PATEY PRIZE 1

O1 NOVEL BIOMARKERS IN CANCER - ROLE OF LINE-1ASP IN THE DIAGNOSIS AND PROGNOSIS OF COLORECTAL CANCER

C Neophytou (1,2), E Theophilidou (1,2), C Parr (1), J Williams (1,2), C Tufarelli (1), J Lund (1,2)
(1) School of Health Sciences, University of Nottingham (2) Royal Derby Hospital

Introduction: Long interspersed nucleotide elements (LINEs) are retrotransposons with the ability to jump, copy and paste themselves in the genome. In normal tissues, they are usually silenced. Activation of LINE promoters (LINE-1ASP) at inter- and intragenic locations has been shown to drive transcription of isoforms of cellular genes that can have oncogenic properties and have been implicated in colorectal cancer (CRC). We hypothesise that these aberrant proteins formed as a result could act as biomarkers for CRC detection. Our aim is to examine the role of four such proteins (c-MET, RAB3IP, CHRM3 and GNGT1) as biomarkers in the diagnosis of CRC.

Method: Tumour mucosal biopsies with matching non-tumour mucosal tissue (>5cm from the tumour) and blood serum were collected from 36 CRC patients undergoing elective resection. Serum samples from 45 healthy volunteers were used as controls. qPCR and ELISA tests were performed.

Result: Expression of GNGT1 and RAB3IP proteins were significantly increased compared to controls in tumour tissue ($p=0.0008$ and $p=0.0006$ respectively) and serum ($p=0.034$ and $p=0.0002$ respectively). GNGT1 gene expression was also significantly increased ($p=0.0268$). Expression of CHRM3 and c-MET proteins were significantly decreased compared to controls in tumour tissue ($p<0.0001$ and $p=0.0038$ respectively). In serum, only c-MET protein expression was significantly decreased ($p=0.0041$). c-MET gene expression was significantly increased ($p<0.0001$) whereas CHRM3 gene expression was significantly decreased ($p=0.0091$).

Conclusion: The above proteins could form a panel of biomarkers obtained by blood testing, to complement currently used screening tests that are linked to low compliance, false results and potentially serious complications.

Take-home message:
Our team has identified four promising blood circulating protein biomarkers as proven with statistical significance in ELISA and qPCR tests. They could complement current screening tests for colorectal cancer and in the future, their role could expand to replace those tests and used for predicting relapse and response to treatment.

O2 HCV POSITIVE ORGANS FOR TRANSPLANTATION: A MISSED OPPORTUNITY

PB Trotter (1,2), DM Summers (1,2), M Robb (2), CJE Watson (1), I Ushiro-Lumb (2), J Powell (3), JA Bradley (1), J Neuberger (2)
(1) University of Cambridge (2) NHS Blood and Transplant (3) Edinburgh Transplant Unit Edinburgh Royal Infirmary

Introduction: Hepatitis positive (HCV+ve) donors are currently contraindicated for transplantation due to the risk of disease transmission. New direct acting antivirals (DAA) mean that HCV is now curable. More organs could be made available for transplantation through the use of HCV+ve donor organs.

Method: The UK Transplant Registry and the Potential Donor Audit were interrogated to identify all HCV antibody positive deceased organ donors from 01/01/2000 to 31/12/2015. Discarded HCV+ve organ quality was assessed using donor quality indices and functional parameters.

Result: 244 HCV+ve deceased donors were identified, of which only 76 proceeded to organ donation. These donors resulted in 93 transplants (63 Liver and 30 other organs). Unadjusted liver recipient graft and patient survival was not adversely impacted by the donors HCV+ve status. 146 HCV+ve organ donors were not used for transplantation,71.4% due to positive virology and only 8.9% due to poor organ function. Median eGFR of discarded HCV+ve kidneys was 103 IQR (70-144) and 49% of them had UK donor risk index scores <1.02 suggesting at least 77% of these kidneys would be functioning at 5 years. Cost analysis demonstrated that transplanting an HCV+ve kidney into an HCV-ve recipient and treating them with DAA would be cost neutral with dialysis 4 years post transplantation. Discussion: Consideration should be given for using HCV+ve donor organs for HCV-ve recipients, as the organ quality is good and it is cost effective compared to dialysis and may greatly reduce time on the waiting list.

Take-home message:
A large proportion of Hepatitis C virus (HCV) positive organs are not used for transplantation. As HCV is now curable, using HCV positive organs for transplantation will help save many lives and will actually save money compared to patients remaining on dialysis.

O3 PREDICTING LIMB VIABILITY FOLLOWING LOWER EXTREMITY VASCULAR TRAUMA

ZB Perkins, B Yet, S Glasgow, W Marsh, TE Rasmussen, NRM Tai
Centre for Trauma Sciences. Queen Mary, University of London

Introduction: Limb viability is a key outcome following lower-extremity vascular trauma and is central to decisions between attempting salvage and amputation. However, the risk to limb viability, and projected outcome of limb reperfusion, is often unclear at the time surgical decisions are made, greatly increasing the difficulty of these decisions and threatening sound judgement. Our aim was to develop a

prognostic model for limb viability to support surgical decision-making.
Method : A Bayesian Network (BN) prognostic model was developed from clinical knowledge and data. Data sources include a meta-analysis of prognostic factors and a large cohort study, the Global War On Terror Vascular Injury Initiative (GWOT-VII). BN Performance (discrimination, calibration, and accuracy) was tested using ten-fold cross validation and compared to the Mangled Extremity Severity Score (MESS).
Result: We developed a 10-variable BN and validated performance on data from 508 threatened limbs. Blast was the most common mechanism of injury (66.7%) and 64 limbs (12.9%) ultimately required amputation for non-viable tissue. The BN had excellent performance at predicting limb viability (AUROC 0.932 (0.898–0.967), sensitivity 90.6%, specificity 85.5%, Diagnostic Odds Ratio 56.8 (43.1–74.9); was well calibrated (Hosmer-Lemeshow statistic: 14.1; p=0.079); and accurate (Brier Score 0.06 (0.05–0.07), Brier Skill Score 0.39 (0.25–0.48)). Furthermore, the BN had significantly better performance than MESS at predicting limb viability (AUROC 0.932 (0.898–0.967) versus 0.723 (0.656–0.790); P<0.0001).
Conclusion: A BN can accurately predict limb viability at the time of initial wound evaluation. This information may complement clinical judgement, support rational and shared treatment decisions, and establish sensible treatment expectations.
Take-home message:
A Bayesian Network prognostic model can accurately predict the risk to limb viability following lower extremity vascular trauma.

O4 EVALUATION OF A NOVEL MITOCHONDRIA-TARGETED ANTI-OXIDANT THERAPY FOR ISCHAEMIA REPERFUSION INJURY IN A MODEL OF KIDNEY TRANSPLANTATION

M Hamed (1), A Logan (2), A Dare (2), A James (2), A Barlow (1), J Martin (1), N Georgakopoulos (1), A Gane (2), A Gruszczyk (2), K Crick (1), D Fouto (1), C Fear (1), E Bolton (1), JA Bradley (1), G Pettigrew (1), S Hosgood (1), M Nicholson (1), M Murphy (2), K Saeb-Parsy (1)
(1) Department of Surgery, Addenbrooke's Hospital, Cambridge NIHR Biomedical Research Centre and NIHR Blood and Transplant Research Unit in Organ Donation and Transplantation, Cambridge Biomedical Campus, CB2 0QQ, UK (2) MRC Mitochondrial Biology Unit, Hills Road, Cambridge CB2 0XY, UK
Introduction: Ischaemia reperfusion injury (IRI) makes a major contribution to graft damage during kidney transplantation and increases the risks of primary non-function, delayed graft function and rejection. As mitochondria play a central role in the generation of reactive oxygen species during IRI, we examined the efficacy of the novel mitochondria-targeted antioxidant MitoQ in amelioration of renal IRI using porcine and human kidneys.
Method: Pairs of kidneys were retrieved from anaesthetised pigs after 10min of warm ischaemia, flushed with preservation solution ± MitoQ (concentration range 50nm–250μM), stored at 4°C for 10h and underwent ex-vivo normothermic perfusion (EVNP) with oxygenated autologous blood. Pairs of human kidneys retrieved for transplantation but subsequently declined were flushed and stored in preservation solution ± MitoQ, stored at 4°C for 6h and underwent EVNP with ABO group matched blood. Data were compared using paired analysis.
Result: In the porcine model, renal blood flow and urine output were significantly higher in the 50μM MitoQ-treated group after 6h of EVNP compared to controls (115±15 vs. 33±7 mL/min/100g, p=0.001 and 678±208 vs. 309±112 mL/100g; p=0.007 respectively; n=5 pairs). Human kidneys demonstrated a numerically higher urine output and creatinine clearance after 3h of EVNP with 50μM MitoQ treatment compared to controls but the difference did not reach statistical significance (196±139 vs.74±90 mL/100g; p=0.054, 4.0±4.1 vs. 1.5±2.1 mL/min/100g, p=0.152 respectively; n=7 pairs).
Conclusion: Our data suggest that treating kidneys with MitoQ during the cold preservation may ameliorate the detrimental effects of IRI. MitoQ has the potential to improve graft and patient outcomes after kidney transplantation.
Take-home message:
Our data suggest that administration of a mitochondria-targeted antioxidant during human kidney storage may improve the outcomes for transplant recipients.

O5 MECHANISMS UNDERLYING HUMAN VENOUS VALVE DISEASE CAUSED BY MUTATIONS IN FOXC2 AND CONNEXIN47

OTA Lyons (1), P Saha (1), C Seet (1), A Kuchta (2), A Arnold (2), S Grover (3), V Rashbrook (1), A Sabine (4), G Vizcay-Barrena (5), A Patel (1), F Ludwinski (1), S Padayachee (2), T Kume (6), B Kwak (7), G Brice (8), S Mansour (8), P Ostergaard (9), P Mortimer (9), S Jeffery (9), N Brown (10), T Makinen (11), T Petrova (4), B Modarai (1), A Smith (1)
(1) Academic Department of Vascular Surgery, Cardiovascular Division, BHF Centre of Research Excellence, King's College London, St Thomas' Hospital, UK (2) Department of Ultrasonic Angiology, Guys & St Thomas' NHS Foundation Trust, UK (3) Division of Hemostasis and Thrombosis, Beth Israel Deaconess Medical Center and Harvard Medical School, USA (4) Department of Fundamental Oncology, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Epalinges, Switzerland (5) Center for Ultrastructural Imaging, King's College London, UK (6) Feinberg Cardiovascular Research Institute, Northwestern University School of Medicine, USA (7) Department of Pathology and Immunology, University of Geneva, Switzerland (8) SW Thames Regional Genetics Service, St George's Hospital,

United Kingdom (9) Cardiovascular and Cell Sciences Institute, St George's University of London, UK (10) Institute of Medical & Biomedical Education, St George's University of London, United Kingdom (11) Rudbeck Laboratory, Department of Immunology, Genetics and Pathology, Uppsala University, Sweden
Introduction Venous valves (VVs) prevent blood reflux that can give rise to chronic venous hypertension and ulceration. Patients with mutations in the genes encoding the transcription factor Foxc2 and gap junction protein, connexin47 (Cx47), have venous reflux. We sought to examine VV phenotypes in these patients and to use a murine model to elucidate the function of these proteins in VV development.
Method Human VV number and length were quantified by ultrasound. Murine VV phenotype (marked by expression of Prox1) was examined using confocal microscopy, in wild-type and mice with complete or conditional deletion of genes expressing transcription factors and connexins.
Result Patients with Foxc2/Cx47 mutations had reduced valve number ($P<0.0005$) and shorter valves ($P<0.0005$). VV initiation in mice was marked by elongation/reorientation of Prox1hi endothelia by postnatal day 0. Expression of Foxc2 and Nfatc1, and the gap junction proteins, Cx47, Cx43 and Cx37, were temporo-spatially regulated during this process. Combined Foxc2 deletion with calcineurin-NFAT inhibition disrupted endothelial organisation, suggesting co-operative Foxc2-NFATc1 patterning. Deletion/knockout of each of the connexins also disrupted endothelial organisation. Specific deletion of endothelial Foxc2 had no effect on VV maintenance.
Conclusion Patients with mutations in Foxc2 and Cx47 have globally reduced VV numbers and shorter VV leaflets. Foxc2 and Nfatc1 likely cooperate to organise the initial ring of VV-forming cells. Connexins are critical for early organisation of valve-forming cells at P0 and failure of this process may underlie abnormal VVs identified in patients with mutated Cx47. Foxc2, in endothelia, is not required for valve maintenance.
Take-home message:
Structural defects in venous valves can now be systematically quantified in patients, and genetic defects (for example, mutations in the genes encoding Foxc2 and Cx47) causing valve failure can now be determined. Transgenic murine models have been used to show the functions of the proteins encoded by these genes in normal valve development and maintenance.

O6 THE ASSESSMENT OF DIFFERENT OXYGEN TENSIONS DURING EX-VIVO NORMOTHERMIC KIDNEY PERFUSION

TD Adams, SA Hosgood, ML Nicholson
University of Cambridge, Department of Surgery
Introduction Kidney Ex-Vivo Perfusion (EVNP) has historically used a gas mixture of 95% O_2 and 5% CO_2, however evidence suggests that supra-physiological oxygenation can be deleterious. We hypothesised that excess oxygenation during EVNP may abate its conditioning effects.
Method Porcine kidneys, subjected to 10min warm ischaemia and 2hr cold static storage, underwent 1hr of EVNP with either 95% (n=8), 25% (n=4), 12% (n=5) or 6% (n=5) O_2 with 5% CO_2 and N_2 balance; or Air (n=6). Continuous functional measurements were taken; blood and cortical wedge biopsies were taken at 30min intervals.
Result Oxygen content (CAO_2, ml/100ml) was higher in the 95% group (9.27±0.22) vs. 6% (5.39±0.9, p=0.006), 12% (7.55±0.6, p=0.04) and 25% (6.59±0.43, p=0.003) groups. Oxygen Delivery (DO_2, ml/min/100ml) was lower in the air group (1.99±0.45, p=0.025) and 6% (1.59±0.47, p=0.012) versus 95% group (4.17±0.71). Oxygen Consumption (VO_2, ml/min/100g) was higher in the 95% group compared to the Air group (1.74±0.26 vs. 0.96±0.21, p=0.04). There were no differences in functional measurements. Mean pH in the Air group was higher after 60min EVNP (7.48±0.05, p=0.0001).
Conclusion Despite higher CAO_2 and DO_2, VO_2 in kidneys given 95% O_2 appears to have no effect on renal function. A high pH during Air perfusion suggests 5% CO_2 is essential to maintain acid-base homeostasis. Further investigation of oxidative stress, histology and organ behaviour is needed to determine ideal gas perfusion during EVNP.
Take-home message:
Ex-Vivo Normothermic Perfusion conditions transplant kidneys. Giving supra-physiological oxygen does not improve the kidneys function, and may indeed be harmful.

O7 THE STUDY OF A SELF-ADMINISTERED INTRA-PROCEDURAL CHECKLIST ON THE PERFORMANCE OF SURGICAL TRAINEES DURING LAPAROSCOPIC CHOLECYSTECTOMY

M El Boghdady (1), W Wang (2), A Alijani (1)
(1) Cuschieri Skills Centre, Ninewells Hospital and Medical School, Dundee, UK, (2) Institute of Motion Analysis and Research,TORT Centre, Ninewells Hospital and Medical School, Dundee, UK
Introduction : Surgical checklists are in use to reduce errors for safer surgery. We aimed to study the effect a previously designed performance based selfo-administered intra-procedural checklist on the performance of trainees during elective laparoscopic cholecystectomy.
Method: Twenty-four laparoscopic cholecystectomies were enrolled into the study. Six surgical trainees each performed four cases, two cases without the checklist and directly followed by two cases with the checklist. A soft beeping sound reminded each trainee to apply the checklist every 4 minutes during the procedures. The unedited videos were analysed using the human reliability analysis technique for the number of consequential and inconsequential errors, number of interventions by the trainer, number of

instrument movements and time execution. Non-parametric test was used for data analysis.
Result: Participants performed statistically better with fewer number of errors per time with the application of the checklist compared to when no checklist was used respectively: Median [IQR] total number of errors 1.51 [0.80] vs 3.84 [1.42] (p=0.002), consequential errors 0.20 [0.12] vs 0.45 [0.42] (P=0.005) and inconsequential errors 1.32 [0.75] vs 3.27 [1.48] (p=0.006). After the introduction of the checklist, the number of interventions by the trainer per time decreased from 2.79 [1.85] to 0.43 [1.208] (p=0.003) and the number of instrument movements per time decreased from 11.90 [5.34] to 10.38 [5.16] (p=0.04).
Conclusion: The performance based self-administered intra-procedural checklist improved the performance of surgical trainees and decreased the number of interventions by the trainer during laparoscopic cholecystectomy.
Take-home message:
The performance based self-administered intra-procedural checklist improved the performance of surgical trainees during laparoscopic cholecystectomy.