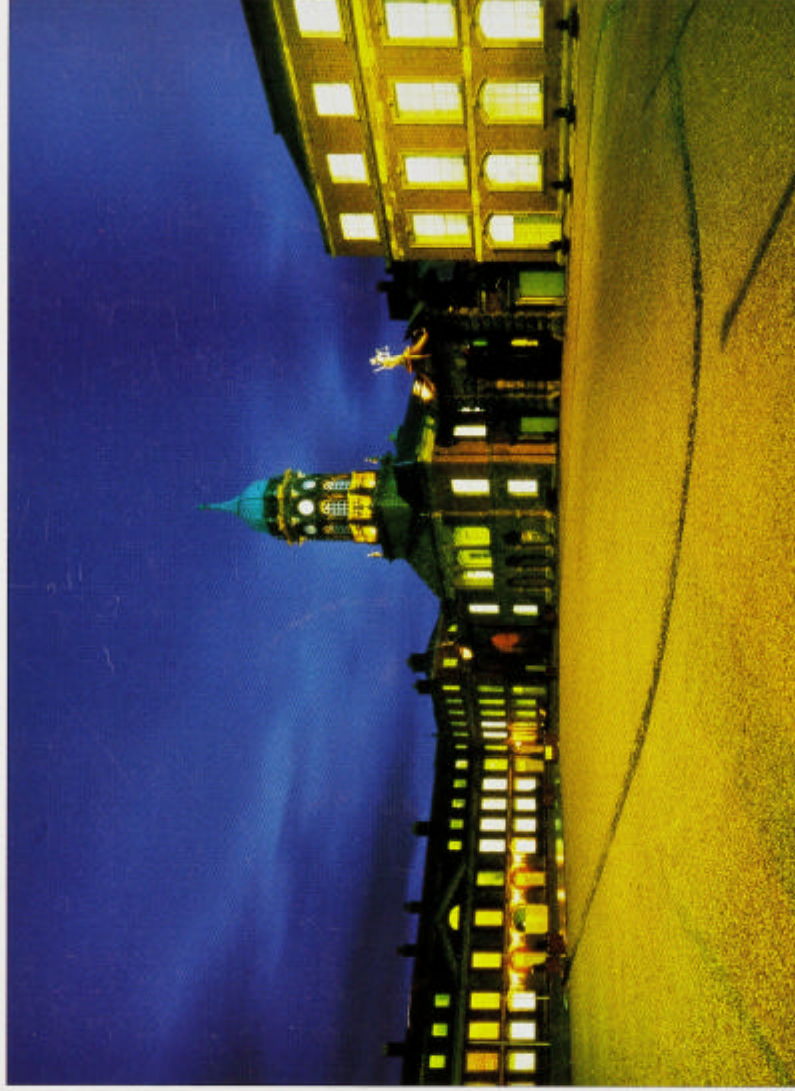


**THE BRITISH TRANSPLANTATION SOCIETY**

**1<sup>ST</sup> ANNUAL CONGRESS 1998**

**DUBLIN**



**1<sup>st</sup> - 3<sup>rd</sup> April 1998**

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Mr Lodge noted that the TTAC was not formally mentioned in the Society constitution. It was confirmed by Mr Johnson that the constitution would be amended to include this.

Mr Lodge reported that the TTAC would now meet at least three times a year apart from around the annual meeting

**8 Ethics Committee**

In the absence of Mr Brown there was no report from the Ethics Committee

**9 Any other business**

***Elective Ventilation***

Prof John Fabre reported that to date the government refused to discuss the matter of elective ventilation, until such time as the medical profession was in unanimous agreement over the matter.

**10 Date of next meeting**

April 1998

**ABSTRACTS SELECTED FOR PRESENTATION**

*[Faint, illegible text, likely bleed-through from the reverse side of the page]*

## THE EFFECT OF HLA-A, B, DR MISMATCHING ON KIDNEY TRANSPLANT SURVIVAL IN THE UK

Fuggle, S on behalf of Members of the Kidney Advisory Group

A collaborative study involving 23 centres in the UK investigated the effects of HLA-A, B and DR mismatching on the outcome of kidneys transplanted between 1986 and 1993. 6363 adult first cadaveric kidney only transplants were analysed, for which the one year follow-up rate was 99.6% and the five year rate was 97.8%.

Analysis using a multivariate (Cox) model showed both recipient and donor age to affect transplant survival, in addition to year of graft, kidney exchange, donor cause of death and recipient diabetes. These factors were included in the model investigating the HLA-A, B, DR mismatching effect. The model was stratified by centre to allow for inherent centre differences.

Analysis of HLA-A and B mismatches was based on the following antigens HLA-A: 1, 2, 3, 9, 10, 11, 19, 28, 29, 36, 43, HLA-B: 5, 7, 8, 12, 13, 14, 15, 16, 17, 18, 21, 22, 27, 35, 37, 40, 41, 42, 46, 47, 48, 53, 59, 67, 70, 73, 78 and HLA-DR: 1, 103, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12. Analysis of mismatches of HLA-DR13-18 gave no significant improvement to the model.

When looking at the results of the 27 possible HLA-A, B, DR mismatch combinations it was clear that '000' HLA-A, B, DR mismatched grafts had a significantly superior outcome. A second group also associated with improved outcome could be identified, including transplants with '100', '010' and '110' HLA-A, B, DR mismatches. One year transplant survival rates (95% confidence intervals) for the 3 groups 000, 100/010/110, and the rest were 88% (84-90%), 83% (81-85%) and 79% (78-81%) respectively.

The improved outcome of renal transplants in the presence of no mismatches for HLA-A, B and DR ('000') is striking. The effect of lesser degrees of matching ('100', '010' and '110') also has a beneficial effect on outcome compared to all other degrees of mismatching. This model for HLA-A, B, DR mismatching held for all adult recipient ages, for re-grafts and for patient survival.

*Very good presentation .. lacking info on mismatch first graft v second, re-graft results*





A COMPARISON OF EXTRACELLULAR MATRIX GENE EXPRESSION IN  
RENAL TRANSPLANT BIOPSIES FROM PATIENTS RANDOMISED TO  
CYCLOSPORIN OR FK506

BICKNELL GR, WILLIAMS S, FURNESS PN\*, NICHOLSON ML

DEPARTMENTS OF SURGERY AND PATHOLOGY\*, LEICESTER GENERAL  
HOSPITAL, LEICESTER LE5 4PW, UK

Human studies of the influence of new immunosuppressive agents on chronic rejection (CR) are difficult to perform as the long term studies of large numbers of patients are required. The histopathology of CR is dominated by an excessive deposition of extracellular matrix proteins and these may provide useful ad interim surrogate endpoint in studies of CR. The aim of this study was to compare expression of the genes controlling matrix deposition in renal transplant recipients entered into a control trial of cyclosporin versus tacrolimus (FK506).

Renal transplant recipients were randomly allocated to receive either neoral cyclosporin 15 mg/kg/day (n=16) or tacrolimus 0.2 mg/kg/day (n=17). Tissue core transplant biopsies were performed 1 week, and 3, 6 and 12 months post transplant. Single glomeruli were plucked from the surface of transplant biopsies and total mRNA was extracted using oligo dT dynabeads. Complementary DNA was synthesised by reverse transcription and specific mRNA species were amplified by PCR and quantified using an ELISA method. 180 biopsies were analysed for the mRNA of TGF $\beta$ , collagen type III, collagen IV (alpha 2 chain), tenascin, matrix metalloproteinases and their tissue inhibitors TIMP1 and TIMP2.

TGF $\beta$  expression correlated with collagen III (r=0.18, p=0.015), collagen IV alpha 2 (r=0.35, p<0.1), Tenascin (r=0.73, P<0.01), TIMP1 (r=0.61, p<0.01) and TIMP2 (r=0.57, p<0.01) suggesting that TGF $\beta$  is a fibrogenic influence in human renal transplants. There were no differences in the levels of mRNA for TGF $\beta$  between recipients treated with cyclosporin and those treated with tacrolimus. Levels of mRNA for collagen III, TIMP1, TIMP2 and tenascin levels were consistently higher in patients immunosuppressed with cyclosporin (p<0.01).

TGF $\beta$  would appear to exert a profibrotic influence in human renal transplants. The finding that cyclosporin is associated with the expression of a number of genes controlling fibrosis suggests an important mechanism by which long term cyclosporin nephrotoxicity may be mediated. Further studies need to concentrate on these effects at the level of protein synthesis.

### Extracellular matrix deposition

In early days the cyclosporin treated patients have profibrotic balance of forces.  
Some evidence that the NHB donors are less fibrotic producing.

## RECIPIENT-DONOR AGE MATCHING FOR KIDNEY TRANSPLANTS IN THE UK AND REPUBLIC OF IRELAND

Belger, M A on behalf of Members of the Kidney Advisory Group (Morris P J)

It is general knowledge that the age of kidney donors has been rising during the last 10 years and that this trend is still continuing. The mean age of donors of kidneys used in 1986 was 33 years whereas for 1996 this figure has reached 40 years. The proportion of donors aged over 60 has increased from 5% in 1986, 7% in 1990 and 11% in 1993 before declining to 8% in 1996. This trend to use more older donors is cause for concern as it has been shown that post transplant survival is significantly inferior to that with younger donors. We have examined the use of older donors together with the ages of the patients who receive their kidneys and to look at age-matching effects on survival.

The degree of age matching seen in locally transplanted organs is greater than that for organs transplanted after allocation through the national sharing scheme, which is based on HLA. However, despite the national scheme taking no account of recipient or donor age, evidence suggests that a selection process occurs such that kidneys from donors more than 25 years older than the potential recipient are rarely accepted from the national scheme (6.5% of national allocations 1994-96).

Analysis of a potential effect on transplant survival of age-matching the donor and recipient has been carried out on 6363 adult cadaveric kidney transplants in the UK between 1986 and 1993. In a multivariate (Cox) model both recipient and donor age were found to be highly significantly related to outcome but these effects were additive and no interaction effect of these variables was found. Table 1 gives one year transplant survival estimates by donor and recipient age groups. Other studies have found improved survival for older recipients when using older donors but this was not the result of the UK data.

Table 1 One year transplant survival (percent) by recipient and donor age

Recipient age (years)	Donor age (years)			
	0-39	40-54	55+	
18-39	86	82	74	
40-54	85	82	73	
55+	81	77	67	

Although age matching of donor and recipient occurs in practice for both locally and nationally used kidneys, evidence suggests that the effects of recipient and donor age on survival are independent. Matching no doubt occurs on ethical grounds: matching the life expectancy of the donor kidney as closely as possible to the life expectancy of the recipient. Therefore the decision to allocate organs according to age matching is an ethical issue rather than one based on transplant outcome.

Recipient age did not affect survival  
Donor age ↓ survival  
The age of donor & recipient does not affect match grade  
Donor age ↑ survival - Recipient age marginal increase  
25% donors allocated to recipients 15 or more yrs their senior. Budget: should give younger grafts to younger recipients





## ASYSTOLIC RENAL TRANSPLANTATION: AN ANALYSIS OF 157 GRAFTS

PA ANDREWS, RWS CHANG

St Helier and St George's Hospitals,  
on behalf of the South Thames Renal Transplant Group

The use of non-heart-beating (NHB) donors for renal transplantation is controversial. Wijnen et al have reported good results from Maastricht, and these have been supported by results from Guy's Hospital. Others, however, have been more reserved, including from within the South Thames Region. We have analysed results from all NHB kidneys retrieved from this area between 1988-94, with a three year follow up. These 147 adult and 10 paediatric grafts represent the largest series to date (cf 57 reported by Wijnen).

**Results** Patient survival was 86 and 81.6% at 1 and 3 years, crude graft survival 62.1 and 43.6%; and censored graft survival 62.1 and 62.1% respectively. Results improved with time, perhaps due to better donor selection. Univariate Kaplan-Meier analysis identified length of warm ischaemic time, donor age, and the origin of the donor organ as significant predictors of censored graft survival (all  $P < 0.05$ ). Cold ischaemic time  $< 36$  hours and DR matching were not significant. Surviving grafts functioned well, with mean creatinine at 1 and 3 years 180 and 160  $\mu\text{mol/l}$  respectively. Mean time on dialysis for functioning grafts was 22 days (SD 17.3), median 18 days. Surprisingly, warm ischaemic time  $< 65$  minutes did not correlate with delayed function or eventual creatinine in surviving grafts. Grafts from the same donor had similar outcomes.

Using multivariate analysis and logistic regression, a series of equations was then generated to predict those donors with poor graft outcomes. Resultant predictive values depend upon the entry criteria applied, but are around 73%. For example, using one formulation, 16 of 22 kidneys predicted to be discarded would not have worked.

**Conclusion** NHB cadaver grafts are an important resource, comprising 11% of the grafts performed in South Thames over this period. Predictive equations such as these may be an important tool to assist the selection of viable organs for transplantation.

Primary non function increased, and 91% required dialysis of 40% in HB, longest period of dialysis 98 days. Once functioning mean cr similar to HB at 3, 5, 10 yr

20% of Helier donors in past yrs were living related



Then results of NHB donors

Surv. 1988-1994

Prediction of grafts that should not be used

Circulatory arrest  $< 30$  min

total warm  $< 60$

age  $< 65$

Match ABO, A, B, DR X male

50% need ALS / ATG

157 pts.

Pts surv 87% @ 1 yr 133  
82% 3 yr  
5 yr

Overall graft 65% @ 1 yr - 99  
61 3 - 85  
60 5 yr - 64

Pts death loss is excused

65% @ 1 yr 99

61 3 yr

60 5 yr

Improved 1992-1994 over 1990-91, better evaluation of donor & recipient

warm isch  $< 40$  70% surv.  $>$  longer warm isch.

Donor age over 55-60 deleterious

Grafts survived acute (R.A) 80% @ 1 yr of others

Old time longer probably deleterious  
Probably also true for 2 DR mismatch (numbers in groups small)



Important cause of graft loss / patients seems to be graft thrombosis.

US impression that apart from the risk of thrombosis in very young, if they get an acute rejection, seldom recovered

#### FACTORS AFFECTING PAEDIATRIC KIDNEY TRANSPLANT SURVIVAL

Radhak

Johnson R, Postlethwaite B, on behalf of Members of the Paediatric Task Force and the Kidney Advisory Group, UKTSSA Bristol

Cadaveric transplants carried out in recipients aged under 18 years in the calendar years 1986 to 1995 in the UK and Republic of Ireland have been analysed to identify the factors affecting post-transplant survival. 1252 such transplants have been carried out by 34 centres and one year follow-up information was available for 97.2% of the full cohort of transplants. At five years post-transplant 94.8% of expected follow-up was available. There are 13 dedicated paediatric units with the remaining non-specialist centres mostly transplanting 15 to 17 year olds rather than the younger patients.

A multivariate analysis of these data investigated potential effects of recipient factors (age, sex, CMV status, blood group and primary disease) and donor factors (age, sex, CMV status, blood group and cause of death) in addition to year of graft, graft number, cold ischaemic time, HLA-A, B, DR matching and whether the organ was locally retrieved or imported from another centre.

Of the factors analysed only recipient age, primary disease, donor age and HLA mismatching were found to be significantly related to outcome. Cold ischaemic time (CIT) was available for only a limited data set and the results suggested that increased CIT was associated with inferior outcome ( $p=0.09$ ) but further data are being sought to clarify results.

The effects of the significant factors varied over time post-transplant and thus an epoch analysis was carried out to look separately at 0-3 months, 3-12 months and beyond 12 months post-transplant. Results showed that different recipient age groups were affected at different stages post-transplant with the highest risk of failure in the earliest epoch being associated with the youngest recipients whilst the 16-17 years age group were those most at risk of failure between 3 and 12 months post-transplant. Other findings were that the youngest donors were a highly significant influence on outcome in the first 3 months post-transplant only, that patients with obstructive uropathy had inferior survival throughout the post-transplant period and that HLA matching exerted most influence between 3 and 12 months post-transplant when 2 DR mismatched grafts had particularly poor outcome (Relative Risk=3.4,  $p=0.0008$  compared with favourable matched grafts).

1264 in 1986-95 in 34 centres.

About 120 / yr over the 10 yr. Trend to fewer adult kidneys used in children

Matching improved over the years.

Best results 15-16 yr recipient  
Risk of very young donors high, up to age 5, but still more than in adults up to 15 yr. Some of this perhaps due to small numbers

Recipient age but outcome 8-13 yrs  
Matching results very small nos in groups  
Improved graft survival first 5 yrs of second 5 yrs but donor age & recipient age changes confound results.



Attention drawn to use of <sup>SOCIETY</sup> in bloc kidneys  
Doesn't know if BTS was used in the study



FACTOR V LEIDEN MUTATION - A POTENTIAL RISK FACTOR IN RENAL VEIN THROMBOSIS.

Benson S, Garnham A, McPake D, Wilde J\*, Lipkin G, Ready A

The Renal Unit and Department of Haematology\*, Queen Elizabeth Hospital, Birmingham.

Renal Vein Thrombosis (RVT) remains a cause of early renal allograft loss, which is unpredictable in occurrence and enigmatic in aetiology. Hyper-coagulability states are recognised risk factors for deep venous thrombosis and thrombosis in reconstructive arterial grafts. They may also increase the risk of RVT particularly if other unfavourable factors, e.g. poor graft preservation, are also present. Recently, a new prothrombotic factor, the *Leiden mutation* in the gene for Factor V (Factor V Leiden - FVL), has been described which causes resistance to activated Protein C. This has an incidence in the general population of approx. 3.0% [1]. Since the associated prothrombotic tendency is easily reversed by warfarin it is important to establish whether this factor contributes to the development of RVT. The aim of this study was, therefore, to assess the prevalence and significance of FVL in renal patients with a history of RVT.

16 adult patients (5 females, 11 males) with an mean age of 38 years (range 16-63 years) were identified who, in the past 6 years, had experienced renal allograft loss due to RVT. The mean time from transplantation to thrombosis was 9 days (range 1-28 days). These patients were screened on peripheral blood for FVL. 2 of the 16 (12.5%) patients exhibited the FVL mutation. In 12 patients other factors were present (eg technical difficulties, long cold ischaemia, poor perfusion) which may account for the development of RVT. However, in 4 patients no other factors were evident. These included both the patients with FVL.

In this limited study FVL was four times more common in patients with a history of RVT than in the general population. Whilst it may therefore constitute a preventable risk factor for the development of RVT statistical significance could not be established ( $p=0.87$ ) because of the small number of RVT patients available. A larger study would be required to fully establish the level of risk posed by FVL and the desirability of including a FVL screen in the pre-transplant work-up. Since other individual units will also have only small numbers of RVT patients such a study would have to be multi-centered.

1. Brown et al. B. J. Haematol 1997; 98: 907-909.

Meris: increased incidence of thrombosis in cyclosporin treated pts.  
Over 5 yrs have given low dose aspirin, first dose before tx, continued for a month - virtually eliminated thromboses.

Lancaster: used aspirin, similar experience  
Roseanne: - du Bey knows of the thrombosis renal vein pts have had vascular access problems?



Heparin does as well.  
Someone also used 150 mg aspirin in adults, 75 in under 14 yr olds.

## PAEDIATRIC LIVER TRANSPLANTATION AT KING'S COLLEGE HOSPITAL

Rela M, Muiresan P, Vilca Melendez H, Hadzic D, Dhawan A, Baker A, Mieli Vergani G, Heaton ND.

Liver Transplant Surgical Service and Department of Child Health,  
King's College Hospital, Denmark Hill, London, SE5 9RS, UK

Between 1989 and December 1997, 302 liver transplants were performed on 267 children at King's College Hospital/London. The mean age was 5.8 years (5 days-16 years). Overall 176 transplants were performed in children less than 5 years of age, 74 in less than 1 year and 16 in less than 3 months of age.

Seventy children were transplanted for acute liver failure (23.2 %) and 190 were for chronic liver disease (62.9 %) the most frequent aetiology being biliary atresia (45.8 % of chronic liver disease). Forty-two retransplants were carried out on 35 children. The main indications for retransplantation were chronic rejection (18) and hepatic artery thrombosis (15).

Whole grafts were used in 96 (31.8%) and segmental grafts in 206 (68.2 %) transplants. Of these, 135 were reduced, 47 split, and 13 auxiliary grafts (in 4 auxiliary transplants a split graft was used). Fifteen living related liver transplants were also performed.

Actuarial survival at 1 and 5 years for children transplanted for chronic liver disease was 88 % and 82 % and for acute liver failure 72% and 63 % respectively.

Over the course of the programme there have been significant improvements in 1 year survival and incidence of hepatic artery thrombosis and retransplantation.





CONTRASTING EFFECTS ON HEPATITIS B VIRUS ANTIGEN  
EXPRESSION OF IMMUNOSUPPRESSIVE AGENTS.

HOBBINS, B. L., GREER, S., HOWARD, J. AND ALEXANDER, G. J. M.

Department of Medicine, University of Cambridge Clinical School, Cambridge,  
CB2 2QQ

Significant life threatening liver damage caused by the hepatitis B virus following transplantation appears to be related to the accumulation of viral antigens in tissue rather than being immunologically mediated. The basis for antigen accumulation is unexplained, but might be a direct effect of the immunosuppressive agents on the virus, as already reported for corticosteroids.

Using an HBV transfected HepG2 cell line that has the ability to produce viral antigens, but cannot replicate, we have already confirmed that corticosteroids increase HBsAg production. In addition we have recently reported that Cyclosporin A increased HBsAg expression even further, with no evidence of saturation, and that Tacrolimus had no effect on HBsAg production. To continue our study in this area we looked at the effects of three cyclosporine analogues, three cyclosporin metabolites and a rapamycin analogue on HBsAg production, using clinically relevant concentrations.

HBV transfected cells and control (plasmid only) transfected cells were incubated with a range of concentrations of the appropriate compounds for 6 days. All three cyclosporine metabolites had a major stimulatory effect on both secreted (203%-365%,  $p=0.003$ ) and intracellular HBsAg (95%-222%,  $p<0.02$ ). One of the metabolites, AM1, caused significant intracellular accumulation of HBsAg and this was the only compound associated with a reduced cell number (37%,  $p=0.03$ ). All of the cyclosporin analogues had a massive stimulatory effect on secreted HBsAg (276%-1343%,  $p<0.02$ ) and also caused an increase in intracellular HBsAg (99%,  $p=0.03$  and 445%,  $p=0.0005$ ); changes which were proportional and had no effect on cell growth. The rapamycin analogue had an inhibitory effect on secreted HBsAg and reduced it by 45% ( $p<0.05$ ) at 3 ng/ml, and reduced it further by 64% ( $p<0.02$ ) at 300 ng/ml, cell associated HBsAg showed no significant change.

These *in vitro* observations provide a rational explanation for increases in HBsAg production and accumulation following graft infection and suggests that the type of immunosuppressive agent used should be tailored to the primary liver disease requiring transplant.

*Now use Tacrolimus and have had no hepatitis  
following transplant.*



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## IS A PATCH NECESSARY FOR HEPATIC ARTERIAL ANASTOMOSIS IN ORTHOTOPIC LIVER TRANSPLANTATION ?

S. Gupta, N. Ahmed, JPA Lodge, SG Pollard

Department of Transplantation, St. James's University Hospital  
Beckett Street, Leeds, LS9 7 TF

Since January 1994 we have carried out 271 adult liver transplants using a standardized technique. Arterial revascularisation is usually by anastomosis of common hepatic artery to common hepatic artery. In our standard method the donor hepatic artery is cut short to avoid kinking. No attempt is made to either preserve the patch or create a new one using the donor splenic artery stump, if by doing so, there would be a redundancy in length. The suture material used is 6-0, 7-0, or 8-0 prolene or Gore-Tex (continuous). Some variation is clearly needed for donor livers with accessory arteries and arterial conduits (iliac) are used for recipients with a non useable hepatic artery.

Following the transplant we keep the Haemoglobin <10g/dl(PCV 25-30) venesecting if necessary. If the PT is < 24s, 6 hours after transplantation then i/v heparin is started (40 units/kg/day) for 6 days. A protocol biopsy is done on day 7 and the recipient is then placed on clexane 20 mg s/c until discharge. Graft function is monitored by serial liver function tests and doppler ultrasound with early recourse to arteriography for abnormal results. Our experience with this anastomosis technique are summarized below.

Number of transplant since 1994	271
Arterial conduit	23 (8.5%)
Early hepatic artery thrombosis(<30 days)	6 (2.2%)
Late arterial thrombosis (> 30 days)	2 (0.74%)
Retransplants	6 (2.2%)

There were no thromboses or stenoses in the conduit group. Three patients required revision of non thrombotic stenoses by surgery (2) or by angioplasty (1).

An acceptable arterial thrombosis rate can be achieved by this approach of sacrificing the patch to prevent kinking and the use of low dose heparin.





## ARTERIAL REVASCULARIZATION IN ADULT LIVER RETRANSPLANTATION

Afonso RC, Gur U, Gunson BK, Mayer AD, McMaster P, Buckels JAC, Mirza DF.

The Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham.

Arterial blood supply is indispensable for liver graft function and usually the recipient hepatic artery provides adequate arterial inflow. In orthotopic liver retransplantation (ROLT), the arterial anastomosis is usually performed proximal to the site of the initial anastomosis, excising all previous donor artery. From 1982 to 1997, 116 ROLTs were performed in 101 adults. Eleven patients received a third graft and two patients a fourth graft. The indications for retransplantation were: irreversible rejection (41), hepatic artery thrombosis (HAT) (38), primary nonfunction (12), massive haemorrhagic necrosis (12), recurrent disease (6), and others (7).

Fifteen retransplants required aortic arterialization by infrarenal antepancreatic iliac conduit anastomosed to the donor artery and 101 had standard arterial reconstruction utilising the recipient coeliac axis. The latter group was divided in 4 subgroups according to the level of the recipient vessel. Fifty six (50.4%) ROLTs had an arterial anastomosis at or distal to gastroduodenal bifurcation (group A), 31 (28%) to the common hepatic artery (group B), seven (6.3%) to the coeliac trunk (group C), and two (1.9%) had interposition donor iliac grafts between donor and recipient hepatic or splenic arteries (group D). Overall 8/116 (7%) ROLTs developed HAT. Post ROLT HAT developed in 2,1,1 and 1 transplants in groups A, B, C, and D respectively ( $p=ns$ ). In comparison, there were 3 HAT events among 15 ROLTs in patients with infrarenal aortic conduits. One patient had 2 events of HAT after ROLT and received further ROLTs. Two patients are alive with HAT, one of them with an anastomotic biliary stricture treated with percutaneous biliary dilatation (group B) and the second one is asymptomatic (aortic inflow group). Five patients died after HAT associated complications.

In conclusion, the rate of HAT after ROLT is similar compared to HAT after primary grafts. The use of an aortic conduit does not eliminate this complication.



AN ELISA FOR ANTI-VIMENTIN ANTIBODIES AS A NON-INVASIVE METHOD TO MONITOR TxCAD IN HEART TRANSPLANT PATIENTS

Jurcevic, S., Ainsworth, M., Banner, N., Yacoub, M., Dunn, M. and Rose, M.

National Heart and Lung Institute, Imperial College School of Medicine, Heart Science Centre, Harefield Hospital, Harefield, Middlesex, UB9 6JH

Transplant associated coronary artery disease (TxCAD) is the most frequent complication of heart transplant and it is a major factor in determining patient longevity. TxCAD is difficult to diagnose at an early stage and the treatment of an established disease is very limited. Hence, there is need for a non-invasive test which would be able to identify patients at risk or detect disease at an early stage. We have previously shown a strong correlation between TxCAD and presence of IgM anti-endothelial antibodies in patients sera with Western blot reactivity towards 58-60 kDa antigen, recently identified as vimentin. Here we have developed an ELISA test using recombinant human vimentin and measured anti-vimentin IgM antibodies in sera of heart transplant patients. The ELISA assay allows quantitative detection of antibody titres as end point dilutions (highest sera dilution giving absorbance (OD) > average healthy serum + 3SD) with some of the high titre sera showing activity at dilutions > 1/1000. We report on two studies in which 72 patients were studied prospectively and 68 were followed retrospectively. In order to follow the temporal pattern of antibody formation, 4 or more samples were tested in the first year and 1 or 2 samples in the following year. Of a total of 140 sera taken prior to transplantation only 21 were antibody positive. Of the 72 patients studied prospectively, 24% produced antibodies. As none of the patients had abnormal coronary arteriograms measured 1 year post-transplant, we were unable to evaluate ELISA results. However, 11 of these patients were also investigated by a more sensitive intravascular ultrasound angiography (IVUS). Within this limited group of patients 2/11 patients had TxCAD (stenosis >25%) at 1 year and both of them had high titre (>1/400) anti-vimentin antibodies. In contrast, 9/11 patients who were without TxCAD had either no antibodies or only low titres (<1/100). Interestingly, in patients with TxCAD detected by IVUS, high titre antibodies preceded morphological changes. The retrospective study was of 68 patients transplanted between 1987-1991 using sera collected at a time of biopsies. Within this group 30/68 patients developed TxCAD in the 5 year period. Significantly, 86% of them (26/30) had persistently high titre (>1/100 for more than 6 months) of anti-vimentin antibodies. Typically, the peak of antibody production was around 6 months post-transplant, but some patients developed antibodies only later (at 2 or 3 years post-transplant) which in 4 out of 6 cases correlated with the late onset of TxCAD. We have noticed that patients with very high antibody titres (>1/400) tend to develop more severe TxCAD manifested in an early onset and a rapid progression. In contrast, only 7/38 (18%) patients who did not develop TxCAD had antibodies and these were of moderate titre (1/100 to 1/200). In conclusion, ELISA for anti-vimentin antibodies may provide an important additional test which can help in identifying patients at risk of developing TxCAD. Furthermore, as antibodies to vimentin precede visible morphological changes in coronary arteries, this novel non-invasive test may offer an opportunity for early intervention aiming at slowing down or blocking further development of TxCAD.



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## MEDIUM TERM RESULTS OF TOTAL LYMPHOID IRRADIATION AS RESCUE THERAPY AFTER CARDIAC TRANSPLANTATION

BP Madden, L Backhouse, D Tait, L Reynolds, J Barros, A Murday.

St George's Hospital, London, SW17 0QT

### BACKGROUND

Allograft dysfunction develops in a proportion of heart transplant recipients without cellular infiltrate in endomyocardial biopsies and with normal coronary arteries at angiography. The mechanisms responsible for this presentation are unclear and the prognosis poor. We report encouraging experience with total lymphoid irradiation (TLI) in the management of this condition.

### METHODS

Ten patients (seven male, three female, median age 53 years) developed severe biventricular failure (NYHA Class 4) one-62 (median five) months post transplantation in spite of immunosuppression with Cyclosporin A, Azathioprine, oral Prednisolone, Cyclophosphamide and intravenous Methyl Prednisolone therapy. Endomyocardial biopsies and coronary angiography were normal in each patient. TLI was given with standard mantle and inverted Y fields over ten treatments to achieve a cumulative dose of 8Gy.

### RESULTS

Each patient had a significant improvement in clinical response (NYHA Class 1 or 2) and in ventricular performance after TLI. Nine patients are currently alive and well with a range of follow-up from six to 43 (median 20) months. One patient died as a consequence of chronic renal failure. Two patients developed cytomegalovirus infection (one systemic, one retinitis) and two developed pneumonia (one pneumocystis carinii, one pseudomonas aeruginosa). Treatment was successful in each case with appropriate antimicrobial therapy. One patient developed an Epstein Barr virus associated B cell lympho-proliferative disorder which was successfully treated by reduction in immunosuppression and Acyclovir.

### CONCLUSIONS

TLI is well tolerated and should be considered as adjunct therapy to conventional immunosuppression for heart transplant recipients with poor graft function in the absence of cellular rejection or coronary artery disease.



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## INTERLEUKIN 8 AND ITS MODULATION IN LUNG REPERFUSION INJURY

CLARK S., SUDARSHAN C.D., ALI S., KIRBY J., DARK J.H.

Cardiothoracic Centre, Freeman Hospital, Newcastle upon Tyne, NE7 7DN.

### Background

Interleukin 8 (IL8) is the principle neutrophil chemoattractant during reperfusion injury of transplanted lungs. We studied the effects of controlled pressure reperfusion, Pentoxifylline, inositol polyaniions (InSP6) and SIN 1 (3-morpholinosydnonimine), a potent nitric oxide donor, on the expression of IL8 to determine the importance of this mechanism in the pathophysiology of reperfusion injury.

### Methods

Donor lungs were harvested and preserved with Euro-Collins solution for 18 hours. Five groups of 45kg pigs (n=5 in each) subsequently underwent left single lung allotransplantation with 12 hour follow up. Group 1 was reperfused at a high pulmonary artery pressure (PAP) of 45mmHg, Group 2 was reperfused at a low PAP of 25mmHg, Group 3 was reperfused as for Group 2 but with intravenous Pentoxifylline (2mg/kg/h), Group 4 with intravenous InSP6 (0.02mg/kg/hr) and Group 5 with intravenous SIN1 (0.02mg/kg/hr).

Open lung biopsies were taken from normal lung, unperfused donor lung and at 2 minutes, 10 minutes, 30 minutes and 12 hours post reperfusion. IL8 expression was determined by RNA isolation (RNAzol method), followed by reverse transcription polymerase chain reaction and gel electrophoreses. Pulmonary venous oxygenation, neutrophil elastase and MDA (malonyldialdehyde) - a free radical marker were also assessed at each time point.

### Results

In Group 1 reperfused at high pressure, IL8 was expressed at 2 minutes after reperfusion and maximally at 10 minutes. In contrast, lungs reperfused in Groups 2, 4 and 5 showed minimal expression at 10 minutes and maximal expression at 30 minutes. In the Pentoxifylline treated group (Group 3) only minimal IL8 expression was observed at 30 minutes. No groups showed IL8 expression at 12 hours. Pulmonary venous oxygen tension was best in Group 3 and worst in Group 1. Similarly the highest levels of neutrophil elastase and MDA were seen in Group 1, and the lowest in Group 3. Groups 2, 4 and 5 were similar.

### Conclusions

IL8 is important in the pathogenesis of neutrophil mediated reperfusion injury. Controlled pressure reperfusion alters the expression of IL8 and may act through this mechanism. Pharmacological agents such as InSP6 and SIN 1 also modulate IL8 expression, but Pentoxifylline has the most profound effects, which correlate with the observed improvements in pulmonary graft injury.





## COPING WITH MULTI-ORGAN THORACIC DONORS - UNITED KINGDOM TRANSPLANT PRACTICE

AC ANYANWU, CA ROGERS, AJ MURDAY on behalf of the Steering Group, UK Cardiothoracic Transplant Audit.

Surgical Epidemiology and Audit Unit, The Royal College of Surgeons of England, London WC2A 3PN

**Background:** Cardiopulmonary transplant units are occasionally challenged with donors providing organs for 2 or 3 recipients; accepting all organs implies 2 or 3 simultaneous transplants, otherwise 1 or more organs are 'exported'. We examine the use of organs when a retrieval unit is faced with 2 or 3 organs.

**Methods:** Multi-centre prospective cohort study involving all 9 UK transplant units. Group medians compared using Wilcoxon 2-sample test.

**Subjects:** 757 UK thoracic organ donors between April 1995 and March 1997. Organs retrieved but not used do not contribute to this analysis.

**Results:** 630 (83%) donors provided organs for 1 recipient only, 99 (13%) 2 recipients while 28 donors (4%) provided organs for 3 recipients. In 537 (70.9%) retrievals, no organs were exported, 192 (25%) had 1 organ exported, while in 28 (4%) 2 or 3 were exported. Proportion exported per organ were, 23% Heart (HT), 48% Lung (LT) and 16% for Heart-lung (HLT). The proportion of retrieved organs exported per unit was variable (range 9 to 47%). Where two organs were retrieved, the retrieving unit used both organs in only 35% of cases; with 3 organs, all three were used locally in 25% (Where one organ was retrieved 79% were used locally). Of 48 'domino' hearts retrieved from HLT recipients, 9 (20%) were exported. The ischaemic times were longer for exported organs, HT (median 216 mins Vs 166.5 mins when used locally,  $P=0.0001$ ), LT (257.5 Vs 212,  $P = 0.006$ ) and HLT (255 Vs 198,  $P=0.02$ ). Exported domino hearts showed a considerable increase in ischaemic time (226 Vs 73,  $P= 0.006$ ).

**Comments:** While some organs are exported because there are better suited recipients elsewhere, our results suggest that resources to undertake simultaneous transplants may often be lacking in the UK. Long ischaemia times are associated with reduced graft survival. Although resource intensive, ability to perform simultaneous transplants in the local unit is ideal as this would reduce ischaemic times and, theoretically, could transform to increased graft survival.



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## HAEMATOPOIETIC STEM CELLS TRANSDUCED WITH A SINGLE DONOR CLASS I MHC GENE CAN INDUCE OPERATIONAL TOLERANCE TO FULLY ALLOGENEIC CARDIAC ALLOGRAFTS

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Bone marrow cells (BMCs) are a useful vehicle for pretransplant alloantigen delivery to prolong allograft survival. We have shown that recipient derived bone marrow cells transduced with a single donor class I MHC molecule can facilitate acceptance of a fully allogeneic donor cardiac allograft. Haematopoietic stem cells have the potential for self-renewal, persistence in the recipient and differentiation into all haematopoietic lineages. These properties make haematopoietic stem cells attractive targets for gene therapy. In this study, we have isolated recipient stem cells and transduced them using a replication defective retroviral vector carrying a single donor class I MHC gene. The ability of the transduced stem cells to induce operational tolerance to fully allogeneic cardiac allografts was investigated.

BMCs were harvested from recipient strain mice CBA.Ca (H2<sup>b</sup>) after intravenous injection of 150mg/Kg of 5-Fluorouracil (5-FU) to deplete the more mature clonogenic cells and to recruit primitive stem cells into cycle. Haematopoietic stem cells were enriched by flow cytometry using c-kit as positive and Cd4, Cd8, B220, Mac-1 and GR-1 as negative markers. Haematopoietic stem cells enriched using this protocol showed extensive potential to rescue lethally irradiated recipients.  $3 \times 10^4$  purified haematopoietic stem cells were able to rescue all irradiated recipients while  $5 \times 10^5$  (200-fold higher) unfractionated bone marrow cells were required to achieve the same effect.

The mouse class I MHC gene, K<sup>b</sup>, was inserted into a replication defective LNSX retroviral vector (K<sup>b</sup>YF). Recipient type CBA stem cells were purified, transduced with K<sup>b</sup>YF and re-injected into recipient CBA mice together with 2 doses of anti-Cd4 monoclonal antibody. Twenty eight days later, heterotopic cardiac grafts from fully allogeneic donors, C57BL/10 (H2<sup>b</sup>) expressing the full complement of allogeneic major and minor histocompatibility antigens including the class I molecule K<sup>b</sup> were transplanted into the recipients. As few as 3 000 recipient CBA haematopoietic stem cells transduced with K<sup>b</sup>YF were able to induce indefinite survival of fully allogeneic C57BL/10 cardiac grafts in 50% of recipients. When unfractionated bone marrow cells were used,  $5 \times 10^5$  transduced cells were required to induce graft prolongation.

Haematopoietic stem cells have extensive potential to repopulate recipients and are suitable target cells for retroviral gene transfer. When transduced with a single donor class I MHC gene, recipient stem cells can induce operational tolerance to fully allogeneic cardiac allografts, further demonstrating the potential of this strategy to facilitate the development of linked unresponsiveness *in vivo*.





## RISK FACTORS FOR CHRONIC ALLOGRAFT FAILURE IN HUMAN RENAL TRANSPLANTATION - A MULTIVARIATE ANALYSIS

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Chronic allograft failure (CAF) is a leading cause of late graft loss in renal transplantation. The pathogenesis is unclear, as are the risk factors for its development. Several centres, but not all, have implicated acute rejection as the major risk factor. We sought to analyse these in a single centre using a multivariate approach.

**Methods:** Sequential transplants, treated with conventional triple therapy, between 1985 and 1996 were studied (n=836). The data was censored to exclude early graft losses (n=117) giving a study population of 719 grafts. Data was collected on pre- and post-transplant variables including patient and donor HLA type, age, gender, CMV status, transplant function, cardiovascular risk factors and acute rejection episodes. CAF was defined according to the Alexis Carrel definition. The data was initially analysed in a univariate fashion and then in a multivariate analysis using logistic regression with CAF as the dependent variable.

**Results:** 77 (10.7%) of the study population had biopsy proven CAF. The major risk factors in the multivariate analysis are shown in the table below.

Variable	Odds ratio	Confidence interval (95%)	P value
Acute rejection > 3 months	5.914	2.65 - 13.2	<0.0001
Proteinuria	5.069	2.58 - 9.98	<0.0001
Triglyceride > 2.7mmol at 1yr	3.061	1.59 - 5.90	0.0008
Recipient age < 50	2.506	1.22 - 5.18	0.013
Early acute rejection	2.310	1.17 - 4.57	0.016
Recipient gender (male)	0.347	0.18 - 0.67	0.0015
Creatinine at 6 months	1.004	1.00 - 1.01	0.032

Further univariate analysis of acute rejection showed that any acute rejection episode increased the risk of developing CAF (4.2% if no rejection, 14.1% if mild early rejection; p<0.001), the risk is substantially greater for grafts with steroid resistant rejection episodes (26.2%; p<0.0001).

**Conclusions:** Our findings indicate that the major risk factor for CAF is acute rejection, at any time point. Metabolic factors such as proteinuria and hypertriglyceridaemia are also relevant. The metabolic factors may not be causative, but are certainly of predictive value in identifying patients at risk of developing CAF. Younger recipients would appear to be at increased risk. This may be due to a qualitative difference in the severity of acute rejection in these patients.



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**CA REPEAT ALLELE IN THE FIRST INTRON OF THE INTERFERON GAMMA (IFN- $\gamma$ ) GENE IS ASSOCIATED WITH THE DEVELOPMENT OF FIBROSIS IN LUNG TRANSPLANTS.**

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<sup>1</sup>Tissue Typing Laboratory, St.Mary's Hospital, <sup>2</sup>Cardiothoracic Unit, Wythenshaw Hospital, and <sup>3</sup>Immunology Research Group, Manchester.

Damage due to inflammation results in graft fibrosis following lung transplantation. The inflammatory cytokines including IFN- $\gamma$  have been implicated in this process. IFN- $\gamma$  specifically plays a role in the modulation of fibroblast collagen matrix deposition, collagen synthesis as well as upregulating other genes implicated in matrix deposition including fibronectin. Our group has previously described five size alleles of the microsatellite, CA repeat element (allele #1=11CA repeats, #2=12, #3=13, #4=14, #5 =15) in the first intron of the human IFN- $\gamma$  gene. A parallel study, analysing this polymorphism in a group of healthy controls, has also correlated high IFN- $\gamma$  production as defined by *in vitro* stimulation followed by ELISA, with the 12 CA repeat allele (allele #2).

PCR primers were used to amplify a 150 bp fragment of the IFN- $\gamma$  gene encompassing the repeat element and size polymorphism was observed following electrophoresis on polyacrylamide gels (12%, 1:19 bis/acrylamide). Using this method we have analysed eighty-two lung recipients transplanted in a single centre between 1990 and 1997 and sixty-nine healthy controls.

When the genotype frequencies for both groups were analysed, no significant differences were observed. However, when we compared the transplant recipients with and without fibrosis and the presence of the allele associated with high IFN- $\gamma$  production we found a significant correlation (see table).

Genotype	Allograft fibrosis	No allograft fibrosis
High producer	56	8
low producer	10	8

$p < 0.005$

We postulate that IFN- $\gamma$  is implicated in the development of lung fibrosis and subsequent chronic rejection following lung transplantation. Further, we suggest the CA repeat polymorphism identified in this study may have prognostic significance in a wider range of fibrotic and or sclerotic conditions.





FINDING MORE ORGAN DONORS: AN INVESTIGATION INTO THE REFERRAL PATTERNS OF PATIENTS DYING IN HOSPITAL WITH INTRA-CRANIAL EVENT OR INJURY.

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**Introduction:** A major limiting factor of organ transplantation is the supply of donor organs, the consequence of which is a continual quest to identify patients with the potential to donate but who are not referred for donation. Several studies (UKTCA, BACCN, MORI 1995 & Gore et al 1992) have reported the incidence of brain stem death testing on intensive care. This study sets out to investigate the admission and treatment of patients who die with broadly similar diagnoses to those who donate but are not admitted to the intensive care unit (ICU).

**Method:** All deaths occurring in a single year (1<sup>st</sup> January 1996-31<sup>st</sup> December 1996) at one NHS Trust were reviewed using the clinical coding data to define causes of death. This list was then refined by excluding all patients dying over the age of 70, of malignant disease or HIV. The relevant clinical codes were identified by matching codes to causes of death of all donors in one NHS Executive region during the study period. Suitable patients were identified using the donor codes as selection criteria. The medical records of the selected patients dying on the wards were then reviewed for the following criteria:

1. Provisional diagnosis and prognosis on admission.
2. Glasgow Coma Score on admission.
3. Resuscitation status or evidence of Do Not Resuscitate (DNR) order.
4. Age of patient and past medical history.
5. Length of time from admission to death.
6. Mode of death.

**Results:** A total of 1499 patients died in the Trust during the study period. Application of initial exclusion criteria identified 211 patients dying in ICU and 348 dying in other wards and departments. Using the clinical codes, 57 (27%) deaths in ICU and 42 (13%) deaths in other departments were matched to the 'donor codes'. 21 (50%) of medical records from the latter group were available for review.

67% (14/21) had a CT scan and in 76% (11/14) of cases the findings of the scan were recorded as influencing the subsequent course of treatment. In 3 cases initial management was aggressive but changed as the patient deteriorated and the remaining 8 patients had a DNR order recorded. None of the patients with a DNR order had any record of the death except routine certification. 3 of the patients having CT scan were recorded as being intubated and ventilated for this purpose, ventilation was discontinued in all 3 cases although they remained intubated. GCS was recorded in 43% (9/21) of cases however full neurological assessment was performed on 90% (19/21) of patients, with motor deficit and/or sluggish or absent pupillary response recorded in all these cases. During the study period there were 88 solid organ donors in the region the average age of whom was 39.5 years (range 8mths.-74yrs). The average age of patients whose records were reviewed was 60.4 years (range 31-70).

**Conclusion:** There are a group of patients dying on the wards in this Trust from the same causes as those who became organ donors from the regional ICUs. Admission to ICU was not considered for these patients due to their poor prognosis on initial assessment. An increase in the number of ICU beds is unlikely to alter this without a change in the guidelines for admission to ICU.



**THE EFFECT OF DIFFERENT IMMUNOSUPPRESSANTS ON IMMUNOLOGICAL AND NON-IMMUNOLOGICAL FACTORS INVOLVED IN THE DEVELOPMENT OF CHRONIC REJECTION IN THE RAT AORTIC MODEL**

Cole OJ, Rigg KM and Shehata M

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**Introduction.** Both immunological and non-immunological factors have been suggested to play a role in the development of chronic rejection (CR). In this study we describe a new model to investigate the effects of different immunosuppressive drugs on the reduction of vascular changes in syngeneic and allogeneic rat aortic transplants subjected to cold ischaemia.

**Methods.** Segments of abdominal F344 rat aorta preserved in Marshalls solution for 1, 4 or 24 hours at 4°C, were transplanted orthotopically into either F344 or Lewis rats (6/group = 36 untreated transplants). Further groups (n=36) then received different immunosuppressants by gavage daily at the following doses: - CyA 12mg/kg/day; FK506 0.15mg/kg/day; MMF 20mg/kg/day; SDZ RAD 2.5mg/kg/day. Aortas were retrieved at 2 months and examined by standard histology and computerised morphometry.

**Results.** F344-F344 controls of 1 hour cold ischaemic time (CIT) in all groups showed little histological changes - there was a single cell layered intima and a thick, cellular media. With increasing ischaemic time in the syngeneic grafts, however, interruptions to the internal elastic lamina were observed, along with reduced medial thickness and cellularity and increased intimal thickness. There was also evidence of smooth muscle cells within the intima. Similar changes occurred in the untreated allogeneic grafts but were more pronounced, changes being most marked in the 24hr CIT allografts. In the treatment groups the severity of histological changes paralleled their morphometry results. Morphometric analysis showed that CyA resulted in no significant reduction in intimal or medial changes in either the syngeneic or allogeneic groups. Whilst FK506 had a significant effect in reducing damage due to immunological mechanisms (ie. F344-LEW 1hr CIT), its effect on grafts with longer CIT's was less marked. MMF appeared to reduce immunological damage the greatest and also reduced ischaemic damage. However, SDZ RAD resulted in the most significant reduction in combined damage from immunological and ischaemic mechanisms.

**Conclusions.** This is a reproducible model in which to study the pathogenesis and treatment of CR. By using orthotopic abdominal aortic transplants, we were able to halve the donor aorta and use each half for two recipients with different CIT, thereby reducing animal numbers.

Cyclosporin has no effect on the development of CR in this model. Fk506 significantly reduces the development of CR only when there is no ischaemic insult. Mycophenolate has the greatest effect in reducing CR when immunological insults only are involved, and also reduces CR caused by ischaemic damage. SDZ RAD results in a significant reduction in CR in the presence of ischaemic injury, and also significantly reduces changes caused by combined immunological and ischaemic insults.





ABNORMALITIES IN INTACT AND SPLIT PROINSULIN SECRETION IN ISLET AUTOTRANSPLANTATION AND CHRONIC PANCREATITIS: A PRELIMINARY STUDY

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Islet autotransplantation can be combined with pancreatectomy for the treatment of chronic pancreatitis (CP). However, in some recipients, exogenous insulin therapy is still required to maintain normoglycaemia. Studies on these patients demonstrated abnormally high secretion of intact proinsulin (IPI) and 32/33 split proinsulin (SPI) in the islet autotransplant recipients relative to normal controls and chronic pancreatitis. The SPI secretion was also elevated relative to IPI secretion. A preliminary *in vitro* study has been undertaken to attempt to elucidate the cause of these anomalous results.

Islets of Langerhans were isolated from normal cadaver pancreata (n=4) and pancreata removed from CP patients (n=2) unable to undergo autotransplantation due to abnormal oral glucose tolerance test (OGTT) results. Aliquots of the islets were cultured in media containing basal or stimulatory concentrations of glucose. Samples of medium were taken at regular intervals over a period of 24 hours, stored at -80°C and analysed for insulin, IPI and SPI. The results tabulated below are the ratios of IPI:insulin, SPI:insulin and IPI:SPI secreted by islets incubated at 5mM or 20mM glucose after 1 and 24 hours.

5mM glucose	1 hour			24 hours		
	IPI:Ins (%)	SPI:Ins (%)	IPI:SPI	IPI:Ins (%)	SPI:Ins (%)	IPI:SPI
Control islets	3.54	2.76	1.59	6.42	5.27	1.37
CP1 islets	0.56	0.56	1.06	1.22	0.57	1.67
CP2 islets	1.03	1.00	1.02	2.13	0.56	3.87

20mM glucose	1 hour			24 hours		
	IPI:Ins (%)	SPI:Ins (%)	IPI:SPI	IPI:Ins (%)	SPI:Ins (%)	IPI:SPI
Control islets	3.65	2.70	1.52	6.45	5.47	1.22
CP1 islets	0.57	0.56	1.22	1.96	1.10	1.83
CP2 islets	1.20	0.74	1.68	2.83	1.19	2.39

These results demonstrate differences in the profile of insulin, IPI and SPI secretion, between control islets and those from patients with CP. The IPI:insulin and SPI:insulin ratios for both CP1 and CP2 are considerably lower than for control islets, indicating a decrease in the secretion of IPI and SPI relative to insulin. The 24 hour results also demonstrate an increase in the IPI:SPI ratio. Both of these trends are opposite to the results of elevated IPI and SPI secretion seen in the autotransplant patients. Of further interest is the difference between the results from the two CP patients, as their aetiologies were different: CP1 was idiopathic and CP2 was alcoholic.

In conclusion, the preliminary data presented here demonstrate differences in the secretory function of islets isolated from normal donors and from patients with CP. The reason for the opposite effect on IPI and SPI secretion *in vitro* compared with the results from the autotransplant patients is unknown, although may in part reflect the abnormal OGTT results in the CP patients from whom the islets were isolated. However, in the light of these results, damage to the islets during isolation is an unlikely cause of the abnormalities seen in the autotransplant recipients. Differences in islet function relative to the aetiology of CP may indicate additional factors for consideration prior to selection of patients for islet autotransplantation.



## TUMOUR NECROSIS FACTOR ALPHA (TNF- $\alpha$ ) & INTERLEUKIN-10 (IL-10) GENE POLYMORPHISMS PREDICT ACUTE RENAL ALLOGRAFT REJECTION

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The pro-inflammatory cytokine TNF- $\alpha$  has been implicated in acute rejection while evidence from animal transplant models suggests a role for IL-10 in promoting graft survival. It has also been shown that polymorphisms in the TNFA gene promoter (at position -308) and in the IL-10 gene promoter (at position -1082) correlate with differential production of the cytokines *in vitro*.

In this study, we have investigated the possible relationship between TNF- $\alpha$  and IL-10 gene polymorphisms and the incidence of early acute rejection episodes (RE) upto 3 months post-transplant. One hundred consecutive first cadaveric kidney recipients transplanted at a single centre were studied. Forty of the 100 patients received HLA-DR mismatched transplants. Acute rejection episodes were defined clinically and confirmed histologically where possible. The TNF- $\alpha$  and IL-10 genotypes of these recipients were determined using a simple PCR-based genotyping technique. Recipients' genotypes were then correlated with the RE.

Seven out of 20 recipients (35%) with the IL-10 high producer genotype had multiple RE ( $\geq 2$ ) compared with 12/80 (15%) IL-10 low producers ( $p < 0.05$ ). In HLA-DR mismatched transplants, 5/11 (45%) of recipients with the TNF- $\alpha$  high production genotype had multiple RE compared with 7/29 (24%) recipients with the TNF- $\alpha$  low producer genotype ( $p < 0.005$ ). There was no association between TNF- $\alpha$  genotype and RE in patients with no mismatch at HLA-DR.

When TNF- $\alpha$  and IL-10 genotypes were analysed in combination and in the context of HLA-DR mismatching, 3/3 (100%) patients with the *high TNF- $\alpha$ /high IL-10* genotype had multiple RE as compared to 9/28 (32%) with all other genotypes ( $p < 0.05$ , RR=4.11, 95% CI 2.33-7.26). Conversely, significantly fewer patients with the *low TNF- $\alpha$ /low IL-10* genotype had multiple RE (3/22, 25%) as compared to all other genotypes (9/18, 68%) ( $p < 0.05$ , RR=0.27, 95% CI 0.09-0.86).

Predicted Phenotype	Number of patients and cytokine production profile			
	High TNF- $\alpha$ High IL-10 (n=3)	High TNF- $\alpha$ Low IL-10 (n=7)	Low TNF- $\alpha$ High IL-10 (n=8)	Low TNF- $\alpha$ Low IL-10 (n=22)
$\leq 1$ RE	0	3	6	19
multiple RE	3	4	2	3

We conclude that TNF- $\alpha$  and IL-10 gene polymorphisms are determinants of acute rejection following renal transplantation. These findings support the potential role for cytokine genotyping in predicting transplant outcome, and so aid in the tailoring of immunosuppression to individual patients.





LUNG TRANSPLANTATION : PRODUCTION AND FUNCTION OF  
β-CHEMOKINES GENERATED BY LUNG EPITHELIAL AND ENDOTHELIAL  
CELLS

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Following lung transplantation, donor lung tissue is the target of the host immune system. However, the donor endothelial and epithelial cells are not simply targets, but also play an active role in leukocyte adhesion, recruitment and antigen presentation.

In this series of experiments we investigated the ability of endothelial and epithelial cells from the lung to produce the chemokine MCP-1 at biologically relevant concentrations.

Primary human alveolar epithelial cells (AEC) were isolated from clinical lobectomy specimens using a method developed by this group. Primary cultures of human small airway epithelial cells (SAEC) and human lung microvascular endothelial cells (HLMVEC) were purchased from Clonetics (Biowhittaker) and the endothelial cell line Eahy.926 was used for the transmigration model. Peripheral blood mononuclear cells (PBMC) were isolated from normal blood by density gradient centrifugation.

The results showed that under normal culture condition, HLMVEC produced low levels of MCP-1. AEC did not express mRNA for MCP-1 until stimulated with the pro-inflammatory cytokines IFN $\gamma$  and TNF $\alpha$ . No MCP-1 was detected in SAEC culture supernatant but these cells responded rapidly to IFN $\gamma$  stimulation by producing MCP-1 as measured by ELISA. MCP-1 was detected after two hours stimulation and the concentration reached a peak level after 24 hours ( $974 \pm 25$  pg/ml). In contrast, TNF $\alpha$  (100 U/ml) alone did not induce MCP-1 production following 48 hour stimulation by the addition of TNF $\alpha$  (100 U/ml) strongly synergised with IFN $\gamma$ , resulting a significantly increased MCP-1 concentration after 48 hours ( $4647 \pm 52$  pg/ml)

The MCP-1 containing culture supernatants could stimulate a significant level of PBMC transmigration across a monolayer of an endothelial cell line, Eahy.926. Analysis of the phenotype of transmigrated cells induced by cytokine treated SAEC supernatant demonstrated that the majority were monocytes ( $77.16\% \pm 2.89$ ). Lymphocytes comprised only  $15.05\% (\pm 3.58)$  of the population. This was in sharp contrast to the starting population which had only  $15\% (14.77\% \pm 5.26)$  of monocytes and approximately  $80\% (79.13\% \pm 3.97)$  of lymphocytes. The T to B lymphocyte ratio showed no significant change before and after transmigration. Interestingly, this transmigration was inhibited by immunosuppressive concentrations of both CyA and FK506

In conclusion, primary human lung endothelial and epithelial cells can produce leukocyte chemoattractants that effectively stimulate monocyte transmigration across endothelial monolayers. This transmigration can be inhibited by immunosuppressive drugs.



**MOLECULAR WEIGHT AND DEGREE OF SULPHATION ARE IMPORTANT CHARACTERISTICS GOVERNING THE ABILITY OF HEPARINS TO BLOCK THE ACTIVATION OF HUVEC BY IFN- $\gamma$ .**

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Recent work has demonstrated the potential immunomodulatory effects of heparins on endothelial cells (EC) in preventing interferon- $\gamma$  (IFN- $\gamma$ ) induced EC activation probably by competitive blockade of IFN- $\gamma$  binding to proteoglycans. However clinical application would be restricted by the unwanted anti-coagulant properties of unfractionated heparin. Therefore, we have investigated which characteristics of heparin mediate this immunomodulation, assessing molecular weight, antithrombin III affinity (i.e. anticoagulant potency), and the degree of sulphation. A variety of low molecular weight heparins (LMWHs), a low affinity heparin and unrelated polysaccharides were studied.

Triplicate wells of human umbilical vein endothelial cell (HUVEC) monolayers (at passage 3 to 6) were incubated with IFN- $\gamma$  (100 U/ml) for 72h - 96h in the presence of control serum or a test compound. EC activation was assessed by flow cytometry (FACScan BD) for ICAM-1, MHC Class I and II. Results were standardised using molecular equivalents of soluble fluorescein (MESF) units based on Dako Fluorospheres and analysed using WinMDI and Excel 97. The effect of molecular weight was investigated using commercial LMWHs and molecular weight fractions prepared from the 2nd international standard heparin. The influence of increasing sulphation was compared using dermatan sulphate (SO<sub>4</sub><sup>-</sup>/COO<sup>-</sup> ratio 1), LMWHs (SO<sub>4</sub><sup>-</sup>/COO<sup>-</sup> ratio 2 to 3), lung heparin (SO<sub>4</sub><sup>-</sup>/COO<sup>-</sup> ratio 3) and pentosan polysulphate (SO<sub>4</sub><sup>-</sup>/COO<sup>-</sup> ratio 4) whilst the importance of antithrombotic activity was assessed by comparing immunomodulation by a LMWH (Fragmin®) with Low Affinity Heparin Abl-4 (Kabi-Pharmacia). The requirement for the basic heparin structure was tested using unrelated polysulphated polysaccharides such as sulphated oat-spelt xylan, pentosan polysulphate and fucoidans.

We confirm previous findings that unfractionated heparin blocks IFN- $\gamma$  induced EC activation although at a slightly higher dose than previously reported (9.3 nM/ml vs 6.6 nM/ml). Dose dependent abrogation of both upregulation of ICAM-1 and MHC Class I and induction of MHC Class II was seen using LMWHs; of which dalteparin (Fragmin®) showed the greatest inhibitory effect at both equi anti-thrombotic and equimolar doses ( $p < 0.05$ ). Low Affinity Heparin Abl-4 (0.16aXaU/ml (15nM/ml)) also inhibited EC activation. The effectiveness of immunomodulation was found to increase with increasing degrees of sulphation (pentosan polysulphate >> lung heparin >> dermatan sulphate). Finally polysulphated polysaccharides unrelated to heparin were also effective immunomodulators at 0.3±0.05 nM/ml. In increasing order (measured as percentage inhibition of MHC II induction) they were: crude fucoidan (45% inhibition), sulphated oat-spelt xylan (63.5% inhibition), pure fucoidan (91.1% inhibition) and pentosan polysulphate (93.2% inhibition).

In conclusion both LMWHs and Low Affinity Heparin Abl-4 block IFN- $\gamma$  activation of EC to the same extent as unfractionated heparins. The effect is not heparin specific and is also observed with other anionic polysaccharides. This property is not dependant on antithrombotic activity but molecular weight and degree of sulphation are important. Therefore, clinical use is possible if controlled trials were to demonstrate a significant reduction in rejection in human transplantation.



## RENAL TRANSPLANTATION -A SURVEY OF SURGICAL TRAINEES AND THEIR ATTITUDES TOWARDS TRANSPLANTATION SURGERY AS A CAREER.

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At present there are a number of unfilled consultant posts in Renal Transplantation in the United Kingdom. A recent survey reported that most vascular trainees would prefer not to undergo a period of training in Transplant Surgery<sup>1</sup>. Unless this problem is addressed it seems highly probable that the number of unfilled posts will continue to increase. This survey aimed to highlight the reasons underlying trainee's reluctance to enter the field of Transplant Surgery and, in addition, to assess how the specialty might be changed to attract new trainees.

Questionnaires were sent to 102 surgical trainees requesting details on age, sex, training grade, research interests and chosen specialty. They were then required to consider ten specified reasons commonly thought to influence a trainee's decision on whether or not he/she will enter their chosen specialty, and to grade each of these according to their relative importance (e.g. on-call commitment, operations performed, private income, unpredictable workload, lack of information/ experience etc.). The survey then suggested five changes in training/ structure and the trainees were again asked to grade the relative importance of each with regard to whether it would attract them towards Transplant Surgery (e.g. reduction in on-call, more structured training, increased salary, experience at SHO level.).

Questionnaires were received from 61 trainees (60%). The results are categorised according to Basic Surgical Trainees (BST) and Higher Surgical Trainees(HST). Among HSTs the reason that Transplantation Surgery remains unattractive appears to be lack of exposure to transplantation during early training and a lack of information on careers in transplantation. This was also borne out by BSTs.

Both groups felt that increased exposure and improved training structure would attract them to the specialty.

It appears that in order to address the problem of attracting new trainees into Transplantation Surgery, greater opportunities should be made available to expose surgical trainees to the specialty early in their training. In addition, the British Association of Transplant Surgeons should consider following other specialties in sending career information direct to the trainees.



## PRE-EXISTING GLOMERULOPATHY IN THE DONOR KIDNEY AND ITS INFLUENCE ON GRAFT SURVIVAL AND FUNCTION

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Between 1986 and 1997, as a routine policy, we performed intraoperative biopsy on 388 kidneys obtained after reestablishment of blood flow. In each patient, 3 samples were taken and submitted to light, electron microscopy and immunofluorescence. Morphologic assessment of biopsy specimens was carried out by the same experienced renal pathologist. All patients received a cadaver renal transplant. We found unexpected evidence of pre-existing renal disease in 15 donors (3.9% of donor population). IgA nephropathy was found in 7 grafts, mesangioproliferative glomerulonephritis (MPGN) in 4 grafts, crescentic glomerulonephritis (CGN) in 2 and focal segmental glomerulosclerosis (FSGS) in 2. All donors with abnormal histology had a serum creatinine within the normal range. We analysed renal function in the recipients of these kidneys at 1, 3 and 12 months. The data is shown in the table.

	Donor Serum Creatinine ( $\mu\text{mol/L}$ )	1 month	3 months	12 months
IgA nephropathy n = 5	92 (67-128)	190 (132-273)	149 (117-200)	157 (116-201)
MPGN n = 4	84 (63-114)	193 (164-242)	180 (127-250)	210 (102-277)
CGN n = 2	94 (80-108)	334 (283-385)	285 (154-416)	228 (144-312)
FSGS n = 2	96 (81-112)	178 (134-224)	195 (136-254)	154 (86-222)

Two patients lost their kidney shortly after transplantation (renal vein thrombosis and hyperacute rejection). Four patients died with a functioning kidney and 2 kidneys failed 3 and 4 years after transplantation (cellular rejection and end stage kidney without diagnostic features). Seven kidneys are still functioning with a mean serum creatinine of 179  $\mu\text{mol/L}$  (range 118-252  $\mu\text{mol/L}$ ) with a mean follow up of 3.2 years (1-7 years). No patient lost their kidney due to the pre existing disease in the donor kidney.

In conclusion there is no contraindication to use kidneys from donors with the renal diseases referred to above, provided the serum creatinine is normal.





SUPPRESSION OF HUMAN ANTI PORCINE T CELL IMMUNE RESPONSES BY CLASS II TRANS ACTIVATOR (CIITA) CONSTRUCTS LACKING THE AMINO TERMINAL DOMAIN

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The class II trans-activator (CIITA) is a bi- or multi-functional domain protein which acts as a transcriptional activator and plays a critical role in the expression of MHC class II genes. We have previously reported to this Society that a mutated form of the human CIITA gene, coding for a protein lacking the amino-terminal 151 amino acids, acts as a potent dominant-negative suppressor of HLA class II expression. Porcine MHC class II antigens are potent stimulators of direct T cell recognition by human CD4+ T cells, and are therefore likely to play an important role in the rejection responses to transgenic pig donors in clinical xenotransplantation. We have therefore examined our mutated constructs for their effect on porcine MHC class II expression. In preliminary studies, we demonstrate that transient and stable transfection of the porcine PIEC vascular endothelial cell line with full-length human CIITA constructs results in strong expression of SLA-DR and SLA-DQ antigens, thus establishing the cross-species effectiveness of the human CIITA protein in the pig. The mutated human CIITA constructs were therefore tested in the pig. PIEC clones stably transfected with one of these constructs showed up to 99% suppression of SLA-DR and SLA-DQ antigen induction by recombinant porcine interferon gamma, and marked suppression of SLA-DRA mRNA induction, in spite of normal induction of endogenous porcine CIITA mRNA. Moreover, transient transfection of the porcine L23 B cell line showed up to 90% suppression of constitutive SLA-DR and SLA-DQ antigen expression. In functional studies, we demonstrate that interferon gamma stimulated PIEC clones transfected with this mutated CIITA construct failed to stimulate purified human CD4+ T lymphocytes. The incorporation of the mutated CIITA gene under the control of a vascular endothelial cell-specific promoter in transgenic pigs might markedly reduce the capacity of transgenic pig organs to stimulate human rejection responses.



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## PHYSIOLOGICAL IMPACT OF RENAL DOSE DOPAMINE ON THE EARLY POST TRANSPLANT KIDNEY

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In spite of the wealth of experimental data, the clinical role of "renal dose" Dopamine as a renal protective agent remains controversial. Furthermore, its effect on the post transplant denervated kidney is unknown.

We measured the effects of low dose Dopamine (2.5 micrograms/ kg/ min) on twenty consecutive patients on the first post transplant day. They were sequentially allocated to two groups, each with three study periods of three hours. Hence each patient was acting as its own control. All patients had primary function.

The following parameters were measured for each study period. Renal plasma flow (RPF) using Para Amino Hippuric acid clearance, Glomerular Filtration Rate (GFR) based on creatinine clearance, urine output, urinary Sodium). Hourly Blood pressure and heart rate were recorded.

	Period 1	Period 2	Period 3
<b>Group 1</b>	OFF	ON	OFF
Urine flow (mls/ min)	3.4 (1.5-6.4)	5.8 (3.0-8.9)**	1.8 (1.4-3.1)***
GFR (mls /min)	24.6 (14.7-401.)	33.6 (25.3-43.5)*	25.6 (15.9-6.1)***
RPF (mls /min)	96 (71-150)	138 (108-230)*	105 (56-174)***
<b>Group 2</b>	ON	OFF	ON
Urine flow (mls/ min)	4.3 (1.9-5.1)	1.9 (1.3-2.3)***	3.5 (2.4-4.2)***
GFR (mls /min)	27.3 (19.2-37.9)	23.6 (14.3-2.5)**	27.4 (18.0-4.6)***
RPF (mls /min)	114 (68-255)	73 (41-134)**	106(59-297)***

All values are median (inter-quartile range)

ON = Dopamine infusion, OFF = No Dopamine infusion

Statistics Wilcoxon Rank Sum matched pairs test, 2 tailed

Each group statistically compared with the one preceding it

\*p < 0.05 \*\* p < 0.01 \*\*\* p < 0.005 \*\*\*\* p < 0.001

There were substantial and significant increases in RPF, GFR, and Urine output associated with the infusion of dopamine in both groups. These changes were reversed when dopamine was discontinued.

There were no significant changes in heart rate or Blood pressure.

The physiological effect of renal dose dopamine on the transplanted kidney indicates that it may improve the quality of short-term renal function. It provides the scientific basis for a controlled study to evaluate its clinical impact.



HIGH PRODUCER INTERFERON GAMMA (IFN- $\gamma$ ) AND INTERLEUKIN 10 (IL-10) GENOTYPE IS ASSOCIATED WITH INCREASED FREQUENCY OF ACUTE REJECTION EPISODES IN KIDNEY TRANSPLANT RECIPIENTS.

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IFN- $\gamma$  is a Th1 effector cytokine produced by CD4, CD8 and NK/LGL cells. It is a major regulator of MHC expression in macrophages, endothelial cells and epithelial cells. It increases IL-2 receptors on Tc cells, enhances cytotoxic effects on LGLs and promotes B cell differentiation. Our group has previously described five size alleles of the microsatellite, CA repeat element (allele #1=11CA repeats, #2=12, #3=13, #4=14, #5=15) in the first intron of the human IFN- $\gamma$  gene. A parallel study, analysing this polymorphism in a group of healthy controls, has also correlated high IFN- $\alpha$  production as defined by *in vitro* stimulation followed by ELISA, with the 12 CA repeat allele (allele #2). Further, our group has previously described IL-10 polymorphism defining *in vitro* high and low IL-10 producer status.

PCR primers were used to amplify a 150bp fragment of the IFN- $\gamma$  encompassing the repeat element and size polymorphism was observed following electrophoresis on a polyacrylamide gel (12%, 1:19 bis/acrylamide). We have analysed eighty first cadaveric kidney recipients transplanted between 1990 and 1991.

In this particular group only the #2, #3, #4 and #5 polymorphisms were found. Their frequencies were comparable to a control group of healthy individuals.

Out of 80 patients 41 (51.2%) had at least one rejection. When IFN- $\gamma$  gene polymorphism was correlated with acute rejection episodes in the first three months post transplant, no significant association was found. However, when IFN- $\alpha$  genotypes were analysed in combination with IL-10 polymorphism, patients with the high IFN- $\alpha$  /high IL-10 genotype were shown to reject more often. Of those recipients that had the high IFN- $\gamma$  /high IL-10 genotype, 11/15 rejected compared to 30/65 with other genotypes (RR=1.59, 95% confidence limits 1.06-2.38). When the result was corrected for the presence of DR mismatch the Mantel-Haenzel RR was 1.50 (95% confidence limits 0.98-2.28). It also seems that the increased risk was higher within the DR mismatched group, but numbers were too small for any conclusive result.

From this study, we demonstrate that IFN- $\gamma$  and IL-10 genotype affect acute rejection of kidney transplants even if such confounding factors as DR mismatch are removed. These results along with other recent reports change our traditional view about the suppressive role of IL-10. We suggest that these findings are a further step towards the prediction of renal transplant rejection.



**RANDOMISED CONTROLLED TRIAL COMPARING EFFECTS OF CYCLOSPORIN (NEORAL) AND TACROLIMUS (PROGRAF) ON BONE MINERAL DENSITY AFTER CADAVERIC RENAL TRANSPLANTATION – PRELIMINARY REPORT.**

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**Background:** Cyclosporin-based therapy is associated with a reduction in bone mineral density (BMD) in the initial period following transplantation. A non-comparative study of seven heart transplant recipients, treated with tacrolimus triple therapy suggested that tacrolimus also has a deleterious effect on BMD in the early post-transplant period. The relative effects of these two agents on BMD have not been compared.

**Aim:** Comparison of BMD in patients receiving Neoral or Prograf based triple therapy.

**Methods:** At transplantation, patients were randomised to receive either Neoral (NEO) (8mg/kg/day) or Prograf (PRO) (0.2mg/kg/day). Additional immunosuppression consisted of Azathioprine 1.5mg/kg/day and Prednisolone 20mg/day (tapered to 5mg/day by the end of 3<sup>rd</sup> post-transplant month). Anti-rejection pulse therapy, when indicated, consisted of Methylprednisolone (MP) 500mg/day for 3 days. Base-line DEXA scans were performed within the first few days after transplantation and repeated at 3 months. Results were expressed as Z-scores (a measure of the distance in standard deviations between the patient's BMD and the mean value for age and sex matched controls).

**Results:** There was no difference in a total dose of steroids or in the frequency of methylprednisolone pulses for the 2 groups. Results of DEXA studies are presented in the table.

Z scores (+ S.E.M) for 2 treatment groups for total body and selected regions.

	NEORAL (n=12)	PROGRAF (n=17)
Whole body, 0 months	0.12 +/- 0.35	0.40 +/- 0.41
Whole body, 3 months	0.09 +/- 0.36 <sup>1</sup>	0.39 +/- 0.41 <sup>1</sup>
Lumbar spine, 0 months	0.57 +/- 0.59	-0.13 +/- 0.34
Lumbar spine, 3 months	0.41 +/- 0.57 <sup>2</sup>	-0.44 +/- 0.41 <sup>3</sup>
Femoral neck, 0 months	-0.73 +/- 0.32	-0.53 +/- 0.37
Femoral neck, 3 months	-0.80 +/- 0.33 <sup>1</sup>	-0.66 +/- 0.37 <sup>2</sup>
Radius, 0 months	-1.40 +/- 0.51	-0.16 +/- 0.52
Radius, 3 months	-1.38 +/- 0.51 <sup>1</sup>	-0.30 +/- 0.50 <sup>2</sup>

<sup>1</sup> no significant difference, <sup>2</sup> p < 0.05, <sup>3</sup> p < 0.01, for 0 vs 3 months, Student's paired t test.

There was no significant difference between Z scores for Neoral or Prograf groups at time 0 or 3 months for any region.

**Conclusions:** The majority of patients showed pre-existing reduced BMD in the regions most vulnerable to fractures. Both Neoral and Prograf based therapy were associated with deterioration in Z scores for the lumbar region. Patients in the Prograf group also demonstrated significant early reduction in bone mineral density at the femoral neck and radius. These findings represent the initial results of a continuing long term study.





SECONDARY KIDNEY TRANSPLANTATION  
PROGNOSTIC FACTORS OF AN EXCELLENT OUTCOME

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**OBJECTIVE:** To determine whether the appropriate use of scarce donor resources is accomplished by renal re-transplantation by reviewing the initial and long-term outcomes of second renal transplant recipients and more importantly by defining factors that affect this outcome.

**PATIENTS AND METHODS:** One hundred and thirty eighty patients who had both their first and second transplant performed during a 16 year period were analysed. Ten out of those transplants did not receive triple immunosuppression. Graft survival was the primary outcome measured whereas the number of rejections and DGF were secondary outcomes. These were used because of both their immediate cost and long term effects. Survival was analysed using Kaplan-Mayer curves and differences among groups were confirmed by the log rank test. Mann-Whitney and Kruskal-Wallis tests were used for multiple comparisons and analysis of variance, whereas relative risk used for comparison of risk factors.

**RESULTS:** The 1, 5 and 10 year graft survival was 83%, 69% and 60.5%. This compares favourably with the overall actuarial survival of first transplants in our centre. In contrast with results of others the survival of second grafts was not affected by their respective Previous Graft Survival Time (PGST). When PGST was <1 month the 1 and 5 year graft survival of the second graft was 80 and 64%; when the PGST was between 1 and 6 months it was 89 and 81%; when PGST was between 6 months and 2 years it was 76 and 50%, and when PGST was above 2 years the second graft survival was 84 and 72% respectively.

When HLA-DR matching was taken into account it was found that the subgroup of patients whose PGST was 1-6 months had an excellent survival irrespective of mismatch in this locus.

The percentage of transplants with 0 DR mismatch was 58.7% and was even higher among recipients with PGST>2 years (Mann-Whitney  $p=0.06$ ). Thus it was no surprise that rejections (40% in the whole study group) were less common in the group with PGST>6m and especially >2years ( $p=0.013$ ). In fact the number of rejections was negatively correlated to the PGST (Spearman correlation coefficient -0.2,  $p=0.05$ ).

DGF was higher in these 138 secondary transplants, namely 28.1% (dialysis dependent 13%) than in our primary kidney transplant population. Recipients with PGST>2 years had a relative risk of having DGF of 0.46 (95% CI 0.23-0.92) compared to those with PGST<2 years.

**CONCLUSION:** Secondary kidney transplantation can result in excellent graft survival comparable to that of primary grafts. PGST<6 months results in a higher DGF and a higher rejection rate but is not associated with worse graft survival. 0 DR mismatch was associated with better survival in the recipients with PGST>2 years but not in those with PGST<2 years. The excellent graft survival of second transplants that lost their first grafts between 1 and 6 months, mainly due to immunologic reasons, is surprising but might be partly explained by the use of flow cytometry crossmatching for all secondary transplants.



## DO SEQUENTIAL CHANGES IN PERIPHERAL T-CELL IL-10/IL-4 GENE EXPRESSION RATIO REFLECT ALLOREACTIVITY IN HUMAN RENAL TRANSPLANTATION?

Tan LC, Howell WM\*, Smith JL\*, Sadek SA

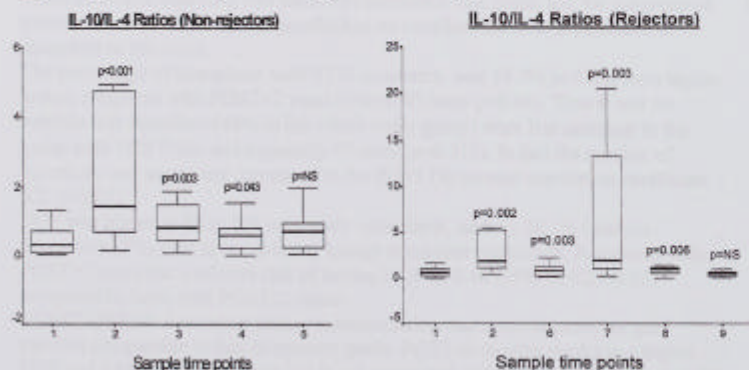
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Cytokines are important immunological determinants in transplantation (Tx). Many "single time point" gene expression studies have produced varied and often conflicting results. In this study, we monitored sequential changes in the ratio of IL-10 to IL-4 gene expression in peripheral blood T-cells in the early post renal transplant period because of the controversy surrounding Th2 cytokines.

**Methods:** 28 recipients without rejection and 15 recipients with histologically proven acute rejection were studied. Peripheral blood T-cells were extracted at time points: [1] pre-Tx, [2] early post-Tx, [3] 1 week post-Tx, [4] 2 weeks post-Tx and [5] 4 weeks post-Tx. In rejectors, additional samples were taken:- [6] before anti-rejection therapy (Th), [7] early post-Th, [8] 1 week post-Th and [9] 4 weeks post-Th. RT-PCR ELISA detection of IL-4 and IL-10 cDNA sequences were performed.

**Results:** IL-10/IL-4 gene expression ratio was examined because the two cytokines showed opposing trends. The Wilcoxon matched-pairs signed-ranks test was used and the p values refer to each time point and its preceding one.

The significant rise in IL-10/IL-4 ratio early post-Tx in both groups may reflect the effect of induction immunosuppressive therapy. The ratio falls significantly during acute rejection prior to anti-rejection therapy but rises sharply following treatment.



**Conclusion:** The sequential changes in IL-10/IL-4 gene expression ratios of peripheral T-cells can closely mirror clinical changes in alloreactivity following renal transplantation. As IL-10 and IL-4 expression levels appear to show opposing patterns, monitoring the balance of these cytokines may be more relevant than monitoring the changes within individual cytokines.





## REVERSAL OF DIASTOLIC FLOW ON DUPLEX SCANNING OF RENAL ALLOGRAFTS IS A SIGN OF POOR PROGNOSIS

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Duplex scanning has become an important investigative tool in the evaluation of post-transplant renal dysfunction. Besides detecting ureteric obstruction, lymphocele, haematoma/abscess, the use of doppler can detect blood flow in the allograft. Whilst a raised resistive index may indicate rejection, its lack of sensitivity and specificity for this diagnosis makes its use questionable. In our experience, however, reversal of flow on duplex scanning appears to infer substantial risk to the graft and we review the data here.

Over a 5 year period, 379 renal allograft recipients underwent duplex scanning regularly in the post-operative period using an Acuson 128XP/10 colour flow doppler scanner. After ultrasonography, the doppler arterial waveform, flow in diastole and venous trace were recorded. In 40 patients (10.5%) reversal of diastolic flow was observed. These patients were shown to have a range of pathologies on further investigation or surgery including acute rejection (majority), vascular complications, acute tubular necrosis with or without acute rejection, ureteric obstruction, pyelonephritis and cyclosporin nephrotoxicity.

In 8 patients (2%) reversal of flow occurred throughout diastole (severe reversal) and in 5 of these patients vascular complications occurred leading to immediate graft loss in 4. In the remaining 3 patients acute severe vascular rejection was diagnosed on biopsy leading to graft nephrectomy in 2 patients later. Thus, in 6 of 8 patients (75%) severe reversal of diastolic flow led to early graft loss.

In the remaining 32 patients, reversal of flow occurred in early diastole, but there was a return to baseline before the next cycle. Of these patients, one patient never gained renal function, 3 lost their grafts within 3 months due to rejection, 1 patients died with functioning graft, 14 patients successfully achieved good function (serum creatinine <200 micromol/l) after intervention, but 13 patients continued with moderate graft function.

Thus, overall, of 40 patients with reversal of flow on duplex scanning 11 grafts were lost at 3 months (28%). Reversal of flow on duplex scanning indicates a poor prognosis for the renal transplant particularly if occurring throughout diastole.



**A PROSPECTIVE RANDOMISED STUDY OF CSA MONOTHERAPY VERSUS CSA + MYCOPHENOLATE MOFETIL IN CADAVERIC RENAL TRANSPLANT RECIPIENTS.**

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Cyclosporine A (CSA) monotherapy in kidney transplantation is used in many centres to avoid steroid use. CSA, however, is nephrotoxic. We report the use of mycophenolate mofetil (MMF 3g) to permit CSA dose reduction following renal transplantation. This randomised parallel group, patient and pathologist blind study, enrolled 99 patients in two centres in the UK and Spain. 50 of these received CSA monotherapy & placebo with blood levels titrated to 250-350 ng/ml and 49 received 3 g MMF daily with CSA, levels titrated to 150-210 ng/ml. Results at three months are as follows:

	CSA monotherapy	MMF & CSA
Acute rejection	46%	32.7%
Graft loss	5 pts (2 from AR)	1 pt (thrombosis)
Death	2	1
Graft Dysfunction - CSA tox	11 pts (22%)	6 (12.2%)
ATN (delayed function)	13 (26%)	9(18.4%)
CMV	3	9

Serum creatinines over this period were consistently lower in the MMF arm and creatinine clearances higher. The need for interim or maintenance steroids was lower in the MMF arm. Overall adverse event rates were comparable between the two groups (92% vs 96%) but more patients in the MMF group cited adverse events as a reason for withdrawal from the study. The commonest reason for withdrawal in the monotherapy arm was unsatisfactory therapeutic effect.

**SUMMARY**

The addition of MMF to a CSA monotherapy regimen permitted reduction of CSA dose accompanied by lower acute rejection rates, better graft function at 3 months and reduced steroid use. However, the infection rate using 3 g MMF was higher than in CyA monotherapy.



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Parameter	CSA Monotherapy	MMF & CSA
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CMV	3	9



## ORGAN DONATION- THE REALITY IN THE U.K.

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As organ transplant waiting lists rise and organ donor numbers remain static, the transplant community look for new ways to improve organ donation rates. Several initiatives which have originated in Europe have been considered by some U.K. units.

This paper reviews some of the factors which affect organ donation in the U.K. and Europe, and discusses initiatives taken by the UKTCA to maximise organ retrieval. Factors which can affect organ donation rates include the incidence of road deaths, ICU bed availability and consent legislation. Table 1 compares several European countries and demonstrates areas of disadvantage in the U.K.

Table 1.

Country	Road Deaths p.m.p (1994)	ICU beds in hosps. With >400 beds	Opting out legislation	Kidney TP rate p.m.p (1994)
Austria	23.3	10+3	Yes	42.4
Germany	17.0	12+5	No	23.4
Spain	19.8	14+11	Yes	42.0
France	22.3	11+6	Yes	26.4
UK	9.9	6+ 2	No	28.6

The incidence of brain stem deaths (bsd) was reported by Gore (1992) who found 2389 in a two year period in England. The Relatives Refusal Study (RRS) (UKTCA et al 1995) calculated 3063 cases of bsd in Great Britain in a similar period. Of these confirmed cases of bsd 50% and 57% respectively became donors, the major reason for not retrieval of organs being refusal of relatives (Gore 30%, RRS 26%). These data do not support there being large numbers of potential donors being missed in ICU's. However, there are wide variations between donation rates in the U.K. regions (11.6-24.1 donors p.m.p in 1996) and this must be addressed.

Initiatives by the UKTCA to maximise the available donors include

1. Implementation of a national education strategy to ensure identification and referral of all potential donors
2. Provision of donor maintenance guidelines to ensure retrieval of all suitable organs
3. Production of Standards of Practice for TC's to ensure support to hospitals



## CAN WE INDUCE XENOGRAFT TOLERANCE? FUNCTION OF PORCINE ADHESION MOLECULES IN A HUMAN MARROW MICROENVIRONMENT

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One way to circumvent the need for chronic immunosuppression in solid organ xenografting may be to induce donor-specific tolerance using bone marrow transplantation. If this approach is to succeed in the pig-to-human species combination, pig marrow must be capable of maturing into relevant tolerance-inducing cells and replenishing itself in host human marrow. One possible barrier to these activities is adhesion molecule incompatibility between the species. We have studied the compatibility across the pig-human species barrier of three ligands of potential importance in haematopoiesis, CD44, VLA-4 and ICAM-1. *In vitro* long-term bone marrow cultures were studied in which the effects of blocking antibodies were assessed by measuring cell numbers and colony-forming units. The blocking of CD44 had a comparable inhibitory effect on the haematopoiesis of human and pig marrow, even if the latter was maintained on a human stromal layer. Both cellular proliferation and colony-forming activity was inhibited by anti-CD44 monoclonal antibodies. By contrast, the blocking of ICAM-1 had no inhibitory effect on haematopoiesis of either human or pig marrow on human stromal layers. (In static adhesion assays, the direct binding of human ICAM-1 with its ligands on porcine cells was demonstrated.) However, a significant difference was observed in VLA-4 usage by haematopoietic cells of the two species. Blocking VLA-4 markedly inhibited human haematopoietic cellular proliferation, but had no effect on pig haematopoiesis, on either porcine or human stroma. These data suggest that incompatibility of CD44, VLA-4 or ICAM-1 is unlikely to limit the efficiency of porcine haematopoiesis in a human marrow environment. However, the difference in VLA-4 utilisation between these species raises the possibility that other interactions may be important for effective porcine haematopoiesis, and that their inability to function between species may contribute to the poor function of porcine haematopoietic cells in primate marrow microenvironments.



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PREVIOUS TUBERCULOSIS (Y/N)  
PREVIOUS HIV INFECTION (Y/N)  
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PREVIOUS OTHER DISEASES (Y/N)  
PREVIOUS SURGICAL TREATMENT (Y/N)  
PREVIOUS MEDICAL TREATMENT (Y/N)  
PREVIOUS ALLERGIC REACTIONS (Y/N)



## TUMOUR NECROSIS FACTOR IN AQUEOUS HUMOUR FOLLOWING CORNEAL ALLOTRANSPLANTATION: POTENTIAL FOR MODULATING CORNEAL ALLOGRAFT SURVIVAL

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**Purpose.** TNF is a proinflammatory cytokine which has been shown to be elevated following allotransplantation in several organ systems. We investigated bioactive levels of this cytokine in aqueous humour following corneal allotransplantation in a rabbit model. To investigate the role of TNF in allograft rejection, we injected a recombinant protein blocking TNF activity intracamerally in a rabbit model of corneal transplantation. **Methods.** Prevascularised corneas of NZ White rabbits received unilateral 8mm allografts from Dutch Belted strain donors. TNF levels in aqueous humour were measured in untreated allografted animals using a L929 bioassay. Test rabbits received five alternate day intracameral injections of 0.1mg recombinant soluble p55 TNF receptor fused to a human IgG Fc region (TNFrIg) after allografting. One group received injections from day 8 to day 16 post transplant, and a second group received injections from day 14 to 22 post transplant. Mean onset of endothelial rejection in the control untreated group was 21 days. **Results.** TNF levels were elevated in aqueous in 9 out of 10 allografted animals but not in 5 out of 5 autografted animals nor in normal ungrafted eyes. In the first TNFrIg treated group, three out of seven animals showed prolonged graft survival by at least 10 days whereas four rabbits showed rejection times unchanged from controls. In the group receiving later injections, rejection times were unchanged from controls. No toxicity of intracameral injection of the recombinant fusion protein was seen. **Conclusions.** Bioactive levels of TNF are elevated in rabbit aqueous following corneal allotransplantation. There may be a protective effect of blocking TNF during the early stages following corneal allotransplantation, although later injection of TNFrIg has no clear beneficial effect.



## LIVER AND HEART-LUNG-LIVER TRANSPLANTATION FOR CYSTIC FIBROSIS (CF)

Gur U, Gunson BK, Afonso RC, Skiba G, Elias E, Kelly DA\*, Mirza DF, Buckels JAC, McMaster P  
The Liver Units - Queen Elizabeth Hospital and The Children's Hospital\*, Birmingham.

Twelve patients (1987-97) with CF underwent transplantation: six adult (5 male) median age 20 (17-26) and six paediatric patients (5 male), median age 12 (10-14). Four adults with severe pulmonary dysfunction received triple heart-lung-liver grafts, eight patients with moderate or mild lung disease received isolated orthotopic liver grafts. Immunosuppression was based on triple therapy with cyclosporin, azathioprine and steroids.

Three recipients of triple grafts died after transplantation at 1 day, 2 weeks (cardiorespiratory) and at 2 months (CMV pneumonia). The surviving triple-graft recipient was ventilated for 37 days. The liver graft recipients were ventilated for a median of 2 days (range 1-3). The remaining nine patients are alive at a median follow-up of 24 months (2 weeks - 10.5 years). The longest survivor is the triple-graft recipient. Median age of survivors is now 24 (19-36) -adult and 14 (12-21) - paediatric.

Acute liver graft rejection requiring treatment was seen in 3/9 patients and cardiac and lung rejection in 1 patient. Eight patients currently require pancreatic enzyme replacement and 4 paediatric patients require long-term nasogastric feed supplements. Anorexia and failure to thrive despite supplements remains a problem for only one paediatric patient 16 months post-transplant. The long-term triple graft survivor has gained 25kg since transplantation. Quality of life has, however, improved for all 8 long-term patients with a reduced incidence of chest infections and an improvement in respiratory function. One adult was diabetic pre-transplant and one paediatric patient became an insulin requiring diabetic 2 months post-transplant following problems with cyclosporin toxicity.

Seven patients are receiving cyclosporin (all now on Neoral). Median dose at one month and one year post-transplant in the paediatric patients was 9.7 mg/kg (6.5 - 10.3). One adult was converted to Tacrolimus at 2 weeks due to cyclosporin malabsorption and ongoing acute rejection, and one patient with cyclosporin nephrotoxicity was converted to Mycophenolate at 6.5 years.

Liver transplantation (LT) is the treatment of choice in selected patients with biliary cirrhosis and portal hypertension arising from complications of cystic fibrosis (CF). Pulmonary bacterial colonisation and tendency to malabsorption of cyclosporin may result in additional postoperative complications.





## THE IRISH EXPERIENCE WITH PANCREAS TRANSPLANTATION

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**Objective:** Retrospective review of the outcome to date of pancreatic transplantation in the Irish Republic.

Pancreatic transplantation is currently the most physiological and successful method of establishing long-term euglycaemia in insulin dependent diabetes mellitus (IDDM). Between 31<sup>st</sup> December 1992 and 31<sup>st</sup> October 1997, we have performed 25 whole organ pancreas transplants. Twenty three simultaneous kidney pancreas transplants and 2 pancreas alone transplants were performed in 25 patients with Type 1 IDDM. Exocrine output of all but one pancreas was managed by primary vesical drainage via a duodenal segment anastomosed to the urinary bladder- one patient underwent primary enteric drainage. Induction quadruple immunosuppression with cyclosporine, azathioprine, steroids and anti-thymoglobulin (Fresenius) was employed in all cases.

### Pre-operative Patient Demographics

Male : Female

Mean Age (years) 38.5 (range 27 - 48)

Mean Duration of IDDM 24.6 (range 10 -34)

Pre-operative HbA1c 8.4% (range 5.7 - 10.9%)

All patients are alive and well after a mean follow-up of 28.7 months (range 5.6 - 55): Twenty-two patients remain insulin independent with a mean HbA1c of 6.0% (range 4.6 - 7.6%). There were two pancreatic graft failures - one patient developed anti-insulin antibodies 9 months post-transplantation and requires exogenous insulin therapy. A second patient underwent allograft pancreatectomy following the development of a duodenal-vesical leak associated with sepsis. One kidney was lost to chronic rejection at 49 months. The mean serum creatinine of the 22 functioning kidney allografts is 168mmol/L (range 123 - 307).

**Conclusion:** Pancreas transplantation is a highly successful, safe therapeutic option in selected patients with IDDM.



**IMPACT OF TRANSPLANTATION ON NON-TRANSPLANT SURGICAL RESOURCES - A MULTICENTRE AUDIT OF PROCESSES INVOLVED IN CARDIOTHORACIC TRANSPLANTATION IN THE UNITED KINGDOM**

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**Background:** Audit often focuses on outcomes; audits of structure and process are also important as they help guide health care planning and resource allocation. Because of low volume ( $\approx 50$  thoracic transplants per unit/year) and unpredictable timing, it is unfeasible to set aside resources for sole use of thoracic transplantation; resources are therefore often shared with the general cardiothoracic service. This should ideally cause minimal disruption to elective non-transplant surgery.

**Aim:** To examine some aspects of surgical provision for transplantation.

**Methods:** Multicentre prospective audit involving all 9 transplant units in the UK.

**Subjects:** 573 heart (HT), 210 lung (LT) and 101 heart-lung (HLT) transplants performed in the UK within a 2-year period between April 1995 and March 1997.

**Results:** Consultants performed 90% of orthotopic and 100% of heterotopic HT, 95% of LT and 86% HLT transplants. Only 7% of HT, 1% of LT, and 8% of HLT were performed by surgeons in training. In contrast, few retrievals were performed by consultants (16% HT, 9% LT, 2% HLT). The preservation method for most HT and LT was flush perfusion, but for HLT up to a third used core-cooling which involves additional manpower and equipment. Transplants were most frequently carried out at night – in 75% of cases, the organs were reperfused between midnight and 8 a.m. making infringement on the next day's theatre activities inevitable. 28% of operations were performed over the weekend. The median (interquartile range) duration of intensive care bed occupation post-transplant was HT 1 (1-3), LT 2 (1-5), HLT 2 (1-5) and hospitalisation HT 21 (15-28), LT 24.5 (17-39), HLT 26 (17-36) days. 9% of patients needed haemofiltration. The reoperation rates (for complications) reported were 11% (HT), 13% (LT) and 18% (HLT); reoperations cause further infringement on elective workload.

**Comment:** In planning surgical services in transplant units, the impact of transplantation on staff, theatres, intensive care, and other surgical resources should be considered. Extra resources are necessary if disruption of non-transplant work-load is to be minimised.





## CHANGES IN THE CORNEAL ENDOTHELIAL CELL LAYER DURING GRAFT REJECTION IN THE RAT

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**Aim.** To study infiltration of leukocytes and changes in expression of associated molecules on the endothelial cell layer of the cornea during graft rejection.

**Methods.** Corneas from female LEW or PVG strain rats were transplanted orthotopically to PVG strain recipients. Animals were killed just before and during rejection (days 10, 15 and 18 after transplantation), transplanted corneas were removed and the endothelial layers were detached and mounted on glass slides. They were stained by the avidin biotinylated peroxidase method using monoclonal antibodies for the major subsets of leukocytes and a polyclonal antibody to FAS-ligand. Stained infiltrating cells were counted. Normal corneas were also examined. **Results.** In allografts, on day 15, the endothelium was disrupted and infiltrated with large numbers of cells, consisting predominantly of CD8<sup>+</sup> cells and macrophages. By comparison, there were very few CD4<sup>+</sup> (W3/25<sup>+</sup>) cells ( $p < 0.05$ ), although large numbers of CD4<sup>+</sup> cells adhered to fibrin in the anterior chamber. CD25<sup>+</sup> cells, NK cells and/or granulocytes were few in number. On day 18, the number of CD8<sup>+</sup> cells remained unchanged, but the numbers of W3/25<sup>+</sup> cells had increased. Rejection was associated with relatively low levels of MHC class I on endothelial cells and *de novo* expression of MHC class II and ICAM-1. FAS-ligand was expressed on the endothelial cell membrane of normal corneas and syngeneic grafts, but, in endothelial cells of grafts undergoing rejection, appeared predominantly intracellular and perinuclear. **Conclusions.** This is a new and useful method for assessing changes to this critical layer of cells after transplantation. FAS-ligand-mediated apoptosis may be responsible for reducing the numbers of CD4<sup>+</sup> cells in the early stages of rejection, but this mechanism cannot prevent rejection, which appears, in this model, to be predominantly mediated by CD8<sup>+</sup> cells or macrophages. Rejection is associated with loss of cell membrane expression of FAS-ligand.



COMPUTERISED HISTOMORPHOMETRIC ASSESSMENT OF RENAL TRANSPLANT BIOPSIES FOR SURROGATE MARKERS OF CHRONIC REJECTION

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Chronic transplant rejection has emerged as the commonest cause of long term renal allograft failure. Early identification of those grafts at risk could allow targeting of specific therapies aimed at delaying this process.

A consecutive series of 48 renal transplant recipients with five year follow up data were studied. All patients underwent protocol needle core transplant biopsies at 1, 3 and 6 months postoperatively. All patients were immunosuppressed with cyclosporin and prednisolone ( $\pm$  azathioprine). In this study the 6 month protocol biopsies were analysed by computerised histomorphometry. No patient had clinical rejection at the time of the biopsy. Immunostaining for collagen III, tenascin and infiltrating leukocytes was performed using an indirect immunoperoxidase technique. Interstitial area stained (%) was measured by a blinded research associate using a semi-automatic image analysis system. Images from biopsy sections were transferred in digital form by a microscope mounted video camera to a microcomputer fitted with a frame grabber card. The area fraction occupied by the substance under study was computed using the programme *NIH image* on non-counterstained sections. The results were related to glomerular filtration rates measured at 6, 12 and 24 months post transplant using rank correlation coefficients.

Area fraction of immunostained collagen III correlated with 6 month GFR ( $r=-0.42$ ,  $p=0.005$ ) and was predictive of 12 months GFR ( $r=-0.32$ ,  $p=0.03$ ). Neither staining for tenascin nor leukocytes correlated with renal functional parameters at any time point. An area fraction of immunostained collagen III of  $>40\%$  at 6 months was associated with a significantly lower GFR at 24 months than a percentage area of  $\leq 40\%$  ( $31 \pm 4$  versus  $45 \pm 4$  ml/min/1.73 m<sup>2</sup>,  $p=0.01$ ). Furthermore a collagen III of  $>40\%$  at 6 months identified patients who were at risk of progressive deterioration in graft function. Donor age correlated with collagen III staining at 6 months ( $r=0.33$ ,  $p=0.03$ ).

In conclusion, those grafts with poorer long term function can be predicted using 6 month protocol biopsies immunostained for collagen III. This should prove to be a useful ad interim surrogate marker of allograft damage in studies addressing the effects of new immunosuppressive agents on the development of chronic rejection.





## THE OUTCOME OF RENAL TRANSPLANTATION IN THE UNDER FIVES

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### INTRODUCTION

The optimum timing of renal transplantation in young children remains controversial, as does the age of the donor most suited to their needs.

### PATIENTS

We report the long-term results of a consecutive series of transplants in children less than five years of age in a single centre. Since 1979 eighty renal transplants have been performed in sixty-five such children. Thirteen patients subsequently received a second and two patients a third allograft whilst all still aged under five. This represents 21% of the unit's overall transplant activity during this period. 51% of the transplants were performed pre-emptively.

### RESULTS

The mean age at first transplant was 2.8 years (range 0.8 - 4.9). The mean cadaveric donor age was 8.8 years (range 1-43). Actuarial patient survival at 1, 5 and 10 years was 89%, 84% and 82% respectively, whilst actuarial first cadaveric graft survival was 63%, 54% and 54%. Only seven patients (11%) received first grafts from live related donors with actuarial graft survival for this group being 71% at 1, 5 and 10 years. Donors aged less than five years accounted for 34% of all first cadaveric grafts. Actuarial graft survival from these young donors was 50%, 45% and 45% at 1, 5 and 10 years, compared with 70%, 57% and 57% from donors over five years. These results should be compared to those in recipients of first grafts aged more than five years in our unit during the same time period (n=308) who show actuarial graft survival of 79%, 62% and 53%. The chief causes of graft loss in the under five group were chronic rejection (n=19), graft thrombosis (n=8) and acute rejection (n=5).

### CONCLUSION

The relatively poor short-term outcome in the very young may therefore be due to both donor and recipient factors. The use of younger donors is associated with an increased risk of graft thrombosis and younger recipients appear to have an increased propensity to rejection.



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Survival	1 year	5 years	10 years
Actuarial patient survival	89%	84%	82%
Actuarial first cadaveric graft survival	63%	54%	54%
Actuarial graft survival from young donors	50%	45%	45%
Actuarial graft survival from donors over five years	70%	57%	57%

Following the introduction of cyclosporin, the short-term outcome of renal transplantation in young children has improved. However, the long-term results are still poor. The use of younger donors is associated with an increased risk of graft thrombosis and younger recipients appear to have an increased propensity to rejection.

**A CA REPEAT ALLELE IN THE FIRST INTRON OF THE HUMAN INTERFERON GAMMA (IFN- $\gamma$ ) GENE CORRELATES WITH *IN VITRO* IFN- $\gamma$  PRODUCTION**

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IFN- $\gamma$  is a principal Th1 effector cytokine which is considered to be a controlling factor in the polarisation of the immune response. We have examined potential regulatory regions of human IFN- $\gamma$  gene, specifically a previously described CA repeat sequence in the first intron of this gene. In a group of randomly selected healthy individuals (n=164) and in a cohort of transplant recipients (renal, lung, liver) (n=764) we have observed five alleles of the microsatellite polymorphism resolved by polyacrylamide gel electrophoresis (12% 19:1 acrylamide/bis, 1XTBE) and visualised by staining with ethidium bromide. The alleles were subsequently sized by direct cycle sequencing with allele #1 composed of 11 CA repeats; allele #2, 12 CA repeats; allele #3, 13 CA repeats; allele #4, 14 CA repeats and allele #5, 15 CA repeats.

The frequency distribution of the CA repeat alleles within first intron of the human IFN- $\gamma$  gene are shown in the table (n=1856)

Allele	Size (bp)	Frequency
#1	22	0.00054
#2	24	0.521
#3	26	0.375
#4	28	0.045
#5	30	0.058

In the control group, when the genotypes were correlated with production of IFN- $\gamma$  as measured by ELISA following *in vitro* stimulation of PBMC, we were able to show a significant correlation ( $p < 0.01$ ) between presence of allele #2 and high IFN- $\gamma$  production ( $>100$  IU/ml).

Since IFN- $\gamma$  has been implicated in the development of inflammation and fibrosis, we suggest the genotype/phenotype association identified in this study could be used in the analyses of a wide range of conditions including transplant rejection.





PHARMACO-ECONOMIC STUDY OF TACROLIMUS AND NEORAL IN  
CADAVERIC RENAL TRANSPLANTATION.

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**Introduction** The growing demands on health care systems in the United Kingdom has highlighted the importance of cost-effectiveness of alternative therapeutic regimens. The aims of this study were to assess the pharmaco-economics of tacrolimus and neoral based triple therapy regimens in renal transplant recipients.

**Methods** Data was obtained from 100 consecutive adult cadaveric renal recipients undergoing transplantation at a single institution. Eighty-nine patients had at least 6 months follow-up and these patients were analysed.

**Results** The age and sex distributions were similar as were the number of first grafts vs re-transplant and the pre-operative creatinine. There were 6 patients with a PRA>50% in the tacrolimus group compared with Neoral.

Parameter	Neoral		Tacrolimus		Cost per Patient
	Cost per Unit £'s	Number of Units	Cost per Patient	Number of Units	
Transplant Operation	3000 per op.	45	3000	44	3000
Days of Postoperative Dialysis	150 per day	153	510	7	24
ICU Stay	1200 per day	42	1120	3	82
Median Hospital Stay	200 per day	15	3000	14	2800
Median Number of Inpatient Drug Levels	5/15 per level	10	50	5	75
Ultrasound Scans	35 per scan	124	96	122	97
Transplant Biopsies	30 per biopsy	51	34	78	53
Other Investigations/Interventions	175 per test	24	93	17	68
Methylprednisolone		18 *	4	20 *	7
Mycophenolate Mofetil		3 *	128	6 *	187
Monoclonal Antibody (OKT3)		1 *	62	3 *	306
Ganciclovir		5 *	111	2 *	51
Cost of Return to Haemodialysis	150 per day	127	423	0	-
Graft Nephrectomy	2000 per op.	4	178	0	-
Median Number of Outpatient Visits	60 per visit	22	1320	22	1320
Median Number of OPD Drug Levels	5/15 per level	24	120	22	330
Re-admission Days	200 per day	339	1507	337	1532
Immunosuppression Costs		45 *	1301	44 *	2891
Cost of Other Medications		45 *	159	44 *	159
<b>Total cost per patient for 6 months</b>			<b>£13200</b>		<b>£12982</b>

\* = number of patients taking medications.

**Conclusions** Neoral and Tacrolimus appear to have similar pharmaco-economic profiles. Further studies are ongoing to assess the precise merits and disadvantages of these two drugs for patients undergoing cadaveric renal transplantation.



## IS LONG TERM RENAL GRAFT SURVIVAL PREDETERMINED BY RECIPIENT CYTOKINE PROFILE?

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**OBJECTIVE:** To investigate the role of cytokine polymorphisms in determining outcome after renal transplantation.

**DESIGN:** Population based study of frequencies of multiple cytokine polymorphisms of theoretical relevance to renal transplant outcome. Polymorphisms in TNF, Lymphotoxin- $\alpha$ , TGF- $\beta$ , IL1 $\alpha$ , IL1 $\beta$ , IL4, IL6, and IL10 were determined by PCR-SSP analysis under universal conditions and where applicable as haplotypic sets.

**SUBJECTS:** 112 patients with long-surviving first renal transplant (>10 years) from a single centre. Control groups were: a) 101 consecutive solid organ donors and b) 62 renal transplant recipients who had chronic rejection as defined by Alexis Carrel criteria.

**RESULTS:** Frequency disturbances were observed in 3/8 genes investigated. The most significant findings related to Lymphotoxin- $\alpha$  and IL-10. Homozygosity for the TCC LT $\alpha$  haplotype was found in 21/112 long term survivors in comparison with 8/101 controls and 4/62 chronic rejectors. Homozygosity for the ACC IL-10 haplotype appeared to act as an additional risk factor in TCC LT $\alpha$  haplotype patients. A TNF association was also observed but could be explained by linkage disequilibrium with LT $\alpha$ .

**CONCLUSIONS:** Polymorphisms in the proinflammatory cytokines LT $\alpha$  and TNF are associated with long term allograft survival and their effects may be significantly modulated by IL-10. Thus we conclude that long term survival of renal allografts is partly determined by recipient cytokine genotype. The association detected is consistent with that described for granulomata production in common variable immune deficiency and also rejection in lung allografts.



## TRANSPLANT SURGEONS IN TRAINING - IS ANYBODY OUT THERE ?

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**Introduction** Little is known for certain of the number of trainees currently aspiring to a career in transplant surgery in the United Kingdom and Ireland. Previous audits had suggested that there were significant differences between the career intentions of the trainees and the job descriptions of consultant posts being advertised. This has subsequently transpired into a shortfall of suitable candidates eligible to be appointed to available posts. This presentation will report the results of the latest audit and will examine whether recent changes to the structure of training have improved the outlook for potential transplant surgeons.

**Methods** The names of all trainees currently working in transplant posts in the UK and Ireland were obtained by correspondence with a nominated surgeon in each transplant unit. In addition, names were obtained from membership lists of the Carrel Club and the British Transplantation Society. A total of 87 potential candidates were identified. A postal questionnaire was dispatched to all candidates and a second questionnaire to all those not responding. Furthermore, telephone calls were made to persons still outstanding after the second postal survey.

**Results** The total number of replies was 26 of 87 (30%). Equal numbers of those replying were graduates of British/Irish Medical Schools and Overseas institutions. The majority had training at a single grade and the median length of training was 1 year (range: 0.5-6 years). Half of the responders had completed or were in the process of preparing a higher degree in the transplantation sciences. Ten of the 13 overseas trainees were hoping to obtain a substantive post in the UK or Ireland. Of the 23 intending to practice in the UK or Ireland, 20 (87%) were aiming for NHS consultant posts and only 3 for academic appointments. Only a minority, 5 of 23 wished for a pure transplantation post whilst the remainder wanted a mixed post: transplant and general (n=9), transplant and vascular (n=4), transplant and hepatico-pancreatico-biliary (n=3) and transplant and urology (n=2).

**Conclusions** There appear to be few trainees in the UK and Ireland who are intent on following a career in transplant surgery. Whilst the numbers of responders are low they are entirely in keeping with previous audits. The results show that the majority of trainees wish to have transplantation surgery incorporated with a second speciality and do not wish to work in the field of transplantation alone.



CHRONIC RENAL TRANSPLANT REJECTION AND LONG TERM RENAL FUNCTION CAN BE PREDICTED BY THE AREA UNDER THE SERUM CREATININE VERSUS TIME CURVE

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Chronic allograft rejection has become one of the most important challenges in renal transplantation. Early prediction of its development would greatly facilitate research into a number of the logical therapeutic interventions which are becoming available.

The area under the serum creatinine time curve (AUC) over the first 30 days following transplantation was calculated using the "Excel" spreadsheet for 341 consecutive renal allografts. Needle core biopsies were performed at 3 months and 6 months post transplant and annually thereafter and glomerular filtration rate (GFR) was measured using a single shot isotope technique at the same time points. Chronic rejection was defined by biopsy evidence of neointimal hyperplasia, interstitial fibrosis and glomerulosclerosis with or without sustained allograft dysfunction.

Of 341 transplants, 1 year follow up data was not available in 73 (33 failed, 16 died, 1 transferred to another centre and 23 shorter follow up times). 88 of the remaining 268 transplants (33%) demonstrated biopsy evidence of chronic rejection. This group had a significantly higher AUC than the group without biopsy evidence of chronic rejection (mean  $\pm$  SEM =  $11500 \pm 780$  vs  $7900 \pm 430$ ,  $p < 0.001$ ). 48 renal transplants (18%) had biopsy evidence of chronic rejection associated with sustained allograft dysfunction as measured by the reciprocal of creatinine plotted against time. AUC was higher in this group of 48 patients when compared to the group of 220 patients without evidence of renal dysfunction ( $10000 \pm 900$  vs  $8900 \pm 440$ ,  $p = 0.07$ ). Furthermore there were negative correlations between AUC and GFR at 1 month ( $r = -0.31$ ,  $p = 0.035$ ), 6 months ( $r = -0.27$ ,  $p = 0.05$ ), 12 months ( $r = -0.33$ ,  $p = 0.016$ ) and 24 months ( $r = -0.34$ ,  $p = 0.017$ ).

The area under the creatinine time curve calculated in the first 30 days correlates with long term renal function and predicted development of biopsy proven chronic rejection.





A NOVEL STRATEGY FOR THE DETECTION AND DEFINITION OF HLA SPECIFIC ANTIBODIES IN PATIENTS AWAITING RENAL TRANSPLANTATION

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The definition of HLA specific antibodies is a vital aspect of pre-transplant work-up. By defining the specificities of a patient's antibodies, HLA antigens unacceptable in a donor for that patient can be identified and hence unnecessary crossmatching avoided. Screening sera for HLA specific antibodies has traditionally been by complement dependent cytotoxicity (CDC) which is time consuming, laborious and absolutely dependent on supply of viable lymphocytes from a spectrum of donors. More recently flow cytometry and ELISA based methods have been described for the detection HLA specific antibodies and ELISA kits are now available commercially. These techniques have generally been considered as alternatives to one another but we have devised a screening strategy that employs each method in turn to maximise the information obtained whilst minimised the amount of CDC screening required.

The strategy is to screen all sera from patients awaiting renal transplantation with the ELISA QUIKSCREEN (GTI) kit to detect HLA class I (-A, -B and -Cw) specific antibodies. Positive sera are then screened by CDC and when indicated with the ELISA kit PRA-STAT (SangStat) for specificity definition. Sera negative by GTI are screened by flow cytometry (FCS) using lymphoblastoid cell line pools to detect HLA class II (-DR,-DQ) specific antibodies and confirm negativity for class I. FCS positive sera are also screened by CDC for specificity definition. The aim is to screen out the negative sera which comprise the majority and focus on specificity definition for only those sera known to contain HLA specific antibodies.

4222 sera have been GTI screened of which 989 (23.4%) were positive. Of the 3233 GTI negative sera 409 (12.6%) were FCS positive. Therefore only 1398 of the 4222 (33.1%) sera contained HLA specific antibodies that required specificity definition. 13.7% of the 1398 sera were negative by CDC. This is under further investigation but preliminary work has identified HLA specific antibodies (DR7, DQ3, DR4, DR15, A9, B35, B44, B45 and A2) in 8 sera of the 17 that have undergone further testing. This probably reflects the greater sensitivity of the ELISA and FCS assays and their ability to detect non-complement fixing antibodies. Also as the use of flow cytometry crossmatching is becoming increasingly prevalent it is important to use equally sensitive screening techniques.

This novel screening strategy has significantly reduced the CDC workload of the laboratory whilst enabling the detection and definition of additional HLA specific antibodies.



BRITISH TRANSPLANTATION SOCIETY  
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## PREDICTION OF CYTOMEGALOVIRUS DISEASE IN RENAL TRANSPLANT RECIPIENTS USING LABORATORY MARKERS

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**Objective:** To compare eight laboratory markers, viz. the pp65 direct antigenemia test (DAT), a quantitative cytomegalovirus (CMV) specific IgG assay (CMVG - Biomerieux VIDAS), a CMV specific IgM assay (CMVM - Biomerieux VIDAS), the Hybrid Capture system (HCS - Murex), an in-house polymerase chain reaction (PCR) tested on plasma (P-PCR) and leukocytes (L-PCR) and a commercial PCR (Roche AMPLICOR) on plasma (P-AMP) and leukocytes (L-AMP) for their ability to predict CMV disease in renal transplant recipients

**Materials and Methods:** A prospective study of 37 renal transplant recipients (13D+R-, 4D-R+, 20D+R+) with weekly blood sampling for 12 weeks. CMV infection was defined by laboratory criteria (two or more of the markers positive) and CMV disease was diagnosed by combined clinical/pathological criteria.

**Results:** 22 (8/13 D+R-, 1/4 D-R+, 13/20 D+R+) patients were identified to have CMV infection and 13 of these (8/8 D+R-, 1/1 D-R+, 4/13 D+R+) had CMV disease. Three patients did not have positive laboratory markers before onset of disease and prediction of disease was only possible in 10/13 (77%) patients. PCR of leukocytes were the most sensitive tests and positive at a mean duration of 10.8 days (L-AMP) and 4.8 days (L-PCR) before the onset of disease. PCR of plasma was less sensitive than PCR of leukocytes and were positive at a mean duration of 3.5 days (P-AMP) and 7.9 days (P-PCR) before the onset of disease. P-AMP had the best overall positive and negative predictive values, had a simpler procedure, no PCR inhibition and less sporadic non-consecutive positive results. The non-PCR methods (DAT, CMVG, CMVM, HCS) generally had high specificity but often gave late positive results.

**Conclusions:** Despite the use of sensitive PCR methods, prediction of CMV disease is possible only in 77% of patients. The non-PCR methods are generally not sensitive enough for use as predictive tools. Of the PCR methods, leukocyte PCRs are the most sensitive but plasma PCR, in particular P-AMP has the best overall performance.



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## DOES ADDITION OF LACTOBIONATE IN A PRESERVATION SOLUTION CONFIRM ANY BENEFIT IN THE PREVENTION OF CELL SWELLING IN KIDNEY CELLS?

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Lactobionate is an important component of University of Wisconsin Solution. Its main role is as an impermeant to prevent cell swelling. It has recently been incorporated into phosphate buffered sucrose (PBS) to yield a solution sodium lactobionate sucrose (SLS) that provides successful liver transplantation. The effects of lactobionate upon kidney cells are less clear. In these experiments the effects of adding varying concentrations of lactobionate to PBS upon swelling of kidney tubule cells were studied in a cell model which uses strophanthidin (a Na/K pump blocker), to simulate warm ischaemia. Sodium lactobionate (40, 70 and 100 mmol) was introduced into PBS by replacement isosmotically for sucrose (PBSL40, PBSL70 and PBSL100 respectively).

Kidneys of anaesthetized NZW rabbits (1.4-2.4kg) were flushed with and stored for 72 hours at 4°C in PBS140 or one of the test solutions. Isolated proximal tubule segments were then set up unperfused on micropipettes and bathed in oxygenated physiological saline (containing NaCl 114, NaHCO<sub>3</sub> 25, K<sub>2</sub>HPO<sub>4</sub> 2.5, MgSO<sub>4</sub> 1.2, CaCl<sub>2</sub> 2.0, glucose 5.5, alanine 6.0, Na lactate 4.0 and Na citrate 1.0 mmol/l, pH 7.4) at 37°C for 15 min to equilibrate cell volume. The bathing fluid was then exchanged for the test solution also containing 10<sup>-3</sup> M strophanthidin for 35 min, and finally returned to oxygenated physiological saline for 20min. Outside tubular diameter (um) was measured at 5 min intervals and cell volume was calculated mathematically (pl/mm) and are shown as mean ±SEM (n=6) for the end of each of the three periods following 72 hour preservation. The percentage changes in the cell volume in the second and third period are compared to the first period in saline.

Test solutions	Cell volume (pl/mm)				
	Physiological saline	Test solution + 10 <sup>-3</sup> strophanthidin		Physiological saline	
		Cell volume	% change	Cell volume	% change
PBSL100	289 ± 29	245 ± 28	-15	412 ± 31	+43
PBSL70	242 ± 16	255 ± 25	+5	409 ± 21	+69
PBSL40	278 ± 19	269 ± 16	-3	459 ± 37	+65
PBS	323 ± 27	254 ± 23	-21	344 ± 45	+6

Lactobionate in combination with sucrose in a wide range of concentrations were equally effective in the prevention of cell swelling during the period of simulated warm ischaemia. Whilst PBS facilitated restoration of cell volume to control values on return to physiological saline (simulated re-perfusion), all three PBSL solutions were associated with an increase in cell size. These results suggest that lactobionate may not be advantageous for kidney preservation.

## POSTERS SELECTED FOR PRESENTATION

## ARTERIOTOMY USING THE AORTIC PUNCH IN KIDNEY TRANSPLANTATION

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The arterial anastomosis in kidney transplantation can be difficult either because the artery is without a patch (such as in living related transplantation) or when the artery is too long compared to the vein and ideally should be shortened preventing a kink which can occur and cause the loss of a graft. In order to easily perform an end to side anastomosis of the renal artery to the external iliac artery we used an aortic punch, an instrument routinely used in coronary aortic bypass grafts surgery.

**Patients and Methods:** We have performed 30 cadaveric kidney transplants (14 females, 16 males, mean age  $\pm$  SD:  $47 \pm 14$  years) using the aortic punch. Those kidneys had a single renal artery not on a Carell patch, either because it was inadvertently cut during the procurement or it was electively shortened to match the length of the vein. In all cases the kidney was transplanted extraperitoneally and the renal artery and vein were anastomosed to the recipient's external iliac vessels. Using an 11 blade an arteriotomy was performed in the external iliac artery small enough to allow the insertion of a single-use 4 mm aortic punch into the lumen providing a clean accurate circular incision. The renal artery was then anastomosed end to side to the external iliac artery of the recipient using 6/0 prolene.

**Results:** All kidneys were well perfused immediately after unclamping. All patients have currently normal serum creatinines (ranging between 80-186, 134-170, 105-164 and 95-160  $\mu\text{mol/L}$  at 2, 3, 6 and 12 months posttransplant respectively). An MR angiogram (MRA) was performed 2 months posttransplant. In 29 patients the MRA was normal with no evidence of arterial stenosis. In one patient the MRA demonstrated an area of signal loss in the proximal part of the transplant artery suggesting a stenosis, however a subsequent angiogram did not demonstrate any abnormalities.

**Conclusions:** The use of the aortic punch in renal transplantation is a technique which facilitates the arterial anastomosis and results in excellent kidney function without stenosis of the renal artery. It is especially useful in living-related transplants and in cases where the renal artery has to be shortened to match the length of the renal vein.



## EX-VIVO LIVER PERFUSION SYSTEM: AN EFFECTIVE AND SUCCESSFUL METHOD FOR HEPATIC SUPPORT

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**INTRODUCTION:** There is a well recognised need for a system capable of supporting patients with hepatic failure, either pending regeneration of their own liver, or while awaiting a liver transplant. Recent attempts of using columns containing charcoal or porcine hepatocytes have failed to show consistent and successful outcome. The technique of ex-vivo hepatic perfusion developed and successfully used by ourselves in the 1970s, has now been modified and redesigned. Prior to its human application, this technique was tested in animal models with surgically induced acute hepatic failure.

**METHODS:** Acute hepatic necrosis and liver failure were induced in mongrel dogs weighing 15-20 kg by an end-to-side portacaval shunt followed 24 hours later by occlusion of the common hepatic and gastroduodenal arteries for two hours. All animals were medically supported with intravenous fluids and glucose administration and were divided into two groups. In the control group (N=5), only medical support therapy was given throughout the period of survival. In the experimental group (N=5), the animals were connected to the ex-vivo liver support apparatus via an arteriovenous shunt through the femoral artery and vein. Ex-vivo support was carried out when the animal developed acute hepatic failure at 12-14 hours after clamping of the hepatic artery. Ex-vivo liver was removed from a dog of a similar weight and placed in the specially designed liver chamber, which mimics the physiological conditions of a normal liver. Hepatic perfusion was carried out at 37° using an arterial roller pump which perfused the liver via heat exchanger through the hepatic artery at a pressure of 80-100mm Hg and also through the portal vein at a pressure of 10-15cm of water, at a flow rate of 0.8-1 ml/min/gram of liver. The blood was returned to the animal by a venous pump. During perfusion the following observations were made: Clinical state of animal, hepatic blood flow, oxygen consumption, bile flow and bile bilirubin concentration, oxygen consumption by the liver, and level of hepatic enzymes, ammonia and prothrombin times in the recipient animal before and after perfusion. Histological studies of the animal's own liver were carried out at the time of death or sacrifice.

**RESULT:** All control animals showed progressive hepatic failure and died at 14-19 hours (mean 16.2 hrs) after clamping of hepatic artery. The animals treated with ex-vivo liver perfusion survived for 36, 48 and 60 hours and two animals recovered completely and became long-term survivals. During the 8 hour of ex-vivo liver perfusion. All animals continued to show clinical and biochemical improvement. Bile output was 8-10ml/hr with bile bilirubin concentration of 1000-5000 µmol/L. At the end of perfusion blood ammonia fell from 160 to 90 µmol/L and the prothrombin time from 22 to 12 seconds. Liver histology in the controlled animals showed severe necrosis and vascular degeneration, while in the treated animals there was improvement in the hepatocyte architecture with restoration of glycogen and chologen within the liver parenchyma.

**CONCLUSION:** These results and our previous observations confirm the safety and marked effectiveness of ex-vivo liver perfusion as a bridge to liver regeneration. This technique should be widely used as a temporary support for patients with acute, but reversible liver failure as well as for the long-term intermittent support of patients who develop hepatic insufficiency while awaiting liver transplantation.

## IMPROVING THE THERAPEUTIC MONITORING OF CYCLOSPORIN THERAPY

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The narrow therapeutic window of cyclosporin A (CsA) and the variable pharmacokinetics of the traditional preparation, CsA-SIM (Sandimmune™), have made it difficult to establish its optimal use. The introduction of a microemulsion preparation, CsA-ME (Neoral™), with less variable pharmacokinetics has made it possible to attempt to define its use more closely. 101 renal allograft recipients were converted from CsA-SIM to CsA-ME. Absorption was monitored using a standard pharmacokinetic "profile": measuring serum CsA levels at 0, 2, 4, 6 and 8 hours following CsA-SIM administration, and 0, 1, 2, 3 and 4 hours following CsA-ME. Areas under the resulting time-versus-serum CsA concentration curve (AUC) were calculated. We were surprised how many patients showed very little fluctuation in CsA concentration after CsA-SIM administration: 31% showed a difference between the pre-administration ( $t_0$ ) and maximal concentration ( $t_{max}$ ) ( $S_{min-t}$ ) that was <200 mg/l. Retrospective analysis of earlier profiles performed on these patients showed a consistency to this pattern. There was no correlation between  $S_{min-t}$  and length of time since transplantation, age, sex, renal function, number of rejection episodes or cause of underlying renal failure. However, this observation may justify revisiting the use of CsA in numerous clinical contexts. Given its much lower pharmacokinetic variability, there was a much better correlation on CsA-ME between AUC and a series of parameters incorporating elements of both trough and peak values. We found the best correlation was with  $t_0 + (t_1/4)$  ( $r^2 = 0.779$ ). This parameter may be of practical clinical use.



## PREVENTION OF BILIARY COMPLICATIONS BY A SIMPLE SUTURE TECHNIQUE IN LIVER TRANSPLANTATION

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Biliary complications following liver transplantation have plagued most units with incidences of up to 15%. In the beginning years of our programme we used a variety of methods for biliary anastomosis but since 1994 we have adopted a standardised method using interrupted 5-0 PDS as suture material with knots placed outside. All recipients have had a choledochocholedochal (duct to duct) anastomosis unless the indication for transplantation was Primary Sclerosing Cholangitis or tumour or if the bile duct was surrounded by multiple varices. When a Roux loop was used the method of anastomosis remained the same. A t-tube is not used routinely. The donor duct is cut as short as possible in order to preserve its blood supply. Our results are presented below.

Number of transplants since 1994	271
Roux-en-Y biliary anastomosis	18
Biliary complications	15 (5.5%)
Biliary leaks	3 (1.1%)
Biliary strictures: early (within 3 months)	8 (2.95%)
Biliary strictures: late	5 (1.85%)

There were no complications in the Roux-en-Y group. In the duct-to-duct group 5 patients had initial radiological management of strictures or leaks and three of these required surgery. Eleven patients were treated initially by surgery. There were no complications from either early or late hepaticojejunostomy.

Our results show that this simple technique of biliary anastomosis can give satisfactory results following liver transplantation.

## EVALUATION OF THE ARTERIAL FLOW OF THE PANCREATIC GRAFT WITH DUPLEX-DOPPLER ULTRASONOGRAPHY

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Adequate blood supply to the transplanted pancreas is essential for the normal metabolic function of the graft and for prevention of graft thrombosis. The normal range of arterial blood flow in the transplanted pancreas that results in normal metabolic graft function and prevents graft thrombosis has not been investigated.

**Purpose:** To evaluate the arterial supply to the transplanted pancreas in relation to its metabolic function by using Duplex-Doppler ultrasonography (US).

**Patients and Methods:** Eleven patients who received a cadaveric whole organ pancreaticoduodenal transplant (10 bladder-drained and 1 enteric-drained) were studied. In all patients an arterial extension iliac Y graft of donor common, internal and external iliac artery was used for the arterial reconstruction of the superior mesenteric artery and splenic artery. The common iliac artery of the Y graft and the portal vein of the pancreatic graft were anastomosed end to side to the external iliac vessels of the recipient. All patients were maintained on Aspirin (75mg o.d.). Six months posttransplant the pancreatic arterial circulation was evaluated with Duplex-Doppler US examination using a low frequency probe of 2.5 MHz (ATL HDI 3000). The volume flow (VF; ml/min), peak systolic velocity (PSV; cm/sec), end diastolic velocity (EDV; cm/sec), resistive index (RI) and pulsatility index (PI) in the splenic artery of the pancreatic graft and only the VF in the splenic artery of the native pancreas were measured.

**Results:** All patients were insulin independent immediately postoperatively and at six months posttransplant and the HbA1c varied between 4.2 and 5.6%. The results (mean±SD) for the VF, PSV, EDV, RI and PI were as follows:

VF	Pancreatic Grafts				Native Pancreases
	PSV	EDV	RI	PI	VF
24±13	52±16	16±7	0.6±0.1	4±2	98±7

The VF in the splenic artery of the pancreatic grafts was 11 to 56% of the VF in the splenic artery of the native pancreases.

**Conclusions:** The Duplex-Doppler US demonstrated that the arterial reconstruction of the pancreatic graft with a Y graft provides adequate blood supply to the pancreatic graft, however not as good as in the native pancreas ( $p < 0.05$ ). The flow in the transplant splenic artery results in a PSV, EDV, RI and PI sufficient enough to provide a normal graft function and no thrombosis. The maintenance of an adequate blood pressure and appropriate anticoagulation is important to prevent graft thrombosis.



## USE OF VASCULAR CLOSURE STAPLES IN RENAL AND PANCREATIC TRANSPLANTATION

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Vascular Closure Staples (VCS), a relatively new technique in vascular surgery, has shown encouraging results in a variety of different vascular anastomoses. The VCS do not penetrate the vessel or disrupt the endothelium and do not have an intraluminal component. Experience from our centre as well as from others has proven that the use of the VCS in creating arterio-venous fistulae for dialysis access has been correlated with reduced early thrombotic complications, less peri-operative bleeding and decrease in anastomotic and operative times.

**Purpose:** To test the use of the VCS in creating the venous and arterial anastomosis in renal and pancreatic transplantation.

**Patients and Methods:** Seven recipients of cadaveric kidney transplants (the first operation was the first application of the VCS in kidney transplantation in Europe) and three recipients of cadaveric kidney-pancreas transplants (the first operation was the first pancreas transplant with VCS in the world). For the creation of venous and arterial anastomosis (donor vein and artery to recipient's external iliac vein and artery respectively) the vessels were initially approximated with four 5/0 prolene stay sutures. Approximation and symmetrical eversion of the walls of the donor and recipient vessels were done with the tissue approximation forceps and the VCS were applied with the disposable VCS Autosuture stapler applicator. Large VCS (2 mm staple span) were used for the venous and extra large (3 mm staple span) for the arterial anastomosis. The anastomotic time was  $\approx 7$  min for the vein and  $\approx 8$  min for the artery. There were no leaks from the venous or arterial anastomosis.

**Results:** All organs were well perfused after revascularization. Coloured doppler ultrasonographic angiography performed immediately post-operatively, demonstrated no anastomotic abnormalities. There were no postoperative complications. Currently, all kidney transplant recipients have normal creatinines (follow up 1-6 months) and the recipients of pancreatic transplants are insulin independent (follow up 1-5 months).

**Conclusions:** The VCS technique allows shorter warm ischaemia time with equal or possibly improved operative outcomes. A prospective randomized clinical trial is required to evaluate the VCS technique in organ transplantation.

## REVISITING CORRELATES OF CMV INFECTION - NEW OPPORTUNITIES FOR CLINICAL MANAGEMENT

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How reliable are clinical symptoms in comparison to laboratory tests in the diagnosis of CMV infection? In order to address this, 37 patients were monitored for CMV infection using a range of laboratory tests including PCR, direct antigenaemia test, hybrid capture and serology on a weekly basis for 3 months and also for the presence of leukopaenia (WBC  $< 3 \times 10^9/l$ ), thrombocytopenia (a persistent fall of a least 20% compared to preoperative values), fever and acute rejection episodes. Donor/recipient CMV status was as follows:

D+/R- 13                      D- R+ 4                      D+ R+ 20

A total of 22 patients had laboratory evidence of CMV infection and 13 of these patients developed CMV disease.

### Results

Clinical correlate of CMV infection	Total no of patients n	Lab marker +ve for CMV		Lab marker -ve for CMV n
		n	Mean time in days after 1 <sup>st</sup> CMV lab marker +ve	
Fall in WBC	8	8	14.4*	0*
Fall in platelets	12	12	12.2*	0*
Fever	23	16	10.1*	7
Acute rejection	23	17	4.9	6

\*p = < 0.05

This study confirms the associations of CMV infection with the clinical correlates leukopaenia, thrombocytopenia and fever, although the latter is only a weak correlate. However, most changes in these parameters occurred at least 10 days subsequent to a positive CMV laboratory marker and, therefore, these tests are more reliable indicators of CMV infection than clinical symptoms and give a window of opportunity for pre-emptive therapy against CMV.



## FRUCTOSE IMPROVES LIVER METABOLISM DURING COLD HYPOXIA

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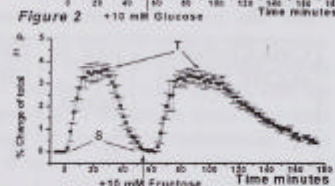
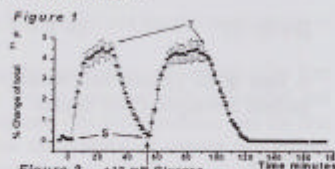
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**Introduction:** Following transplantation, the donor liver must quickly regenerate ATP to sufficient levels to re-establish solute and ion exchange. It has been suggested that fructose is a good substrate for maintaining liver energetics during hypoxia, but results have been variable. Experiments using fructose both *in vivo* and in isolated organs have shown a converse detrimental depletion of ATP levels. However, adding fructose to livers at hypothermia (which may alter relative rates of uptake or metabolism) has not been considered. We have added either glucose or fructose to a hypothermic reperfusion (HtR) system following brief cold hypoxia to establish baseline ATP depletion. <sup>31</sup>P NMR was employed to monitor ATP content in the intact organ.

**Methods:** Livers were harvested from Land Race Large White cross pigs after fasting for 12h (n=5/group), and perfused with UW solution. At 2h storage, the liver was placed in a 1.5 Tesla MRS machine & <sup>31</sup>P spectra collected. Each liver underwent 2 periods of HtR, the first with a gluconate/raffinose/O<sub>2</sub> solution containing Hespan starch (HtR1). At steady state, HtR was stopped to allow complete ATP depletion. The liver was HtR for a second time (HtR2) with either, 10mM glucose or, 10mM fructose, added. At steady state HtR was again stopped.

**Results:** HtR1 ATP regeneration rates were approximately the same at  $7.0 \times 10^{-3} \text{ s}^{-1}$  for both groups (S=start of HtR). During HtR2 the fructose group ATP regeneration rate was 38% (p<0.001) less than the glucose group, however both groups regenerated equivalent amounts of total ATP compared with their HtR1 values. Following cessation of HtR2 (T), the glucose group showed a fall in ATP at a rate which was 13% (p<0.001) slower than when glucose was omitted. The fructose group produced a rate of  $-0.7 \times 10^{-3} \text{ s}^{-1}$  which was 74% (p<0.001) slower compared with the glucose group. The glucose group took 30 min to reduce ATP levels to zero, whereas, the fructose group at 90 min of cold hypoxia, still contained a significant amount of ATP.

**Discussion** Fructose produced a 4-fold reduction in the rate of ATP depletion. It is probable that fructose enters the glycolytic pathway predominantly *via* fructokinase and hence, by-passes phosphofructokinase which is the key glycolytic regulatory enzyme. During ischaemia, accumulated glycolytic intermediates from the fructose load may slowly buffer ATP hydrolysis. We propose that fructose may provide an important means of sustaining ATP levels in the stored donor organ, but it may be best administered at hypothermia to avoid rapid energy depletion as the sugar is initially metabolised.



## HIGH INTRACELLULAR HEPATITIS B SURFACE ANTIGEN CONCENTRATIONS RESULT IN LIVER CELL DEATH: A MODEL FOR POST TRANSPLANTATION LIVER DISEASE.

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Graft infection with hepatitis B virus following transplantation for chronic liver disease due to HBV is associated with high levels of viral replication and intracellular accumulation of HBsAg as seen in Fibrosing Cholestatic Hepatitis. The occurrence of severe liver damage in the post transplant immunosuppressed state is unexplained given that HBV related liver damage is thought to be largely immune mediated. It has been proposed that in the presence of extreme viral loads, HBV becomes cytopathic. We have developed an HBV transfected HepG2 cell line that permits synthesis of viral protein without the production of infective virions. To explore the hypothesis that HBsAg accumulation damages the cell we incubated HBV transfected and control (plasmid only) cell lines with Brefeldin A (0-0.01 nM) for 6 days, which blocks the binding of proteins to golgi membranes. Brefeldin A leads to HBsAg intracellular accumulation and has an effect on cell proliferation/survival.

HBsAg released into the supernatant was reduced by 96% with concentrations of brefeldin up to 0.005 nM, p<0.001, the amount of cell associated HBsAg was unaffected. Intracellular and extracellular LDH were used as an indication of cell number and as a measure of cell death, respectively. There was a similar decrease in cell number for both control and HBV transfected cell lines (p<0.001), while there was an increase of 40% (control cell line, p<0.002) and 75% (HBV transfected cell line, p=0.0002) in extracellular LDH. This indicates that the HBV transfected cell line accumulates HBsAg and is, as a result, more susceptible to the effects of Brefeldin A. To study further the effect of intracellular HBsAg we increased the quantity of HBsAg produced by co-incubating with Brefeldin and Cyclosporin A (this immunosuppressive drug increases HBsAg production greatly in this system). HBsAg release into the supernatant was inhibited by 90% (p=0.0005) while cell associated HBsAg increased by 117% (p<0.04). In both cell lines the cell number dropped by 94% (p<0.001) as in the previous experiment, but the extracellular LDH levels were increased to 109% (control cell line, p<0.001) and 120% (HBV transfected cell line, p<0.001).

Brefeldin increases the quantity of intracellular HBsAg and this can be increased further by co-incubation with Cyclosporin A, resulting in accumulation within the cell. It can be postulated that HBV could contain mutations that act in a similar manner to brefeldin, which when combined with immunosuppressive therapy in the post transplantation situation results in liver damage. In combination with our cell culture system, these experiments could provide a suitable model to study post transplantation liver damage.



## CONTRIBUTION OF RENAL SECRETED COMPLEMENT C3 TO THE CIRCULATING POOL IN HUMANS

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Accumulating evidence indicates that complement C3 produced within the kidney is an important mediator of renal inflammatory and immunological injury. Meaningful interpretation is limited by the lack of data on the amount of C3 the kidney can produce in normal and inflamed states. This was investigated by utilizing the conversion of complement proteins from recipient to donor allotype (C3S to C3F) that occurs following renal transplantation.

We examined the C3 allotypes of 80 consecutive renal donor-recipient pairs (148 individuals) by amplification refractory mutation system analysis. The extent of allotype conversion in C3 F/S mismatched recipients was quantified by enzyme-linked immunosorbent assay at different stages post-transplantation and confirmed by Western blotting.

Twenty-one of the 80 donor-recipient pairs were informative (C3 FF or FS donors; C3 SS recipients). In the early post-operative period, the level of donor-derived C3F was undetectable, increasing to 9.6 percent of the total circulating C3 at times of acute allograft rejection. When graft dysfunction occurred from causes other than rejection, namely acute tubular necrosis, cyclosporin toxicity, thrombosis, urinary tract or cytomegalovirus infection, its levels remained undetectable. After stable graft function was attained (3-13 months post-transplant), donor-derived C3 contributed to 4.5 percent of the total C3 pool.

Our findings demonstrate that the kidney in the resting state is a significant source of extrahepatic C3. Its heightened local synthesis during rejection episodes provides evidence for a significant pathogenic role of C3 in this immunological process. The concept of local C3 synthesis may have potential implications for strategies directed against intrarenal inflammatory injury.

## HOMOCYSTEINE INDUCED ENDOTHELIAL CELL DYSFUNCTION AS A PUTATIVE MECHANISM FOR GRAFT ATHEROSCLEROSIS IN RENAL TRANSPLANTATION

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Hyperhomocysteinemia is a common finding among renal transplant recipients, and may contribute to the excess incidence of atherothrombotic sequelae in this population. Endothelial cell (EC) dysfunction is known to play a central role in graft atherosclerosis. This study looked at the effect of homocysteine on (EC) reparative capacity by measuring EC's proliferation and apoptosis. Endothelial cell function was also assessed by measuring nitric oxide (NO) and endothelin-1 (ET-1) concentrations.

Human umbilical vein endothelial cells (HUVECs) at passage 2-3 were treated with *d,l*-Hcy at concentrations of 0.00 mM, 0.01 mM, 0.1 mM, 1.0 mM, 2.5 mM for 24 hours. EC proliferation was assessed using 5-Bromo-2'Deoxy-Uridine Labelling and Detection kit. Apoptosis was assessed using Cell death detection ELISA kit. Nitric oxide production was measured using the Griess reaction after 24 and 48 hours. ET-1 production was measured in the supernatant at 24, and 48 hours using human ET-1 ELISA kit. Constitutive NO synthase expression is assessed by western blotting using anti-human EC-NOS antibody.

Hcy inhibited HUVECs proliferation, induced apoptosis and decreased NO concentration independent of eNOS expression. It increased endothelin-1 concentrations up to 1 mM Hcy concentration and declined thereafter suggesting EC toxicity.

Impairment of EC reparative capacity and induction of endothelial cell dysfunction are recognised early events in atherosclerosis. These events are seen secondary to exposure to homocysteine and may therefore suggest the contribution of homocysteine induced endothelial cell dysfunction in graft atherosclerosis in renal transplantation.

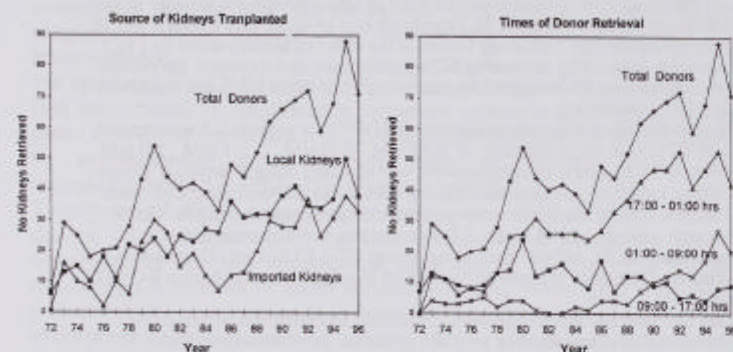


25 YEARS OF CADAVER ORGAN PROCUREMENT - EXPERIENCE IN A SINGLE TRANSPLANT UNIT.

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Cadaver organ retrieval between 1972-1996 was reviewed to assess the changing patterns over time. All donations were documented as "local" or "imported", and the time of cessation of ventilation, the organs retrieved, the extent of damage to kidneys and the grade of surgeon operating from the "local" team were noted.



Whilst there has been a steady increase in organ donation and kidney sharing over the 25 years, the donor rate appears to be plateaued over the last 8 years, reflecting National trends. Multi-organ retrieval started slowly in 1983, but latterly forms approximately 60% of all donations, with liver, liver/heart, liver/heart/lung being the commonest combinations. Renal damage of any degree still occurs in approximately 11% cases. Latterly there has been a trend towards the liver retrieval team taking out more donor kidneys without the presence of a "local" surgeon and, worryingly, far more donations take place overnight. These trends have a major implication for training and exacerbate the unattractive image of transplant surgery as a career.

EFFECT OF COLD STORAGE ON EXPRESSION OF ELAM, ON CULTURED HUVEC

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The response of the renal vasculature after cold storage and reperfusion by recipient blood is critical in determining initial graft function and possible susceptibility to rejection. Factors such as Interleukin 1 (IL1) that affect the adhesion of white cells to the endothelium within this early phase, may be important in this process.

The level of ELAM-1 expression by endothelial cells is a marker of activation in response to inflammatory products such as Interleukin 1 (IL1). We have used an in vitro cell culture model to investigate the responsiveness of endothelium to IL1 after varying periods of cold storage. Endothelial cell cultures, grown from collagenase treated human umbilical veins, were stored at 4°C for 1, 3, 4 or 6 hrs followed by exposure at 37°C to an optimal concentration of IL1. Expression of ELAM-1 was determined by flow cytometry and fluorescence values converted to MESF units.

The following table presents the results:

	Storage times (hrs)							
	1		3		4		6	
Storage temp	37°C	4°C	37°C	4°C	37°C	4°C	37°C	4°C
Control	17957	18763	14793	12266	22306	22134	18873	20985
IL1 100ng/ml	700788	849585	404717	192638	115755	91415	125990	39459

Over the 6 hr cold storage period, ELAM expression by control cells only varied between 83% (2 hrs) to 111% (6 hrs) from the 37°C control. However, responsiveness to IL1 was diminished to 48% after 3 hours of cold storage. The cells remained unresponsive after 6 hrs storage at 4°C. This work indicates that endothelial cell responses are reduced following cold storage, but by a magnitude seemingly unrelated to the storage interval. However the remaining responsiveness to IL1 was sufficient to produce detectable levels of ELAM 1, allowing white adhesion that may be detrimental to subsequent graft function.



## INDUCTION OF ALLOSPECIFIC T CELL TOLERANCE BY TREATMENT WITH FAS LIGAND (CD95L) POSITIVE CELLS.

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Transplantation of Fas ligand (FasL) expressing cells can induce functional allograft tolerance in some model systems. In this study we used mixed lymphocyte culture to investigate the basis for this tolerance.

Allospecific T lymphocytes were activated by mixture with an allogeneic EBV-transformed B cell line (EBV-BCL). It was shown that T cells required prolonged culture before they developed sensitivity to Fas-mediated apoptosis. After culture for 7 days, Fas was expressed by the T cells at a high level whilst intracellular bcl-2 expression was reduced. At this time the T cell-line was mixed with FasL positive SW620 colonic cancer cells or was treated with agonistic anti-Fas antibodies. Significantly greater T cell apoptosis was detected by TUNEL staining and the JAM assay in treated than in control cultures. The remaining T cells were re-challenged with antigen presenting cells and both lymphoproliferation and IL-2 production were assayed after 3 and 5 days. A significantly reduced specific T cell re-activation response was observed in cultures which had shown a high level of apoptosis. However, the remaining T cells from both anti-Fas treated and control cultures showed a similar response to stimulation by a third-party EBV-BCL.

These data indicate that stimulation of cell-surface Fas with ligand or antibody can be used to induce apoptotic tolerance of allospecific T cell lines.

## ABSENCE OF BRADYCARDIC RESPONSE TO APNOEA AND HYPOXIA IN CARDIAC TRANSPLANT RECIPIENTS WITH OBSTRUCTIVE SLEEP APNOEA

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### BACKGROUND

In patients with obstructive sleep apnoea (OSA) the vagal stimulation caused by inspiration against the upper airway obstruction results in sinus bradycardia during the apnoea followed by a reflex tachycardia at apnoea termination.

### METHODS

Five male cardiac transplant recipients with OSA and two male patients with OSA who had coronary artery bypass surgery had overnight polysomnography. The transplant patients were immunosuppressed with azathioprine and cyclosporin A and no patient had evidence of allograft rejection or vasculopathy.

### RESULTS

The cardiac transplant recipients demonstrated no change in base line heart rate in spite of marked haemoglobin oxygen desaturation (below 65% in each case) presumably on account of parasympathetic denervation of the allograft. The patients who had coronary artery bypass surgery demonstrated a typical variation in heart rate response. Each patient obtained good symptomatic relief and apnoea control with nasal continuous positive airway pressure and each was placed on a weight reducing diet.

### CONCLUSIONS

Cardiac transplant recipients with OSA may be at an increased risk of developing potentially fatal ventricular arrhythmias if the allograft is unable to respond appropriately to hypoxia. Should cardiac parasympathetic re-innervation occur, prospective polysomnography may be a marker for this process.



## PREDICTION OF GRAFT AND PATIENT SURVIVAL FROM EARLY GRAFT FUNCTION

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**Introduction.** Acute rejection (AR) is generally recognised to have an adverse effect on long-term graft survival but the impact of delayed graft function (DGF) is controversial. The influence of AR and DGF on long-term patient survival is not well established.

**Methods.** To determine the relationship between early graft function and long-term outcome, we performed a retrospective analysis of 589 first cadaveric transplants at our centre between 1 Jan 1984 to 31 Dec 1993 (Cyclosporin era). The influence of AR and DGF on graft and patient outcome were studied by univariate (Kaplan-Meier) and multivariate (Cox proportional hazards) analyses.

**Results.** There were 360 (61%) patients with immediate graft function (IF), 181 (31%) with DGF and 48 (8%) patients whose grafts never functioned (PNF). **Graft Survival.** Graft survival did not differ between IF and DGF ( $p=0.51$ ), but was significantly worse in the AR group ( $p<0.001$ ), particularly with late and multiple episodes. Serum creatinine concentration at three months was a strong simple predictor of long-term graft outcome ( $p<0.001$ ). Younger patients were at increased risk of graft loss ( $p=0.023$ ) but primary renal disease, sex of recipient and duration on renal replacement therapy prior to transplantation had no significant influence on graft survival. **Patient Survival.** Increasing age ( $p<0.001$ ) and diabetes ( $p<0.001$ ) were the strongest predictors of adverse outcome. Serum creatinine at three months was inversely correlated with patient survival ( $p=0.002$ ). Other factors associated with poor patient survival were rejection ( $p=0.001$ ) and adult polycystic kidney disease ( $p=0.005$ ). Long-term survival did not differ between the groups with IF and DGF but was significantly worse in patients with PNF ( $p=0.002$ ).

**Discussion and Conclusions.** Poor early graft function and rejection but not DGF are strong predictors of both long-term patient and graft survival. Their association with increased mortality in renal transplant recipients has not been widely reported and merits further study.

## COMPARATIVE EFFICACY OF LIPOSOMAL FK506 WITH FK506 WITH AND WITHOUT ANTI CD4+/CD8+ MONOCLONAL ANTIBODIES.

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**Objective:** Liposomal FK506 (LFK) is a novel immunosuppressant formulation with an altered biodistribution when compared with FK506 (FK) injection solution. Here we test, for the first time, the efficacy of LFK *in vivo*.

Monoclonal antibodies have been used to induce tolerance in the mouse cervical heart allograft model. Here we investigate the potential interaction between novel and conventional immunosuppressive agents.

**Methods:** The murine cervical heart allograft model was used in experimental groups ( $N=6$ ). CBA recipients were grafted with BALB/c hearts. LFK506 or FK506 was administered daily, *i.p.*, at 1mg/kg on Day 0 to Day 14. Nondepleting anti-CD4+ (1 mg) and anti-CD8+ (1 mg) antibodies (YTS 105 and YTS 177 respectively) were given *i.p.* on Day 0 and on alternate days thereafter for a total of 6 doses.

Group 1 received LFK506. Group 2 received FK506. Vehicle Control Group 3 received liposomes alone *i.p.* Group 4 received antibody and LFK506. Group 5 received antibody and FK506. Group 6 received mabs only. All heart grafts were palpated daily and cessation of beating was taken as rejection. Serum FK506 levels were measured.

### Results:

Group	Median Graft Survival (days)	Range (days)	Mean Serum FK506 (micrograms/l/day 7)
1	25	14,25,25,25,27,66	2.4
2	29	25,27,29,45,47	21
3	7	7	N/A
4	Ongoing: > 36	10,11,35,35,36,36	6.9
5	Ongoing: > 40	40,40,40,41,42	15.5
6	>100	>100	N/A

**Conclusions:** LFK treated allografts have a significantly prolonged survival over liposomal controls. Thus effective immunosuppression was achieved although serum FK506 levels were significantly lower in LFK treated recipients than those treated with the same dose of the conventional formulation of FK506.



USE OF AN IN VITRO IMMUNISATION SYSTEM TO GENERATE ALLOTYPE-SPECIFIC ANTI-CD45 MONOCLONAL ANTIBODIES

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CD45, the highly-abundant glycoprotein seen on the surface of all bone-marrow derived cells, with the exception of erythrocytes, has been shown in rats and pigs to exist in two allotypic forms. Several different isotypic forms are present within the allotype, these being specific for the cell type on which they are expressed. The isotypic forms of CD45 seen on human leukocytes are very similar to those seen in the rat and it would seem reasonable to assume that, this being the case, human CD45 will also exist in two allotypic forms.

The aim of this study was to generate human monoclonal antibodies specific for the putative allotypes in order to confirm their existence. Leukocytes isolated from the blood of volunteers were immunised with CD45 and used as antigen presenting cells for autologous lymphocytes in culture. These cells were then fused with a human/mouse heteromyeloma cell line (K6H6B5) using PEG and the appearance of HAT resistant clones monitored.

Emergent clones were tested for anti CD45 production by ELISA using CD45-coated plates and culture supernatant as a source of antibody. Positive clones were then tested further using whole blood in a flow cytometric technique with FITC anti human IgG as the second layer antibody. It was found that when the clones were tested with blood from different individuals, some of them exhibited positive antibody staining of all leukocytes from some individuals whilst staining none of the cells from others. This indicates that these antibodies were directed against an epitope found on all leukocytes of a given individual, the expected result for an anti-allotypic antibody and thus provides firmer evidence for the existence of human CD45 in allotypic forms. Antibody directed against an epitope specific for one of the isomeric forms of CD45 would show positive staining of specific populations of cells from all volunteers tested.

Allotype specific monoclonal antibodies have a potential clinical application in the field of transplantation. It has been shown that passenger leukocytes present in renal grafts can stimulate the onset of rejection. Therefore, if graft recipients could be passively immunised with allotype-specific antibody, this would remove passenger leukocytes and thus reduce the possibility of graft rejection.

SUCCESSFUL RAT LIVER PRESERVATION WITH PHOSPHATE BUFFERED SUCROSE: DEMONSTRATION IN AN ISOLATED PERFUSED LIVER MODEL

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Phosphate-buffered sucrose (PBS140) is a clinically proven and inexpensive kidney preservation solution but it has not been fully evaluated in liver preservation. We report successful liver preservation for 24 hours with PBS140 in a model of isolated perfused rat liver (IPRL) with results comparable to University of Wisconsin (UW) solution.

Male Wistar rats (200-250g) were anaesthetised with intraperitoneal pentobarbitone (50mg kg<sup>-1</sup>). After a midline laparotomy, the common bile duct was cannulated with a fine bore polyethylene catheter. Fifty units of heparin in 2 ml of normal saline was injected via the penile vein. The aorta was cannulated and the liver flushed in situ with 20 ml of Ringer Lactate (control), UW or PBS140. Five ml of the test solution was next perfused via the portal vein. The liver was removed and stored in the flush solution at 4°C. After 24 hours of storage, the liver was set up on a recirculating circuit system at 37°C. The basic solution for reperfusion was Ringer lactate to which bovine red cells were added to increase the oxygen carrying capacity and bovine albumin to maintain the oncotic pressure. Taurocholic acid (4.84%) was infused at 5 ml.hr<sup>-1</sup> to the inflow system to support bile production. Bile was collected during the reperfusion period. Liver enzymes (LDH, AST and ALT) were determined after preservation and reperfusion. The concentration of bilirubin in the bile was also measured and the percentage of the bile acid used by the liver was calculated. The results are shown below as mean ± SEM.

Test solutions	Bile flow (µl/2h/g)	Bile acid used in the liver (%)	Bilirubin (mg/dl)	Enzymes (U/L)		
				ALT	AST	LDH
UW (n=6)	145±7	55.2±3.42	4.41±0.49	†17.0 ± 0.3 *24.3 ± 1.1	†23.2 ± 5.4 *30.2 ± 3.5	†180 ± 20 *84 ± 8
PBS140 (n=8)	147±16	50.8±4.52	6.65±0.74	†33.3 ± 2.8 *43.9 ± 3.3	†30.1 ± 3.5 *62.2 ± 5.5	†161 ± 15 *181 ± 16
R. Lactate (n=6)	45±7	8.3±2.74	1.55±0.36	†171 ± 29.4 *118 ± 19.4	†225 ± 36.3 *331 ± 34.4	†1640 ± 132 *1107 ± 182

†:After 24-hour preservation (first flush) \* :After reperfusion (last flush)

These results suggest PBS140 is worthy of further investigation for preservation of organs other than kidney.

## WARM ISCHAEMIC INJURY IN THE RAT KIDNEY: EVALUATION OF THE PROTECTIVE ROLE OF THE CURRENT PRESERVATION SOLUTIONS

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We report on the protective role of the current preservation solutions in the prevention of warm ischaemic and reperfusion injury in a rat kidney model. The solutions studied, included: Euro-Collins (EC), Hyperosmolar Citrate (HOC), Phosphate Buffered Sucrose (PBS140) and University of Wisconsin (UW) solution.

Male Wistar rats (300-350g)\* were anaesthetized by intraperitoneal injection of inactin (120 mg.kg<sup>-1</sup>). An intravenous infusion was set up at 6ml.hr<sup>-1</sup> (NaCl, 125mmol.l<sup>-1</sup> and HCO<sub>3</sub>, 25 mmol.l<sup>-1</sup>) also containing 37MBq.l<sup>-1</sup> of <sup>3</sup>H inulin (for inulin clearance). Both ureters were cannulated for serial urine collection. An equilibration period of 1 hour was allowed following surgery after which urine was collected from each kidney for one hour (control). The left kidney was then flushed with 0.5 ml of 0.9% saline, EC, HOC, PBS140 or UW at 37°C. A clamp was then applied to the left renal pedicle. After 45 minutes the clamp was released to allow reperfusion and a right nephrectomy was performed. Urine flow and composition from the left kidney were observed for 4 hours.

The results are presented below for the second hour post-ischaemia as some experiments had to be terminated in the third and fourth hour because of rats dying, particularly in saline and EC groups. Inulin clearance and urine flow are expressed as percentage of pre-ischaemic control values. Urine osmolality is expressed as percent above plasma osmolality (n=6 for each group)

Function	Saline	EC	HOC	PBS14	UW
GFR μl.min <sup>-1</sup> .100g <sup>-1</sup>	5	15	47	51	66
Urine flow μl.min <sup>-1</sup> .100g <sup>-1</sup>	156	190	573	462	1149
Osmolality mOsm.kg <sup>-1</sup>	0.6	13	25	46	36

\* Home Office guidelines were strictly followed in all animal experiments.

45 minutes of warm ischaemia to rat kidney was shown to be a severe ischaemic insult. Euro-Collins failed to provide effective protection against warm ischaemia when compared to HOC, PBS140 and UW.