ABSTRACTS FROM THE SURGICAL RESEARCH SOCIETY OF AUSTRALASIA ANNUAL SCIENTIFIC MEETING Sydney Hospital, 14 May 2006

Convenor: Dr. Andrew Biankin

POST-OPERATIVE NITRIC OXIDE RELEASE IS ASSOCIATED WITH LOWERED CIRCULATING CHOLESTEROL AND ALBUMIN LEVELS - IMPLICATIONS FOR SURGERY

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Introduction: Nitric oxide in cell culture reduces cholesterol and protein synthesis in vitro without altering energy metabolism (1,2). The action of NO in vivo is unknown. As surgical operations raise nitric oxide production acutely, changes of cholesterol and albumin levels were measured post-operatively.

Methods: Ten patients undergoing elective laparoscopic cholecystecomy were enrolled. Serial blood samples under fasting conditions were collected pre-operatively and 30 mins, 24 h and 48 h post-operatively. Serum samples were centrifuged using Microsep 30 KD Omega columns and eluate processed for HPLC separation. Nitrite/nitrate was measured by the Griess reaction after appropriate conversion. Albumin and cholesterol were measured by Olympus AU2700 autoanalyzer.

Results: Serum nitrite-nitrate levels rose from 6.7±1.7 to 12.4±2.4 after 30 mins ($p<0.02$) and then returned to normal. Cholesterol levels dropped from 4.99±0.27 to 4.12±0.17 at 24 hrs ($p<0.02$) with similar changes in albumin level. Peak NOx levels inversely correlated with changes in cholesterol/albumin suggesting rather than peak levels, time/duration exposure to NO were more important. A direct correlation was found between changes in albumin and changes in cholesterol.

Conclusion: Dietary and dilutional factors were excluded in demonstrating elevated nitric oxide production in vivo which was associated with lowering of cholesterol and albumin levels. In vitro and in vivo observations are supportive of the concept that NO co-regulates cholesterol and albumin synthesis in human subjects with implications for surgical repair processes.

THE NEW PERSPECTIVE TO THE UNDERSTANDING OF COLORECTAL CANCER WITH PROTEOMIC MAPPING BY TWO DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

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Introduction: It is well documented that the development of colorectal cancer (CRC) from normal mucosa is mediated through complex interactions of genetic and environmental factors. Genetic studies have identified several possible pathways involving numerous gene abnormalities. Multiple, cumulative molecular events lead to the progression of CRC. Proteomic studies using two dimensional polyacrylamide gel electrophoresis (2D-PAGE) provide a new perspective in the investigation of the protein molecules expressed in CRC.

Methods: All CRC specimens from the Colorectal Unit at Concord Hospital are collected after resection and frozen immediately for subsequent analyses with 2D-PAGE. Routine histopathological examination and clinicopathological staging of the tumour is carried out and compared with 2D-PAGE protein patterns.

Results: Preliminary results have identified intracolonic regional variations. Comparison of protein expression in CRC with remote and site-specific normal mucosa showed cancer-specific patterns with significant differences between colonic and rectal tumours. Analysis of protein expression patterns from various CRC indicated possible stage-dependent sequential expression changes. Certain proteins spots have shown marked variation between early and late CRC, indicating possible associations with prognosis.

Conclusions: Proteomic study with 2D-PAGE provides a valuable route in improving our understanding of CRC. Malignancy-specific protein patterns can be obtained. The further correlation of the protein patterns with clinicopathological stage may lead to a new molecular method of staging CRC. Analysis of distinct, abnormally expressed, proteins may identify new disease markers, potential targets for treatment and provide more accurate prognosis in the management of CRC. Further analysis within different stages and prognostic indicators in a larger series is necessary to confirm our findings.

INDUCTION OF LONG-TERM ALLOGRAFT SURVIVAL USING ANTI-LYMPHOCYTE ANTIBODIES IN THE RAT MODEL

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Introduction: Anti-CD3 monoclonal antibodies such as G4.18 and anti-lymphocyte serum (ALS) are potent immunosuppressives in transplantation however their immune mechanism might be different.

Methods: The immunosuppressive effects of ALS and G4.18 were studied in a high-responder rat heart transplant model of PVG strain donor to Lewis recipient. In vivo immunosuppressive properties of G4.18 and ALS were investigated by flow cytometry and immunohistochemistry. Real time polymerase chain reaction (PCR) analysis for splenic cytokine expression was also performed.

Results: Untreated animals rejected the heart transplant in 8.5 days while treatment with 1 ml ALS prolonged survival to 11.5 days (p=0.008) while treatment with 7mg/kg G4.18 on days 1 and 3 prolonged survival to >100 days (p=0.0006 vs. control), which was significantly longer than survival in ALS (P=0.0007). Despite the greater survival of G4.18-treated animals, ALS led to longer and more extensive depletion of T cells and myeloid cells. G4.18 treatment stimulated an increased level of inducible nitric oxide synthase (iNOS) and a decreased level of interleukin (IL)-2 in the spleen compared to ALS.

Conclusions: G4.18 induced long-term cardiac allograft survival, associated with down-regulation of IL-2 and up-regulation of iNOS, an inhibitor of T cell proliferation. ALS was more effective for depletion of T cells but much less effective for prolongation of survival. G4.18 might inhibit T cells directly or by indirect effects on other cells.

MANAGEMENT OF CRANIAL DURAL ARTERIOVENOUS MALFORMATIONS- A GRADING SYSTEM TO PREDICT ADVERSE OUTCOME

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Introduction: Dural arteriovenous malformations are relatively uncommon lesions, representing 7% of all intracranial vascular malformations. Although the natural history of these lesions has been described, no satisfactory model or classification system exists to predict adverse outcome following management. Many published series have described the treatment of cranial dural arteriovenous malformations with minimal morbidity and mortality. These highly selected series may underestimate the inherent difficulty in treating these complex lesions. This study proposes a new grading system to predict the characteristics of dural arteriovenous malformations associated with an adverse outcome from treatment.

Methods: Between January 1991 and January 2005, the Sydney Aneurysm and AVM Neurosurgical Centre prospectively collected data on all consecutively enrolled AVMs treated at the Royal North Shore Hospital, Dalcross Private Hospital, and North Shore Private Hospital. Information included demographic characteristics, clinical features before and after treatment, modified Rankin Scale (mRS) score before treatment, mRS following treatment (at 6 weeks, 12 month and last review), and radiological features.

Results: A total of 112 patients with DAVMs were enrolled. Twenty-nine patients (25.9%) experienced a poor response to treatment, including 13 patients who died or developed a new permanent neurological deficit. The mode of presentation, location, and mRS on presentation were predictive of a poor response to treatment in univariate analysis. Permanent neurological morbidity or death occurred in 2.6% of patients who were asymptomatic or presented with bruit alone (Group 1), compared to 12.3% of patients presenting with intracranial haemorrhage or complications of focal venous hypertension (Group 2), and 44.4% of patients with complications of global venous hypertension (Group 3). These results were statistically significant and the grading system was confirmed to have good interobserver agreement.

Conclusions: The mode of presentation and clinical state at presentation were associated with the risk of adverse outcome following management using multivariate analysis. This three-tiered grading system is highly predictive of the likelihood of adverse outcome following treatment of cranial dural AVMs.

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RE-EVALUATION OF THE EXTENT OF NEUTROPHIL ACTIVATION ASSOCIATED WITH CARDIAC SURGERY

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Introduction: Cardiac surgery with cardiopulmonary bypass (CPB) has long been considered to induce a marked systemic acute inflammatory response associated with circulating neutrophil activation. Improvements in CPB techniques and materials may have reduced the severity of this inflammatory response. We have investigated the extent of circulating neutrophil activation during coronary artery bypass surgery (CABG) using standard (eg. upregulation of CD11b, shedding of L-selectin) and novel markers of neutrophil activation status (eg. conformational activation of CD11b, neutrophil-platelet aggregate formation).

Methods: Blood samples were collected from CABG patients (n=16) before, during and after CPB. Circulating neutrophil CD11b (total protein and an activation-specific neo-epitope) CD18, CD10, CD16, L-selectin and P-selectin Glycoprotein Ligand-1 (PSGL-1) expression and neutrophil-platelet aggregates were quantified using flow cytometry. Soluble L-selectin (sL-selectin) levels were determined using ELISA.

Results: Total CD11b increased transiently during CPB (p=0.01) whereas CD18, CD16, CD10 and PSGL-1 remained unchanged, however the proportion of neutrophils expressing the CD11b neo-epitope increased intra-operatively and remained elevated post-operatively. Neutrophil L-selectin expression was increased intra-operatively (L-selectin MFI: 108.8 ± 15.1 at baseline vs 167.4 ± 21.6 during CPB/myocardial ischemia), concurrent with reduced sL-selectin levels. The proportion of circulating neutrophils with adherent platelets decreased at the end of surgery (5.1 ± 0.6 % at baseline vs. 3.8 ± 0.6 % during reperfusion) and post-operatively (1.9 ± 0.1 %).

Conclusions: CABG is associated with a distinctive and mild profile of neutrophil activation characterized by transient CD11b upregulation, unchanged expression of other major cell surface markers and unexpected upregulation, rather than shedding, of L-selectin. Expression of a CD11b neo-epitope appears to be a sensitive marker of circulating neutrophil activation relevant to CABG.

DO URETERIC ACCESS SHEATHS CAUSE URETERIC STRICTURES?

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Introduction: Ureteric access sheaths have been designed to facilitate access to the ureter and protect the ureter and flexible ureteroscope during flexible ureteroscopy. Concerns have been raised about the risk of these access sheaths causing ureteric strictures.

Methods: Patients who had undergone flexible ureteroscopy for the treatment of renal tract stones between January and April 2005 were included in the study. Each patient had a limited IVU 3 to 5 months post their procedure. These were assessed by a Radiologist and an Urologist to detect the presence or absence of ureteric strictures.

Results: 33 consecutive flexible ureteroscopies using an access sheath were performed during this time. 30 of these subsequently had an IVU performed. The average time post ureteroscopy to IVU was 4.5 months (3-7 months). 5 patients had a JJ stent in-situ prior to the procedure. No strictures were found in the 30 patients.

Conclusions: The use of ureteric access sheaths does not result in ureteric strictures at early follow-up.

PATHOLOGICAL PROGNOSTIC FACTORS IN STAGE II COLON CANCER

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Background: Guidelines for the use of adjuvant chemotherapy in stage II colorectal cancer state this treatment may be considered to patients whose tumours show features of poor prognosis. The aim of the current study was to evaluate the prognostic significance of commonly reported clinical and pathological features of this disease.
Methods: A population-based observational study encompassing all stage II colon cancer patients diagnosed in the state of Western Australia from 1993-2003 inclusive. A total of 1306 cases treated by surgery alone were identified and had a median follow-up of 59 months (range 0-145).
Results: Multivariate analysis revealed the only independent prognostic factors for disease-specific survival were T4 stage (HR=1.75, 95%CI [1.32-2.32], P<0.0001) and vascular invasion (HR=1.63, 95%CI [1.15-2.30], P<0.0001). In the younger patient group (<75 yrs) who are more likely to be considered for chemotherapy, the same two features showed independent prognostic significance but with higher HR values (1.96 and 2.73 respectively). T4 and/or the presence of vascular invasion identified a “poor” prognosis group comprising 26% of younger cases and having a 5-year survival rate of 71%. The remaining “good” prognosis group showed 84% survival at 5 years follow-up.
Conclusion: This study highlights the importance of accurate pathological assessment of T stage and vascular invasion for the prognostic stratification of stage II colon cancer and their subsequent consideration for adjuvant chemotherapy.

SKILLS ACQUIRED ON VIRTUAL REALITY LAPAROSCOPIC SIMULATORS TRANSFER INTO THE OPERATING ROOM IN A BLINDED, RANDOMISED, CONTROLLED TRIAL

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Introduction: Virtual reality (VR) laparoscopic simulators are emerging as a significant resource for those seeking to impart and assess surgical skills. A major determinant of their success is whether skills learnt on the simulator can immediately be applied to operating on a live human patient.

Aim: The purpose of this study was to determine whether skills learnt on a VR laparoscopic simulator could be transferred into the real operative setting.

Methods: Ten basic surgical trainees, each of whom had performed fewer than 30 laparoscopic procedures, were randomised into two groups. Participants in the experimental group were trained on a commercially available VR laparoscopic simulator to perform a laparoscopic clipping task, akin to clipping and dividing the cystic duct or artery during laparoscopic cholecystectomy. They were required to continue training until they achieved a level of proficiency equivalent to a pre-determined consultant surgeon standard. Trainees in the control group, however, did not receive VR training. All participants were then required to perform the same task in a live human patient, while being proctored by a consultant surgeon assisting them during the laparoscopic procedure. These procedures were recorded on DVD and distributed to five experienced laparoscopic surgeons, who were blinded as to whether or not each candidate had received training on the VR simulator. They were asked to rate the participants' performance using a *pro forma* based on a validated instrument for assessing laparoscopic performance. Comparison was made between the two groups in four parameters: time to task completion, number of errors committed, bimanual coordination, and global assessment. These parameters were analysed statistically for significance at the 5% level using Fisher's unpaired *t*-test.

Results: Mean time to task completion was shorter for the experimental group (mean 106.4 s, SD 28.553 s) than the control group (mean 170.2 s, SD 65.163 s), although this failed to reach statistical significance (p = 0.0799). Statistically significant improvements were found, however, in the mean number of errors committed (9.68 *vs* 24.60; p = 0.0487), bimanual coordination (3.21 *vs* 2.04; p = 0.0175), and global performance scores (3.29 *vs* 2.00; p = 0.0149).

Conclusion: Skills acquired using VR laparoscopic simulators readily transfer to the real operative setting. Virtual reality simulators are an important resource for surgical training, and should be incorporated into surgical training programmes.

THE USE OF A PORCINE MODEL OF THE BRAIN-DEAD MULTI-ORGAN DONOR TO ASSESS THE EFFECTS OF HORMONE RESUSCITATION TO IMPROVE DONOR ORGAN QUALITY

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Introduction: Hormone Resuscitation (HR) may ameliorate the negative effects of brain death on the organ donor, and has been advocated as a treatment to increase organ quality and utilisation. Our aim was to investigate the effects of HR on transplantable solid organs.

Methods: Brain death (BD) was induced in pigs by inflating a Foley catheter balloon in the subdural space. One hour after BD, animals were commenced on noradrenaline (NA; n=5), HR (triiodothyronine, methylprednisolone, vasopressin and insulin; n=5) or continued IV fluids (FL; n=5) to maintain haemodynamics, and then monitored for 5 hours. Hemodynamic data, blood flows, and other physiological markers of organ function were assessed.

Results: At 6 hours post-BD, mean arterial pressure (MAP) was highest in the HR group compared with FL and NA ($p<0.05$). Cardiac output was similar across groups. Cardiac contractility was highest in HR animals followed by FL and then NA ($p<0.001$). There was no difference in lung function (Aa gradient and PaO2/FiO2) between groups. Renal arterial flow was higher in HR than NA animals ($p<0.05$), as was the creatinine clearance ($p<0.05$). There was no difference in hepatic arterial or portal venous flow. There was no difference in liver function tests, amylase or lipase between groups.

Conclusions: These results demonstrate that HR can improve MAP, and cardiac and renal function in the brain dead donor. HR does not appear to have any detrimental effects on the lungs, liver or pancreas. This study supports the use of HR to resuscitate and support the brain-dead donor.

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NINE YEAR FOLLOW-UP OF CROSS LEG TRANSFER FOR BILATERAL LEG AMPUTATION.

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Introduction: Traumatic bilateral lower limb amputation means a daunting postoperative rehabilitation program for the amputee. It is sometimes possible to transfer a viable limb to the contralateral side after such injuries, with good long term results.

Methods: This is a 9-year follow-up of a 54 year old male who suffered bilateral lower limb amputations after being caught in a paper pulping machine. The right limb suffered a crush amputation at the level of the knee joint preserving the lower leg. The left limb was crushed and amputated at mid-calf level rendering the lower leg useless for salvage. His viable right lower leg was transplanted to the left limb stump with rigid bony fixation and neurovascular anastomosis.
The bony fixation of the limb transplant involved matching the left lower limb stump and the right tibia 11 cm distal to its tubersosity. A10 hole DCP was bent to accommodate surfaces and plated under compression and autologous bone grafting was used. The fibula was screwed to the tibia. An end-to-end anastomosis of post tibial nerve, artery and veins was performed. A peroneal tenodesis and tibial anterior and posterior tendons and triceps surae were sutured.

Results: Immediate postoperative recovery saw no trauma related complications. Further surgery was required to debride necrotic tissue leaving a part of the rigid fixation and bone exposed. There was, however no deep infection and after two years solid bony union had occurred. The rigid fixation was removed and minor surgery to correct claw toes was performed concurrently.
The patient has active plantar and dorsiflexion. He lacks protective sensation on the sole of his foot which resulted in a superficial burn. The most significant delay seen in his recovery has been the fitting of a comfortable prosthesis for his right above knee amputation. The patient prefers his cross leg transplant to his artificial limb.

Conclusions: Fortunately bilateral amputation is rare in our community. This case, the first of its kind reported in the Western literature represents an acceptable alternative to bilateral amputation.

THE ROLE OF CELL PHENOTYPE IN AN ORTHOTOPIC MODEL OF TRANSITIONAL CELL CARCINOMA

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Aim: To develop an orthotopic model of muscle-invasive transitional cell carcinoma (TCC) of the bladder which models primary tumour growth and metastasis.

Methods: Cell lines were derived from the TCC cell line T24 (Tsu-Pr1) using in vivo selection for metastatic ability1. Each of these cell lines (Tsu-Pr1 and sub-lines, B1 and B2) was then injected intramurally into the mouse bladder wall (n=25 x 3). The cell lines were also injected intratibially (n=29) and intraperitoneally (n=25). The presence of systemic cancer spread was indicated by the amounts of PCR product for the human Alu repeat sequence.

Results: After orthotopic injection, primary bladder tumours formed in 73.9% of animals. Macroscopic metastases were observed in lymph nodes (38%), adrenal (18%), liver (16%), pancreas (22%) and on other intra-abdominal mesenteric surfaces. Parentals had a significantly greater incidence of micrometastatic deposits in the lung when compared with the B1 and B2 sub-lines (p=0.0461, non parametric ANOVA) In contrast, B2 mice were more likely to develop bone lesions after intra-tibial injection and have increased numbers of tumour deposits after intraperitoneal injection. The difference in sub-line behaviour after various injection techniques may be explained by differences in cell phenotype.

Conclusion: The TSU-Pr1 cell lines will be a useful model of invasive bladder TCC to study both primary tumour growth and the process of metastases. Advantages of this orthotopic model include macroscopic metastases, lymphatic as well as haematogenous spread, and the use of lineage related cell lines. In addition, we believe the TSU-Pr1 lineage-related cell line is the first reported cell line used in vivo that demonstrates MET in the metastatic process, thus providing an excellent platform to study the later stages of the metastatic cascade.

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SURVIVAL RATES FOR STAGE II COLON CANCER IN PATIENTS TREATED WITH OR WITHOUT CHEMOTHERAPY IN A POPULATION BASED SETTING

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Background: There is considerable uncertainty as to whether adjuvant 5-fluorouracil-based chemotherapy provides survival benefit for colon cancer patients with stage II disease. Consequently, the current rates of chemotherapy use for this disease are low, despite 5-year survival rates of only 70-80%. The aim of the present study was to compare the survival rate of stage II colon cancer patients treated by surgery alone with that of patients also treated by chemotherapy.

Methods: A population-based, observational study was conducted on the survival of stage II colon cancer patients (n=812) diagnosed in Western Australia from 1993-2003. The study was restricted to patients aged <75 years, of whom 18% (n=142) were treated with chemotherapy. Only 0.9% of patients older than 75 years received chemotherapy.

Results: Patients who received chemotherapy were significantly younger (mean age 60 years) than those treated by surgery alone (65 years, P<0.001) and their tumors were more often positive for vascular invasion (P=0.007). Multivariate analysis that included all prognostic factors revealed adjuvant chemotherapy was associated with improved survival (HR=0.62, 95%CI [0.39-0.98], P=0.043), with females gaining more benefit (HR=0.48, 95%CI [0.20-1.22], P=0.09) than males (HR=0.94, 95%CI [0.54-1.64], P=0.8).

Conclusions: In view of the apparent survival benefit from chemotherapy for stage II colon cancer, the present study raises concerns about the current low rates of adjuvant treatment for this disease in the community, particularly for female patients.

DOES EARLY COMPUTERISED TOMOGRAPHY EXCLUDE FRACTURE IN 'CLINICAL SCAPHOID FRACTURE'?

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Introduction: The diagnosis of fractured scaphoid remains notoriously difficult to make because initial plain radiographs of the carpus may not always identify a fracture. As a result of the uncertainty and to reduce the risk of complications such as avascular necrosis, it is well-accepted policy to over-treat these patients in a scaphoid plaster followed by repeat radiological examination at a later date. The present study examines whether an early CT excludes scaphoid fracture and provides a relatively cheap and accessible alternative to the gold standard MRI. This would hopefully safeguard patients against unnecessarily rigid follow-up and protracted plaster immobilisation.

Methods: A prospective observational study was performed on Emergency Department patients presenting to Ballarat Hospital Victoria from October 2004 to September 2005 and fulfilling the inclusion criteria. This includes those with a suspected clinical scaphoid fracture based on mechanism of scaphoid trauma and anatomical snuffbox tenderness, but with normal initial plain radiographs. A CT was performed within 48 hours with the results blinded until the patient was re-evaluated after 10 days of standard plaster immobilisation.

Results: 7 of 35 (20%) occult scaphoid fractures were identified by early CT.
CT demonstrated a very high negative predictive value of 96.4% for ruling out a scaphoid fracture.

Conclusion: The strategy of early computerised tomography can exclude fracture in a patient with a suspected clinical scaphoid fracture at a lower cost than other diagnostic modalities. It should be considered an alternative to the conservative approach of unnecessary plaster immobilisation and 2-week review.

PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) INHIBITS OSTEOSARCOMA IN A SYNGENEIC RAT MODEL

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Introduction: Pigment epithelium-derived factor (PEDF) has been shown to be the most potent inhibitor of angiogenesis and recent studies have demonstrated that decreased expression of PEDF is associated with increased tumour angiogenesis and metastasis and also poorer prognosis in several different types of neoplasms.

Methods: In this study we investigated both the in vitro and in vivo tumour characteristics of a rat osteosarcoma cell line, UMR 106-01, which has been (1) stably transfected to overexpress human PEDF or (2) treated with varying concentrations of recombinant PEDF (rPEDF).

Results: Although *in vitro* proliferation, migration and invasion did not demonstrate any difference in the PEDF-transfected tumour cells, collagen adhesion was significantly increased. In UMR 106-01 cells that were treated with rPEDF, dose dependent inhibition of cell proliferation was noted, together with increased collagen adhesion and decreased migration. *In vivo*, overexpression of PEDF resulted in marked inhibition of tumour growth of UMR 106-01 cells that were orthotopically injected into the proximal tibias of nude mice, when compared to the parental and vector clones. These results suggest that PEDF might be a promising therapeutic agent for the treatment of patients with osteosarcoma.

COLONIC MANOMETRY IN PAEDIATRIC SLOW TRANSIT CONSTIPATION SHOWS CONSISTENT DEFICIENCIES IN PROPAGATING SEQUENCES: MANOMETRIC EVIDENCE OF A NEW DISEASE

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Introduction: Slow Transit Constipation (STC) is a poorly understood and debilitating paediatric condition. Pathophysiological derangements of motility patterns have been proposed. We developed a per-appendicostomy approach to the manometric assessment of the colon. A catheter is passed through an appendix stoma created for antegrade continence enemas. This produces minimal discomfort with no need for anaesthesia, enabling 24hr data collection.

Methods: 18 children (12M, mean 11y, range 6-18y) with scintigraphically proven STC underwent 24hr manometry. Data were compared with 24hr naso-colonic studies from 14 healthy young controls (range 18-25y).

Results: When comparing contraction frequency over 24hr in STC children with controls, STC children had [mean (standard error)]:
(1) fewer antegrade propagating sequences (APS) [26(5) vs 52(4), $p < 0.01$]
(2) similar retrograde propagating sequences (RPS) [11(3) vs 16(3)]
(3) similar high amplitude propagating sequences (HAPS) [9(2) vs 8(2)]

When comparing contraction amplitude (mmHg), STC children had:
(1) similar APS pressure waves [53(7) vs 57(4)]
(2) similar RPS pressure waves [24(4) vs 28(1)]
(3) similar HAPS pressure waves [92(6) vs 117(3)].

STC children did not demonstrate the increased colonic motility observed in controls post-prandially and post-waking.

Conclusions: STC children had reduced APS frequency compared to controls. A reduction in antegrade activity would contribute significantly to poor colonic motility. The lack of post-prandial and post-waking response suggests a central signalling defect. This work provides further evidence for a pathophysiological basis to paediatric STC using an innovative, safe and well-tolerated technique.

ELASTOGENESIS WITH THE APOE -/- MOUSE WITH AII INFUSION MODEL OF AORTIC ANEURYSM

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Introduction: Disordered elastin formation (elastogenesis) has recently been demonstrated in human AAA and may be an important determinant of aneurysm formation and rupture. The Apolipoprotein knockout mouse (ApoE-/-) infused with angiotensin II develops supra renal aneurysms, however it has been suggested that AAAs within this model may resolve following a period of AII withdrawal. We sought evidence of elastogenesis within this model and examined the natural history of elastin injury and repair in a cohort of mice 6 months after completion of AII infusion.

Methods: 13-week male ApoE-/- mice were infused subcutaneously with AII (1.4 mg/ kg /day) for 28 days (n=46). Group A mice were sacrificed at 17 weeks (n=26) and group B mice were sacrificed at 43 weeks (n=20). At termination the aorta was PBS perfused, photographed for morphometry, and stored for frozen section. Approximately 5000 serial cryostat sections were examined to identify areas of elastin damage. Adjacent sections were then immunostained using markers of elastogenesis (tropoelastin GA 317 1:100, Elastin Products) and macrophage infiltration (MAC 3 1:10, Abcam).

Results: Tropoelastin was detected in sections from 31 of the 46 mice, and strong immunostaining within aneurysms was found in areas of medial elastin degradation and adventitial remodelling. Co staining with MAC 3 was present in sections from 6 mice. The AAA incidence did not differ between groups A and B (14/26 and 14/20 respectively, $p>0.05$). Markedly disordered elastin was noted in sections from the long term study group.

Conclusions: This study demonstrates a capacity for new elastin formation within the ApoE-/- AII-infusion model of AAA, though an inability to fully repair AII induced injury. This model of AAA provides a unique in vivo model of disordered elastogenesis which should facilitate further investigation of this process and testing of potential modifiers which may have therapeutic applications in the management of small aortic aneurysms.

PRE-OPERATIVE CARBOHYDRATE LOADING IN PATIENTS UNDERGOING MAJOR ABDOMINAL SURGERY: RESULTS OF A DOUBLE BLIND RANDOMISED CONTROLLED TRIAL

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Introduction: Carbohydrate (CHO) loading for patients undergoing major surgery reduces post-operative insulin resistance. This metabolic change may have clinical benefits in terms of reduced fatigue, length of stay (LOS), and complications. We conducted a randomised trial to examine the effects of CHO given shortly before surgery on these clinical parameters.

Methods: 142 patients (73M, 69F; median age 63, range 22-80y) were randomised to CHO (n=73, Nutricia PreOp®) or placebo (n=69) with 800mL taken the night before surgery and 400mL 2 hours prior to anaesthesia. Patients underwent elective colorectal surgery (n=97) or liver resection (n=45). Fasting glucose, insulin and cortisol were measured at baseline and post-operative day (POD) 1. An insulin resistance index (IRI) was calculated as [insulin][glucose]/22.5. Post-operative fatigue was evaluated by visual analogue scale (0 to 100) at baseline and on POD3 and POD5.

Results: On POD1 cortisol was lower in the CHO group (136, range 16-343 vs 431, range 11-751 nmol/L; P<0.0001). IRI at POD1 was not different between the CHO and placebo groups (6.3 ±1.5[SE] vs 5.9±0.8, P=0.71). Change in fatigue score from baseline to POD3 was 16.6±4.8 with CHO *vs*. 18.1±5.0 with placebo (P=0.83), and to POD5 was 11.0±5.4 *vs.* 17.9±4.7 (P=0.34). Median LOS was 6 (range 2–43) d in the CHO and 7 (1–92) d in the placebo group (*P*=0.27). Number of infectious complications was, respectively, 13 and 19 (*P*=0.31).

Conclusions: This study confirmed improved postoperative stress hormone response with preoperative CHO but not improved insulin resistance. Significant clinical benefits were not observed.

IDENTIFICATION OF PANCREATIC ADENOCARCINOMA SERUM BIOMARKERS USING SELDI-TOF MS

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Introduction: Pancreatic adenocarcinoma is a most devastating cancer that presents relatively late and is rapidly progressive. The extraordinarily large mortality rates associated with pancreatic adenocarcinoma are most certainly a result of the lack of effective early detection methods. This study aimed to identify unique protein biomarkers from serum that could effectively distinguish between pancreatic adenocarcinoma and non-cancer groups.

Methods: Serum from patients with pancreatic adenocarcinoma (n=25; PC), benign pancreatic disease (n=44; B) and age and sex matched healthy volunteers (n=48; HV) was analyzed on hydrophobic (H50) protein chip arrays by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Purification was performed by FPLC using size separation and further purified using RP-HPLC. The purified proteins were analyzed by peptide mass fingerprinting and MS-sequencing (BMSF, UNSW). Training models were developed on 20/25 PC, 35/44 B and 38/48 HV randomly selected samples using logistic regression and validated using the 10-fold cross validation approach as previously described[1]. The models were then tested on an external set of samples (PC, n=5; B, n=9; HV, n=10). Discriminatory power of these panels was assessed using Receiver Operator Characteristic Area Under the Curve (ROC AUC) values and compared to the tumor marker CA19.9.

Results: Differential expression profiles were demonstrated between serum from PC, B and HV groups using univariate analysis (Mann-Whitney U Test). Logistic regression and 10-fold cross validation identified overlapping panels of peaks to develop training models that distinguished PC from B (70% sensitivity; 88.6% specificity) and PC from HV serum (85.0% sensitivity; 97.4% specificity). When tested on external samples, the final selected PC vs. B panel correctly classified 4/5 PC and 9/9 B samples (ROC AUC: 0.91) while the PC vs. HV panel correctly identified 4/5 PC and 9/10 HV samples (ROC AUC: 0.98). For PC v B, ROC AUC values improved in the 4-peak panel (0.89) compared to CA19.9 alone (0.84), and increased significantly when CA19.9 was added to the panel (0.96; $P<0.05$). For PC v HV, ROC AUC values improved in the 3-peak panel (0.94) compared with CA19.9 (0.87) and significantly increased when CA19.9 was added (0.99; $P<0.05$). The 6.63 kDa protein was identified as Human Apolipoprotein CI, a protein associated with the infiltrating macrophages present within the stroma[2]. Identification of the remainder of these proteins is currently underway.

Conclusions: This study used high-throughput SELDI-TOF MS to identify putative biomarkers expressed in serum of patients with pancreatic adenocarcinoma compared with serum from non-cancer controls. The identification of individual biomarker proteins may provide an approach to early diagnosis and monitoring of this disease.

A MODIFIED CLASSIFICATION FOR ADENOCARCINOMA OF THE GASTRO-OESOPHAGEAL JUNCTION ASSOCIATED WITH INTESTINAL METAPLASIA

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Introduction : Incidence of gastro-oesophageal junction (GOJ) adenocarcinoma is increasing. Siewert's classification subdivides junctional adenocarcinomas anatomically. Cytokeratin (CK) 7 and 20 immunophenotypes differentiate Barrett's intestinal metaplasia (IM) from gastric IM. Comparing CK immunostaining with Siewert's classification may establish tumour origin and influence surgical choice.

Method : In this experimental study, 57 patients with GOJ adenocarcinoma were subdivided endoscopically into 15 type 1, 26 type 2 and 16 type 3 adenocarcinoma. Representative biopsies were immunostained for CK7 and CK20.

Results : IM was associated with type 1 adenocarcinoma in 12 of 15 patients, 80%; with type 2 in 13 of 26 patients, 50% and type 3 in 6 of 16 patients, 37.5%. All type 1 patients demonstrated Barrett's CK7/20 phenotype within IM; type 2 a mixture: 69% (n=9) Barrett's CK7/20 and 31% (n=4) gastric CK7/20 while type 3 patients had a gastric CK7/20 pattern in 83% (n=5). Immunostaining within adenocarcinoma was variable.

Conclusion : Siewert's type 1 adenocarcinoma express Barrett's CK7/20 pattern, type 3 a gastric CK7/20 pattern and type 2 tumours a mixture of Barrett's and gastric CK7/20 patterns within associated IM. CK immunostaining may refine Siewert's classification into oesophageal type 1 or gastric type 2 adenocarcinoma with IM.

A SPONTANEOUS PULMONARY METASTASIS MODEL OF OSTEOSARCOMA USING NON-TRANSFORMED HUMAN CELLS TRANSPLANTED ORTHOTOPICALLY IN MICE

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Introduction: A model for osteosarcoma is long overdue given the devastating demographics (second highest cause of cancer-related death in the paediatric age group) of the ailment and the lack of proper options for control, if not cure, for the disease, as it also is the most common primary tumour of bone.

Methods: We have established a model of this disease using non-transformed human osteosarcoma cells. Orthotopic injection of SaOS-2 cells within a biomatrix into the proximal tibia of nude mice results in primary growth of tumour and development of pulmonary metastases. The model closely resembles the clinical progression of the disease and macroscopic pulmonary metastases are established by 4 weeks. We also have generated GFP-transfected SaOS-2 cells as this allows determination of lung tumour burden. Proliferation of SaOS-2 cells occurs at a faster rate compared to 2 other human osteosarcoma cell lines, U2OS and 143B, a finding that may explain this line's ability to take *in vivo.* We have characterised the adhesion, migration, invasion, transfectability, colony-formation and bone formation ability of this cell line *in vitro.* We are currently using this model for testing novel molecular therapeutic agents against osteosarcoma.

EXPOSURE OF OSTEOSARCOMA CELLS TO UPA ANTISENSE PARADOXICALLY UPREGULATES ITS EXPRESSION AND THAT OF UPAR: A WORD OF CAUTION

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The uPA/uPAR system is involved in tumour progression and metastasis of a variety of cancers. Previously, we have shown that increased expression of urokinase plasminogen activator (uPA) correlated with malignancy grade in certain sarcomas. In the present study, the effects of antisense against uPA were observed in vitro using a 24-hour long transfection. UMR106-01 osteosarcoma cells were transfected with either uPA antisense, sense or scrambled control oligonucleotides for 24 hours. Paradoxically, the antisense demonstrated increased uPA expression 24h post-transfection, despite adequate intracellular delivery as determined by fluorescence microscopy. uPA antisense treatment also elevated uPAR expression. Understandably, uPA antisense treatment increased both adhesion (collagen and polylysine) and migration in cell-based assays in vitro ($p < 0.05$). However, cellular invasion in fact was decreased as a result of uPA and uPAR overexpression, suggesting that the uPA/uPAR upregulation response incited by antisense treatment includes an element that reduces the ability of these cells to traverse through matrix-coated pores. Proliferation of cells was not affected by uPAR downregulation. This study cautions against using antisense technology without proper checks and balances for both research therapeutic purposes or as a molecular dissection tool.

VITAMIN D DEFICIENCY IN FRACTURE OF THE PROXIMAL FEMUR: IT IS NOT TOO LATE TO TREAT.

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Purpose: Fracture of the proximal femur remains a major public health concern with Vitamin D deficiency an important contributing factor. Despite being labelled the sunburnt country, it is a fallacy that Australians receive adequate vitamin D from casual sun exposure.
This study aims to report the prevalence of Vitamin D deficiency in fractured proximal femur and challenges orthopaedic surgeons to be more vigilant in its treatment.

Methodology: A prospective observational study was performed at two major hospitals in Ballarat Victoria on all patients presenting with fractured proximal femur or fixation failure between 1/2/05 and 30/6/05. Plasma 25-hydroxy vitamin D was measured and all deficiencies were treated as per current recommendations.

Results: Of 80 patients presenting with fractured proximal femur, 60 (75%) were vitamin D deficient. This includes 45% with a mild deficiency, 22.5% moderate, and 7.5 % severe. 2 cases of fixation failure occurred, one with moderate vitamin D deficiency and the other with a severe deficiency.
When comparing patients from different places of residence, it was also found that vitamin D deficiency was 100% in institutionalised accommodation compared to 64.3% in patients living independently.

Conclusion: Vitamin D deficiency is high in Australia despite high relative sun exposure and the resultant osteomalacia may contribute to fixation failure and re-operation. Given that vitamin D deficiency first presents itself one third of the time with a fracture, orthopaedic surgeons are in a prime position to be proactive in its treatment.

HEREDITARY HAEMORRHAGIC TELANGIECTASIA – ACTIVIN AND RELATED PEPTIDES ARE INVOLVED IN THE FORMATION OF NASAL TELANGIECTASIAS

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Introduction: A pathophysiologic study of HHT was undertaken looking for the presence of activin and its related peptides and abnormalities in their expression. Activin and its binding protein follistatin are associated with angiogenesis. This study has investigated the contribution of activin and its related peptides to the formation of nasal telangiectasias in patients with HHT.

Methods: Tissue samples from patients were used to evaluate the nature of the vascular pathology using the techniques of routine histology and immunohistochemistry. A comparison with normal tissues was made.

Results: Normal nasal tissue expressed the subunits for activin β_A, β_C and follistatin in the basal layer of the epithelium, around glandular epithelium, blood vessel walls and periostium. Inflammatory cells were also found to express activin and follistatin. In HHT tissue there was a significantly increased expression of both activin β_A ($p<0.045$) and activin β_C ($p<0.0021$) compared to normal tissue. There was no difference found in the expression of follistatin. The use of topical oestriol did not change the expression of activin or follistatin in HHT affected mucosa.

Conclusions: This study has demonstrated that activin and its related peptides are related to angiogenesis in HHT and may potentially be modulated in the treatment of the condition.

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