O101 THE UTILITY OF TIME-ZERO BIOPSY SCORING OF ISCHAEMIA/REPERFUSION INJURY AFTER LIVER TRANSPLANTATION
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Introduction
The utility of time-zero biopsies after orthotopic liver transplantation (OLT) remains unclear. The aim of this study is to evaluate histological grade of ischaemia/reperfusion injury (IRI) on time-zero biopsy as a prognostic indicator following OLT.

Methods
Between October 1996 and February 2010, 883 OLT were performed at our centre. Time-zero biopsies were available for 572 patients. Patients were divided into four groups based on histological grade of IRI: nil (121), mild (303), moderate (124) and severe (24) and clinical data compared for each.

Results
Biopsy score severity was strongly associated with recognised risk factors for IRI, including donation after cardiac death, donor age, donor BMI and allograft steatosis (p<0.001). Higher IRI grades also correlated closely with the incidence of post-perfusion hyperkalaemia (p=0.001) Interestingly, neither cold nor warm ischaemic times were significantly different between the groups (p=0.5). The degree of IRI on biopsy correlated closely with graft outcome. In particular, a severe IRI grade was associated with significantly greater post-transplant morbidity compared to the other 3 groups. The severe group demonstrated markedly higher rates of primary non-function (13% vs 6%; p=0.02), early graft dysfunction (57% vs 32% p<0.0001) and the need for re-transplantation within 90 days (13% vs 2%; p=0.01). One year graft survival in nil, mild and moderate groups were significantly better than in the severe group (82%, 85%, 90% and 61% respectively; p=0.003).

Conclusion
Time-zero biopsies have value in predicting adverse clinical outcomes following OLT and allow identification of patients at risk of a complicated post-operative course.

Take-home message
Assessment of ischaemia reperfusion injury on time zero biopsy after liver transplantation is an accurate prognostic indicator of post-operative outcome.

O102 THE FIRST CLINICAL SERIES OF EX-VIVO NORMOTHERMIC PERFUSION (EVNP) IN MARGINAL DONOR KIDNEY TRANSPLANTATION
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Introduction
Ex-vivo normothermic perfusion (EVNP) is a novel method of preservation that restores circulation and allows an organ to regain function prior to transplantation. The aim of this study was assess the effect of EVNP in kidneys from marginal donors.

Methods
Twenty two kidneys from marginal donors underwent a short period of EVNP immediately before transplantation. Kidneys were perfused with a plasma free red cell based solution at a mean temperature of 34.7°C. The outcome of these kidneys was compared to a control group of 53 marginal donor kidneys that underwent static cold storage (CS).

Results
The average donor age was 57 ± 11.7yr in the EVNP and 60 ± 9.5yr in the CS group (P=0.335). EVNP kidneys were perfused for an average of 63.2 ± 14.2min, all produced urine and were transplanted successfully. The total cold ischaemic time was 12.6 ± 4.6h in the EVNP group and 11.8 ± 3.7h in the
CS group (P=0.918). The delayed graft function rate (DGF) defined as the requirement for dialysis within the first 7 days was 1/22 patients (4.8%) in the EVNP group versus 18/53 (33.9%) in the CS group (P=0.008). There was no difference in graft or patient survival at 12 months (P=0.510, 1.000). There was a similar incidence of acute rejection in both groups (EVNP 22.4% vs CS 26.4%; P=1.000).

**Conclusion**

This first series of EVNP in marginal donor kidneys suggests that this technique of restoring circulation and function prior to transplantation is a safe and feasible method of preservation.

**O103 HOW HELPFUL ARE SERUM MARKERS IN EVALUATING GRAFT PANCREATITIS FOLLOWING SIMULTANEOUS PANCREAS KIDNEY TRANSPLANTATION?**

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**Introduction**

Graft pancreatitis, with or without underlying acute rejection, remains difficult to diagnose using clinical assessment alone, leading to indiscriminate use of cross-sectional imaging. The aim of this study is to evaluate the accuracy of routine serum markers in predicting allograft inflammation following simultaneous pancreas-kidney transplantation (SPK).

**Methods**

A retrospective analysis of 109 SPK performed at our centre between January 2005 and December 2010 was undertaken. All 299 post-operative CT scans performed in this period were blindly assessed by 2 radiologists for features of pancreatitis: graft enlargement (normal/enlarged), graft perfusion (normal/heterogenous), ascites (absent/present) and peri-pancreatic fat changes (mild/moderate/severe). Serum markers measured at the time of each scan were recorded to assess correlation with radiological severity of pancreatitis.

**Results**

Serum C-reactive protein (CRP) levels correlated closely with radiological evidence of graft pancreatitis. Mean CRP levels were significantly higher in patients with CT findings suggestive of graft pancreatitis, including enlarged vs. normal graft size (114+/−74 vs. 76+/−80 mg/dL), heterogenous vs. normal perfusion (132+/−81 vs. 82+/−76 mg/dL), presence vs. absence of ascites (125+/−81 vs. 74+/−73 mg/dL) and mild vs. moderate vs. severe peri-pancreatic fat changes (23+/−34 vs. 53+/−59 vs. 107+/−80 mg/dL). Mean white cell counts were also significantly higher in patients with enlarged grafts (10+/−5.1 vs. 8.7+/−5.5) and ascites (11+/−5.2 vs. 8.7+/−5.6). In contrast, neither serum amylase nor lipase had any correlation with radiological grading of pancreatitis.

**Conclusion**

CRP and white cell count appear to reflect pancreas graft inflammation and may be useful in diagnosing graft pancreatitis and avoiding overuse of radiological investigations.

**Take-home message**

CRP and white cell count are useful markers of graft pancreatitis and as such may allow avoidance of repeated radiological investigations to assess graft inflammation.

**O104 SIGNIFICANT RESPONSE TO LOCAL ABLATIVE BRIDGING TREATMENTS FACILITATES ACCEPTABLE RATES OF SURVIVAL FOLLOWING LIVER TRANSPLANTATION FOR HCC**

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**Introduction**

Liver Transplantation (LT) is a well-recognised treatment option for selected patients with hepatocellular carcinoma (HCC). However there is concern regarding tumour progression whilst on the waiting list. UK guidelines recommend local ablative therapy for all HCC patients being considered for LT.

**Methods**
All consecutive patients with HCC who have undergone LT between 2001 - 10 were identified from our prospectively maintained database. All patients are discussed at LT assessment meeting and at a separate HPB MDT for consideration of bridging treatment [trans-arterial chemo-embolisation (TACE) and/or radio frequency ablation (RFA)] whilst on the waiting list. We have sought to evaluate the benefits of this.

Results
55 HCC patients underwent LT (M:F = 43:12 ). Bridging treatments were TACE n=31, RFA n=28, or both n=4. Six patients did not undergo any form of bridging treatment as they rapidly progressed to LT. The response to bridging treatment was complete (n=8), good (n =10), moderate (n = 18), poor (n = 4) or no response (n =15). There were 2 deaths within 100 post-operative days. At last follow-up n= 21 were disease free. Overall survival [median (95% CI)] was 62 (53 -71) months [good response to bridging treatments it was 67 (55 – 79) months and those with poor/no response it was 53 (42 – 64) months (log-rank p=0.059)].

Conclusion
This study demonstrates the feasibility of various bridging treatments for patients with HCC who await liver transplantation in the UK. In combination with careful patient selection and surveillance acceptable rates of survival can be achieved.

Take-home message
Bridging treatments for HCC are feasible and response to bridging treatment suggests improved survival following liver transplantation.

O105 WHAT ARE THE IMPLICATIONS OF 12-MONTH SURVEILLANCE BIOPSIES FOLLOWING RENAL TRANSPLANTATION?
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Introduction
Surveillance biopsies are performed in renal transplant patients with stable renal function at set times following transplantation. Biopsies are performed to detect sub-clinical rejection (SCR), which is evidence of inflammation with no change in renal function. Surveillance biopsies are not routinely performed in all centres. We reviewed our 12-month surveillance biopsies and their impact on patient management.

Methods
A retrospective review of patients who underwent a kidney transplant between 2008 and 2011 was performed. Data were collected from patient case notes, histology reports and clinical databases. Patients who underwent a 12-month surveillance biopsy were included. Exclusions included clinical trial patients and those undergoing diagnostic biopsies. Histology was scored according to Banff classification and chronic damage was classed as mild, moderate or severe.

Results
Ninety-six patients had a 12-month biopsy and 81 were included for analysis. 44 were living donor and 37 were cadaveric transplants. Eleven (14%) biopsies showed normal kidney. Thirty-six biopsies (44%) showed chronic damage, in 9 cases it was severe and 3 had immunosuppression altered. In total 14 (17%) patients had medication altered of whom 3 (4%) were admitted for intravenous methylprednisolone. SCR was found in 2 of these 3 patients. Only 4 of 17 patients with Banff borderline rejection had medications altered. There were no adverse events requiring hospital admission.

Conclusion
Twelve-month surveillance biopsies have an impact on immunosuppressive therapy and patient management and SCR is an important finding resulting in admission for treatment.

Take-home message
Surveillance biopsies are safe and help direct immunosuppressive therapy in renal transplant recipients.
O106  MICE ON TRIAL: IS XENOGRAFTS USING HUMAN TISSUE A BETTER MODEL OF CANCER THAN XENOGRAFTS FORMED FROM CELL LINES?
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Introduction
Xenograft tumours are validated models using either patient samples (primary xenografts) or cell lines (secondary xenografts) grown in a murine host. Repeated culturing of cancer cell lines results in epigenetic changes and subsequent behaviour changes distinct from primary human cancers. The aim of this study is to form both models and compare their responses to chemobiologic therapy.

Methods
Fresh primary colorectal adenocarcinoma tissue or colorectal cell lines (SW480 non metastatic line / SW620 metastatic line) were inoculated subcutaneously into immunodeficient mice to form primary and secondary xenografts respectively. Both models were subjected to either a 5-fluorouracil (5-FU) or Irinotecan trial. The clinical response and biological features were evaluated.

Results
Secondary xenografts were more aggressive with a shorter time to tumour formation, higher uptake rate and more aggressive histological features compared to primary xenografts. The mice were randomised to either the control group (saline) or to the treatment group [60mg/kg of 5FU (SW620 / primary xenografts) or 60 mg/kg of Irinotecan (SW480 / primary xenografts)] via intraperitoneal injection. In the 5FU group, the primary xenografts began regressing after Day 2 but the secondary xenografts continued to grow until Day 8. The decrease from the maximal tumor volume to the study end point was evaluated with primary xenografts showing a greater decrease compared to secondary xenografts (56.6% vs. 10.4%; p = 0.002).

Conclusions
Primary xenografts are a better model for the in vivo testing of new chemobiological agents and allows for drug screening in order to personalise an individual’s chemotherapy regime.

Take-home message
We demonstrate for the first time the ability to grow tumours in mice from fresh human colonic adenocarcinomas. Tumours formed in mice from patient derived adenocarcinomas produce more clinically accurate testing than tumours formed from cell lines.

O107  WITHDRAWN

O108  TISSUE ENGINEERING OF THE KIDNEY USING A WHOLE ORGAN DECELLULARISATION APPROACH
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Introduction
Renal transplantation is the optimal form of renal replacement therapy. However, it is restricted by the limited pool of organs available from cadaveric donors – over 2500 renal transplants were performed in 2010 but there are more than 50,000 patients with end stage renal failure in the UK.

Objective: To use a new approach in tissue engineering – ‘whole organ’ decellularisation’ – to produce a complete kidney extracellular matrix (ECM) scaffold and recellularised organ construct. This approach has been successfully utilised in the heart, lungs and liver in animal studies.

Methods
Wistar rat whole kidneys were decellularised with 1% w/v SDS. Characterisation of decellularisation included histology (H+E), immunohistochemistry (IHC) for ECM components, vascular corrosion resin
casting (Batson’s no. 17 kit). Recellularisation with i) rat primary renal cells and ii) rat bone-derived mesenchymal stem cells were performed within a bioreactor for 7 days. Recellularisation viability was assessed with LIVE/DEAD staining and Alamar blue assay; characterisation of constructs by histology, IHC for renal cell markers and scanning electron microscopy.

**Results**

Rat kidneys were successfully decellularised to create whole kidney ECM bio-scaffolds. Characterisation of the bio-scaffold demonstrates good decellularisation, preservation of ECM components and architecture, renal-specific architecture and preservation of the vasculature. Recellularisation studies show penetration and distribution of viable cells throughout the bio-scaffold architecture within ‘renal-like’ structures, suggesting an appropriate response to structural and bio-inductive cues within the bio-scaffold.

**Conclusion**

Successful decellularisation of rat whole kidneys with recellularisation studies on this bio-scaffold have shown formation of cellular ‘renal-like’ structures within the matrix architecture.

**Take-home message**

Tissue engineering using decellularised whole organ ECM bio-scaffolds (e.g. in the kidney) may provide a strategy to create an alternative source of organs for transplantation.

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**O109  THE EFFECTS OF ARTERIAL PRESSURE DURING A PERIOD OF EX-VIVO NORMOTHERMIC PERFUSION IN A PORCINE KIDNEY MODEL**

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**Introduction**

Ex-vivo Normothermic Perfusion (NP) is a novel method of kidney preservation. However, there is a risk that higher perfusion pressures may cause endothelial injury. The aim of this study was to evaluate the effects of two different arterial pressures during NP.

**Methods**

Porcine kidneys underwent static cold storage (CS) for 23 hours followed by 1h of ex-vivo NP using leukocyte depleted blood at a mean arterial pressure of either 55 or 75mmHg. Following this, kidneys were reperfused for 3h to assess renal function and injury. This was compared to a control group that underwent 24h CS.

**Results**

During NP kidneys perfused at 75mmHg had a higher renal blood flow (RBF), increased oxygen consumption (55±17 vs 30±16ml/min/g;P=0.026) and produced more urine (P=0.002) than kidneys perfused at 55mmHg. During reperfusion, RBF was significantly higher in the 75mmHg group compared to the control (AUC 75mmHg 447±187, 55mmHg 520±243 vs control 285±82 ml/min/100g.h P=0.040). There was no significant difference in renal function between the groups however tubular injury was significantly reduced in the 75mmHg kidneys (P=0.007). The 55mmHg group kidneys produced significantly less urine compared to the 75mmHg and control (P=0.016). There was no significant increase in levels of endothelin-1 in the 75mmHg group (P=0.190).

**Conclusion**

Kidneys perfused at 75mmHg during 1h of ex-vivo NP had a higher level of renal metabolism compared to kidneys perfused at 55mmHg. 75mmHg is a more favourable arterial pressure with improved haemodynamics, less tubular damage and no evidence of increased endothelial injury during reperfusion.

**Take-home message**

A perfusion pressure of 75mmHg appears to be an optimal perfusion pressure for normothermic kidney perfusion.

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**O110  TRANSCRIPTOMIC CHANGES IN HUMAN RENAL BIOPSIES FROM LIVING AND CADAVERIC DONORS AT 30 MINUTES AND 3 MONTHS POST-TRANSPLANTATION**
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Introduction
Renal allograft survival is >90% at 1 year, but falls dramatically in 10 years, with no single
conventional method allowing timely assessment. To develop novel biomarkers, transcriptomic
signatures in human transplant kidneys were investigated.

Methods
Renal biopsies from living donors (LD) and cadaveric donors (CAD) at 30 minutes and 3 months post-
transplantation (n = 6) were used for whole-geneome profiling. Selected genes were validated by
quantitative PCR in 24 microarray samples and additional 33 biopsies. Relevant clinical data were
analyzed related to gene expression.

Results
The overall gene profiles were clearly different in 30-minute and 3-month samples. Differentially
expressed genes were 681 at 30 minutes, which reduced to 75 at 3 months (1.5 fold change, p<0.05)
between two donor types with significantly longer warm and cold ischemic time in CAD. Many acute
response genes (FGA, VWF, SERPINA1) were up-regulated at 30 minutes, while genes involved in
inflammation, nephrotoxicity and proliferation were up-regulated at 3 months. More interestingly,
up-regulated tissue remodeling associated genes (COL3A1, TIMP1, MMM9) and down-regulated FGA
at 3 months in contrast to 30 minutes in CAD was not seen in LD. A list of 120 genes closely
correlated with serum creatinine and fibrosis at not only early times, but also 12 and 24 months.

Conclusions
Transcriptomic signatures were shifted from acute responses to tissue damage and remodeling by
the time post-transplantation with divergent profiles between LD and CAD. These changed
signatures might be linked to initial donor injury and adaptive immune responses, also potential
biomarkers for diagnosis and interventions.

Take-home message
Transcriptomic signatures were shifted from acute responses to tissue damage and remodeling by
the time post-transplantation with divergent profiles between LD and CAD. These changed
signatures might be linked to initial donor injury and adaptive immune responses, also potential
biomarkers for diagnosis and interventions.

O111 SILDENAFIL ENHANCES RENAL BLOOD FLOW BUT DOES NOT PROTECT AGAINST ISCHAEMIA
REPERFUSION INJURY IN A MODEL OF DONATION AFTER CIRCULATORY DEATH KIDNEY
TRANSPLANTATION
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Introduction
Sildenafil has been used as a pre-conditioning agent to protect against ischaemic injury, although
there is little evidence for its benefit in reducing ischaemia reperfusion (I/R) injury in renal
transplantation. The aim of this study was to assess the effects of sildenafil on I/R injury in a porcine
model of donation after circulatory death (DCD) kidneys.

Methods
Kidneys were subjected to 20 minutes warm ischaemia followed by 18 hours cold storage. After
preservation kidneys were reperfused on an ex vivo perfusion system for 3 hours with an
oxygenated blood based solution. Kidneys were treated with 0.2mg (n=4), 0.7mg (n=4) or 1.4mg
(n=6) or no (control, n=6) sildenafil during reperfusion. Renal function and renal injury markers were measured throughout reperfusion.

**Results**

Renal blood flow was increased in a dose dependent manner with a significantly higher flow in the 1.4mg treated kidneys compared to the controls [mean area under curve (AUC), (1.4mg) 482±99, (0.7mg) 469±123, (0.2mg) 387±115, (Control) 360 ± 47ml/min/100g.h; P=0.021]. There was no significant improvement in renal function [AUC creatinine clearance; (1.4mg) 2.9±0.8, (0.7mg) 2.5±0.6, (0.2mg) 3.0±2, (control) 4.5±2.0ml/min/100g.h; P =0.099], tubular injury [neutrophil gelatinase-associated lipocalin (NGAL); P = 0.060], levels of inflammatory cytokines (IL-6; P=0.357, TNFα; P=0.340) or neutrophil infiltration (P=0.106) between the groups.

**Conclusion**

Sildenafil had a vasodilatory action but did not affect recovery of renal function or protect against I/R injury. This suggests that sildenafil is not renal protective during the early reperfusion phase in an ex vivo DCD model.

**Take-home message**

Sildenafil has a vasodilatory action during reperfusion but does not protect against ischaemia reperfusion injury.

**O112  LHB IN PRESERVATION SOLUTION AND AUTOLOGOUS BLOOD PERFUSATE PROTECTS ISOLATED ISCHEMIC PORCINE KIDNEYS**

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**Introduction**

There is an imperative need to develop new approaches to prevent ischemia reperfusion injury during organ cold storage and reperfusion. A novel levorotatory and cyclic helix B peptide (LHBP) derived from erythropoietin was synthesized recently and its tissue protective roles were evaluated in an isolated ischemic porcine kidney model.

**Methods**

Porcine kidneys subjected to 20 minutes warm ischemia were retrieved and flushed with 500 ml hyperosmolar citrate and cold preserved for 18 hours with or without 10.56 nmol/L LHBP. The kidneys were then reperfused with oxygenated autologous blood with or without LHBP for 3 hours on an isolated organ perfusion system to assess renal function, histology, apoptosis and inflammation.

**Results**

LHBP significantly increased renal blood flow during 2 to 3-hour reperfusion, with increased oxygen consumption and urine output, but decreased serum potassium. The 32 kD precursor of caspase-3, as well as 12 and 17 kD active subunits were all down-regulated by LHBP in after 3-hour reperfusion, while only the 12 kD subunit was decreased post CS. In addition, apoptotic cells were significantly decreased in tubular areas, but increased in lumens and interstitial areas in post CS and reperfusion kidneys. HSP70 was up-regulated; myeloperoxidase+ cells was reduced and renal tissue damage was also ameliorated by LHBP.

**Conclusions**

The administration of LHBP during cold preservation as well as hemoreperfusion improved IRI with better renal blood flow, oxygenation, tubular function and renal tissue damage, which might be due to highly expressed HSP70, but less caspase-3 protein, apoptosis in tubular areas and inflammation.
Take-home message
The administration of LHBP during cold preservation and hemoreperfusion improved IRI, which might be due to highly expressed HSP70, but less caspase-3 protein, as well as apoptosis and inflammation.