the composite outcome of graft and patient survival (63% vs 80 %, p=0.03), and time to halving of year one eGFR (mean 8.84 vs 10.84 years, p=0.008). There were more first year graft failure or mortality events in the Black cohort (14% vs 4.6%, p=0.005). Death censored 5 year graft survival was poorer in the Black population in the heart-beating deceased-donor subgroup (66% vs. 88%, p=0.02), and when compared with the non-Black CYP3A5 expresser group (66% vs. 93%, p=0.001).

Discussion. In this cohort from a UK transplant centre with socialised medicine, Black recipients had poorer long-term outcomes. This could not be explained by CYP3A5 expresser status or proportion of non-heart beating donors.



P57

Variability in HLA Mismatching by Type of Organ Transplanted and by Allocation Algorithm

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HLA mismatching (mm) in organ transplantation is an established risk factor for transplant failure and recipient sensitisation with a hierarchy of increasing risk of

-DR>-B>-A. The degree of HLA mm is influenced by both allocation algorithms which may (eg kidney) or may not (eg heart) include minimising HLA mm, and by the degree of polymorphism at each HLA locus (-B>-A>-DR). We have reviewed the incidence of HLA mismatches in kidney (861 since 2000), kidney & pancreas (91 since 2000) and heart or lung(s) (728 since 1987) transplants supported by a single histocompatibility laboratory.

	HLA-A			**HLA-B**			**HLA-DR**		
HLA mm:	**0**	**1**	**2**	**0**	**1**	**2**	**0**	**1**	**2**
610 A DBD K '00-03 06	34.1	54.1	11.8	25.7	62.9	11.3	88.0	11.3	0.7
120 A DBD K' 04 06-10 07	20.0	48.3	31.7	18.3	69.2	12.5	39.2	54.2	6.7
66 P DBD K '00-03 06	18.2	72.7	9.1	12.1	77.3	10.6	87.9	9.1	3.0
14 P DBD K' 04 06-10 07	7.1	85.7	7.1	14.3	85.7	0.0	78.6	14.3	7.1
91 A SPK '00-10 07	14.3	48.4	37.4	12.1	52.8	35.2	23.1	50.6	26.4
51 A DCD K '00-10 07	15.7	62.8	21.6	7.8	68.6	23.5	47.1	45.1	7.8
728 A DBD HL '87-10 07	10.3	46.8	42.9	3.7	33.9	62.4	7.8	44.6	47.5

A=adult recipient, P=paediatric recipient,

DBD=donation after brain death, DCD=donation after cardiac death,

K=kidney, SPK=simultaneous kidney & pancreas, HL=heart / lung(s)

The degree of mismatch in heart or lung transplantation is that achieved after random allocation ie <10% 0 –DR mm & 45% 2 –DR mm. In the '98-06 UK T allocation scheme for DBD kidneys to adult recipients almost 90% were 0 –DR mm but this fell by over half to 40% in the revised scheme; however 2 –DR mm remained few in number. Interestingly –B mm did not change. The change in mm was less for paediatric recipients. SPK organs are allocated with the aim of minimising HLA mm if possible but with a greater emphasis on waiting time which is reflected by frequencies of 25% for both 0 and 2 –DR mm. Comparative monitoring of HLA mm is important and highlights the risk of increasing numbers of HLA mm transplants.

P58

Outcome after 12 Months Following Antibody Incompatible Transplantation.

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Introduction: Although antibody incompatible transplantation (AIT) can achieve excellent early graft survival, the longer term outcomes are not well defined. It has been suggested that reduced graft function and proteinuria may be frequent.

Methods: We reviewed 28 AIT patients whose transplants functioned for over 12 months. Mean follow up was 24 (range 13-54) months; 26 transplants were performed for HLA antibody incompatibility (HLAi), 1 for ABO incompatibility (ABOi), and 1 for HLAi and ABOi. 19 were regrafts, 7 patients had pre-treatment +ve complement dependent cytotoxic crossmatch (CXM), 12 had +ve flow cytometric CXM, and 8 had HLA antibodies detectable only by microbead testing.

Results: Patient and graft survival from 12 months to latest follow up was 100%. Mean eGFR was 54.4 and 53.5 ml/min/1.73m^2 at 12 and 24 months respectively. Twenty two patients have been stable, and 6 experienced graft dysfunction. In 4 cases there was rejection, associated respectively with CMV; non-adherence; surgery for lymphocoele; recurrent urinary tract infections. In another there was non-adherence, and in another a biopsy performed just after 12 months showed no rejection (CMV and rejection at 11 months). Rejection was treated with methylprednisolone, and also with OKT3 in 1 case. Two of these patients have transplant glomerulopathy. There have been no malignancies.

In the 103 'standard' transplants performed in our unit in the same time period with function for over 12 months, mean eGFR was 51.6 and 54.0 ml/min/1.73m^2 at 12 and 24 months respectively There has been 1 death and 3 graft failures, 5 more had graft dysfunction and 1 had malignancy. Thus 10/103 (10%) 'standard' patients have had significant graft dysfunction or malignancy, compared with 6/28 (21%) AIT patients.

Discussion: 100% of AIT patients with graft function at 12 months were alive with functioning grafts, compared with 96% of patients with 'standard' transplants. However, there was a higher proportion with graft dysfunction compared to 'standard' transplants. Even after successful early outcome, patients transplanted in a programme of AIT should have careful long term follow up.

P59

Does the evidence support the use of mycophenolate mofetil therapeutic drug monitoring in clinical practice? A systematic review.

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Introduction

Mycophenolate mofetil (MMF) is used with increasing frequency as a primary immunosuppressant following transplantation. A number of factors interact to result in variability in blood levels of mycophenolic acid (MPA) increasing the risk of toxicity as well as rejection. This has led to interest in the application of therapeutic drug monitoring (TDM) to optimise its use.

Methods

A systematic literature search was performed using OVID Medline and Embase, the Cochrane Central Registry of Clinical Trials, the Transplant Library and clinical trial registries for studies investigating the clinical role of MMF pharmacokinetic drug monitoring. All studies relating monitoring regimens to clinical outcomes were included.

Results

There were 10 studies (2 RCTs, 8 retrospective) were identified that investigated the relationship between full total MPA (protein bound and free) area-under-the-curve (AUC) and clinical outcomes, showing good correlation with the risk of acute rejection, but not toxicity. Free MPA levels may better predict toxicity. There were 26 studies (1 RCT) of single time-point monitoring strategies, in particular trough levels, which showed poor correlation with the risk of both acute rejection and toxicity. In prospective studies these strategies do not improve clinical outcomes. 6 studies (2 RCTs) of limited sampling strategies using samples from the first few hours post-dose allowed good prediction of the full AUC. The results of one recent RCT suggests that a limited sampling strategy utilising a Bayesian model may improve clinical outcomes when compared with fixed dosing.

Discussion

The current data regarding therapeutic monitoring of MMF is of limited quality. The most promising results to date come from limited sampling strategies, with benefit seen in one prospective randomised trial. Further prospective trials and longer follow-up are required to investigate the optimum sampling strategy and subsets of patients who may benefit from monitoring, but the current evidence in favour of monitoring is weak.

P60

ENTERING A CLINICAL TRIAL: NO INCREASED RISK FOR PATIENT OR GRAFT SURVIVAL

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Introduction

When consent for clinical trials is requested, Patients are faced with a difficult decision. This consent reflects a balance between the unquantifed risk of the unproven intervention against any potential benefit, clinical or scientific. This study addresses the hypothesis that the choice to enter a clinical trial is associated overall with similar clinical outcomes when compared with patients who receive best current treatment outwith any clinical trial.

Methods

From July 1998 to May 2006, 180 kidney transplant recipients were entered into clinical trials. A further 807 patients underwent kidney transplantation outwith trials. Demographic, clinical and biochemical data was prospectively collected for all patients in an electronic database (Microsoft Access). Data was supplemented by review of clinical and laboratory records. Patient demographics, 1 year graft/patient survival, acute rejection rates and delayed graft function rates were compared for trial and non-trial groups (χ^2). Serum creatinine 1year post transplant was also compared (t-test).

Results

There were no significant demographic differences between groups in terms of age, cold ischaemic time or frequency of live donors. The trials group contained a significantly higher proportion of 1st grafts, 166/180 v's 623/807 ($p<0.0001$ χ^2). There were no significant differences in outcome between trial and non-trial groups in terms of incidence of DGF: 18.8% v 16.9% ($p=0.65$ χ^2), acute rejection: 21.2% v's 16.5% ($p=0.24$ χ^2), 1 year patient survival: 98.9% v's 97.8% ($p=0.49$ χ^2) or 1 year graft survival: 94.5% v's 92.9% ($p=0.55$ χ^2). Serum creatinine at 1 year was also similar between the 2 groups: 157.2 +/- 52χM v's 171+/- 70χM ($p=0.41$)

Discussion

Average outcomes in all patients entering clinical trials over an 8 year period are equivalent to those receiving treatment according to the unit protocol. Patients can be reassured that whilst risks associated with entering a clinical trial exist, such a decision is not associated with increased mortality or worse graft outcome.

P61

High Rate of Kaposi's Sarcoma Following Renal Transplant In Black African Patients

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This inner-city transplant centre serves a region which includes areas of high immigrant population with about 10% of those waiting for a transplant from sub-Saharan Africa. The purpose of this study was to examine the rate and outcome of KS in black patients of African origin. Previous studies report rates of 1-2% in Europe to 3.9% in South Africa.

Method: From 1988-2005 25 renal transplant patients of black African origin, and were followed up for at least 6 months, were retrospectively analysed. Triple immunosuppressive therapy was the baseline used in all cases.

Results: 9 (36%) patients developed KS. Mean time from transplantation to diagnosis was 45 months, median 17 months. 3 of the 9 patients received additional immunosuppression for rejection and 1 had received prophylactic ATG. 6/16 non-KS recipients were also treated for rejection.

Treatment for KS until 2006 consisted firstly of reduction of immunosuppression, and if the lesions did not regress, then chemotherapy (Doxorubicin) or radiotherapy considered.

One patient had only a small KS plaque with no oedema and which did not worsen; immunosuppression was already reduced and no further action was taken. This KS remained stable for eight years and behaved similarly to KS seen in 2 Caucasian patients.

The remaining patients had plaques and oedema. One patient with failing graft died following GI bleed. 4 pts had graft loss followed by resolution. 1 pt had graft loss and KS stomach remains, 1pt graft improved and KS resolved, 1 pt stable graft with improving KS. Of these, 2 pts had chemotherapy, radiotherapy and Rapamune; both with graft loss. 1 pt switched to Rapamune with low GFR; graft failed. 2 pts switched to Rapamune with eGFR of 30 and 50 respectively, first has improved graft function and KS resolution; other has stable function with improved KS. In 2006 there were five, and in 2007 seven black African patients transplanted. One 2006 patient was found to have a KS lesion in the lung shortly before death from atypical pneumonia.

Discussion: This is an important issue for patients from this group and must be raised during pre-transplant education and counselling. Since this was instituted no patients have decided against going on the transplant list. In one patient with eGFR 38 switch to Rapamune did not preserve the graft or stop KS. The high risk of developing KS in this group is likely to be due to reactivation of latent virus or less probably de novo acquisition/transmission from allograft or transfusion. Other studies have found HHV-8 DNA in renal transplant recipients prior to developing KS, suggesting that monitoring recipients from this sub-group may allow pre-emptive action such as reduction in immunosuppression or switch to Sirolimus-based regimen. Screening for HHV-8 seropositivity prior to transplantation would assist counselling of the patient and perhaps modification of immunosuppression in the early post transplant period.

P62

Peritubular Capillary C4d: An Uncommon Finding in Early Diagnostic Biopsies from Non Heart Beating Donor Kidney Transplants

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Introduction

Peritubular capillary (PTC) deposition of complement protein 4d (C4d), is recognized as an indirect sign of an antibody response in transplanted kidneys. Organs from NHBDs undergo primary warm ischaemia that increases the expression of MHC and endothelial antigens and may increase the risk of anti-donor antibodies. This study aimed to determine if this was the case by comparing C4d staining in paraffin-embedded, diagnostic biopsies from HB and NHB donors.

Methods

Archival biopsies from a consecutive series of NHB and HB donor kidney transplants were stained for C4d using an anti C4d polyclonal antibody (Biomedica) and immunoperoxidase staining (non-biotin, supersensitive kit, Biogenex). The biopsies were done to investigate early graft dysfunction 1 month post transplant. The sections were read by a single, blinded observer and then later correlated to pathological diagnosis and clinical outcome. Degree of staining in unscarred cortex was graded as follows: Diffuse:>50% of PTC's positive, Focal:>10 PTC's but < 50%, Minimal: 3-10 PTC's and Nil:<3 PTC's.

Results

	No of Recip's (No. of Bx's)	C4d Staining No. of Recipients			Pathological Diagnosis No. of Recipients				
		Diffuse	Focal	Min/Nil	AR	BR	ATN	Ne	Other
NHBD	21(21)	0	1	19	8	1	8	1	3
HBD	21(21)	2	3	15	9	1	10	0	1

AR- Acute cellular Rejection BR-Borderline Rejection Ne- Necrosis

Conclusion

These results suggest that an antibody response is less common in NHBD kidneys however greater numbers are required to confirm this. The early diagnosis and treatment of humoral rejection is vital for graft survival and is greatly enhanced by the detection of PTC C4d.

P63

Cardiovascular Risk Factors And Their Management In Renal Transplant Recipients

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Introduction: The commonest cause of premature death in the renal transplant population is cardiovascular disease. This retrospective study aimed to identify conventional cardiovascular risk-factors and their modification in a cohort of 229 renal transplant recipients from a group of transplant units in a single city who were transplanted in 1999 with five years of follow-up.

Methods: Data on all adult patients transplanted in the calendar year 1999 were collected into an Access database by one clinical research nurse who visited all of the units. Cardiovascular risk factors at the time of transplantation were recorded with annual measures of blood pressure (mean of 3 values closest to annual review), lipid profile and eGFR. A blood pressure standard of < 130mmHg/< 80mmHg was introduced by the Renal Association in 2002. There are no formal standards for lipid management in renal transplant recipients so those used for the general population were employed.

Results: Among the study population, 23% had a previous cardiovascular event. Five year graft survival rate was 84% and death censored graft survival rate was 94%. Mean eGFR was around 50 mL/min/1.73m^2 for the whole study period.

Only 30-40% of patients achived the target systolic blood pressure of <130 mmHg and fewer than 10% had diastolic BP <80mmHg. The mean number of antihypertensive agents was between 1.7 and 1.9. Use of ACE-inhibitors or Angiotensin-II receptor blockers and statins increased over the follow-up period. The percentage of patients with total cholesterol < 5.0 mmol/L increased from 45% to 70% over the follow-up period. Mean LDL was 2.8 mmol/L in 2000 and 2.4 mmol/L in 2005. Aspirin was prescribed for approximately 30% patients.

Discussion: In this cohort, over 50% of patients failed to meet the Renal Association blood pressure targets in spite of a mean of around two antihypertensive agents per patient. This suggests that there is scope for increasing the number of agents in an attempt to reach targets. In contrast, achievement of lipid targets through statin use was more successfully achieved.

P64

Effects Of Serum Calcium After Renal Transplantation on Allograft Function

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Introduction: Abnormalities of bone and mineral metabolism including hypercalcaemia are ubiquitous in end stage renal failure and may persist after successful renal transplantation. Marked hypercalcaemia and persistent hyperparathyroidism post transplantation often prompts parathyroidectomy or more recently, pharmacological reduction with the use of novel calcimimetic agents. However, the effects of abnormal serum calcium on allograft function are unknown. The aim of this study was to evaluate the impact of post transplant corrected calcium on graft function within the first year after successful renal transplantation.

Methods: We retrospectively evaluated the case records of 762 patients transplanted in our unit between August 1998 and July 2007. We selected recipients for which comprehensive records of serum calcium, phosphate and creatinine were available including at time 0, and at 1, 3, 6 and 12 months after transplantation. After excluding patients who were dialysis dependent at 30 days post transplantation or in whom episodes of rejection had been documented, 227 patients were included in our analysis. The effects of corrected calcium 30 days post transplantation (Ca30) on graft function were evaluated.

Results: Consistent with previous reports, graft function at day 30 (1/Cr30) predicted the rate of decline in renal function in the first year ($p<0.0001$). Ca30 did not correlate with graft function at 12 months ($p = 0.72$), nor with the rate of decline in graft function ($p = 0.71$). Surprisingly however, a low Ca30 (<2.37mmol/l) correlated strongly with poor graft function at 12 months ($p<0.0001$). This correlation was not present for Ca30 > 2.37 mmol/l irrespective of the degree of hypercalcaemia.

Conclusions: Moderate post transplant hypercalcaemia at 30 days does not adversely affect renal allograft function at 1 year, or impact on the rate of decline in graft function. However we demonstrate for the first time an association of post transplant hypocalcaemia with worse allograft function at one year. These data are congruent with worsening graft function previously reported after post transplant parathyroidectomy. Furthermore our findings question the merits of pharmacological reduction in serum calcium in the post transplant setting. Further studies are needed to evaluate more fully the role of calcium, PTH and the vitamin D endocrine axis on renal allograft function.

P65

Infective Complication Associated With Ureteral Stent In Renal Transplant Recipients

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Background: Stenting of the ureter is commonly performed during the time of renal transplantation in order to avoid early ureteric complication. However, it predisposes to infections. These infections in the immunosuppressed transplant recipients pose a significant threat to the graft and to the patient. **Aim:** Our study aimed to investigate the incidence of infections associated with stenting in renal transplant recipients.

Material and methods: A retrospective analysis of 100 consecutive renal transplant recipients in a period of one year with the follow up period of 6 months post-transplant.

Results: The patients median age was 46 (19-71 years). 75 patients received organ from deceased donors while 25 from live donors. 79 patients had stents and 18 patients had no stent. Three patients had vascular thrombosis that required early nephrectomy and were excluded from the study. There were 2 cases of ureteric stenosis (following stent removal, one required surgical correction the other was treated radiologically) and no cases of urinary leak. The incidence of Urinary Tract Infection (UTI) was significantly higher in the stent compared to the non stent group (70% vs 39% p=0.02). New episodes of UTI following removal of the stent were more common in patients who had infection whilst having a stent as compared to infection-free stented patients (54% vs 30% p =0.04).

Conclusion: Ureteric stent helps to reduce early postoperative ureteric complications (leak and stricture) but increases the likelihood of UTI. Infection wilst having a ureteric stent was associated with high recurrences rate of UTI even after removal of the stent.

P66

STEROID AVOIDANCE IMMUNOSUPPRESSION : LONG TERM EVALUATION IN LIVE DONOR RENAL ALLOTRANSPLANT RECIPIENTS

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Introduction:

Steroids had the main role in renal transplantation since more than four decades .However; chronic use of steroids was associated with a lot of co- morbidities .So we aimed to assess the long term safety and efficacy of steroid free immunosupression regimen in live donor renal transplant recipients.

Patient and methods:

Ninety eight patients were randomized to receive tacrolimus (FK), mycophenolate mofetil (MMF), and basiliximab (simulect) as an induction. Steroids were given only for 3days in (49 patients, Study group) and was maintained in (49 patients, control group).Median follow up was 36 months.

Results:

By the end of the third year, Patient and graft survivals were 100% in both groups. Biopsy proven acute rejection episodes were 16% in both groups .Mean serum creatinine was 1.34 mg/dl in steroid free group vs.1.33 mg/dl in the control group. Post-transplant hypertension was 4.1 % vs. 14.3 % respectively (p=0.08). Post-transplant D.M. was 0% vs. 26.5 % respectively (p=0.0001). Post-transplant waight gain was 6% vs. 15% respectively (p=0.001). The two groups were comparable regarding cases with hepatic impairment, serious bacterial infections or malignancies (p=>0.05).

Conclusion:

In cases with low immunological risk, steroid free regimen was safe and tolerable without morbidities in live donor kidney transplants, however long term use of steroids was associated with post-transplant diabetes.

P67

The Effects Of Rosuvastatin On Insulin Sensitivity And Secretion In Non-diabetic Renal Transplant Recipients

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Background. Previous studies by this group have examined the impact of interventions to prevent the onset of new onset diabetes after transplantation (NODAT). More recently statins have been demonstrated to have pleiotropic effects beyond lipid-lowering. It is hypothesised that statins may delay the onset of NODAT by improving insulin sensitivity and reducing the decline in insulin secretion. The aim of this study was to analyse the effect of rosuvastatin on insulin sensitivity and secretion in non-diabetic renal transplant recipients.

Methods. 20 non-diabetic renal transplant recipients were randomised to either rosuvastatin 10mg or placebo daily for 12 weeks in a randomised, double-blind, crossover study. Insulin sensitivity, first and second phase insulin secretion was assessed at the end of each 12-week treatment period with a frequently sampled, intravenous glucose tolerance test and meal tolerance test. After a 4-week washout, patients crossed over to the alternative therapy for a further 12 weeks and the investigations were repeated.

Results. Rosuvastatin was a safe and effective lipid-lowering agent, lowering total cholesterol by 31% ($p<0.001$), LDL cholesterol by 48% ($p<0.001$) and triglycerides by 19% ($p=0.018$) when compared to placebo. It also had a significant anti-inflammatory effect, lowering C-reactive protein by 43% ($p=0.042$). However no significant difference was observed in insulin sensitivity or secretion (Table 1).

Change in insulin sensitivity and secretion: placebo vs rosuvastatin (mn ± sem)

	Placebo	Rosuvastatin	*p* value
First-phase insulin secretion (pmol/l.min)	2693 ± 365	2785 ± 455	0.875
Second-phase insulin secretion (pmol/l.min)	67082 ± 8937	67393 ± 11748	0.983
Insulin sensitivity ($10^{-5}min^{-1}$/_U/ml)	4.8 ± 0.5	4.4 ± 0.6	0.580

Conclusion. Short-term rosuvastatin was not associated with an improvement in insulin sensitivity or secretion, despite significant lipid-lowering and anti-inflammatory effects. Whether statins can attenuate the development of NODAT in the long-term is questionable and is not supported by the findings of this study.

P68

Laparoscopic Donor Nephrectomy: Effects of Large volume intraoperative fluid administration

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Introduction: Administering large volumes of fluids to minimise the effects of pneumoperitoneum during Laparoscopic donor nephrectomy (LDN) remains controversial. Such practice frequently leads to fluid overload and increases the risk of developing pulmonary oedema in otherwise healthy individuals. To our knowledge the incidence of pulmonary oedema and risk factors in laparoscopic kidney donors has not been demonstrated clearly. We reviewed our series of LDN to ascertain the adverse effects of high volume fluid administration and ways to minimise the risks.

Methods: Medical records of 117 consecutive donors who underwent LDN at our institution were reviewed. A set criterion was followed in identifying donors who developed pulmonary oedema in the immediate postoperative period. The demographic and perioperative data of donors with pulmonary oedema were compared with donors with no adverse effects to assess any risk factors. We also assessed impact of Oesophageal Doppler used in latter part of the series. Non-parametric tests were used for continuous data and categorical data were compared with Chi-squared and Fisher's exact tests as appropriate.

Results: In our series donors received on average 11.9mls/kg/hr to maintain urine output of over 100mls an hour as per our protocol. Of these donors five (4.3%) of them developed pulmonary oedema in the recovery room needing further treatment. Comparison of intraoperative fluids showed that the donors with pulmonary oedema received significantly higher volumes of fluid (14.1 vs 11.0, p0.05). Interestingly the donors with no adverse effects received far less colloids (2.2 vs. 7.9, p0.001) and more crystalloids (9.6 vs. 6.9) when compared with other donors. The donors developing pulmonary oedema were tended to be older than the other group but showed no statistical significance (57 vs 46 years, p0.106). There was no difference noted in ASA status, comorbidities, length of stay, and discharge creatinine. In donors monitored with oesophageal doppler received slightly less fluids than the group with no monitoring (10.2 vs 12.8, p0.09) but there were no difference in recipient outcomes.

Conclusion: Though all patients with pulmonary oedema had no adverse sequalae, this complication had found to be associated with administration of large volume of colloids as opposed to crystalloids. The benefits of using oesophageal monitoring are not clear in this cohort a randomised controlled trial is needed to assess the merits.

P69

An Audit of Sun-Protection Practices Amongst Renal-Transplant Recipients in Northern Ireland

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Introduction

Non-melanoma skin cancer (NMSC) is the commonest form of cancer following solid-organ transplantation. Renal transplant recipients are often immuno-suppressed for many years, and therefore skin cancer incidence amongst this group is high. Exposure to UV radiation is an avoidable risk, and patient education regarding good sun-protection practices is essential. Northern Ireland has a renal transplant population of 585, with approximately 400 of these patients attending a single tertiary referral centre. We performed an audit of sun protection practices in the renal transplant clinics, with the aim of assessing levels of patient education and compliance. Patients in our centre are given education sessions pre-and post-transplant by a renal education nurse, during which verbal, written and DVD information is given regarding sun-protection and skin cancer risk post-transplantation.

Method

Patients attending the renal transplant clinic were invited to participate in the study by completing a questionnaire consisting of 32 questions.

Results

Two hundred and twelve patients completed the questionnaires, (males=55%, females=39%).Time from transplant ranged from 1-35 years. Multiple immunosuppressive regimes are currently in use. Sun-protection advice was recalled as being given pre-transplant in 64% of patients, and post-transplant in 75%. Fifty-seven percent of patients did not recall warnings of long-term skin cancer risk. No sun-protection was used in 11% of patients. Regular self-examination of skin was performed by 63.5%. Eighty percent of patients grossly over estimated their skin type. Fifteen percent had had surgical excision of skin lesions, and 20% regularly attended a dermatologist. Skin cancer had been diagnosed in 9.5% of patients, most of which were NMSC.

Discussion

As shown in previous studies, poor sun-protection compliance is common amongst this patient population. Patient education needs to be regularly reinforced during the post-transplant follow-up period.

P70

Vacuum-assisted closure therapy (VACT) in the management of wound infection following renal transplantation

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INTRODUCTION: Wound infection in the setting of an immunosuppressed state such as after renal transplantation (RT) causes significant morbidity from sepsis, prolongs hospital stay and is expensive. Vacuum-assisted closure therapy (VACT) is a new technique of management of wound based on the principle of application of controlled negative pressure. The aim of this study was to assess the efficacy of VACT in the management of wound infection following RT.

METHODS**:** This is a prospective study of a cohort of 237 consecutive RTs performed over a period of 5 years, where the data were retrieved from a prospectively maintained computerised database and case-notes.

RESULTS: 10 of 237 (4.2%) patients developed deep wound infection following RT leading to cavitations and dehiscence with copious discharge, which refused to heal with conventional treatment. All 10 cases were treated with VACT. The VACT system was removed after a median of 9 (range 3-30) days when discharge from the wound ceased. Five patients were discharged home with portable VACT device and managed on an outpatient basis, where the system was removed after a median 5.5 (range 3-7) days. The median hospital stay after initiation of VACT was significantly shorter (4, range 2-12 days) than on conventional treatment prior to VACT (11, range, 5-20 days) . Complete healing was achieved in all cases. No complications related to VACT such as haemorrhage and intestinal fistulae were observed in this series.

DISCUSSION: The use of VACT is an effective and safe adjunct to conventional and established treatment modalities for the management of wound infection and dehiscence following RT.

P71

Transperitoneal hand-assisted laparoscopic live donor nephrectomy : a single centre experience

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INTRODUCTION: Laparoscopic live donor nephrectomy is being increasingly practised. It reduces the disincentives to live kidney donation and is associated with less post-operative pain, shorter hospital stay, early return to work and better cosmesis. The purpose of the study was to assess the impact of hand-assisted laparoscopic live donor nephrectomy (HLLDN) on donor and recipient outcomes and compare these with the established technique of open donor nephrectomy (ODN).

METHODS: Over a period of 7 years, commencing 2000, 72 live donor nephrectomies (HLLDN = 33, and ODN = 39) were performed. Data was retrieved from a prospectively maintained departmental computer database and case notes. HLLDN was performed through transperitoneal and ODN through retroperitoneal flank approaches.

RESULTS: Donor age (HLLDN 42 ± 11 vs. ODN 43 ± 10 years) and sex (HLLDN 16 males, 17 females vs. ODN 14 males, 25 females) was similar in both groups. The total anaesthetic time was longer in HLLDN group (267 ± 52 vs. 174 ± 42 minutes, $p < 0.001$). The mean serum creatinine of the donors at discharge and at 1 year was higher in the HLLDN group (116 ± 22 vs. 101 ± 21 µmol/l, $p<0.05$ and 115 ± 21 vs. 103 ± 20 µmol/l, $p<0.05$, respectively), which would correlate with higher body mass index in the HLLDN group (28 ± 3 vs. 26 ± 3 kg/m^2, $p<0.05$). The recipient serum creatinine at discharge and at 1 year was similar in both groups (140 ± 88 vs. 147 ± 66, $p=0.72$ µmol/l and 128 ± 52 vs. 146 ± 41 µmol/l, $p=0.17$, respectively). Duration of post-operative hospital stay was shorter in the HLLDN group (3.7 ± 1.1 vs. 6.4 ± 1.4 days, $p<0.001$) as was time to return to work (6.0 ± 1.6 vs. 12.5 ± 4.7 weeks, $p<0.001$). Delayed graft function was observed in 4 of the HLLDN group in comparison to 3 of the ODN group. No significant post-operative complications occurred in the HLLDN group. Three patients required conversion to an open procedure due to dense adhesions between the kidney and perinephric fat. All three were athletic young males.

DISCUSSION: HLLDN is a safe alternative to ODN. It has distinct advantages over ODN with respect to shorter hospital stay, early return to work and cosmesis without compromising graft function.

P72

Initial Tacrolimus Dose In Black Renal Transplant Recipients

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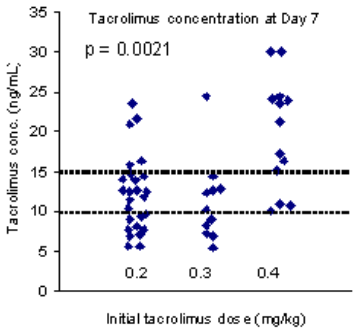
Introduction: Black renal transplant recipients require higher tacrolimus doses to achieve therapeutic blood concentrations compared to Caucasians, resulting in delay in achieving target blood concentrations with increased risk of rejection.

Methods: Sequential cohorts of patients transplanted at a single centre were studied. An initial daily dose of 0.2 mg/kg (n=26) was employed from 1995-2002. Increase in the dose to 0.4 mg/kg (n=13) from 2002 in response to most patients being under immunosuppressed resulted in initial blood concentrations in the toxic range (as reported previously). In 2005 an initial dose of 0.3 mg/kg was adopted.

Results: An initial dose of 0.4 mg/kg resulted in higher d7 blood concentrations (Kruskall-Wallis p=0.0012) than 0.2 mg/kg but an initial dose of 0.3 mg/kg did not (Fig). On day 7, 5/11 patients in the 0.3 mg/kg group had concentrations below the minimum target of 10 ng/mL. By the second week the influence of therapeutic drug monitoring eradicated the difference between the dosing regimens. Acute rejection occurred in 50% of the 0.2 mg/kg group, 36% of the 0.3mg/kg group and 38% of the 0.4 mg/kg group. Interpretation is rendered complex by the introduction of routine use of basiliximab in the 0.3 and 0.4 mg/kg cohorts.

Discussion: In Black patients, there appears to be a flat dose-response curve in the 0.2-0.3 mg/kg range that steepens with higher doses. This would be compatible with a saturation of an active barrier to drug absorption by higher doses. This sequence of protocol changes has failed to identify an optimal initial dose of tacrolimus for black patients and an alternative strategy such as blocking the barrier to drug absorption with diltiazem may be more successful.



P73

A Comparison Of Laparoscopic Donor Nephrectomy (LDN) And Open Donor Nephrectomy (ODN): A Systematic Review Of The Literature

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Introduction: 21% of renal transplants come from a living donor and surgical removal was primarily an ODN retroperitoneal procedure[1]. However, Ratner and colleagues[2] performed the first LDN in 1995 and this method of organ retrieval has been gaining acceptance. Concern has been expressed as to the mortality, warm ischaemic time, graft survival and cost implications of LDN. A systematic review of the current literature was performed to compare these outcomes between ODN and LDN.

Methods: A systematic search of medical databases (PubMed, Medline, Cochrane Controlled Trials Database, PsychINFO, EMBASE and CINHAL, 2002-2007) were performed to obtain all studies comparing ODN versus LDN, which were appraised critically and used to grade recommendations for evidence-based good practice[3].

Results: Mortality was not found to be significantly different between ODN and LDN in three systematic reviews (SR) or 7 randomised control trials (RCTs). Three SRs and three RCTs showed LDN have similar 1 year survival rates compared with ODN, even though warm ischaemic time was longer in the LDN group in three SRs and 6 RCTs. Three SRs and two RCTs found that direct operative costs were significantly increased in LDN. When this was compared to the number of days saved as patients are discharged earlier than ODN patients the costs are much more equivalent.

Conclusions: LDN is certainly feasible to perform and desirable to patients with an equivalent mortality rate [Grade A recommendation]. LDN has not effect on 1 year graft survival compared to ODN [Grade A recommendation]. Adoption of LDN should not be discouraged due to cost implications [Grade B recommendation].

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P74

Cadaveric kidney retrieval damage: how accurate is the data?

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Introduction

Most data relating to the injury of organs at retrieval come from registry sources; however the accuracy of these data are unknown. The damage recorded on the national transplant database includes that which has been centrally reported by retrieval and/or transplanting teams. The aim of this study was to compare the information held nationally on damage to the vasculature of kidneys retrieved from cadaveric heart beating (CHB) donors with that recorded locally by a single centre.

Method

We identified all adult CHB kidney transplant recipients between 1st Jan 2000 and 31st Dec 2005 in our centre. The operative notes were interrogated to record the assessment of the donated kidney by the transplanting surgeon. Locally recorded vascular damage was compared with the central data held on the national transplant database.

Results

291 adults underwent CHB renal transplantation; of these 267 (92%) sets of notes were interrogated, and this was taken as the study population. 225 (84.3%) had no retrieval damage identified either on the operative note or the national database. 15 (5.6%) had retrieval damage identified and recorded both locally and centrally. 16 (6.0%) had retrieval damage which was not recorded centrally, but was recorded on the operation note. 11 (4.1%) retrieved kidneys were recorded as damaged on the national database but as non-damaged on the transplanting surgeon's operative record. The majority of these grafts had lacerations to the Carrel patch, and not the renal vein or artery itself.

Conclusions

In our centre half the damage to retrieved organs identified by the transplanting surgeon is not being reported to the national transplant database. Improved reporting by surgeons and co-ordinators must be encouraged and facilitated to maintain the validity of the national database.

P75

Development Of A Nomogram That Predicts The Probability Of Graft Survival Following Kidney Transplantation From Living Donors.

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Introduction: The goal of this project was to develop a nomogram that predicts the probability of graft survival at 5-year.

Patients & Methods: Out of our dataset; 1581 patients were utilized for construction of the nomogram (modeling group), the remainder 319 patients (testing group) were utilized for its validation. Initially, the modeling group variables were correlated to the graft survival by univariate analysis. Significant factors were subjected to a multivariate statistics using Cox model; their result was the basis of our nomogram construction. Internal validation was carried out; first by discrimination using the Concordance index (C-index). Second, calibration was assessed graphically. Finally, external validation; the nomogram was utilized to predict the graft survival using testing group. The predicted probability(s) was compared with the actual survival estimates.

Results: The validation of the nomogram yielded a concordance index of 0.77 and the observed correspondence between predicted and actual outcomes suggested a high level of calibration. Nomogram predictions of the testing group revealed no differences in means of predicted and observed graft survival at 5-year with a high correlation coefficient and accepted predictive accuracy (C-index was 0.72).

Conclusions, We have developed a well validated and a reasonably precise nomogram for prediction of the 5-year graft survival among patients who receive a kidney from a living donor. This model provides several advantages: it is simple to interpret by clinicians. Accordingly it can be used in decision making without a need to carry out any sophisticated calculations. In addition it provides a more tailored probability than classification by risk group. It is flexible to incorporate new predictors when indicated.

P76

The Incidence And Management Of Kaposi's Sarcoma After Kidney Transplantation Reported By UNOS Between 1994 And 2005

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Introduction

The aim of this study was to identify all cases of post-kidney transplant Kaposi's Sarcoma (KS) reported by the United Network for Organ Sharing (UNOS), to assess their management and review the graft and patient survival.

Methods

Data on the incidence and treatment of KS in the US between 1994 and 2005 were retrieved and analysed by UNOS*.

Results

Based on OPTN/UNOS data as of December 14, 2007, there were 57 (0.04%) reported cases of KS in 158,008 kidney transplant recipients. Of these, 43 (75.4%) patients were male. The mean time to diagnosis was 728.1 days (SD=652) after transplantation. Almost all patients (53, 93%) were receiving a calcineurin inhibitor and/or an antimetabolite agent as maintenance immunosuppression. Upon diagnosis of KS, management was as follows: immunosuppression was stopped for 7 (12.3%) patients, reduced for 40 (70.2%), not adjusted for 5 (8.8%) and not reported for 5 (8.8%). Graft survival was 91.2% (52/57) and 56.6% (24/57) at one and 5 years respectively. Patient survival was 98.2% (55/57) and 75.8% (27/57) at one and 5 years respectively.

Conclusions

UNOS data suggest that the incidence of KS in kidney transplant recipients is low (0.04%) as compared to the rates of 0.5-5% found to be associated with organ transplants general (Marcelin *et al*, 2007). The patient survival rates described above seem to be better than US figures published previously by the Israel Penn International Tumour Registry (Hannaway *et al*, 2001), where patient survival was 88% and 66% at one and five years respectively. This group described 63 patients, of which 52 had kidney transplants that occurred between 1985 and 2001.

*This work was supported in part by Health Resources and Services Administration contract 234-2005-370011C. The content is the responsibility of the authors alone and does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

P77

Maximisation of the living donor pool: Incidental stones and metabolic abnormalities in potential kidney donors, an increasingly common challenge.

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Introduction;

An increasing renal failure population requires maximisation of the living-donor pool. UK Transplant guidelines exclude donors with a current stone but we consider them extended criteria donors and are keen to facilitate safe use of these kidneys. We examine potential donors with asymptomatic stones since 2004.

Methods:
A retrospective analysis of 377 consecutive potential donors that proceeded to CT angiography were evaluated at our institute. A non-contrast phase of the CT was performed, reported by a consultant radiologist. Patients found to have incidental stones were further investigated for metabolic abnormalities with serum and urinary stone screen. Donor assessment was by a multi-disciplinary living donor team and patient records examined for this study.

Results:

19 (5%) had asymptomatic stones (size 1 - 8.5mm) including 3 bilateral and 2 multiple unilateral. 13 had no previous stone history. 15 proceeded to have a metabolic screen (4 were excluded from the donor program for multifactorial reasons); 7/15 had hypercalciuria (46.6%) of whom 3 had no previous stone history. 3 people donated with <3mm stone and no metabolic abnormality, 3 are still being evaluated and in the others an alternative donor was found. None underwent pre-donation stone treatment. Donor and recipient follow up (3 to 36 months); no stone-related complications or detrimental effect to graft outcome.

Conclusions:

The frequency of incidental stones and metabolic abnormalities emphasises the importance of multidisciplinary management. Hypercalciuria was common even in those with no stone history. Donors and recipients should be fully counselled of potential risks. There are contraindications to potential stone donation to protect both donors and recipients against stone-related morbidity and mortality. These include lack of emergency urology expertise in managing stones in solitary kidney; bilateral stones; infection stones and untreatable metabolic problems.

Donation with a small (<3mm) stone in situ or a correctable metabolic abnormality may be appropriate. Larger patient cohort and longer term follow up are needed for firm conclusions.

P78

Soluble CD30 and Acute Rejection in Renal Transplantation

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Introduction

The CD30 molecule is a transmembrane glycoprotein structurally similar to tumour necrosis factor. The physiological role of CD30 is not completely understood but it is known to co-regulate the balance between T-Helper cell 1 and T-helper cell 2. Serum levels of the soluble form of the molecule (sCD30) have been shown to correlate with T cell activation state. We examined the association between sCD30 levels prior to renal transplantation and incidence of acute rejection.

Methods

This was a retrospective study of 118 renal transplants carried out in one centre. A commercially available ELISA was used to analyse time of transplant sera. A 'high' sCD30 level was set at ≥100U/ml. Clinical data including glomerular filtration rate(GFR) at 4 weeks and 12 months as well as diagnosis of rejection during the first year post-transplant was compiled and examined for associations with sCD30 levels using Fisher's exact test.

Results

Thirdteen patients experienced at least one episode of acute rejection within the first 6 months. Four patients were excluded (three adults died with functioning grafts and one adult experienced immediate primary graft failure). There was a significantly higher levels of sCD30 observed in patients with rejection than those who did not have any episode of rejection(p=0.008). Other details are summarised in Table 1.

Table 1 shows Renal Transplant Recipient Demographics

Patient characteristic	No rejection	Rejection in 1st 6 months
Number	95	13
Average age in years	46 (4 to 75)	40 (17 to 63)
Male:Female	57:38 (1.5:1)	7:6 (1.2:1)
Anti-HLA IgG at time of transplant	5	1
Average HLA mismatch*	2.01	2.46
sCD30 level high:low	55:40	10:3

*HLA mismatch score out of 6 (across HLA-A, B, DR)

Conclusion

Measuring sCD30 levels in renal transplant recipients prior to transplantation is simple, non-invasive and could potentially aid in predicting patients who may have increased risk of acute rejection. Patients with high sCD30 being twice more likely to have rejections than those with low sCD30. Interestingly, no association of sCD30 levels and reduced GFRs was observed between the two groups at 12 months post-transplantation.

P79

What happens after a third, fourth and fifth kidney transplant?

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INTRODUCTION

Encouraged by the outstanding results of primary kidney transplantation, more and more patients are being subjected to re-transplantation. Transplantation becomes increasingly more demanding at each subsequent attempt both due to immunological and technical issues. We present an analysis of outcome after third, fourth and fifth kidney transplants.

PATIENTS AND METHODS

Patients undergoing third, fourth or fifth kidney transplants at our centre from 1993 to 2005 were included. Data were collected from hospital records about patients' demographics, HLA mismatches, PRA, and donor type. Graft survival estimates were done by Kaplan Meier method.

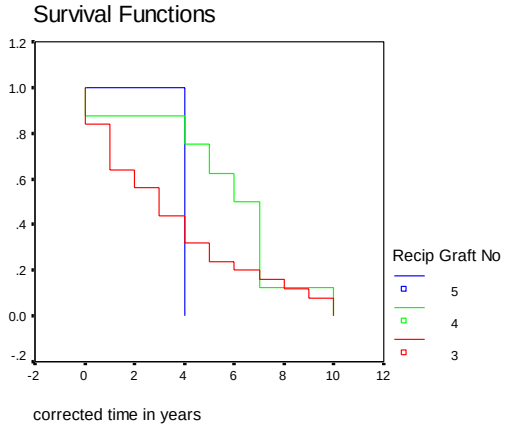
RESULTS

There were 34 patients (20 males and 14 females) with mean age 31.6 (SD±14) years. Twenty-nine (85.3%) were white, 3 (8.8%) black, and 2(5.9%) oriental. Deceased donors were 28 (82.3%) and living donors were 6 (17.6%). Mean HLA mismatches were 1.4±1.8. Mean PRA was 52.8±40.1. Twenty-five had 3rd, 8 had 4th and one had a fifth transplant. Sixteen grafts (47%) failed. Graft survival at one, three and five years after a third transplant was 84%, 64%, and 32% and after 4th or 5th transplant together was 87%, 75% and 62 % respectively.

Further analysis of 4th and 5th transplants showed to have a very high PRA level (mean 80.14), rejection being the main cause of their previous graft failure (three in patient with 5th transplant and all in four out of 8 patients with 4th transplant), a higher incidence of primary graft failure (33.3%) and renal artery stenosis (33.3%). They also had a high incidence of transplant nephrectomy. At mean follow up of 4 years, 67% of their current grafts were still functioning with mean serum creatinine of 147mmol/L.

CONCUSION

Graft outcome after 3rd, 4th, and 5th transplants is quite satisfactory despite previous graft failure. Therefore multiple transplants failures may not necessarily preclude further transplantation.



P80

Pre-operative cardiac assessment of potential renal transplant patients does not alter survival

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Introduction: Patients with ESRF have increased risk of premature cardiovascular (CV) death is. Pre-transplant cardiac assessment is practised commonly but benefits and predictive value of intervention remain uncertain. We examined results of cardiovascular assessment and outcome.

Methods 300 ESRF patients underwent cardiac investigation prior to renal transplantation. After clinical history was obtained, examination, ECG, Bruce protocol exercise tolerance testing (ETT) and ventricular assessment by cardiac MRI were performed. Patients with high index of suspicion of coronary artery disease (CAD) from non invasive assessment underwent coronary angiography and percutaneous coronary intervention (PCI) if indicated.

Results: 222 of tested patients were placed on the renal transplant waiting list; 80 patients were transplanted during the follow-up period and 60 died (7 following transplantation). Successful transplantation was associated with improved survival (mean survival 4.5±0.1 years vs listed not transplanted 4.1±0.1 years vs not listed 3.1±0.2 years;$p<0.0001$). Ninety nine patients underwent coronary angiography; 49 had no or mild CAD and 50 (16.7%) significant CAD. However only 14 patients (4.6%) were treated with PCI. There was no apparent survival difference between patients who underwent PCI or CABG compared to those who underwent angiography without intervention (n=85) or no angiography(n=201;$p=0.692$). Univariate Cox survival analyses demonstrated older age at screening, past medical history of IHD, ETT performance, history of diabetes mellitus (type 1 or 2;DM) and previous peripheral vascular disease (PVD), TIA or CVA as factors significantly associated with death. Multivariate analysis identified older age, history of diabetes mellitus, IHD, and inability to exercise for more than 6 minutes as independent predictors of death. We formulated a score based on multivariate analyses (ETT duration: >6mins =0, <6min =1, unable to exercise=2; presence of IHD=1 and DM=1). Survival was significantly higher in patients with a score of 0 (n=79, 4.6years SD 0.1) compared to patients with scores of 1, 2 or ≥3 (4.0years SD.2, 4.0 years SD 0.2 and 2.8 SD 0.3 years respectively; $p<0.001$ log rank test)

Conclusion Pre transplant CV screening provides little benefit in identifying and treating CAD. However it provides information regarding survival.

P81

Transplanting the Obese Kidney Transplant Recipient – Current UK Opinion

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Obesity is an increasing problem in Western populations. End stage renal failure patients are as likely to be afflicted as any one else. Kidney transplantation in the morbidly obese recipient presents many challenges to the multi-disciplinary management team. At present there is no consensus opinion in the transplant community. There are no BTS guidelines for the management of obese patients and only suggestions in the Renal Association guidelines.

A postal/e-mail questionnaire was sent to all the consultant surgeons involved in kidney transplantation nationally to gauge their current views.

65/96 (67.7%) questionnaires were returned. 16 consultants were also involved in liver transplant programmes and 23 in pancreas transplantation. A majority (n=54) stated that their local unit had a policy for listing of obese kidney transplant recipients but it was unclear whether people knew of any guidelines that were provided by the renal association or BTS. 16/65 would be happy to transplant the morbidly obese recipient (BMI>35kg/m^2) but most specified other criteria such as no associated cardiovascular morbidity. 28 consultants felt we had a “duty of care” to offer all patients live donation even if they were morbidly obese. 60 agreed that these patients would have an increased post-operative stay but there was no consensus opinion for other complications or outcomes. It was not felt necessary for transplantation in the morbidly obese to be centralised to specific units (51/65).

Transplantation in the morbidly obese recipient is a controversial topic of discussion. It will become more of a burden on the transplant team as the incidence of obesity increases in Western populations. In order to be able to provide a reproducible service to the patient that offers not only equity of access to all but also good results, opinions need to be unified and appropriate guidelines potentially formulated. The results of this study have demonstrated the lack of consensus in the consultant transplant community, which is an issue that needs to be addressed.

P82

Impact of Cytomegalovirus (CMV) Status on Outcome in Two Immunosuppression (IS) Eras

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Introduction: Previous studies have shown conflicting effects of CMV serological status on renal transplant outcome. We investigated the impact of differing donor (D) and recipient (R) CMV status pre-transplantation on 1 year graft survival in steroid using and steroid avoiding immunosuppression regimes. **Methods:** All adult recipients of 1st renal allografts were included. High immunological risk recipients were excluded. Cohort 1 (2001-3, n=217) mainly received CyA/Aza/Pred as IS. Cohort 2 (2004-6, n=258) mainly received a steroid avoidance regime with Tac/MMF. All received methylprednisolone 1g IV at induction. Cohort 2 patients received IV basiliximab 20mg (days 0 & 4), as did living donor transplants and diabetics in Cohort 1. Mismatched recipients (D+R-) received prophylaxis with oral ganciclovir (Cohort 1) or valganciclovir (Cohort 2) dosed according to GFR, for 3 months. Suspected CMV disease was confirmed with DNA-PCR. Treatment consisted of IV ganciclovir, and reducing the antiproliferative agent. Rejection was treated with 3 consecutive pulses of IV methylprednisolone. After rejection episodes in the steroid avoidance group, oral prednisolone was given. We analysed graft and patient survival at 1 year by mismatch category (D-R-,D+R-,D-R+,D+R+) and compared between IS cohorts. **Results:** Demographics were similar between the Cohorts. Data on CMV status for both donor & recipient were available for 142 Cohort 1 patients (65%), and 188 Cohort 2 patients (73%). 90/142 (63%) Cohort 1, and 170/188 (90%) Cohort 2 received IS as expected. Variations in intended IS regimes meant 25% of Cohort 1 were exposed to MMF, and 4% of Cohort 2 were not. CMV disease occurred in 5% Cohort 1 and 1% Cohort 2 (p=0.042). Acute rejection rates were 12% Cohort 1 and 18.6% Cohort 2 (p=0.075). In Cohort 1, D+R+ graft survival was significantly lower than in the rest of the Cohort (88% vs 97%, p=0.007), and lower than survival of D+R- grafts (p=0.021). D+R+ combinations had reduced patient survival compared to the rest of Cohort 1 (92% vs 99%, p=0.045). There were no significant differences comparing other mismatch groups, nor in graft or patient survival at 1 year. **Discussion:** D+R+ combinations had poorer graft and patient survival in the earlier, steroid using, regime, but not in the steroid avoiding regime. D+R+ grafts could be considered for CMV prophylaxis, especially where steroids are routinely used.

P83

Factors Affecting Outcomes From Transplants With Non-Heart Beating Donor Kidneys

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Introduction: Kidneys from non-heart beating donors are used to increase the number of kidneys for transplantation. We analysed our results to determine the factors most likely to affect outcomes from transplants using these kidneys.

Methods: Retrospective analysis of 56 consecutive transplants using kidneys from *controlled* non-heart beating (NHB) donors at a single center between 2000 and 2006. Kidneys were subjected either to pulsatile machine perfusion (n=32) or static cold storage (n=24). A tacrolimus and steroid based immunosuppression regimen was used. Delayed graft function (DGF) was defined as the need for dialysis after transplant or a Creatinine Reduction Ratio (CRR) at day 2 of less than 30%. Comparison of outcomes were made against 223 heart beating (HB) cadaver kidneys transplanted during the same period and also against 17 static cold storage preserved *uncontrolled* NHB kidneys retrieved from the Accident and Emergency Department between 1997 and 2000.

Results: There were more male NHB donors who were also older than HB donors. There was as no difference in the recipient age or the cold ischaemic times among the 3 groups. Warm ischaemic time was significantly longer among controlled NHB than HB kidneys, 17 mins and 0.7 mins (p=0.000), respectively, but not different among *controlled* and *uncontrolled* NHB kidneys. HB cadaver grafts had significantly better (p=0.019) 1 year graft survival than *controlled* NHB grafts, 95% and 84%, respectively. Although *controlled* NHB grafts had better 1 year graft survival than *uncontrolled* NHB grafts, 84% and 78%, respectively, this difference was not statistically significant. Pulsatile machine perfusion reduced the incidence of DGF defined by CRR from 91.7% to 71.9%. Although machine perfusion had no impact on graft outcome at 1 year, calculated GFR at 1 year was significantly better (p=0.043), being 54.3 and 40.3 mls/min, respectively. Among grafts from *controlled* NHB donors that were functioning beyond 1 year, the calculated GFR was maintained at above 45 mls/min for at least another 3 years for majority of the grafts. An intriguing finding was that *controlled* NHB grafts with CCR defined DGF and dialysed had better outcomes as measured by the 1 year graft survival than those that were not dialysed, 93.1% versus 56%, respectively (p=0.003). This was also reflected in the calculated GFRs at 2 years among the functioning grafts. This was not seen among grafts from HB donors. This was confirmed by multivariate logistic regression analysis which showed that the odds ratio for graft survival was 12.3 among those dialysed.

Discussion: Grafts from *controlled* NHB donors have better outcomes than those from *uncontrolled* NHB donors. Calculated GFR of surviving grafts from NHB donors are as good as those from HB donors at various time points from 1 to 5 years. Pulsatile machine perfusion reduced the incidence of DGF but had little impact on 1 year graft survival. Dialysing patient with CRR defined DGF appeared to improve outcomes among grafts from controlled NHB donors. If confirmed, CRR DGF may be a trigger to initiate dialysis in order to improve the outcome of transplants using these grafts.

P84

Complement activation in antibody incompatible renal transplantation

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Introduction: HLA antibody incompatible transplantation (AIT) is associated with an increased risk of acute antibody-mediated rejection (AMR). Complement activation plays a pivotal role in AMR and the Banff classification recognises C4d deposition in peritubular capillaries as a cardinal feature of AMR. We sought to look at complement activation in AIT patients.

Methods: 19 patients received AIT and most were treated with pre-transplant plasmapheresis (PP). Samples were obtained at various time points and analysed by ELISA for C3a and C4a (plasma), complete classical (CCP) and alternative complement (ACP) pathways activation (serum). Urine complement factors C3a and C4a excretion were also measured. .

Results: ACP: Mean activation post-surgery was 62% but no activation was seen at other time points.

C3a: Normal level is <0.2µg/ml. Mean C3a level were persistently elevated but there was a drop post-PP ($p=0.026$) and post-operatively ($p=0.041$) but no change at other time points. 7 of 16 patients developed an episode of rejection. No significant difference was seen between the two group at any time.

CCP: There was no mean systemic activation of the classical pathway observed.

C4a: Normal C4a level is <0.9 µg/ml. Mean C4a levels were within the normal range. There was no fall in mean plasma C4a levels with PP but a drop was seen post-surgery ($p=0.013$) and with HLA resynthesis ($p<0.001$). 9/19 patients developed rejection – no significant difference was seen between the 2 groups. However, C4a was significantly higher in rejectors with C4d on renal biopsy than in non-rejectors if measured at peak anti-HLA antibody levels ($p = 0.016$).

We did not detect any urine complement excretion even during episodes of proteinuria.

Discussion: Whilst there was activation of complement factor C3, we found this to be uniformly raised with no correlation to clinical events. The alternative but not the classical complement way appeared to be activated with surgery. A significant finding was the rise in C4a levels in the group of rejectors with C4d staining on biopsy. More samples need to be analyzed to arrive at a more definitive conclusion

P85

Hand-Assisted Laparoscopic Donor Nephrectomy: Results from Single Centre

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Background: Live kidney donation is increasingly required to meet the demand for renal transplantation. To reduce the morbidity associated with traditional open donor nephrectomy (ODN) a shift towards minimally invasive donor procedures has occurred. In this presentation we compare our experience with hand-assisted laparoscopic donor nephrectomy (HALDN) and ODN.

Methods: Data were prospectively collected from 145 HALDNs and outcomes compared against 35 historic ODNs. All procedures were performed at our center. HALDN was introduced in early 2004 and offered to all donors thereafter irrespective of factors such as body habitus and vascular anatomy. SPSS package was used to determine t test and chi square test ($p < 0.05$ was considered significant).

Results: Between April 2004 and September 2007, 145 HALDNs were performed. All ODNs were left sided. Right HALDN was performed due to the presence of a left-sided inferior vena cava. Both groups were matched for age, body weight, preoperative haemoglobin and pre-op serum creatinine. Between the groups, there was no statistical difference in operative time, haemoglobin drop or delayed graft function. However, the mean hospital stay was 2.8 days shorter for HALDN ($p<0.05$), which was also associated with less pain, faster return to normal activity and higher levels of patient satisfaction. This has lead to an increase in donors presenting to the programme.

Conclusion: HALDN is as effective as ODN in providing good allograft outcomes but has the benefits of shorter hospital stay and more rapid return to normal. HALDN is applicable to most donors, makes donation more attractive and helps promote living donor transplantation.

P86

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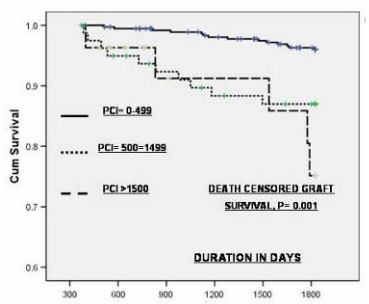
Introduction: High grade proteinuria has been clearly linked to adverse graft outcomes. Here we examine the possibility of a step wise relationship between various grades of proteinuria and longer term graft outcomes after renal transplantation

Methods: Electronic records were searched for 471 adult patients transplanted at a single centre in the United Kingdom between 1986 and 2002. All patients had Protein/creatinine indices (PCI) measured at every clinic visit. They were divided into three groups according to PCI at one year – Group-1, (PCI = 0-499), Group-2 (PCI = 500-1499) and Group-3 (PCI > 1499). The baseline demographics were compared in all the three groups and various outcomes including e-GFR at 1 year and 5 years, graft survival at 5 years were assessed and results statistically analysed.

Results: All the baseline demographics were comparable across the three groups. The graft survival for the three respective groups is shown in the graph below. The median e-GFR for group-1 at 1 and 5 years is 51.9ml/min and 49.6ml/min respectively whereas group-2 has a median e-GFR of 43.6ml/min and 40.0ml/min and group-3, 45.5ml/min and 30.9ml/min respectively. These GFRs were significantly different at both 1 year and 5 years ($p<0.001$).

Discussion: This study demonstrates a stepwise increase in long term risk with increasing proteinuria. It re-establishes the impact of high-grade proteinuria (PCI > 1499) and also demonstrates the significant impact of low grade proteinuria (PCI= 499-1499). This represents a simple and cheap marker of poor graft outcome and should prompt early allograft biopsy.



P87

Laparoscopic Or Open Live Donor Nephrectomy: Meta-Analysis Of Randomised Controlled Trials

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Background: The aim of this study was to compare laparoscopic and open donor nephrectomy techniques using Cochrane meta-analysis methodology.

Methods: Data was collated and analysed using RevMan® software. Primary outcome measures were donor operative variables and graft outcomes were secondary.

Results: Five trials randomising 532 patients were included in this meta-analysis. Selected significant results are present below:

Outcomes		Open (n=256)	Lap (n=276)	p-value
Donor	Operative time (mins)	167 ±36	217 ±51	<0.001
	Analgesic requirements * (mg morphine)	47 ±40	30 ±30	0.02
	Hospital stay (days) *	4 ±1	3 ±1	0.001
Graft	Warm ischaemia (mins)	2 ±1	5 ±2	<0.001
	Delayed graft function *	9	12	ns
	Acute rejection*	30	24	ns
	Graft loss	4	7	ns
	Serum Cr at 3 mths (µmol/l)*	132±62	123±53	ns
	Serum Cr at 6 mths (µmol/l)*	132±53	123±88	ns

Mean ±Standard deviation * Data not available for all trials

Conclusion: Laparoscopic donor nephrectomy is a longer operation with greater graft warm ischaemia time. However, recovery is faster and there does not appear to be any effect on early graft outcomes.

P88

Lower Rejection Rates For Laparoscopically Procured Kidneys In Paediatric Renal Recipients Compared To Open Donation

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Introduction

Although laparoscopic donation has become the method of choice in living donor transplantation in adults, few data exist about its efficacy in paediatric recipients. Small studies have found no difference in graft survival when compared with open techniques, but previous data from UNOS have suggested a higher incidence of rejection in laparoscopically procured kidneys (Troppman et al 2005).

Methods

We examined the outcome in 85 consecutive paediatric renal transplant patients. We compared 46 recipients of laparoscopically (lap) procured kidneys performed over a 3 year period (2004-2007), to a historical control group of 39 recipients of open donors. 37 of the lap donors were by the hand assisted technique. Chi-square test and Fisher's exact test were used to analyse nominal data according to sample size. Mann Whitney U test was used to analyse numerical data.

Results:

Mean follow up in the lap and open group was 13 and 26 months respectively. The mean recipient age (yrs) was 9.78 (s.d 5.04) in the lap group and 10.38 (s.d4.67) in the open group (p=0.617). Two patients had delayed graft function in the lap group (4.3%) and one (2.5%) in the open group (p=0.562). At the latest mean follow up there was 100% graft survival in the lap group compared to 92 % (p=0.093) in the open group (3 failures). The incidence of acute rejection within 1 year of transplant was 26% (16 episodes in 12 patients) in lap group compared to 41% (29 episodes in 16 patients) in the open group (p=0.219). Incidence of operative complications (both intra and post operative) was 23 % (11 pts) in the lap group and 31 % (12 patients) in the open group (p=0.643). There were no deaths in the lap group but 3 deaths (7.6%) in the open group 2 of which were from PTLD and the third from cerebral coning post op (p=0.093).

Conclusions

Our experience of laparoscopic kidney donation for paediatric recipients suggests excellent outcome with a lower rate of rejection compared to that after open donation, contrary to other studies.

P89

A retrospective review of Tacrolimus levels in renal transplant recipients and graft rejection

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Introduction

In the absence of national guidelines, a retrospective audit was undertaken to review the current renal transplant immunosuppression regime. There was a perception that the new Tacrolimus target level (8–10ng/ml) introduced in May 2007 was too low and more rejections observed. Our current maintenance protocol includes prednisolone, Mycophenolate Mofetil and Tacrolimus.

Methods

All transplant recipients (Feb 2003-Oct 2007) were reviewed for the highest C0 (trough) Tacrolimus level achieved within 7 days post-transplant. Tacrolimus level of 10ng/ml was considered a cut-of as it represents the highest target in the current protocol. Rejection episodes within the first 10 days post-transplant were recorded.

Results

This audit included 277 renal transplant recipients. Tacrolimus levels were ≥10ng/ml (high group) in 223(80.5%) and <10ng/ml (low group) in 54(19.5%) recipients. Of the low group, 11(20.4%) had rejection compared to 22(9.9%) of the high group (p=0.03). There was a difference between recipients with high and low Tacrolimus levels with respect to GFR (MDRD) levels at one year post-transplant, with a mean of 36.7±21.2 in 31 recipients with high Tacrolimus levels compared to 40.7±24.5 in 246 recipients with low Tacrolimus levels (p=0.4). Lower GFR levels (31.1±22.5) in rejecting compared with non-rejecting (41.4±24.1) recipients observed (p=0.02).

Conclusion

Trough Tacrolimus level <10ng/mL was associated with more rejection episodes. The authors feel that the current Tacrolimus target, 8-10ng/mL is low and needs revision. While rejection and high Tacrolimus level have adverse effects on the GFR at 1 year, this was not statistically significant when comparing Tacrolimus level groups (sample size).

P90

Establishing Indigenous Living Donor Kidney Transplantation In West Africa

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Three visits have been made to a west African teaching hospital to help set up a locally provided kidney transplant service. The hospital (400 beds) provides specialist surgical services to 25M, Moslem population. The city has a total of 1200 hospital beds in three hospitals. Dialysis is not free and there are only 8 dialysis machines. During each visit two live donated kidney transplants were performed with increasing independence of the local surgical team. BTS/RA guidelines were followed for donor work up, where possible. Access to CT (150Km away) was difficult due to the poor maintenance and renal angiography was only available for two donors. Colour doppler ultrasound was offered as alternative imaging. 10 patient pairs were worked up to preparation for surgery. Four patients were transplanted abroad. The visits identified significant problems. Electricity power cuts are frequent (4 – 10 per day) and last 20 minutes or more. The theatre lights were old, had few bulbs and could not focus. There is no back up power generator for the theatre suite, but sunshine is plentiful. The air-conditioning is archaic. Anaesthetics were given by nurses using Boyles machines, but two modern vaporisers were bought for the final visit. Bore hole water in a large bucket and carbolic soap was used for hand cleansing. The gowns and drapes were perforated in many sites. The surgical instruments were crude but the sutures were of European standard. Tissue typing is unreliable and written detail requires the tests (including crossmatches) to be performed in Egypt. Cyclosporine is the only 'modern' immunosuppressant , and levels are measured in another city: the turnaround time is <24 hours. Luckily all the patients had BMIs of 20 – 22. There were no significant post-operative complications in donors or recipients. They have all done well. Their care was funded by a generous grant from the state governor and this covered all costs and drugs for 1 year post transplant. Historical experience suggests that training African surgeons in the UK will result in a skills drain, due to good opportunities in Britain for career advancement. These options are now reduced by competition with immigrant European medical staff. The available technology and facilities do not prepare them for the limited resources back home and therefore this form of training and mentorship is best provided by senior surgeons exporting their expertise.

P91

Development of HLA DSA in the Modern Era of Immunosuppression; An Overstated Problem?

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Background: Donor Specific Antibodies (DSA) are considered the natural result of Transplantation. They pose a formidable risk to overcome in particular in the setting of Pancreas after kidney transplantation where a new graft possessing an antigen against which the recipient has previously been sensitised could cause an anamnestic reaction with consequences to both the kidney and the pancreas.

Aim: To define the real extend of the problem of HLA DSA in patients that have had a kidney transplant previously and come forward for PAK or SPK if the kidney had previously failed.

Patients and Methods: 15 patients with functioning kidney transplant received PAK and 2 patients received SPK following failed previous kidney and one SPK following failed pancreas alone transplant. We analysed the incidence of DSA (against their previous donor) that the patient had at the moment of the second transplant.

Results: The mean HLA mismatches for the first organ were 2.1 and the total mismatches for all patients 38. There was only one patient that developed DSA against the first donor (2.6% of mismatched antigens). There were also 6 non DS HLA Ab developed that were of no consequence to the second transplant. All first offer Crossmatches were negative for the subsequent organ and there was no immunologic failure among those organs (SPK or PAK).

Conclusion: There is a surprisingly low incidence of DSA among PAK transplants against their previous donor in the modern era of immunosuppression. That shows, if verified, that the anxiety about the degree of potential sensitisation is not justified.

P92

A Study On Postoperative Infection In Transplant Recipients

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Background:

Transplant recipients have a higher risk of developing infection compared to the general public. The risk is multifactorial. Infections pose a significant threat to the graft and to the patient as well.

Aim:

In our study we assessed the infection rate and the type of infections in our transplant recipients and tried to assess the risk factors involved.

Patients and methods:

We performed a retrospective study on 103 transplant recipients (of kidney or simultaneous kidney pancreas) in a period of one year from 2006 to 2007 with minimum follow up of 6 months.

Results:

Median age was 46. (19-71). 76% of the patients received organ from deceased donors while 24% from live donors. Urinary Tract Infection (UTI) was found in 55% of patients, systemic infection-9% (including bactermia, viral infection), Gastro intestinal infection-8%, respiratory infection-3%, wound infection-4%. Bacteria were responsible in 82% cases while virus for 8% and fungus for 10% of the infections. 77% of the patients who had an initial UTI got a recurrent one. In UTIs, Coliforms are the main organism isolated from the culture (41%), followed by Enterococcus (24%). UTI was higher in females as compared to males (62.5%vs37.5%p=0.001). Age or origin of the kidney (cadaveric or live donor) were not associated with the incidence of UTIs or other infections (p=0.6 and p=0.8 respectively). Diabetic recipients had got increased incidence of UTI (15 /23-65%). There was no death or immediate graft loss associated with infections.

Conclusion:

Urinary tract infection is the most common infection in Transplant recipients in our centre. Female gender and diabetes mellitus are independent risk factors for UTI whereas age and kidney origin are not. Recurrent infections are a serious problem in a large kidney, kidney/pancreas program centre.

P93

Low Rate of Rejection After Transplantation in HIV Positive Recipients With Living Related Donors

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Introduction

Data from the US have suggested high rates of severe rejection after transplantation in HIV positive recipients. Treatment of such patients with polyclonal antibody therapy is difficult due to the risk of life threatening sepsis. However, in most series the majority of patients have received organs from cadaveric donors. We report on our early experience of rejection in HIV positive recipients.

Methods

Four patients (3 male, 1 female, 3 Afro-carribean and 1 Caucasian), who were HIV positive underwent renal transplantation. All met the US HIVTR study criteria for inclusion, and in particular had negligible viral loads and CD4 counts over 200. 3 patients were diagnosed as having HIVAN although only 1 had had a biopsy. Baseline immunosuppression consisted of mycophenylate, prednisolone and cyclosporine in 3 patients, with the use of tacrolimus in the 4^{th}. 1 patient (A) received a deceased donor organ, whilst 3 had a live donor. Routine surveillance for CMV was performed in all patients.

Results

After a mean follow-up of 11 months, all grafts were working. Patient A sustained 2 episodes of biopsy proven rejection (Banff 1b) at 8 and 9 months post-operatively, which responded to steroid therapy. No other episodes of rejection occurred.

3 patients developed CMV viraemia and 1 had pneumonia requiring admission.

There was one needlestick injury to a member of the surgical team

Discussion

In conclusion, our early experience suggests good results after transplantation in HIV positive recipients. Significant CMV viraemia indicates more than adequate immunosuppression, and we postulate that the high rejection rates seen in other centres may be a reflection of the use of deceased donor organs.

P94

Evaluation of patients' and carers' views at the First National Kidney Transplant Patients' Forum

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Introduction: The first Kidney Transplant Patients Forum held in Manchester in June 2007 was an opportunity to support and consult with transplant service users in a departure from the traditional method of using individual patients to represent groups or populations. It enabled transplant patients with their carers and transplant multidisciplinary team (MDT) members to meet in a neutral, non-clinical setting. Attendees came from across the UK (including Ireland) and a number belonged to established patient organisations. Patients and carers set the agenda by discussing their experiences and by suggesting improvements for transplant patients' treatment. We exploited the event to identify common themes in the views expressed by attendees. Patients were involved at all stages from initial organisation to evaluation/analysis.

Methods: The format of the Forum was open discussion and small group workshops with patients and carers outnumbering professionals 3:1. Semi-structured questionnaires distributed at the start of workshops were used to stimulate and focus discussion. The questionnaires asked for views on positive and negative aspects of their transplant experience and to suggest improvements. A facilitator recorded the workshop discussion on flipcharts. Out of 55 attendees, 14 patients and 6 carers submitted completed questionnaires, which, together with flip chart data were analysed using the principles of thematic analysis.

Results: Transplant patients and their carers had varied and interlinked experiences and suggestions for treatment improvements. The strongest theme that emerged was the recognition of the positive aspects of receiving a kidney. However subsequent themes dealt with negative experiences, the most prominent of which was the emotional impact of receiving a transplant and this interlinked with suggestions for more emotional support and counselling. Another key theme focused on a need for better contact, liaison and support from the MDT post transplant and this was distinct from, but related to, a theme of concerns about drugs and their side effects. Less prominent themes focused on problems during the transplant process, the benefits of meetings with other patients and carers, a need for more information/knowledge, perceived social problems experienced post transplant and problems at national or structural level outside the immediate patient and area. Finally there was unanimous support and positive feedback about the Kidney Transplant Forum itself.

Conclusions: Transplant patients and their carers valued the opportunity to discuss their views with transplant healthcare professionals. Several interlinked themes emerged which could be used to shape the development of kidney transplant patient treatment in the future. The key themes identified by the analysis will form the basis of planning for the next forum.

P95

Differences in gastrointestinal side effects among cohorts of transplant recipients receiving mycophenolate mofetil

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Introduction: Mycophenolate mofetil (MMF) is a useful component of modern immunosuppression but is known to be associated with gastrointestinal side effects in some patients. This project investigated differences in gastrointestinal tolerability of MMF in patients receiving liver, kidney, or simultaneous pancreas kidney transplants (SPK).

Methods: 283 patients who were prescribed MMF for immunosuppression were studied (133, renal, 61 liver and 89 SPK). Data were retrieved and verified from computer and patient records and through patient interview. GI disturbance was defined as clinically significant diarrhoea or nausea and vomiting. The primary outcome event was withdrawal, reduction in dose or change of scheduling of MMF from the original prescription.

Results: Gastrointestinal side effects were more common in SPK recipients (42%) than other groups (liver 26% and kidney 15%) Chi =9, d.f=2, $p<0.007$. The likelihood of MMF withdrawal was not statistically significantly different between liver (26%), kidney (25%) and SPK (39%) recipients Chi = 4.7 d.f=2, $p<0.09$, however, SPK or kidney patients were significantly more likely to need a dose alteration than liver patients Log rank =14.3 $P<0.001$. Overall female patients were significantly more likely to require dose reduction than males Chi=14.3 df=1, $p<0.0001$, although there was no relationship between requirement for dose reduction and body weight.

Conclusions: Patients receiving MMF for immunosuppression following SPK transplantation are significantly more likely to develop gastrointestinal side effects than liver or kidney transplant recipients. SPK recipients and females in general are more likely to require dose reduction with MMF. The precise reasons for this are unclear and merit further investigation.

P96

The Value Of Hyaluronic Acid Measurement In Predicting Survival

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Introduction and Aim:

Serum fibrosis markers have been compared with liver histology repeatedly in studies which assume the biopsy as the gold standard. Sampling and interpretation variation limits the accuracy and a better, and clinically relevant, reference might be survival.

The aim of this study is to look at the value of serum hyaluronic acid (HA) levels in predicting survival in patients with chronic liver disease.

Methods

Single centre retrospective observational study. From an HA database, a cohort of 236 patients with HA values ≥ 100ng/ml was initially derived. We identified those patients who have subsequently died (or underwent liver transplantation(OLT)) and compared HA values with survival (in months) using Spearman, non-parametric correlation.

Results

From the 236 patients, we identified 25 patients (15 M: 10F) from 1995 who have subsequently died (or undergone OLT). Mean age was 62.2 years with a range of 31-86 years. 4 patients had more than one HA value in excess of 100ng/ml. An inverse correlation of r= -0.411 with a p value of 0.024, was found between the HA and survival in months. Of the 11 patients in this cohort with values over 800, 10 had died within 16 months. Of the 7 patients (8 values) with values less than 200, 5 patients survived >16 months. The mean survival for values >800, 601-800, 401-600, 201-400 and 100-200 was 11.6 (11 samples); 27.5(4); 15.5(4); 47.5(2) and 31.1(9) months respectively. Only 3 patients with values greater than 200 survived >30 months.

Conclusion

In this study looking at all cause mortality (or OLT) in a cohort of patients with at least one HA value who subsequently died, HA levels accurately predicted survival in the majority. In particular, levels >800 have a mean life expectancy of less than 1 year in this cohort.

POSTERS

Pancreas Clinical

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The impact of prolonged cold ischemia time on the outcome of pancreas transplantation. A single centre experience.

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Background: Prolonged cold ischemia time (CIT) is presumed to have higher complication rate in patients with pancreas transplantation. Ideally the CIT should be kept below the optimal 12 hours time, however due to logistic and centre factors that is not usually achieved.

Objective: The aim our study was to compare the outcome and complications in pancreas transplant recipient transplanted within the optimal CIT and beyond.

Material and method: From July 2001 to study analysis date (mid December 2007) 133 pancreas transplantation was performed in our unit. 105 simultaneous pancreas kidney (SPK), 23 pancreas after kidney (PAK) and 5 pancreas transplantation alone (PTA). Clinical data was collected prospectively into an electronic database (Microsoft Excel). Recipients were grouped according the CIT. Group I: CIT< 12 hrs, Group II CIT 12-15 hrs and Group III CIT>15 hrs and data was analysed. Clinical outcomes including early and long term surgical (e.g., bleeds, thrombosis, infections, leaks and interventions) and medical co-morbidity (e.g. urinary and respiratory tract and CMV infections), graft, patient survival and hospital stay were compared between all groups.

Results: The one year patient survival rate in Group I was 100%, 90% in Group II and 93% in Group III. The one year pancreas graft survival rate was 86%, 75% and 71% respectively.

Major surgical complications	Group I	Group II	Group III
Number of Transplants	22	69	42
Graft thrombosis (%)	5	17	17
Bleed/Haematoma (%)	9	13	10
Wound infection (%)	18	16	21
Radiological collection drainage (%)	9	13	14
Major fistula (%)	5	9	17
Peritonitis/Intraabd. Abscess (%)	9	26	26

The frequency of non-surgical complications (sepsis, respiratory complications, cardiac complications, CMV infections, Ileus, DVT, pulmonary emboli and acute rejection) was similar in all groups. The incidence of UTI was higher in Group II and III. The median HDU (2days) and ITU stay (1 day) was shorter in group I compare to other groups so as the median hospital stay which was 15, 17 and 17days respectively.

Summary: Both patient and graft survival rate was higher in the group with the optimal CIT (<12hrs). Similarly the rate of major surgical complication and overall hospital stay was lower in patients receiving pancreas transplants within 12 hours of CIT.

Conclusion: Cold ischemia time should be kept bellow 12hrs to optimize graft function, patient survival and to avoid major surgical complications in pancreas transplant recipients.

P98

What are the consequences of graft failure after 140 consecutive Simultaneous Pancreas Kidney (SPK) Transplants in a single centre?

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Introduction: SPK transplantation is an established replacement therapy for patients with insulin dependent diabetes leading to renal failure. There are concerns that after such a major procedure, failure of one or both organs may have irrecoverable and detrimental effects on long-term patient outcome. We aimed to investigate whether there is any evidence to corroborate this.

Methods: Retrospective analysis of 1 and 10 year outcome in 140 consecutive SPK transplants (1996-2007) in a single institution. Subgroup analyses per organ failure.

Results: Patient, pancreas and kidney survival at 1 year and 10 years were 96% and 92%, 92% and 88%, 76% and 73% respectively.

22/140 (16%) pancreatic grafts failed, 5/22 (23%) due to technical complications (thrombosis, infection, bleeding), 9/22 (41%) due to immunological causes and 8/22 (36%) due to patient death. 11/22 pancreas grafts failed within the first year (range: 1-355 days; median 56 days) and 11/22 after the first year (range 366-2026 days, median 812 days). Of the 14 patients with pancreas graft failure, 6 (1 pancreatic thrombosis, 5 immunological losses) have continuing kidney function for 453-3026 days (median 1124 days). 1/6 of these are being considered for PAK and 2/6 have expressed an interest and are potentially suitable to have PAK in the future.

21/140 (15%) renal grafts failed, 11/21 (52%) due to patient death. Of the 10 alive patients 5 remain insulin independent 298-1689 days later (median: 692 days). 4/10 will remain on dialysis replacement therapy (3/4 have a functioning pancreas graft), 2/10 received a second kidney transplant, 2/10 are on the renal deceased donor list and the other 2/10 (also with pancreas functioning) are being assessed for re-transplantation. 16/21 patients lost both organs (11/16 due to patient death). 1/5 alive patients will remain on dialysis, 2/5 have received a second kidney transplant and the other 2/5 are on the renal deceased donor list.

Conclusion: Long-term outcomes in SPK transplantation give reason for optimism. Failure of one graft does not preclude function of the other graft for a significant time. To date retransplantation with a kidney is more likely than with a pancreas.

P99

Simultaneous Pancreas Kidney Transplantation (SPKT) Enhances Quality And Quantity Of Life In Patients With Chronic Kidney Disease (CKD) And Type-1 Diabetes Mellitus (DM)

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Introduction: DM is the leading cause of CKD, however diabetic patients with advanced CKD are less likely to be listed for transplantation than non-diabetic patients[1]. SPK offers patients with DM to achieve normoglycaemia, prevent neurovascular complications, and improve patient and graft survival[2].

Methods: A systematic search of medical databases (PubMed, Medline, Cochrane Controlled Trials Database, PsychINFO, EMBASE and CINHAL, 1997-2007) were performed to obtain all studies comparing SPKT versus kidney transplant alone (KTA) in patients with DM, which were appraised critically and used to grade recommendations for evidence-based good practice[3].

Results: SPKT offers equal or better long-term kidney graft and patient survival compared with cadaveric and possibly living donor KTA [Evidence level 2b]. Combined pancreas transplantation with a kidney graft may prevent the progression or reverse the cardiovascular complications of diabetes in patients with CKD [Evidence level 2b]. Patients may report improved quality of life associated directly related to the improved management of their diabetes following SPKT [Evidence level 4].

Conclusions: SPKT should be offered to all patients with advanced CKD and DM, ideally prior to initiation of dialysis [Grade B recommendation] and particularly if under the age of 50 years [Grade C recommendation].

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P100

Evaluation of Neural Regeneration after Pancreas Transplantation by Corneal Confocal Microscopy: Updated results from ongoing study

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Introduction

Pancreas transplantation (PTx) in Type 1 diabetes has been shown to prevent progression of retinopathy and nephropathy but to a lesser extent neuropathy. Various methods deployed have looked at large nerve fibres. Small nerve fibres may be the earliest to repair and have not been evaluated. Corneal confocal microscopy (CCM) is a rapid, non invasive in vivo clinical examination technique which quantifies corneal small nerve fibre damage and repair.

Method : 42 Type 1 diabetic patients (pt) within 1 month of PTx, 7 non diabetic renal failure patients within 1 month of renal transplant and 14 non diabetic control subjects (ct) underwent corneal confocal microscopy to quantify corneal nerve fibre density (NFD), nerve fibre length (NFL), nerve branch density (NBD)

Results: Corneal NFD (11.8 ±1.9 pt v 42.7 ct, P =0.0001), NBD (4.3 ±1.3 v 29.0 ±2.9, P=0.0001) and NFL (2.2 ±0.8 v 8.8 ±0.6, P=0.001) were significantly reduced in diabetic patients at transplantation.
6 months after successful PTx, 25 patients underwent repeat assessment and showed significant improvement in NFD (18.2 ±1.3 pt v 10.8 ±1.9 ct, p= 0.001) and NFL (3.50 ±0.3 v 1.9 ±0.4, p=0.002) .

To assess uremic neuropathy, non diabetic renal failure patients have been assessed. Reduction in all the parameters was noted without any significant improvement post successful renal transplant.

Conclusion: Despite severe neuropathy in type 1 diabetics undergoing PTx, small fibre repair can be detected within 6 months of PTx using CCM. ESRF alone can result in the development of nerve damage but successful renal transplant does not provide the benefit. Corneal confocal microscopy is a novel, non-invasive, in vivo clinical examination technique which may be used to diagnose neuropathy secondary to diabetes and chronic kidney disease and also to assess the benefits of therapeutic intervention in human diabetic neuropathy.

P101

Non Heart Beating Pancreas Retrieval; Graft and Patient Outcome. A Single Centre Experience

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Background:

Pancreas transplantation is a recognised treatment for Insulin Dependent Diabetes Mellitus. Utilisation of organs from non heart beating donors (NHBD) has been a new practice at our centre and in the rest of the UK. The aim of this study was to compare the results of NHBD pancreas transplantation at our centre with the results obtained from pancreas transplantation from heart beating donors (HBD).

Methods:

We analysed the outcomes of pancreas transplantation in patients who have received transplants from NHBD with those from HBD who were transplanted at our institution between June 2001 and December 2007. Donor variables, surgical complications (haemorhage, thrombosis, infections and leaks), medical morbidity (urinary and respiratory tract, CMV infections and rejection), and graft and patient survival were compared between the two groups.

Results:

135 pancreas transplants have been performed at our centre from June 2001 and between July 2005 and December 2007 10 transplants have been performed utilising organs retrieved from NHBD. 5 patients received Simultaneous Pancreas and Kidney (SPK) transplants, 3 received a pancreas transplant alone (PTA) and 2 received a pancreas after kidney transplant (PAK). Mean time from withdrawal to circulatory arrest was 28 minutes (0 to 58), mean warm ischaemia time was 12.9 minutes (10 to 18). There were no statistically significant differences between the two groups in terms of donor age, donor BMI, Cold Ischaemic Time, or post operative HDU, ITU or ward stay. 1 SPK, 1 PTA and 1 PKA in the NHBD group thrombosed compared with 10 SPK, and 5 PAK graft thromboses in the HBD transplant group ($p = 0.136$). There were no mortalities within 30 days of transplantation in the NHBD group.

Conclusions:

NHBD pancreas retrieval for transplantation increases the donor pool for the benefit of patients awaiting pancreas transplant, early results are promising but further evaluation of this technique is warranted to asses its safety and benefits.

P102

A meta-analysis of the impact of the two-layer method of preservation on human pancreatic islet transplantation

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Background – There are conflicting reports about the effectiveness of perfluorocarbons used in the two-layer method (TLM) of pancreas preservation for human islet transplantation..*Design* – a systematic review of the literature regarding mechanism of action of TLM and a meta-analysis of the evidence that TLM improves islet isolation outcomes.*Data Sources* – Pubmed, CENTRAL, EMBASE, Science Citation Index and BIOSIS were searched electronically in May 2007. *Methods* – After selecting the relevant human trials for meta-analysis data relating to donor variables, study design, primary and secondary islet isolation outcomes were extracted. Publications that explained the mechanism of action of TLM were chosen from this search for a systematic review.*Results* – Electronic searches identified seven unique citations. When comparing TLM with preservation in University of Wisconsin (UW) solution, there was a statistically significant higher islet yield (WMD 946.22, 95% CI 485.4 to 1407.04) in the TLM group. The number of transplantable preparations obtained was not significantly different (OR 1.56, 95% CI 0.96 to 2.54) between the two groups. The rate of successful islet isolations for marginal organs was higher in the TLM group (OR 6.69, 95% CI 1.80 to 24.87). Improved oxygenation and preservation of cellular bioengertics is thought to be the main underlying mechanism although no single mechanism has yet been confirmed.*Conclusion* – There is currently no clear evidence that the TLM is beneficial in human islet transplantation. It may improve the preservation of marginal organs.

P103

Staged enteric conversion of the pancreatic duct following duodenal necrosis in a non-heart beating simultaneous kidney and pancreas transplant.

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Case:

A 45 year old male underwent simultaneous kidney and pancreas transplant. The donor was a 22 year old non-heart beating donor and the HLA mismatch was 0:1:0. The cold ischeamic time for the pancreas was 19 hours and for the kidney was 23 hors. The pancreas was implanted into the systemic arterial and venous drainage system in the right iliac fossa. Following reperfusion of the pancreas the head and body of the pancreas perfused well. The graft duodenum did not perfuse and remained ischaemic. Blood sugars normalised following reperfusion.

The ischaemic duodenum was excised and the pancreatic duct was directly anastomosed into the bladder. The pancreas worked immediately. Following a short period of delayed graft function the kidney function returned and the patient was discharged home insulin free. The patient made a progressive recovery but was troubled by recurrent urinary sepsis requiring systemic antibiotics. A decision was made to convert the exocrine drainage of the pancreas from the bladder to the gut seven months post operatively.

Procedure:

The pancreas head was disconnected from bladder and anastomosed directly to the distal ileum. The anastomosis was done over a fine feeding tube used as a stent. The stent was brought out distally though the terminal ileum and the anterior abdominal wall. At this point the terminal ileum was secured to the anterior abdominal wall. A drain was placed in the right iliac fossa. The patient recovered well but developed a controlled pancreatic leak. This leak healed with conservative management. Two months post operatively the patient remains well with stable blood glucose, stable renal function and no further episodes of urinary sepsis.

Discussion:

The duodenal segment is vulnerable to ischaemic necrosis in non-heart beating pancreas transplants. The management of the exocrine drainage of the pancreas in this situation can be difficult.

Conclusion:

We report a method of dealing with ischaemic necrosis of the duodenum following non-heart beating pancreas transplantation which has not been described before.

P104

Modified Technique for Pancreas Procurement From Non Heart Beating Donors When The Liver is Not Being Retrieved

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Introduction: There has been an increase in the use of pancreases from non-heart beating donors in the recent years. However, there have been situations where the pancreas is planned to be retrieved, although the liver is not being retrieved. The technique described will enable safe removal of the pancreas with improved vascularisation to the head. **Materials and methods:** From February 2007, Oxford Transplant Centre procured 11 pancreases from controlled non heart beating donors, of which 3 pancreases were retrieved using a modified technique. After the mandatory stand off time, In situ cold perfusion was constituted using Rusch double balloon transplant perfusion catheter inserted into the right common iliac artery, and the distal balloon is inflated to occlude above the level of the celiac axis. The left common iliac artery was clamped at origin. The abdomen is packed with frozen saline, and then the dissection is modified as follows: the portal vein is identified above the level of the head of the pancreas, and is ligated towards the liver hilum, while the other end is left open. Then, a mass clamp is applied at that level across the liver hilum, and the common hepatic artery as well as the bile duct is tied off towards the head of the pancreas. Subsequently, the supraceliac aorta is identified and a common patch of the celiac and superior mesenteric artery is prepared, taking care not to damage the renal arteries. The rest of the pancreatectomy proceeds in the standard fashion. The bench surgery for the grafts was as follows: the left gastric branch was tied off at the origin from the celiac axis. The splenectomy and trimming the duodenum prepared the graft for implantation. No vascular reconstructions were necessary. **Results:** Two pancreases (from donors aged 30 and 42 years) were implanted, the first into a 56 year old female and the second into a 36 year old male, both with type 1 diabetes and normal renal function. The third graft was discarded as was deemed to be too 'fatty'. Both grafts reperfused well. Both grafts had primary function, and are off insulin (median follow up 3 months). **Discussion:** Duodenal perfusion is of primary concern with pancreases retrieved from non-heart beating donors. This modified technique allows better perfusion of the head of the pancreas, as the gastroduodenal and inferior pancreaticoduodenal arcade is left intact, with less dissection around the head of the pancreas. This technique is safe, and helps retrieve pancreases if the liver is not being utilised.

POSTERS

Cardiothoracic Clinical

P105

Will the formulation of MPA affect ciclosporin pharmacokinetics in *de novo* heart transplant patients?

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Background: There are two formulations of mycophenolic acid (an immediate release formulation MMF (Cellcept®), and an enteric coated formulation of the sodium salt of MPA EC-MPS (Myfortic®) available to prescribe to transplant patients. The possibility of differential influence of two MPA formulations on ciclosporin pharmacokinetics was assessed in this study. This analysis compares the ciclosporin two hours' post-dose concentration (C2) and dose adjusted C2 concentration, as well as the frequency of ciclosporin dose changes for recipient treated with MMF or EC-MPS.

Methods: The data were from a multicenter, single-blind, randomized clinical trial comparing the efficacy and safety of EC-MPS and MMF in *de novo* heart transplant recipients. 150 patients were randomized to either EC-MPS 1080mg bid or MMF 1500mg bid, as part of a triple immunosuppressive therapy including ciclosporin microemulsion with C2 concentration measurements and corticosteroids. CsA C2 and dose-adjusted C2 were compared for the two formulations at the following post-transplant periods: two weeks, one month, three months, six months, and over six months. The frequency of ciclosporin dose changes was examined for the first year. A mixed model was used to analysis if MPA formulation associated with dose change and C2 concentrations.

Results: No difference was found for ciclosporin C2 and dose-corrected C2 for the two formulations over five post-transplant periods (two weeks, one month, three months, six months, and over six months). Total ciclosporin dose changes for the two formulations showed no difference: the mean number of changes for MMF was 13.4 and for EC-MPS was 13.2, 95% confident interval for the mean difference was [-3.1, 2.7]. The formulation of MPA did not contribute to the number of dose changes according to the mixed model.

Conclusion: The pharmacokinetics, as well as the practical use of ciclosporin, was not affected by the formulation of MPA for *de novo* heart transplant patients.

Table 1 Pharmacokinetic results for ciclosporin used with either MMF or EC-MPS in *de novo* heart transplant patients

P106

Bronchopleural Fistula Following Lung Transplantation; A 17 Years-Single Centre Experience

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Introduction:

Lung transplantation is an established therapeutic option for end-stage lung failure. The occurrence of serious airway complications like dehiscence and bronchopleural fistulae is generally low. We observed the incidence of bronchopleural fistula following lung transplantation in our unit.

Methods:

A retrospective review of the case records of all patients who underwent lung transplantation between 1990 and 2007 was performed to determine the occurrence of bronchopleural fistula and its outcome.

Results:

A total of 288 lung transplants (140 single, 110 bilateral and 38 heart and lung transplants) were performed, comprising 398 airway anastomoses. Four patients (1.38 %) developed bronchopleural fistula (two cases of cystic fibrosis, one idiopathic pulmonary fibrosis, and one alpha 1 anti-trypsin deficiency emphysema) at a median of 17.5 days (range 10-28) following transplantation. One patient had interrupted sutures and others had continuous sutures for bronchial anastomoses. Three patients were managed conservatively using aggressive antibiotic therapy, bronchial toilet and chest drainage. The bronchopleural fistula closed spontaneously in two patients, who were discharged with satisfactory outcome. One patient succumbed to worsening sepsis at 3 months post-transplant. The fourth patient underwent successful surgical repair using intercostal and pericardial flaps, and remains well 18 months later.

Discussion:

The incidence of bronchopleural fistula following lung transplantation in our centre is low. The technique of wrapping the bronchial anastomoses with vascular or omental flaps was advocated in the early days of lung transplantation. However, this technique has now been abandoned in most centres and we have never used this technique. In our experience, keeping the donor bronchus as short as possible allows optimum healing of the bronchial anastomoses. The therapeutic options for bronchopleural fistula following lung transplantation can be conservative or surgical, and should be individualized according to the clinical scenario.

P107

Onset of Brochiolitis Obliterans Syndrome Predicted by Elevated Plasma IL-6 and IFNγ levels

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Introduction

Bronchiolitis obliterans syndrome (BOS) significantly limits the survival rate of lung transplant recipients. BOS is usually diagnosed once it is clinically apparent and refractory to treatment. The immune response that initiates the development of BOS is unknown but if described could provide a means of early detection. IL-6, IFNγ and IL-17 have all been associated with inflammatory states including chronic rejection. In particular, Th17 cells have been recently characterized as a unique T helper lineage that regulates tissue inflammation and are associated with IL-6 and IFNγ. The association of IL-6, IFNγ and IL-17 in the development of BOS are unknown.

Methods

Using the Lung Transplant Repository, 8 lung transplant patients meeting the criteria of initial onset of BOS (reduction in FEV1 of 25% and/or tissue biopsy) were identified. A control group of 8 lung transplant recipients from the same time period not having BOS were also identified. Plasma samples were collected at the time of bronchoscopy. IL-6, IL-17 and IFNγ levels were detected using ELISA assays.

Results

IL-6 and IFNγ were both found to be significantly elevated in lung transplant patients at the time of initial diagnosis of BOS as compared to patients who had not developed BOS (**IL-6:** 3.3 +/- 1.33 vs 1.5 +/- 0.73 pg/ml; **IFNγ:** 256.6 +/-235.0 vs 93.7 +/- 36.5 pg/ml, respectively ($p<0.05$)). IL-17 levels were not different in patients with BOS compared to those patients without BOS, but were significantly higher overall than that found in non-transplant patients. (33.5 pg/ml and 25.1 pg/ml versus 0.002 pg/ml)

Conclusions

Plasma levels of IL-6 and IFNγ were significantly elevated in lung transplant patients at the initial onset of BOS. IL-17 levels were elevated in all transplant patients possibly reflecting a persistent inflammatory process that is upregulated by IL-6 and IFNγ at the onset of BOS. This cytokine relationship may serve as a set of plasma markers for the early detection of BOS.

P108

Lung Transplantation From Non Heart Beating Donors Without Pre-treatment

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To increase the numbers of lung transplants, and possibly reduce effects of brain stem death, we commenced transplants from Non-heart-beating donors (NHBD)in 2002. Previous work had suggested the inflated lung was resistant to ischemia after death.

All donors died after elective withdrawal of treatment (Maastrict III) and no pre-treatment was permitted. Lungs were inflated after bronchial toilet and assessed at sternotomy. Thrombus, if present, was removed from PA, followed by ante grade and retrograde pulmoplegia. Implantation and postoperative management was as our standard protocol. Data was collected prospectively

Since 2002, there were 8 recipients, 4 cystic fibrosis, 2 COPD and 2 fibrosing alveolitis, mean age 45, (range 19 to 64.4) Donors, mean age 30.75 years(range 15-47) died of cerebral trauma in 3, miscellaneous causes in 5. Mean time to asystole after withdrawal of treatment was 27.5 min(range 8-84).Mean inflated warm ischemic time was 29.12 min(range 9-95);with mean total warm ischemic time of 51.12 min(range 26-114). Total ischemic time was 348.37 min(range 297-413). Early function was excellent in 7 patients. 1 died on 8th POD with primary graft failure, 1 died on 47th POD of colonic perforation but good early graft function and 1 died at 4 months related to non-compliance. 5 surviving patients have excellent lung function and quality of life.

These excellent early results demonstrate the safety of NHBD lung transplantation in the absence of any donor pre-treatment. Simple inflation of lungs gives adequate protection against warm ischemia for up to an hour.

P109

Procalcitonin In The Multi-organ Donor: A Specific Predictor Of Donor Heart Dysfunction

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Background

Procalcitonin (PCT) is a precursor of the hormone calcitonin systemically released in inflammatory conditions including post-brainstem death. A donor PCT>2ng.ml^{-1} has been associated with worse post-heart transplant(HTx) outcomes. We investigated whether PCT estimation could identify dysfunctional donor hearts haemodynamically unsuitable for transplantation.

Methods

Serum PCT was estimated with a commercial immunoassay using flash chemiluminescence, in 80 potential heart donors. All donors underwent pulmonary artery flotation catheter insertion and echocardiography and were actively managed towards transplantable haemodynamic function (cardiac index ≥2.5L.min^{-1}m^{-2}, RAP and LAP≤12mmHg and minimal inotropic or pressor support). Univariate analysis was performed investigating PCT level with initial haemodynamic and echocardiographic findings and ultimate usage of the heart.

Results

The median donor age was 44 years. PCT was undetectable or ≤2ng.ml^{-1} in a 63/80. At initial assessment 40/80 hearts had suboptimal haemodynamics or echocardiography. PCT levels in this group were 6.23±14.56 versus 1.55±3.43 (p=0.05) and a PCT >2ng.ml^{-1} predicted dysfunction. The sensitivity(Se), specificity(Sp), positive predictive value(PPV) and negative predictive value(NPV) of a PCT identifying initial suboptimal haemodynamic function were 32%, 88%, 71% and 56% respectively. After 6.7±1.65 hours of optimisation management, 40 hearts were suitable for transplantation. On univariate analysis an initial PCT <2ng/ml predicted heart suitability (P<0.01) .

Discussion:

In this limited study, a PCT >2ngml^{-1} appears to be a specific indicator of cardiac dysfunction in the potential heart donor. Although larger studies are required to verify its predictive value PCT has potential as a point-of-care assay in donor heart assessment.

P110

Lung Assist Technology and Lung Transplantation.

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Extracorporeal membrane oxygenation (ECMO) has been previously successfully used before and after lung transplantation. This application has been limited by its relative complexity, expense and associated morbidity. Many of these problems have been overcome by the Novalung, a novel pumpless lung assist device. It relies on the patient's circulation to drive the blood via percutaneously placed cannulae through a heparin-bonded membrane, removing CO_2 by using O_2 as the sweep gas. We describe our initial experience with the Novalung: first in bridging a patient to transplant; and second in 2 patients with Primary Graft Dysfunction (PGD)

Patient 1, a 42yr old female with lymphangioleiomyomatosis and pneumothoraces precluding the use of non-invasive ventilation developed a severe respiratory acidosis (pH7.16 $PaCO_2$ >15 kPa). With her general condition rapidly deteriorating, we employed the Novalung. Her CO_2 was rapidly cleared (pH 7.40 $PaCO_2$ 6 kPa) and her condition stabilized. She received a bilateral lung transplant 36 hours later, making a good recovery being discharged home 34 days later. We believe that without the use of the Novalung she would not have survived to be a recipient. Patient 2, a bilateral lung transplant recipient, and patient 3, a redo-single lung transplant recipient, had rising ventilation requirements and CO_2 retention due to PGD. Using the Novalung enabled reduction of inspiratory pressures, with reduction of $PaCO_2$ and resolution of respiratory acidosis. Both later died from multi-organ failure, after 33 days and 3 days Novalung support respectively. In all patients, cannulation was via femoral artery and groin. Flows of up to 1.2 $l.min^{-1}$ were achieved without circulatory compromise. Using sweep gas flows up to 10 $l.min^{-1}$ obtained excellent clearance of CO_2. One membrane was easily changed for early thrombus formation at 8 days. Anticoagulation was achieved with iv heparin with no bleeding complications.

In summary, we have demonstrated that the Novalung can be used simply and without morbidity in acute respiratory failure in PGD. More controversially we have also shown that the Novalung can be used to bridge patients with Type II respiratory failure to transplant. This has both resource and ethical implications. We have decided to restrict the use of this technology to carefully selected potential recipients who are either intolerant of or unsuitable for non-invasive ventilation.

P111

Depletion of Circulating Th17-Cells Following Anti-Hypertensive Treatment in a Heart Transplant Cohort

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Background

Th17 cells are a novel effector T-cell subset which have been implicated in the rejection cascade following heart transplantation. However, the effects of immunosuppressive and immunomodulatory agents on Th17 cells have yet to be described. This study was designed to determine the effects of routine post heart transplantation medication on circulating Th17 cells.

Method

85 stable heart transplant patients were recruited into the study. Th17 cells were characterised via flow cytometry. Clinical data including dose and trough level (where applicable) of immunosuppressive (Cyclosporine, Tacrolimus, Mycophenolate mofetil, Myfortic, Azathioprine, prednisolone) and immunomodulatory agents (statins – Prava, Simva, Atorva, antibiotics – acyclovir, fluconazole etc, anti-hypertensives – ACE inhibitors, ANGII receptor antagonists, calcium channel blockers) were collected from patient notes.

Results

There were no correlations between dose or trough level of primary, secondary or steroid immunosuppression and circulating Th17 cells. Statin or antibiotic treatment had no effect on circulating Th17 cell counts. However, patients treated with an anti-hypertensive agent had lower numbers of Th17 cells compared to untreated patients (CD4+ CCR2+ CCR5- p=0.011). We then compared no anti-hypertensive treatment with ACE inhibitor or angiotensin II receptor antagonist treatment. Both agents significantly reduced Th17 cell numbers (p=0.017, co-ef -0.440). Systolic and diastolic blood pressure did not correlate with Th17 cell numbers.

Conclusion

This is the first study in the literature reporting the effects of routine immunosuppressive and immunomodulatory agents on circulating Th-17 cells. Our finding that routine immunosuppression has no effect on circulating Th17 cells, yet commonly used anti-hypertensive agents reduce this novel cell type may help to explain the dichotomy of results reported in the literature.

POSTERS

Liver Clinical

P112

The Role of Vascular Stenting in the Management of Venous Complications Following Orthotopic Liver Transplants

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Background: Venous complications are rare but serious complications following liver transplantation. Our aim was to review the management and outcome of venous complications at a large UK transplant centre.

Method: Patients with venous complications following liver transplants were identified from a prospective database. Overall outcome was assessed in terms of success rate, re-intervention rate and mortality. .

Results: Thirty one (31/3160; 0.9%) patients were diagnosed with venous complications following liver transplantation between 1982 and 2007. Majority (25/31; 81%) occurred following the first transplant. All grafts were from heart beating donors and whole size grafts except one case of split graft. Transplant technique was classic technique in 13 (42%) and 'piggy back' in18 (58%). All patients were managed with interventional radiology and stenting with single [22/31; 71%] or multiple [09/31; 29%] procedures at a median (range) 210 (34 – 4178) days following transplantation. Management options were; TIPSS in 17 (55%), hepatic vein stenting in 8 (26%), IVC stenting in 7 (22%) and portal vein stenting 7 (22%). TIPPS was performed more often after classic OLT. The failure rate was 48%. Two patients were re-transplanted post stenting. The 30-days mortality was 16% and the overall mortality was 52%. The median (range) survival post-stenting was 510 (1-5232) days and the main causes of death were sepsis (n=7), bleeding (n=2) and cerebral dysfunction (n=3). Survival post stenting was worse in patients that had a classic OLT compared to those that had a piggyback technique (Log Rank, p=0.02). Survival was significantly worse after TIPPS compared to IVC or hepatic veins stenting (Log Rank, p=0.02).

Conclusion: Stenting for venous complications following liver transplantation is a feasible first line treatment in majority despite higher failure rate. Porto-systemic shunts are associated with a worse outcome compared to other venous stenting procedures.

P113

The accessory or replaced right hepatic artery. Incidence and consequences of its presence in the era of multi-organ retrieval

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Background: In the presence of an anatomical variant i.e. an accessory or replaced right hepatic artery (A/R RHA) with a superior mesenteric artery (SMA) origin, a conflict of interest can arise at organ retrieval between liver and pancreas teams, despite recent national recommendations. We evaluated the anatomy of the inferior pancreaticoduodenal artery and its associtation with the accessory right hepatic artery.

Methods: Mesenteric and coeliac angiograms performed in our institution for unrelated indications were reviewed and relevant arteries, their diameters and distances between origins and when variants were found, the blood supply to relevant solid organs were recorded.

Results: 122 'abdominal' angiograms performed between 2003 and 2007 were reviewed and100 angiograms with cannulation of both SMA and Coeliac axis at the same session were identified which form the basis of this study. The inferior pancreatico-duodenal artery (IPDA) was identified in 98% cases. There were 8 patients with a replaced and 4 with accessory RHA. In all 12 the IPDA had an SMA origin, although there were three wherein it shared a common origin with the A/R RHA, on the SMA. In the rest the mean dist between them was 29mm (Range 17.8 to 48.3). In addition, there were two cases with a left hepatic artery from the left gastric artery. In the presence of an A/R RHA, diameter of the Common HA was 4.98mm versus 5.68mm when absent.

Conclusion**: The A/R RHA incidence in our series was 12%, and no case had an IPDA originating from the A/R RHA. Separate ARHA and IPDA origins potentially allow a feeding artery cuff to each team, but 3 patients in our cohort had common origins which would require vascular reconstruction if both liver and pancreas were transplanted. Compromise of the accessory right hepatic artery is rarely required in organ retrieval to preserve vascularisation of the pancreas.**

P114

Late Mortality Following Orthotopic Liver Transplantation: A Single Centre Experience

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Introduction and Aim:

Studies into mortality following liver transplantation have predominantly focused on first post-operative year. Here the causes of mortality are well defined and identify primary graft non-function, technical complication of surgery, infection and cardiovascular complications as important. Much less data exist regarding mortality in OLT recipients who have survived longer.

The aim of this study was to review the causes of death in all patients who underwent OLT in a single centre and survived at least one-year post transplant. The value of the donor risk index was also assessed in this setting.

Methods:

Single centre retrospective observational study. We examined all-cause mortality in single OLT recipients who survived 1 or more years since 1992.

Results:

From 659 OLT patients (February 2007), we identified 65 patients (M:F, 37:28; median age=53 (24-70) who survived ≥ 1year but subsequently died (mean survival after 1yr=1324days (14-3660). Aetiology of liver disease was ALD 19 (29.2%); PBC 16 (24.6%); HCC 8 (12.3%); acute liver failure 5 (7.7%); Cryptogenic Cirrhosis 5 (7.7%); HCV 4 (6.2%); CAH 3 (4.6%); PSC 3 (4.6%); Others 2 (3.1%).

Causes of death include Malignancy 16 (5 PTLD) (24.6%); Sepsis 15 (23.1%); Recurrent disease 9 (13.9%); Cardiovascular 8 (12.3%). Kaplan-Meier analysis of 3-year survival shows recipient gender (F>M) (p=0.02); PBC (PBC>other aetiologies) (p=0.03) and OLT for non HCC v HCC (p=0.017) as being associated with improved survival. Donor factors analysed included donor age, mean=46yrs (10-71); donor death (Anoxia=1; CVA=6; Other =33); Ethnicity (non Afro-Caribbean (n=40)); cold ischaemic time (mean=11hr (5-19).The donor risk index (mean=2.39 (1.55-3.62, n=40) appeared of little apparent value in this cohort. No relationship between donor risk index and late mortality in this cohort.

Conclusion:

Over 70% of deaths in OLT recipients who survived ≥1 year were from 4 main causes; malignancy, sepsis, recurrent disease, and cardiovascular. These have immunosuppression as a putative common denominator and as graft loss from acute or chronic rejection is uncommon, strategies to progressively reduce immunosuppressive therapy with time post-OLT should be actively considered. Furthermore, Donor Risk Index was of no value in predicting late deaths.

P115

Radiological evaluation of the donor liver for live donor liver transplantation; A single centre experience in an NHS setting

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Background

Live Donor Liver Transplantation is a means of addressing the current organ shortage and thereby reducing mortality on the waiting list. However, it is associated with significant risks to the donor and recipient. Here we describe the radiological evaluation of the donor prior to transplantation in an NHS setting.

Methods:

The Live Donor Liver Transplant programme was established in April 2007. The donor assessment was by CT scanning using contrast agents allowing definition of the portal vein, hepatic artery, hepatic vein and biliary tree anatomy. Scans were sent electronically to MeVis (MeVis Medical Solutions AG, Bremen, Germany) where virtual 3-dimensional reconstructions of CT images allowed detailed studies of the donor anatomy, virtual split liver volumes and analysis of hepatic vein dominance. Based on the electronic videos, images and reports of these findings, the donor operation was locally designed

Results

Of the 38 donors evaluated in the Donor Assessment programme, 14 have undergone radiological liver evaluation. Based on favourable anatomy, 3 successful organ donations have been performed, 3 donors await surgery and 2 patients await independent assessments prior to surgery. Of the remaining potential donors, 1 patient withdrew from the donation assessment, four donations were rejected based on MeVis findings (donor graft too small (n=2), remnant liver too small (n=1), unfavourable venous and biliary anatomy (n=1)) and one donor awaits biopsy to assess steatosis following radiological evaluation.

Conclusion

Live Donor Liver Transplantation is associated with significant risks to a healthy donor. Using the current radiological evaluation protocol, a significant proportion of donations were rejected on anatomical and physiological grounds and the use of preoperative biopsy has been minimal. This early experience emphasises the need for accurate evaluation of the donor liver prior to this surgery.

P116

VASCULAR COMPLICATIONS FOLLOWING ADULT EX-VIVO SPLIT LIVER TRANSPLANTATION- A 10-YEAR EXPERIENCE

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Background

Split-liver transplantation (SLT) is a means of overcoming donor organ scarcity, but results in creation of anastomosis between vessels of smaller calibre, possibly with greater size mismatch compared to whole liver transplants (WLT). As there are few studies dedicated to the vascular complications from such transplants we reviewed our experience.

Methods

An adult SLT program has been pursued in Birmingham since Sept 1994.We analysed the entire cohort of SLT patients using our prospectively collected database, clinical case notes and hospital records. SPSS 13 was used.

Results

Of the 104 patients, 41were male(median age at transplant 50 years). In 96 patients an 5,6,7,8,4(trisegment) graft and in 7 patients a 5,6,7,8 segment graft was used. There were 6 patients (5.7%) with hepatic artery thrombosis(HAT), 5 of whom presented with deranged LFTs. One, who presented with a bile leak was treated with a biliary stent, but died. The others were all re-grafted, and are either alive, or died of unrelated causes. The median time of presentation was 9 days after SLT. There were two patients(1.8%) with post operative intra abdominal 'cut surface' haematomas. There were 3 patients(2.8%) who had significant post operative haemorrhage. One (slipped hepatic vein tie), collapsed and died on the 10th post-op day. The other presented at 3 months with deranged LFTs. The third needed re-operation for arterial cut surface bleeding. In addition, we studied type and number of anastomosis, survival and causes of death.

Conclusions

The incidence of early HAT appears to greater in SLT(5.7%) when compared to WLT(1.9%). The prognosis, provided a suitable donor for re-grafting becomes available, is good. Postoperative haemorrhage and intra-abdominal haematomas, can occasional occur as expected and can be resolved in most cases without re-operation. The aetiology of liver disease does not seem to have an influence on vascular complications or their outcomes.

P117

Heart Rate Variability As A Marker Of Cardiovascular Risk Before Orthotopic Liver Transplantation

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Introduction and Aim:

Heart rate variability (HRV) is known to be impaired in patients with cirrhosis and associated with autonomic dysfunction. It has recently been used to identify patients at greater risk of cardiovascular instability during anaesthesia and may potentially be a useful index of cardiovascular risk.

The long term aim of this study is to assess the value of HRV in patients undergoing assessment for OLT in predicting CV events post-transplant. In this report we aimed to compare HRV with other markers of CV risk.

Methods:

During assessment for OLT patients underwent CT for coronary artery calcification scores, 24 hr ECG's for RR' variability (counts per 24 hrs with increment between successive R-R intervals > 50ms), BP and echocardiography. The Framingham cardiac risk score, HOMA-IR (insulin resistance), BMI and MELD were calculated and all compared with HRV, along with age, using Spearman's correlation.

Results:

114 patients, mean age 53.2 (range 24-69 years) had HRV measured. Mean MELD 14.5 (range 2-38), mean UKELD 54.9 (range 31-74) and Childs score A= 20, B=51 and C=43 patients. The mean HRV counts/24 hrs was 1552 (range 3- 35,297). Taking 1000 counts as the lower limit of normal in this age range, 68 (59.6%) were clearly abnormal. A significant positive correlation was identified between HRV and both SBP (r=0.286; p=0.002) and DBP (r=0.315; p=0.001). The severity of liver disease as measured by PT (r=-0.339, p=0.000), bilirubin (r=-0.223, p=0.017), albumin (r=0.340, p=0.000), Na^+ (r=0.447, p=0.000), MELD (r=-0.304, p=0.001), UKELD (r=-0.450, p=0.000) and Childs score (r=-0.313, p=0.001) also correlated with HRV count. No correlation was found with age, gender or CAC score but a positive correlation was seen with Framingham risk score (r=0.276, p=0.008).

Conclusion:

HRV is markedly abnormal in many patients undergoing OLT assessment and this correlates with SBP and DBP but not with other classical markers of cardiovascular disease. HRV negatively correlates with markers of the severity of liver disease. This supports a relationship between the autonomic nervous system and BP control and CV disease.

P118

A single centre's experience of the incidence, diagnosis and treatment of Roux-loop herniation post liver transplant.

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Introduction: Internal herniation after orthotopic liver transplant (OLT) is a rare complication which is difficult to diagnose but potentially life threatening. This is a single centre experience of internal herniae in OLT patients.

Methods: Between Jan 1992 and Jan 2007, 1806 OLT were carried out at our centre. 240 patients had a Roux-en-Y loop hepaticojejunostomy (RL) either primarily or following biliary complication. Over this period 10 patients were identified with internal herniation following RL biliary reconstruction. A review of these case notes was undertaken.

Results: Six had primary RL and four secondary. In six patients this was their first graft. Six patients presented as an emergency with abdominal pain and vomiting necessitating emergency laparotomy. The remaining four had a more insidious presentation with intermittent pain and, in one case, cholangitis. This group were investigated extensively (CT abdomen, HIDA, small bowel follow through and ultrasound) resulting in a preoperative diagnosis in two. The other two had exploratory laparotomies and definitive treatment.

Mean time between transplant and diagnosis was 1860 days (range 89-3738 days) and mean time between presentation and operation was 554 days (range 1-3675 days).

Of the ten patients, nine had imaging carried out preoperatively. Nine had abdominal CT scans of which one was normal. The other 8 showed small bowel obstruction and four demonstrated internal herniation. Of particular note 5 of the CT scans commented on an abnormal mesenteric vascular pattern. A variety of alternative diagnoses were proposed on the basis of CT including pancreatitis and acute venous or arterial infarction.

Five patients had barium small bowel follow-through imaging. Two showed internal herniation, one small bowel obstruction and two were normal.

Conclusion: Diagnosis of internal herniation is difficult and no one imaging modality reliably delineates what is often an intermittent problem. Therefore a high index of suspicion is essential in liver transplant patients with a RL presenting with intermittent abdominal pain. This series demonstrates that it is important to appreciate that presentation may be many years after transplantation.

P119

Outcome Following Paediatric Liver Transplantation In A New Centre

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Aims: The aim of the present study was to report peri-operative results and patient and graft outcomes following orthotopic liver transplantation (OLT) in paediatric recipients and analyse variables that affect outcomes in a relative new centre.

Methods: Patients undergoing OLT from January 2000 to July 2007 were identified from the paediatric transplantation database. Data analyzed included: demographic factors; donor and recipient characteristics; and operative data.

Results: 102 OLT were performed in 91 patients, with a median age at OLT of 4.8 (0.05 – 17.7) years. The most common indication for OLT was biliary atresia (26%). Patients underwent 36 whole size, 18 reduced size and 48 split-liver transplantation. The overall morbidity rate was 46% and there were 3 post-operative deaths. Blood transfusions were required in 89 of the OLTs performed. Patients that required blood transfusion had a significantly higher morbidity compared to patients who did not (**p=0.019**). The 1-, 3- and 5-year graft survival rates were 86%, 83% and 79%, respectively. 11 patients required re-transplantation. The 1-, 3- and 5-year overall survival rates were 90%, 86% and 76%, respectively. Patients that had an OLT with a shorter warm ischaemia time had a significantly better graft (**p=0.044**) and overall survival (**p=0.024**).

Conclusion: Despite technically challenging, excellent peri-operative outcomes could be achieved in a new program. Minimizing blood loss may influence morbidity and warm ischaemia time does affect graft and patient survival.

P120

Outcomes of Donor Assessment for Liver Transplantation in a National Health Service Setting

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Background:

The United Kingdom continues to see a fall in number of cadaveric donors and a rise in number of patients awaiting liver transplantation. Live Donor Liver transplantation is an alternative that can help address the shortage of organs. We present an initial experience with the outcome of donor assessment in an NHS setting.

Methods:

The Live Donor Liver Transplant programme was started in April 2007. The donor assessment process involves the following stages: initial blood group and questionnaire evaluation, clinical assessment, donor liver evaluation, independent evaluation, approval of the Human Tissue Act and successful completion of donation.

Results:

Since April 2007, 38 donors were evaluated for 20 potential liver transplant recipients. In total 22 patients were excluded at various stages of evaluation; blood group incompatibility (n=5), recipient received cadaveric transplant (n=6), self selected out (n=6), excluded on clinical assessment (n=2) and excluded on liver evaluation (n=3). Eleven patients await further evaluation; awaiting out-patient appointment (n=1), awaiting liver evaluation and results (n=8) and awaiting independent evaluation (n=2). Three donors have successfully donated for live related transplantation and two await surgery.

Conclusion:

Donor assessment for Live Donor Liver Transplantation requires the coordinated effort of a large multi-disciplinary team. A significant number of donors get excluded at various stages of the assessment process. This early experience has increased in efficiency and has offered a real option to reduce mortality on the waiting list.

P121

LIVER TRANSPLANTATION AND PULMONARY THROMBOEMBOLISM. A RETROSPECTIVE REVIEW OF 3000 TRANSPLANTS.

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Aim: As there is paucity in recent literature on the incidence of, and sequelae from pulmonary embolism(PE) in liver transplantation, we conducted a retrospective review of our single-center experience of 3000 liver transplants (LT).

Methods: Our prospective transplant database was reviewed to find all patients who were even suspected to have had a PE during or following LT. We cross checked a part of this database against hospital records to confirm accuracy of the database. Clinical records of these patients were reviewed and relevant aspects including presentation, operative detail, blood product transfusion, complications and survival was collated and analyzed in an attempt to find co-relation and learning points.

Results: In the 25 years since our first LT, there were 36 patients in whom a PE was suspected (Median age 49). Twenty-one suspected within 60 days of transplant (median duration 22 days). PE was ruled out in 10, unconfirmed in 2 and confirmed in 8 patients; in one, air embolism was found. All PEs occurred in hospital, but aetiology of liver failure was varied. Of note, 2 patients died of on-table PE. Of the rest, 5 are still alive, (median survival of 65 months). Although thromboprophylaxis is routine in our unit, its use in these patients could not be confirmed from the clinical records. Fifteen PE were suspected and confirmed after 60 days from transplant (6 within, and 9 outside the first year). The complex interplay of pro and anti coagulation mechanisms during surgery in chronic liver disease is discussed.

Conclusions: Acute PE in the setting of LT has an incidence rate(0.26%) that seems to be lower than one would expect after a ‘major complex’ category operation. No specific liver disorder appears to pose greater risk. The prognosis is good with a median survival of 65 months in our series although, sudden death due to an on-table embolism is a rare(0.06%) but significant risk.

P122

Impact Of Use Of T-Tube For Biliary Reconstruction In Orthotopic Liver Transplantation In Patients With Fulminant Liver Failure

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Introduction: The traditional use of T-Tube for biliary reconstruction in elective Orthotropic Liver Transplantation (OLT) has diminished as trials (including one published from our unit) suggest it lead to more complications. However, in the setting of OLT for fulminant liver failure it has been suggested that a T-tube in situ may facilitate postoperative imaging and decrease the need for postoperative ERCP. Use of T-tube in the setting of fulminant liver failure (where infectious complications higher) has not been evaluated. The use of T-tubes in our centre is based largely on surgeon preference in this group. The aim of this study is to evaluate the incidence of biliary and infectious complications in OLT with and without the use of T-tube for biliary reconstruction in patients with fulminant liver failure.

Methods: Retrospective analysis was performed from prospectively collected data on all sequential primary and redo liver transplants for fulminant liver failure from the years 1990 to 2007.Data collected included demographic details, details of biliary reconstruction, early/late biliary and infectious complication, in addition to other confounding factors such as donor age, cold ischemia time, arterial complications and hospital stay.

Results: Out of 1000 liver transplant performed >15 years at our centre, 154 patients were transplanted for fulminant failure (15.4%).36 had biliary reconstruction with T-Tube (group-A) where as 118 had no T-tube(group-B).Postoperative bile leak in group A was 8.3 % compared to 9.7 % in group B (*p value*=0.3539). There was no biliary stricture in group A compared to 3.2% in group B. The rate of wound infection in group A was 16.6% compared to 28% among group B (*p value*=0.0921). Intra-abdominal sepsis was recorded in 11.1% among group A and 28.8% among group B (*p value*=0.0145). The mean cold ischemia time in group A was 598±33 minutes (range: 152-1169) compared to 604±19 in group B (range: 179-1165) (*p value*= 0.8782). Arterial complications included haemorrhage (16.6% for group A and 15.2% in group B) and hepatic artery thrombosis [2.7% in group A and 10.1% in group B(*p value*=0.1270)]. Median hospital stay in group A was 45 days (range: 19-104) while in group B the median hospital stay was 42.5 (range: 12-141)

Conclusion: Use of T-tube in OLT for fulminant liver failure, in our experience, is not associated with increase in infectious or biliary complication, and may permit avoidance of difficult invasive tests such as ERCP to identify biliary complications early postoperatively in this high risk group. However a larger study is needed to clearly assess the impact of T-tubes in this setting.

POSTERS

Donor

P123

Living-Related Sequential Paediatric Hepatic And Renal Transplantation For Primary Hyperoxaluria

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Introduction

Primary hyperoxaluria type 1 (PH1) is a rare autosomal recessive disorder causing overproduction of oxalic acid, leading to oxalate deposition with nephrocalcinosis, end stage renal failure (ESRF) and systemic oxalosis. Several transplantation options are available including combined or sequential liver-kidney transplantation (LKTx). We present two paediatric cases of living related sequential LKTx (organs from same donors). To our knowledge these are the first two such cases in the UK.

Methods

Two neonates presented with ESRF due to PH1, at 3 and 2 months with plasma oxalate levels of 162 (normal <10) and 231umol/l respectively. Both neonates commenced haemodialysis and received a successful live related liver transplant (left lateral segment) at 13 and 16 months respectively. A sequential living related renal transplant (from the same respective liver donors) was performed at 22 (wt 10.8kg) and 23 (wt 11kg) months respectively. A midline transperitoneal approach to the aorta/IVC for vascular anastomosis was performed in both cases, with single neoureterocystotomy and full closure of the anterior abdominal wall.

Results

There were no intra-operative technical complications although the initial post-renal transplant course was complicated in both children by treatable fluid overload, generalised oedema, breathing difficulties and poor urine output. The second child developed asymptomatic CMV viraemia which was treated. However both children had immediate graft function and at the latest follow up of 6 and 3 months there have been no rejection episodes, with plasma oxalate levels of 14 and 8umol/l and Cr 29 and 68umol/l, respectively. Renal transplant biopsy in the second child revealed one oxalate crystal. Both children were immunosuppressed with steroid, tacrolimus and mycophenolate moeftil (MMF).

Discussion

Sequential living-related paediatric hepatic and renal transplantation is both effective and feasible for paediatric recipients in ESRF due to PH1.In addition these two cases illustrate the challenges in both intra and peri-operative management of transplantation in small babies.

P124

Early Results of a Controlled Non-Heart Beating Organ Donation Programme

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Introduction: The continued mismatch between supply and demand for organs has led to the development of controlled non-heart beating donation (CNHBD) programmes. Between April 2006 and March 2007, 159 CNHBD yielded 307 kidneys in the UK. Key considerations in establishing a CNHBD programme include the difficulty in prediction of asystole, logistical issues relating to the process and outcomes of CNHBD organs. We present our experience of CNHBD in a University Hospital with a mixed critical care unit a transplant centre serving a population of 2.2 million.

Methods: Prior to implementation of CNHBD, a steering group determined that the programme was feasible and an implementation committee produced protocols covering all aspects of the programme. All referrals for CNHBD between January 2005 and January 2008 are included in this analysis. The cause of death, withdrawal-to-asystole, machine perfusion, organ usage, warm and cold ischaemia times, delayed graft function, early and late graft function were analysed.

Results: During the period, 79 patients were referred resulting in 35 proceeding to retrieval and 61 kidneys being successfully transplanted. Forty-four patients did not proceed because of delayed asystole (15), declined/no assent (10), medical unsuitability (14), early asystole (4) with one becoming brain stem dead prior to withdrawal of treatment. Of the 35 donors, 18 had intracranial haemorrhage, 10 hypoxic brain damage and 7 traumatic brain injury. The median time from withdrawal of futile life-sustaining therapy to asystole was 15 min (IQR 10.0-23.0). The median primary warm ischemic time (systolic blood pressure <50 mmHg to perfusion) was 20 min (IQR 16.0-27.0) and the median cold ischaemic time was 18 hours (IQR 11.7-20.00). Forty one percent (16/39) kidneys preserved by machine perfusion showed delayed graft function compared to forty-five percent (10/22) of kidneys preserved in cold storage. The median time to halving of serum was 7 days.

Conclusion: Structured implementation resulted in a successful CNHBD programme providing 61 successful renal transplants from 35 CNHBDs' in 3 years – contributing about 35% to the total number of transplants during the period .We consider early involvement of all stakeholders contributed to the success of the programme. Donors generally reached asystole quickly despite low levels of ventilatory support and relatively little circulatory support.

P125

Locally Staffed Retrieval Teams Should Lead Controlled Non-Heart Beating Donation Programmes

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Introduction: Renal transplant units have successfully introduced and staffed Controlled Non-Heart Beating Donation (CNHBD) programmes. Liver, pancreas and lung transplants are also successful from selected CNHB donors; retrieval of these organs relies on the attendance of specialist teams. It has been suggested that regional multi-organ retrieval teams completely replace the activity of locally staffed CNHBD kidney retrieval teams.

Methods: Two neighbouring renal transplant units introduced CNHBD programmes in 2002 and 2005. Both programmes relied entirely on local staff until 2006 and 2007 when specialist teams were also invited to attend CNHB retrievals for suitable donors.

Results: Overall the teams attended 140 potential CNHB donors, 98 (70%) reaching asystole in the allotted time frame and proceeding to organ donation. Since the advent of specialist teams being selectively invited to attend 68 CNHB donations proceeded to 45 retrievals. Of those 68, specialist teams only attended on 20 (29%) occasions. Donor age, instability and a history of excess alcohol intake were the commonest reasons for specialist teams not attending.

Conclusions: Locally staffed kidney retrieval teams are best placed to attend the majority of CNHB donors and should continue to be resourced accordingly. Specialist teams should continue to attend selected CNHB donations and it is likely that this will become more common as confidence grows in the success of CNHB liver, pancreas and lung transplants. Removing the activity of successful locally staffed CNHBD retrieval teams would be a poor use of existing resources. It would be difficult for regional multi-organ retrieval teams to replace all such activity.