Austin LifeSciences

Research Week
18-22 October 2010

Abstracts

INTRODUCTION

Welcome to the 18th annual Austin LifeSciences Research Week. This is the time of year when we celebrate the quality and the breadth of the research being performed at Austin health and its departments, affiliated universities and research institutes. We also aim to get people talking and thinking about how they can work together better. It is also an opportunity encourage potential future students and scientists, who will take what we have done to the next level and, let's face it, discover the treatments we will all be relying on in the future!

Over the last few years the Research Week Committee has made changes to the format each year to accommodate the growing body of work being done on campus and also to provide more opportunities to present it. We have listened very carefully to the feedback and suggestions you have given us in past years and have tried to accommodate these wherever possible, allowing for necessary legal and anatomical restrictions. In 2010 we have:

- continued the format of two separate poster sessions, to accommodate the high number of outstanding abstracts we have received;
- provided further opportunities for researchers to present their work orally during the mini-oral session;
- continued the RJ Pierce Symposium, an event first held in 2009 to honour the contribution of the late Professor Rob Pierce;
- a Nobel Laureate speaking at the plenary session.

Other successful initiatives such as the Professors Professing seminar have also been kept. Several excellent abstracts have been chosen for oral presentation at the Austin LifeSciences Symposium. We are fortunate to have a streamlined and professional abstract submission and assessment system, thanks to sponsorship by ASN Events.

The Austin Hospital Medical Research Foundation has been a key part of our research community and has provided millions of dollars in research support over the years. There will be a special function during Research Week to celebrate the 40th anniversary of AHMRF.

Our plenary session is highlighted by Laureate Professor Peter C Doherty AC, FAA, FRS. Professor Doherty will speak on the topic of, "Killer *T cells and virus killers*" The Research Week awards will also be presented at the plenary session.

We thank all of our new and returning sponsors, without whom Research Week would not be possible.

I hope you find Austin Health Research Week 2010 to be most interesting and enjoyable.

A/Prof Ian Davis
Chair
Austin LifeSciences Research Week Committee

Austin LifeSciences Research Week gratefully acknowledges the generous support of these sponsors in 2010

Major sponsors

Austin Health

Austin Hospital Medical Research Foundation

ASN Events

Pfizer Australia

The following Institutes and Companies provided support

Gold Sponsors
Amgen
GlaxoSmithKline
Health Super
Miltenyi Biotec Australia
Novartis
Pulse Credit Union Ltd
Servier

Silver sponsors
Aspen
Ludwig Institute for Cancer Research

Prize Sponsors
In 2010 the following supported specific awards:
AHMRF
Austin Health
Austin LifeSciences
Florey Neuroscience Institutes
GlaxoSmithKline Institute for Cancer Research
IBAS (The Institute of Breathing and Sleep)
Ludwig Institute for Cancer Research
sanofi-aventis

AUSTIN HEALTH RESEARCH WEEK
PRIZES 2010

LUDWIG INSTITUTE SCHOLARSHIP
Up to $1000 to be used towards travel to a scientific meeting in Australia

FLOREY NEUROSCIENCE INSTITUTES PRIZE
$1000 towards costs to attend a scientific meeting

AUSTIN LIFE SCIENCES PRIZE FOR BASIC RESEARCH
$1000 towards costs to attend a scientific meeting

ALLIED HEALTH RESEARCH AWARD
$600 towards costs to attend a national research meeting

NURSING RESEARCH AWARD
$600 towards costs to attend a research meeting

ROB PIERCE MEMORIAL AWARD
$1000 as a grant-in-aid, to be used for research related purposes

BEST COMMUNICATION POSTER AWARD
For the poster judged to communicate the message most effectively

THE sanofi-aventis PRIZE FOR CLINICAL RESEARCH IN VASCULAR MEDICINE
$1000 towards costs to attend an overseas scientific meeting

THE sanofi-aventis PRIZE FOR CANCER RESEARCH
$1000 towards costs to attend an overseas scientific meeting

GlaxoSmithKline PRIZE FOR SCIENTIFIC RESEARCH
Two identical prizes of $3,000 reimbursement of costs associated with attendance at a scientific meeting or course

GlaxoSmithKline YOUNG RESEARCHER AWARD
$4,000 reimbursement of costs associated with attendance at a scientific meeting or course

AUSTIN HOSPITAL MEDICAL RESEARCH FOUNDATION Young Investigator Award
$2 500 TOWARDS COSTS TO ATTEND A nation or international scientific meeting or professional development course

AUSTIN HOSPITAL MEDICAL RESEARCH FOUNDATION
Distinguished Scientist Award

All winners will be announced at the Research Week Plenary Session, Thursday 21 October 2010

Certificates will be presented by our guest lecturer,
Laureate Professor Peter C Doherty, *AC, FAA,FRS*

Austin LifeSciences Research Week 2010

Contents

Monday, 18 October 2010

Professors professing

12:00 PM - 1:00 PM John Lindell Lecture theatre

Recent recipients of AHMRF Distinguished Scientist Award.
Prof Louise Burrell (2009 winner) and Prof Sam Berkovic (2002 winner), followed by lunch

Miniorals session - Cardiology

3:00 PM - 4:00 PM Education Precinct Rm 4.9

Mini-oral session - Muscluoskeletal & Physiotherapy

3:00 PM - 4:00 PM Education Precinct Lecture theatre

Concurrent mini-oral sessions featuring the work of junior research staff

Tuesday, 19 October 2010

Afternoon forum - Looking forward, looking back - AHMRF the first forty years

3:30 PM - 4:30 PM John Lindell Lecture theatre

followed by wine and cheese

Wednesday, 20 October 2010

Austin LifeSciences Symposium -

12:00 PM - 1:30 PM John Lindell Lecture theatre

AHMRF Young Investigator Award Finalists present their work, followed by lunch

Thursday, 21 October 2010

Plenary - “Killer T cells and virus killers”

12:00 PM - 1:15 PM John Lindell Lecture theatre

Speaker: Laureate Professor Peter C. Doherty, AC FAA FRS
Research Week Award Presentations, followed by lunch

Poster Session 2

2:30 PM - 3:30 PM Level 4 Austin Tower, Education Precinct, Rms 4.4 and 4.5

Friday, 22 October 2010

R J Pierce Symposium - “The Clinical – Research Interface”

12:00 PM - 1:00 PM John Lindell Lecture theatre

Honouring the contribution of Professor Rob Pierce to research in medicine.
Speakers: Professor Mary Galea, Professor Christopher Rowe and Associate Professor Michael Woodward.
Followed by lunch

Poster Session 1

Anaesthesia

Laurence Weinberg

Perioperative anaesthetic outcomes after open radical retropubic prostatectomy: a 13-year experience in a tertiary care centre *abs#001*

Stephanie Chen

Metabolic acid-base changes after liver resection: observational pilot study *abs#002*

Ian Chao

The Effect of Normal Saline 0.9% on Metabolic Acid-Base Balance After Kidney Transplantation *abs#003*

Philip Peyton

Nitrous oxide diffusion and the second gas effect on emergence from anesthesia *abs#004*

Raje Rajasekaram

Variability in Adequacy of Ventilation during Transport of Cardiac Surgery Patients: A Cohort Study *abs#005*

Critical Care & Emergency Medicine

Leah Peck

Intravenous fluid administration and monitoring for adult ward patients in a teaching hospital *abs#006*

David Taylor

A Simple Clinical Target Associated with a High Level of Patient Satisfaction with their Pain Management *abs#007*

Sue Berney

A classification and regression tree to assist clinical decision making in airway management for patients with cervical spinal cord injury *abs#008*

Carmel Taylor

A survey of ward nurses attitudes to the Intensive Care Nurse Consultant Service in teaching hospital *abs#009*

David Taylor

Complementary and Alternative Medicine Use is Associated with Important Clinical Adverse Events Among Patients on Warfarin *abs#010*

Esther Chan

Pharmacoeconomic evaluation of midazolam versus droperidol in the management of acute agitation in the emergency department *abs#011*

Fergus Kerr

Multi-Sourced Feedback is acceptable to and improves the performance of Emergency Physicians *abs#012*

Esther Chan

Management of acute agitation in Australasian Emergency Departments: variations in practice *abs#013*

Loren Sher

Nature and outcomes of chemical eye exposures reported to the Victorian Poisons Information Centre *abs#014*

David Taylor

Tropisetron is Superior to Metoclopramide for Treatment of Nausea and Vomiting in the Emergency Department *abs#015*

Glenn Eastwood

How critical care nurses report they administer, monitor and manage oxygen therapy: A survey *abs#016*

Critical Care & Emergency Medicine (continued)

Leah Peck

Oxygen administration and monitoring for ward patients in a teaching hospital *abs#017*

Sue Berney

The safety and feasibility of exercise rehabilitation in ICU *abs#018*

Victor Lee

Implementation and Evaluation of a Direct Observation Tool for Emergency Department Trainees *abs#019*

Geriatrics

Michael Woodward

Does executive impairment define a frontal variant of Alzheimer's diesase? *abs#020*

Medical Imaging

Thomas Close

Determination of local fibre configuration using Bayesian neighbourhood tract modeling *abs#021*

Lisa Willats

Validating a Local AIF Method for Improved Perfusion Quantification in Stroke *abs#022*

Kevin Ong

Assessment of Aß deposition in mild cognitive impairment with 18F-Florbetaben *abs#023*

J-Donald Tournier

Improved probabilistic streamlines tractography by 2nd order integration over fibre orientation distributions *abs#024*

David Abbott

An objective approach to assessment of language lateralisation using functional magnetic resonance imaging *abs#025*

Musculoskeletal

Noel Lythgo

Gait symmetry in school-aged children and young adults whilst walking at slow, normal and fast speeds *abs#026*

Xiangyun Xu

Structural Deficit in Children with Forearm Fracture *abs#027*

Peter Lee

Biomechanical evaluation of trans-tibial amputee's socket fabricated using the pressure cast (PCAST) technique *abs#028*

Nicole Lee

Odc1 and Tceal7 are potential mediators of androgen actions in skeletal muscle *abs#029*

Sandra Iuliano-Burns

Reduced Mechanical Loading Results In Deficits In Cortical But Not Trabecular Bone Structure: A Study of Children With Legg-Calve Perthes Disease *abs#030*

Lionel Schachna

Utilisation of the internet among patients with rheumatoid arthritis *abs#031*

Diane Apostolopoulos

Completeness of referral details to rheumatologists from general practice *abs#032*

L. Eduardo Cofré

Lower limb joint powers are modified in aging gait *abs#033*

David McKenzie

Knee flexion precedes initial contact in human gait *abs#034*

Musculoskeletal (continued)

Christina Tsikos

Gait function in incomplete spinal cord injury patients using the C.A.M.P Blue-Rocker, Blue-Rocker Combo and BIONESS L300 FES system *abs#035*

Neurosciences

Kate Lawrence

Early-onset absence epilepsy: 10% of cases are caused by GLUT1 deficiency *abs#036*

Patrick Carney

A novel genetic occipital epilepsy syndrome in siblings showing clinical and EEG-fMRI concordance. *abs#037*

Radwa Badawy

Predicting seizure control with anti-epileptic medication: Value of an early cortical excitability study *abs#038*

Noel Lythgo

Head-neck motion, tremor and muscle volume asymmetry in cervical dystonia *abs#039*

Maryam Zoghi

FES-assisted hand exercises increase the motor cortex excitability in tetraplegic patients. *abs#040*

Nicole Kerr

Spinal Cord Compression Injury – The Roles Of Early Decompression And Hypothermia In Functional Recovery. *abs#041*

Ian Luk

SCN1A Mutation Detection in Myoclonic Astatic Epilepsy (MAE) and Infantile Spasms (IS) *abs#042*

Patrick Carney

EEG-fMRI identifies focal cortical BOLD signal change in some individuals with childhood absence epilepsy. *abs#043*

Jackie How

Blunted splanchnic sympathoinhibitory effects of gastric hormones in obesity may contribute to hypertension *abs#044*

Robert Smith

Automated Clustering of Diffusion MRI Tractography Fibers using Global Information *abs#045*

Susannah Bellows

Severe Infantile Multi-Focal Epilepsy: a focal epileptic encephalopathy due to SCN1A mutations *abs#046*

Saul Mullen

A simple change to photic stimulation markedly improves EEG diagnosis of first seizures *abs#047*

Karen Oliver

Epilepsy-Migraine syndrome due to a missense mutation in *SCN1A* *abs#048*

Belinda Bardsley

Exploring strategies to safeguard the future of clinical research in Australia *abs#049*

Tarishi Desai

PCDH19 mutations cause epilepsy and mental retardation in females (EFMR) *abs#050*

Radwa Badawy

Diagnosis of epilepsy following a first 'seizure' event: Value of an early cortical excitability study *abs#051*

Kaushik Bhaganagarapu

Filtering fMRI using a SOCK *abs#052*

Karl Martin Klein

Autosomal dominant vasovagal syncope: clinical and linkage results *abs#053*

Danya Vears

Receiving genetic results: exploration of this experience in familial epilepsy *abs#054*

Neurosciences (continued)

John Damiano

Acetylcholine Receptor gene alpha 7 (CHRNA7) in Idiopathic Generalised Epilepsy (IGE): Mutation Detection *abs#055*

Anthony Verberne

Effects of nitric oxide synthase inhibition on dorsal vagal stimulation-induced insulin secretion *abs#056*

Radwa Badawy

EEG-fMRI in Focal epilepsy: Cortical and subcortical involvement correlates with distribution of interictal discharges *abs#057*

Todor Arsov

Epilepsy with occipital features due to *SCN1A* missense mutations *abs#058*

Svetlana Pejoska

^{18}F-AV-133 VMAT2 imaging in the differential diagnosis of dementia with Lewy Bodies from Alzheimer's disease *abs#059*

Victor Villemagne

Correlation of ^{11}C-PiB to post-mortem Aß plaque load *abs#060*

Talia Pumpa

The renal sympathoinhibitory effects of gastric hormones are attenuated in obesity. *abs#061*

Nursing

Edith Cinc

Facilitating MS Clinical Research: The NCRESS Perspective *abs#062*

Emma Dicello (Wadeson)

What is the generation Y nurses attitude towards the bed-side nursing role at Austin Health? *abs#063*

Nicholas Gaynor

Title: Mental Health Presentations in the Emergency Department *abs#064*

Nutrition

Katrina Purcell

Does the rate of weight loss impact on achieving target weight? *abs#065*

Kate Desneves

Effect of an arginine-containing nutritional supplement on pressure ulcer healing in community spinal patients *abs#066*

Brooke Chapman

Does oral branched chain amino acid supplementation in patients awaiting liver transplantation improve nutritional outcomes? *abs#067*

Jessica Passador

Identification of malnutrition on the acute oncology wards at Austin Health and determining the impact of documenting malnutrition on hospital funding. *abs#068*

Obstetrics and Gynaecology

Stella Liong

Increased expression of Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) in cervico-vaginal fluid precedes spontaneous human labour. *abs#069*

Physiotherapy

Catherine Granger

Exercise rehabilitation for patients following lung resection surgery: A pilot feasibility randomised controlled trial. *abs#070*

Jannette Blennerhassett

Validity of the VICON to measure 3D scapular motion *abs#071*

Mary Galea

An exercise and education program improves well-being of new mothers: A randomised controlled trial. *abs#072*

Kimberly Miller

The AsTex®: A new device for assessment of texture discrimination in the hand *abs#073*

Phuong Phan

Walking on level and sloped surfaces following stroke. *abs#074*

Psychiatry & Psychology

Carol Newnham

Early Sensitivity Training for Parents of Preterm Infants: Impact of *PremieStart* on the Developing Brain *abs#075*

Nicki Gazis

Preliminary Results: Austin Health-Diabetes Lifestyle Change Program *abs#076*

Quality

Alan McCubbin

The DCAS Assessment Database – An innovative use of IT to provide time-efficient, patient-centred diabetes care *abs#077*

Respiratory and Sleep

Warren Ruehland

Impaired cortical response to a respiratory stimulus in untreated severe ObstructiveSleep Apnoea *abs#078*

Danielle Wilson

Misperception of sleep latency and sleep duration in pregnancy *abs#079*

Peter Rochford

Inter-Rater Reliability in the Interpretation of Lung Function Tests *abs#080*

Peter Rochford

PSG scoring reliability in a large cohort of scorers in Australasian sleep laboratories: Initial results from a novel inter-laboratory proficiency testing program *abs#081*

Gary Nolan

Elevated Slope Phase III from UPG correlates with intra-breath changes in low frequency reactance (X5Hz) in patients with COPD. *abs#082*

Rachel Schembri

The prevalence and severity of sleep apnoea in older adults with self-reported insomnia. *abs#083*

Thomas Churchward

Manual Versus Automated Detection of Oxygen Desaturation Index During Sleep. *abs#084*

Speech pathology

Elizabeth Morkham

Communication impairment in children with anxiety disorders: a pilot study. *abs#085*

Shauna Poole

Identifying barriers to communication: Using the Inpatient Functional Communication Interview (IFCI) in a subacute setting *abs#086*

Surgery

Ahmad Aly

Reflux After Oesophagectomy - Can It Be Prevented? : A Randomised Controlled Trial *abs#087*

Philip Hayward

Off Pump Coronary Artery Surgery is Relatively Kind to Kidneys: Evidence From a Novel Biomarker of Acute Kidney Injury *abs#088*

Ying Yan Zhu

Effect of Lipid Exposure on Graft Patency and Clinical Outcomes: Arteries and Veins are Different *abs#089*

BZ Wang

Liver Transplantation Using Grafts from Carbon Monoxide Poisoned Donors *abs#090*

William Shi

Pulmonary vein isolation for atrial fibrillation using the argon-based cryoablation probe: a report of early experience from an Australian centre. *abs#091*

Peter Chu

Foam echosclerotherapy – results of non-surgical varicose vein treatment at The Austin Hospital. *abs#092*

William Shi

Impact of patient-prosthesis mismatch after mitral valve replacement: an Australian multicentre analysis of early outcomes and mid-term survival. *abs#093*

Linh Nguyen

Tumours Survive Vascular Disrupting Agent OXI4503 Treatment by Undergoing Epithelial to Mesenchymal Transition (EMT) *abs#094*

Shir Lin Koh

The Effects of Captopril in a Mouse Model of Liver Regeneration *abs#095*

Fabiano Viana

Single dose Custodiol versus multidose normothermic blood cardioplegia for myocardial protection: Australian single-centre analysis of 134 matched patients undergoing complex cardiac operations *abs#096*

Ying Yan Zhu

Lipid Management after Coronary Surgery: How Effective are we at Secondary Prevention? *abs#097*

Kevin Ong

Initial Outcomes of Localised Prostate Cancer Treated With High-Intensity Focused Ultrasound (HIFU) *abs#098*

Ying Yan Zhu

Impact of radial artery harvest on forearm function and symptoms: a long-term analysis from a randomised trial *abs#099*

Eleanor Ager

Angiotensin II induced changes in epithelial and mesencymal markers in colorectal cancer cells. *abs#100*

Shu wen Wen

The influence of the Renin-angiotensin system on macrophage plasticity *abs#101*

Joyna Chan

Increased AT1R expression on cancer cells can reduce the efficacy of AT1R-targeted anti-cancer treatments *abs#102*

William Shi

Training in mitral valve surgery need not affect early outcomes and mid-term survival: a multicentre analysis. *abs#103*

Ignatius Hadinata

Comparable patencies of the radial artery and right internal thoracic artery or saphenous vein beyond 8 years: Results from the Radial Artery Patency and Clinical Outcomes trial *abs#104*

Poster Session 2

Cardiology

Terase Lancefield

Predictors of Stoke Following Percutaneous Coronary Intervention (PCI): Findings from a Large Multicentre Australian PCI Registry *abs#105*

Ken Lu

Anaemia is a Significant Predictor of Increased Mortality in Subjects with Heart Failure with Reduced Ejection Fraction: Medium Term Follow Up *abs#106*

Sheila Patel

Plasma NT-BNP is Associated with Systolic but not Diastolic Dysfunction in Type 2 Diabetes *abs#107*

Ali Al-Fiadh

Is Patent Foramen Ovale a Cause of Cryptogenic Acute Coronary Syndrome? *abs#108*

Michael Wong

Evaluation of left ventricular lead output in Cardiac Resynchronisation Therapy (CRT): minimum outputs for maximum gains *abs#109*

Ali Al-Fiadh

Retinal microvascular endothelial function is attenuated in patients with chronic and acute cardiovascular disease *abs#110*

David Taylor

Precipitants of Acute Decompensated Heart Failure: A Comparison between Outpatients and Inpatients *abs#111*

Michael Mok

Trans-radial versus femoral artery access for Percutaneous Coronary Intervention (PCI) - has it improved clinical outcomes? *abs#112*

Sarah Lonie

Depression & Autonomic Nervous System Function in Heart Failure *abs#113*

Andrew Stewart

National survey of Australian cardiologists' beliefs and practice regarding screening, diagnosis and management of depression *abs#114*

K Lu

Predictors of Mortality in Infective Endocarditis in Contemporary Australian Practice Over 13 Years. *abs#115*

Fahd Chahadi

Correlation and Characteristics of Electrocardiographic Changes with Coronary Anatomical Findings in Patients with Acute Circumflex Coronary Artery Occlusion. *abs#116*

K Lu

Chronic Renal impairment is a Significant Predictor of Increased Mortality in Subjects with Heart Failure and Normal Ejection Fraction with Medium Term Follow Up *abs#117*

Melanie Freeman

Angiotensin Converting Enzyme 2: A Novel Marker of Coronary Artery Disease *abs#118*

Endocrinology & Metabolism

Scott Baker

Efficacy of Very-Low-Calorie Diets as a Treatment for Obesity in Individuals with Type 2 Diabetes Compared to Non-Diabetic Controls. *abs#119*

Salvatore Mangiafico

Pathway selective insulin resistance: A potential link between obesity, inflammation and elevated triglycerides in Type 2 Diabetes *abs#120*

Steve Weng

Partial, but not total deletion of SEPS1 leads to improved insulin secretion. *abs#121*

Endocrinology & Metabolism (continued)

Wynne Pong

Inducible muscle-specific UBL5 knockout has no effect on glucose metabolism *abs#122*

Patricia Russell

Identification of target genes in osteoblasts that are regulated directly via the Androgen Receptor *abs#123*

Mike Chang

Detection and Assessment of ProGRP-derived Peptides in Human Colorectal Cancer Cells and the Role they play in Cellular Proliferation. *abs#124*

Maria Stathopoulos

Slowing down ß-cell metabolism and insulin secretion protects ß-cell function under conditions of chronic nutrient overload *abs#125*

M Grossmann

Architectural Decay of the Skeleton and Accumulation of Visceral Abdominal Fat following Androgen Deprivation Therapy for Prostate Cancer *abs#126*

Roger Zebaze

Quantifying Intracortical Porosity Producing Cortical Remnants Identifies Individuals at Risk for Fracture Beyond Existing Parameters *abs#127*

Christos Joannides

Insulin resistance causes a progressive impairment in second-phase but not first-phase insulin secretion. *abs#128*

Ali Ghasem-Zadeh

Defining the surface/volume ratio of bone identifies the susceptibility of bone to being remodelled and lost and Individuals at Risk for Fracture better than Bone Density measurements *abs#129*

Qingju Wang

Properties of Trabecular Architecture at Metaphyseal Region Are Established in Early Life and Decayed Before Menopause *abs#130*

Melissa Gomez

Bone microarchitecture during and after lactation *abs#131*

Bryan Wai

Predictive Value of Left Ventricular Mass and Geometry in Type 2 Diabetes Mellitus for Adverse Cardiovascular Events *abs#132*

Sandra Iuliano-Burns

The Skeletal Response to Vitamin D Supplementation During Sunlight Deprivation: a Randomised Trial in Antarctic Expeditioners *abs#133*

Lin Xiao

Metal Ions Upregulate Gastrin Gene Expression in Gastric Cancer Cells *abs#134*

Bryan Wai

E/e' is an Independent Predictor of Heart Failure Admission in Patients with Type 2 Diabetes Mellitus *abs#135*

Richard MacIsaac

Dietary salt intake and mortality in patients with type 2 diabetes *abs#136*

Nicole Wong

Fructose feeding protects against fat-induced obesity *abs#137*

Tammy Pang

Investigating DNA binding-independent signalling pathways of the androgen receptor *abs#138*

Jarrod Skinner

Identification of physiological role of calcitonin in pregnancy using calcitonin receptor knockout mice *abs#139*

Gastroenterology

Chandana Herath

Angiotensin-(1-7) reduces portal pressure via guanylate cyclase-dependent nitric oxide but angiotensin II receptor independent pathway in cirrhotic rats *abs#140*

Suzana Kovac

Gastrin Increases its own Synthesis in Gastrointestinal Cancer Cells via the CCK2 receptor. *abs#141*

Michelle Goodwin

Absence of RAGE (receptor for advanced glycation endproducts) attenuates liver fibrosis in mice *abs#142*

He Liu

Role of P21-activated kinase 1 in survival of Colorectal Cancer cells *abs#143*

Josephine Grace

Angiotensin-(1-7) is a mesenteric vasodilator in experimental cirrhosis *abs#144*

Kathryn Marshall

Biological Activities of Progastrin Peptides *abs#145*

Kai Mak

Investigating the renin-angiotensin system in the development of non-alcoholic steatohepatitis *abs#146*

Jessica Howell

Toll-Like Receptor 7/8-Induced Type 1 Interferon Responses are Impaired in Hepatitis C Patients with Rapid Fibrosis Post Liver Transplant *abs#147*

Jessica Howell

Donor and Recipient Age and HLA-A Mismatch: Modifiable Risk Factors for Rapid HCV Recurrence Post Transplant *abs#148*

Jessica Howell

Early-Onset versus Late-Onset Non-Anastomotic Biliary Strictures: Risk Factors Reflect Different Pathogenesis *abs#149*

Immunology

Ramya Krishnan

Role of Neutrophil CD64 as an early diagnostic marker of Sepsis *abs#150*

Russell Hodgson

ICOS-Ig prolongs cellular xenograft survival and is associated with CD4+CD25+Foxp3+ T regulatory cells and IL10 expression *abs#151*

Zhanqi Liu

ADCC, CDC and binding activities of anti-Le^y antibody hu3S193 with mutated Fc *abs#152*

WenXu Lin

Immuno-monitoring of T regulatory cells in peripheral blood of renal and liver transplant recipients *abs#153*

Infectious Diseases

Eddy Edelman

An Unusual Finding in Renal Transplant Patient. *abs#154*

Korri El Khobar

Dysregulation of Intracellular Signal Transduction in Hepatitis C and Its Implication in HCV Fibrosis *abs#155*

Douglas Johnson

Novel Adjuvants to Increase Humoral Neutralising Antibody and Cellular Immune Responses to Recombinant Mammalian Cell Derived Hepatitis C Virus-Like-Particles *abs#156*

Natasha Holmes

Vancomycin minimum inhibitory concentration (MIC) predicts outcome in *Staphylococcus aureus* bacteraemia independent of methicillin susceptibility and antimicrobial therapy. *abs#157*

Infectious Diseases (continued)

Paul Johnson

A sustained hospital outbreak of vancomycin-resistant *Enterococcus faecium* bacteraemia due to the emergence of vanB *E. faecium* sequence type 203 *abs#158*

Oncology & Haematology

Ashley Knights

Antigen processing & presentation of immunodominant epitopes from the tumour antigen NY-ESO-1; disparity between melanoma cells and vaccine induced responses? *abs#159*

Nigel Anderson

Utilising the improved accuracy of Monte Carlo based calculation algorithms to greater understand delivered dose in head and neck intensity modulated radiation therapy (IMRT). *abs#161*

Pu-Han Lo

TKTL1- a promising anti-tumour target in melanomas *abs#162*

Andrew Weickhardt

DUX study: A phase II study evaluating dual targeting of the EGFR using the combination of cetuximab and erlotinib in patients with chemotherapy refractory metastatic colorectal cancer *abs#163*

Thomas John

Correlation of engraftment, mutation status and response to chemotherapy in primary tumor xenograft models of NSCLC *abs#164*

Tianlu Li

Role of the c-myc Oncogene in Transcriptional Regulation of Histone Deacetylase 3 *abs#165*

Prahlad Ho

An intensified conditioning regiment with intravenous (IV) busulphan-melphalan (bu-mel) and pharmacokinetic monitoring prior to autografting for poor prognostic lymphoma *abs#166*

Vinochani Pillay

Targeting mutant K-Ras with novel siRNA-antibody conjugates for the treatment of colorectal cancer *abs#167*

Dixon Woon

Evaluation of infiltrating T regulatory cells in prostate cancer tissue *abs#168*

Elly Lynch

Hereditary Non Polyposis Colorectal Cancer and Familial Adenomatous Polyposis: Information days For Families *abs#169*

Andreas Behren

The influence of the microenvironment on gene-expression in melanoma cells *abs#170*

Matthew Burgess

A 12 month audit of BRCA mutations identified by the Austin Familial Cancer Clinic *abs#171*

Anderly Chueh

Sustained IE gene induction and AP-1 activity is linked to HDACi-induced apoptosis in multiple tumour types *abs#172*

Genevieve Whitty

Peripheral blood BDCA-1 (CD1c) positive dendritic cells pulsed with NY-ESO-1 ISCOMATRIX® may be tolerogenic in patients with minimal residual cancer at high risk of relapse: a pilot clinical trial *abs#173*

Sheren Al-Obaidi

Villin Expression is Frequently Lost in MSI Colon Cancer *abs#174*

Georgia Corner

The Alkaline Phosphatase gene cluster is epigenetically regulated in colon cancer. *abs#175*

Weranja Ranasinghe

The role of zinc in prostate cancer *abs#176*

Oncology & Haematology (continued)

Lee-Yean Low

Investigation of the Role of WNT Protein Signaling in CRC Angiogenesis and Metastasis Using RNA Interference (RNAi) *abs#177*

Philip Tan

Sequential Alternating Drug Treatment: Overcoming Resistance in Advanced Renal Cell Cancer *abs#178*

Christopher Hudson

Identification of melanoma sub-populations based on functional properties *abs#179*

Matthew Anaka

Intratumoural functional heterogeneity of melanoma cells with distinct genomic profiles *abs#180*

Aparna Jayachandran

Causative role of epithelilal-to-mesenchymal transition in melanoma progression *abs#181*

Sujitra Detchokul

CD151 expression: A biomarker for prostate cancer progression *abs#182*

Ainsley Campbell

The Relative Utility of Cytology and Histology Specimens in Assigning NSCLC Pathological Subtypes *abs#183*

Drew Smith

A comparative review of 3D-CRT and an IMRT technique for anal cancer using CMS XiO and CMS Monaco. *abs#184*

Lars Tögel

Identification of DUSP5 as a potential mediator of Wnt/ß-catenin - MAPK/ERK signalling crosstalk in colon cancer *abs#185*

Fook Lee

^{177}Lu-CHX-A"-DTPA huA33 Radioimmunotherapy (RIT) in Combination with Chemotherapy and Cetuximab in a Colorectal Cancer Xenograft Model *abs#186*

Hui Gan

Adequacy of data provision and analysis in oncology phase III trials over the past five years *abs#187*

Paul Mitchell

Management of lung cancer in Victoria: have we made progress? *abs#188*

Joe Chang

Validation of ^{11}C-choline PET scans with pathology for radiotherapy target definition in prostate cancer *abs#189*

Pavel Sluka

Stimulation of Prostate Cancer Cell Proliferation by Bone-Derived Factors *abs#190*

Angelo Perani

Production of Monoclonal Antibodies for Clinical Trials and Translational Cancer Research. *abs#191*

Carmel Pezaro

Manipulation of epigenetic control of cancer-testis antigen (CTAg) expression in bladder cancer cell lines using azacytidine *abs#192*

Sandy Nguyen

The expression of calcitonin receptor in the brain tumour glioblastoma multiforme *abs#193*

Sarah Baird

Cancer-associated fibroblast expression of serine protease fibroblast activation protein increases tumour metastatic potential and alters cell death mechanisms *abs#194*

Simon Louis

Expression of a novel growth factor inhibitor, ATIP, in human prostate cancers *abs#195*

Maureen Rolfo

Comparison of Set-Up Error of Two Stabilisation Devices in Head & Neck IMRT and Evaluation of Image Guidance Strategy *abs#196*

Oncology & Haematology (continued)

Glenn Cartwright

In vivo Comparison of the DNA Proliferation Tracer [18F]-FLETT with [18F]-FLT in a Mouse Tumor Model *abs#197*

Chris Hamilton

A Statistical Treatment of the Integral DVH *abs#198*

Paul Mitchell

Cancer testis (CT) antigen (Ag) expression in non-small cell lung carcinoma (NSCLC) paired primary (1°) and secondary (2°) tumours. *abs#199*

Lisa Ho

Optimising posaconazole usage as antifungal prophylaxis in acute leukaemia *abs#200*

Pharmacy

Rohan Elliott

Development of a hospital pharmacist prepared interim residential care medication administration chart to facilitate continuity of medication administration after discharge to residential care *abs#201*

Simone Taylor

Impact of a hospital pharmacist prepared interim residential care medication administration chart on medication administration errors after discharge from hospital to residential care (MedGap Project) *abs#202*

Louise Williams

Determination of medication storage temperatures in hospital-in-the-home (HITH) *abs#203*

Renal

The-Phung To

A comparison of oral and intravenous sodium bicarbonate on urinary pH in patients at risk of contrast-induced nephropathy: a pilot study. *abs#204*

Suet-Wan Choy

Renin secretion is augmented in Acetyl-CoA Carboxylase Knockin Mice in response to a Salt Deficient Diet *abs#205*

Andrew Ellis

The Use of Iohexol Clearance to Calculate Reference GFR in an Evaluation of the Utility of eGFR Equations in the Indigenous Australian Population. *abs#206*

Elena Velkoska

Angiotensin 1-7 Increases Cardiac Fibrosis in Renal Injury *abs#207*

Darren Lee

Defect in Albumin Degradation in the Proximal Convoluted Tubule of Limp2 Knockout Mice *abs#209*

Matthew Roberts

Secular trends in cardiovascular mortality rates of patients receiving dialysis compared to the general population *abs#210*

Permission to include this abstract #160 was granted subsequent to the inclusion of the links within this document. For this reason alone this abstract appears out of sequence

Ashley Knights
Smac mimetics promote cytotoxic T lymphocyte-mediated bystander killing of melanoma cells and enhance the expansion and function of antigen-specific T cells abs#160

001

PERIOPERATIVE ANAESTHETIC OUTCOMES AFTER OPEN RADICAL RETROPUBIC PROSTATECTOMY: A 13-YEAR EXPERIENCE IN A TERTIARY CARE CENTRE

L. Weinberg, S. Chen, I. Dissanayake, A. Ko, L. McNicol, D. Story, P. Peyton

Anaesthesia, Austin Hospital, Heidelberg, VIC, Australia

Introduction: Perioperative anaesthetic outcomes in patients undergoing open radical retropubic prostatectomy are poorly described. Our research questions were: what were the nature and frequency of perioperative events; and how did the patterns change over time?

Methods: We performed retrospective observational study of patients who underwent open radical prostatectomy during a 13-year period (1997-2009). Data recorded included patients' age, anaesthesia technique, operation time, blood transfusion rate, hospital stay, and major and minor 30-day complications.

Results: The records of 442 patients were reviewed. The mean age was 61 years (range 36-76). Four-hundred-and-thirty three (98%) patients were American Society of Anesthesiologists physical scoring system 1 or 2. Epidural analgesia was utilized in 85% of patients in 1997 decreasing to 2% in 2009. Mean duration of surgery was similar each year (148 min; SD 51.1). From 1997 to 2009 there were decreases in transfusion rate (29% to 9%), mean duration of hospital stay (15.8 days to 4.1 days) and major complication rate (57% to 6%). The most common complications were myocardial infarction, pneumonia, wound infection, and ileus. Mortality at 30-days was 0%. There was no association with pre-existing patient comorbidities and adverse events.

Conclusion: Perioperative outcomes after open radical retropubic prostatectomy have significantly improved over the last decade. This change reflects refinements in surgical and anaesthetic technique and improved postoperative care. Rates of complications and length of hospital stay provide good opportunities to evaluate strategies aimed at improving peri-operative care for this group. This study provides planning data for future interventional studies.

002

METABOLIC ACID-BASE CHANGES AFTER LIVER RESECTION: OBSERVATIONAL PILOT STUDY

S. Chen[1], L. Weinberg[1], C. Christophi[2], N. Scurrah[1], L. McNicol[1], D. Story[1]

[1]Anaesthesia, Austin Hospital, Heidelberg, VIC, Australia
[2]Surgery, Austin Hospital, Heidelberg, VIC, Australia

Introduction: We are planning a randomised controlled trial (RCT) of intravenous fluid therapy during liver resection. We conducted this observational study to help plan the RCT. We tested the hypothesis that hepatic resection is associated with metabolic acidosis.

Methods: Thirty patients had both preoperative and postoperative blood gas testing during a previous RCT of interpleural analgesia after liver resection[1]. We used a quantitative Stewart type analysis[2] to compare before and after surgery acid-base status using paired t-tests.

Results: The mean decrease in base-excess was 2.5 meq/L (95% CI: 1.7 to 3.3 meq/L decrease, P <0.001). This was due mainly to an acidifying decrease in the sodium-chloride effect on base-excess: 3.1 meq/L (95% CI: 1.9 to 4.3 meq/L decrease, P<0.001). There was a small alkalinising effect of decreased albumin (weak acid) which increased base-excess by 0.75 meq/L (95% CI: -0.17 to 1.7, P =0.1). The other-ion effect on base-excess was largely unchanged, 0.1 meq/L decrease (95% CI: 1.9 decrease to 1.8 increase, P = 0.92). Consistent with our hypothesis we found increased metabolic acidosis after liver resection; predominately due to a strong-ion acidosis. The maintenance fluid was Hartmann's solution (mean volume 2000 ml, range 500 to 4000 ml) and 21 patients also received colloids (mean 300 ml, range 0 to 2000ml).

Conclusion: Our planned RCT will test the hypothesis that Plasmalyte is associated with similar or improved metabolic status (non-inferiority) than Hartmann's solution after liver resection without lactate rise. This pilot study suggests that Hartmann's solution maintenance, with some additional colloid, is associated with a base-excess decrease of 2.5 meq/L.

(1) Weinberg L, Scurrah N, Parker F, Story D, McNicol L. Interpleural analgesia for attenuation of postoperative pain after hepatic resection. Anaesthesia 2010;65:721-728.
(2) Story DA, Morimatsu H, Bellomo R. Strong ions, weak acids and base excess: a simplified Fencl-Stewart approach to clinical acid-base disorders. Br J Anaesth 2004;92:54-60.

003

THE EFFECT OF NORMAL SALINE 0.9% ON METABOLIC ACID-BASE BALANCE AFTER KIDNEY TRANSPLANTATION

I. Chao[1], L. Weinberg[1], A. Sarrai[1], F. Ierino[2], P. Peyton[1], L. McNicol[1], D. Story[1]

[1]Anaesthesia, Austin Hospital, Heidelberg, VIC, Australia

[2]Nephrology, Austin Hospital, Heidelberg, VIC, Australia

Introduction. Normal saline (NS; 0.9% NaCl) is administered during kidney transplantation to avoid the risk of hyperkalaemia associated with potassium-containing crystalloid solutions. Normal saline can however cause hyperchloraemic normal anion gap metabolic acidosis (NAGMA) with deleterious effects on serum potassium levels. We hypothesized that acid-base changes that accompany NAGMA after kidney transplantation relate more to changes in sodium-chloride difference (ΔNaCl) than changes in chloride (ΔCl) alone. Aim: (1) to determine frequency of metabolic acidosis and incidence of hyperkalaemia post transplantation; (2) to define the impact of serum electrolytes on metabolic acid base status.

Methods: A retrospective observational study of patients who underwent kidney transplantation during a 14-month period (2008-2009). Data recorded: demographic details, cold and warm ischaemia times, intraoperative fluid intervention, preoperative and Day 1 postoperative electrolytes, and 12-hour postoperative fluid intervention.

Results: Perioperative data from 44 patients were collected (living donor n=10, cadaveric donor n=34). Mean age: 51 years (range 25-67). Mean cold ischaemia time: 642 minutes (SD 370 min), warm ischaemia time: 39 minutes (SD 8.6). Mean intraoperative NS: 3.3 Litres (SD 2.65). Mean 12-hour NS: 3.5 Litres (SD 2.64 Litres). Twenty-three patients (52%) developed a postoperative de novo NAGMA: mean HCO3 = 20 mmol/L (SD 2.4). Incidence of postoperative hyperkalaemia requiring medical intervention: 18%. After surgery there was a stronger correlation between the ΔNaCl and HCO_3 ($r^2 = 0.5$, $p < 0.001$) than between ΔCl and HCO_3 ($r^2 = 0.25$, $p < 0.05$). There was no correlation between ischaemia times and acidosis or hyperkalaemia.

Conclusion: Post kidney transplantation, the serum ΔNaCl correlates more strongly with severity of hyperchloraemic metabolic acidosis than ΔCl alone. Clinical implications: Compared to NS, balanced crystalloid solutions with a greater ΔNaCl composition (e.g. Hartmann's or Plasmalyte solution) may have more favourable effects on acid-base homeostasis.

004

NITROUS OXIDE DIFFUSION AND THE SECOND GAS EFFECT ON EMERGENCE FROM ANESTHESIA

P. Peyton[1], I. Chao[1], L. Weinberg[1], G. Robinson[2], B. Thompson[2]

[1]Dept of Anaesthesia, Austin Health, Heidelberg, VIC, Australia

[2]Depts of Anaesthesia & Perioperative Medicine, Allergy, Immunology and Respiratory Medicine, The Alfred, Prahran, VIC, Australia

Background: Rapid elimination of nitrous oxide (N2O) from the lungs at the end of inhalational anesthesia dilutes alveolar oxygen producing "diffusion hypoxia". A similar dilutional effect on accompanying volatile anesthetic agent has not been investigated, but may impact on speed of emergence.

Methods: Twenty patients undergoing surgery were randomized to receive an anesthetic maintenance gas mixture of sevoflurane in air-oxygen (control group) or sevoflurane in a 2:1 mixture of N2O-oxygen (N2O group). After surgery baseline arterial blood and tidal gas samples were taken. Patients were ventilated with oxygen, and arterial blood and tidal gas sampling was repeated at 2 and 5 minutes. A blood gas was sampled at 30 minutes postoperatively. Sevoflurane partial pressure was measured in blood by the double headspace equilibration technique and in tidal gas using a calibrated infrared gas analyzer. Times to eye opening and extubation were recorded.

Results: Relative to baseline, arterial sevoflurane partial pressure was over 35% higher at 2 and 5 minutes in the control group ($p < 0.04$ and < 0.02). This difference persisted at 30 minutes. A smaller but significant difference was present between groups in end-tidal sevoflurane concentration. Time to eye opening and extubation were shorter in the N2O group (8.7 minutes and 10.1 minutes) than the control group (11.0 minutes and 13.2 minutes) ($p < 0.04$).

Conclusions: Elimination of N2O at the end of anesthesia produces a clinically significant acceleration of the fall in concentration of accompanying volatile agent, contributing to the quicker emergence observed following inhalational anaesthesia with N2O.

005

VARIABILITY IN ADEQUACY OF VENTILATION DURING TRANSPORT OF CARDIAC SURGERY PATIENTS: A COHORT STUDY

R. Rajasekaram, M. Reade, R. Bellomo, G. Hart, B. Shortal, M. Shaw

Anaesthesia and Intensive Care, Austin Health, Heidelberg, VIC, Australia

Objective: Inadequate ventilation during transport from the operating theatre (OT) to the intensive care unit (ICU) with attendant hypercarbia may adversely affect haemodynamics. We assessed the incidence of inadequate ventilation during transport from OT to ICU.

Setting: Tertiary teaching hospital.

Patients: 99 consecutive patients having cardiac surgery.

Design: Retrospective observational study.

Methods: Collection of demographic, clinical, arterial blood gas and hemodynamic measurements on arrival in the ICU after cardiac surgery. Statistical assessment of the relationship between PaCO2 and PAP and other relevant hemodynamic variables.

Results: Overall, hypocapnia (PaCO2<35mmHg) occurred in 18.2% of patients. 28.3% of patients had hypercapnia (PaCO2>45mmHg) and 28.3% of patients had acidaemia (pH<7.35) on arrival in ICU, with 8.1% of patients having more marked acidaemia (pH <7.3). There was no univariate association between PaCO2 and PAP (R2 = 0.0076, p=0.39).

In patients with hypercapnia, 21.4% patients had a post operative complication compared to 16.9% of patients without it. Noradrenaline infusion was a risk factor for hypercarbia and increased pulmonary artery pressure

Conclusions: Suboptimal ventilation during transport to the ICU occurred in close to 50% of patients with hypercarbia being most common. Acidaemia was similarly frequent. Noradrenaline administration was a risk factor for hypercarbia and elevated pulmonary pressures.

006

INTRAVENOUS FLUID ADMINISTRATION AND MONITORING FOR ADULT WARD PATIENTS IN A TEACHING HOSPITAL

G. M. Eastwood, L. Peck, H. Young, J. Prowle, D. Jones, R. Bellomo

Intensive Care Unit, Austin Health, Heidelberg, VIC, Australia

Background and Aim: Intravenous (IV) fluids are commonly administered to acutely unwell hospitalised patients. Despite the importance of IV fluid therapy, there is a lack of detailed information on the proportion of patients receiving IV fluids; IV fluid therapy monitoring practices, and patient outcomes associated with IV fluid therapy. We sought to assess differences in characteristics and outcomes between patients who receiving are versus are not-receiving IV fluids

Method: We conducted a prospective clinical audit of all adult ward patients in a tertiary teaching hospital in Melbourne, Victoria on 26th August 2009.

Results: All 320 eligible patients were audited: medical 197, surgical 123; 184 (54%) male; mean age 69 yrs (range 20-96 yrs). At assessment 85 (26%) were receiving IV fluid therapy. Fluid balance charts were being maintained for 186 (58%) patients overall, and in 79 (94%) patients receiving IV fluid. Body weight measurements were documented for 49 (15%) patients overall, and in 15 (18%) patients receiving IV fluid (Table 1). During the previous 24 hours serum electrolyte and renal function measurements were obtained for 224 (70%) patients overall, and in 77 (90%) patients receiving IV fluid. Mean (range) of serum electrolytes and renal function values for those receiving IV fluids were: Na^+ 138 (131-141), K^+ 3.9 (3.2-5.3), Cl^- 103 (91-112), Creatinine 74 (35-429), HCO_3^- 27 (19-41), and Urea 5 (1.4-40.8). There was a non-significant difference between patients not-receiving versus those receiving IV fluid for ICU admission or hospital outcome.

Conclusion: We identified that one-quarter of all adult ward patients in our hospital were receiving IV fluids. Patients receiving IV fluids were more likely to have a daily serum electrolyte and fluid balance chart measurement than those not receiving IV fluids. Continuing educational interventions are needed to improve fluid balance monitoring of patients receiving IV fluids.

007

A SIMPLE CLINICAL TARGET ASSOCIATED WITH A HIGH LEVEL OF PATIENT SATISFACTION WITH THEIR PAIN MANAGEMENT

K. Jao[1,2], D. Taylor[1,2], S. E. Taylor[1]

[1]Emergency, Austin Health, Heidelberg, VIC, Australia

[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

Introduction: We aimed to determine factors that are significantly associated with a high level of patient satisfaction with their pain management.

Methods and Materials: We undertook an observational study in a large metropolitan ED. Adult patients with a triage pain score of≥ 4 (0 -10 scale) were enrolled. Data collected included demographics, presenting complaint, pain scores every 30 minutes, whether nurse-initiated analgesia was administered, the nature of the pain relief administered, time to first dose of analgesia, elements of staff communication, and whether a 'significant decrease in pain' was achieved (defined as a decrease in pain score to <4 and a decrease from the triage pain score of≥ 2). The primary endpoint, determined at follow up within 48 hours, was the level of satisfaction with pain management (6-point scale: very unsatisfied-very satisfied).

Results: 167 (82.7%) of 202 enrolled patients were followed up – mean (SD) age 46.4 (18.3) years, 75 (44.9%) males. Eighty-one (48.5%) patients were very satisfied with their pain management. Only two variables were significantly associated with a high level of satisfaction. 44 (58.7%) versus 37 (40.2%) patients who did/did not have a 'significant decrease in pain' (as defined), respectively, were very satisfied (difference 18 .5%, 95%CI 2.3, 34.7, p=0.027). 77 (53.9%) and four (16.7%) patients who were/were not advised by ED staff that their pain management was important, respectively, were very satisfied (difference 37.2%, 95%CI 17.7, 56.6, p=0.002).

Conclusions: Our 'significant decrease in pain' definition may provide a useful clinical target which, alongside adequate communication, may help maximize patient satisfaction.

008

A CLASSIFICATION AND REGRESSION TREE TO ASSIST CLINICAL DECISION MAKING IN AIRWAY MANAGEMENT FOR PATIENTS WITH CERVICAL SPINAL CORD INJURY

S. Berney[1], I. Gordon[2], H. Opdam[3], L. Denehy[4]

[1]Physiotherapy, Austin Health, Heidelberg, VIC, Australia

[2]Statistical Consulting Service, The University of Melbourne, Melbourne, VIC, Australia

[3]Intensive Care, Austin Health, Melbourne, VIC, Australia

[4]Physiotherapy, The University of Melbourne, Melbourne, VIC, Australia

Background: Following acute cervical spinal cord injury (CSCI), most patients require admission to the intensive care unit (ICU) for intubation and mechanical ventilation to support their respiratory function. Whilst airway management that includes the decision to intubate and/or insert a tracheostomy is an important aspect of care, there is little evidence to guide clinicians in making this decision. The aim of this study was to review airway management of patients with CSCI who are admitted to ICU and to develop a classification and regression tree (CART) to direct clinical decision making in airway management.

Method: All patients with CSCI who required intubation and mechanical ventilation and who were admitted to ICU in three tertiary hospitals in Melbourne between October 2004 and May 2009 and two other interstate hospitals between December 2004 and December 2005 were included. Airway management was recorded. A CART analysis was performed to determine the variables that best predicted whether patients were extubated or required insertion of a tracheostomy. An unbiased estimate of the true misclassification rate was obtained using cross-validation.

Results: 114 patients were included. Tracheostomy insertion occurred in 68 patients (59.6%). Using CART analysis, the variables forced vital capacity, the volume of pulmonary secretion and gas exchange were predictive of airway management on 82.3% of occasions with an 8.7% extubation failure rate.

Conclusion: External validation using an independent data set is necessary but potentially this classification tree is useful in clinical decision-making regarding airway management in CSC.

009

A SURVEY OF WARD NURSES ATTITUDES TO THE INTENSIVE CARE NURSE CONSULTANT SERVICE IN TEACHING HOSPITAL

C. Taylor, T. J. McIntyre, D. A. Jones, G. Eastwood, L. Peck, I. C. Baldwin, R. Bellomo

Intensive Care Unit, Austin Health, Heidelberg, VIC, Australia

Aim: The purpose of this study was to survey ward nurses attitudes towards the Intensive Care Nurse Consultant (ICNC) service. A questionnaire was distributed to assess five domains: a) accessibility and approachability, b) perceived ICNC skill and knowledge, c) perceived influence on patient management, d) usefulness as a resource of clinical information, and e) impact on patient's clinical outcomes.

Background: The development of critical outreach services aims to optimise care of acute, complex ward patients. Despite their implementation, specific assessment of nurses' attitudes towards the ICNC Service is lacking.

Methodology: An anonymous Likert-type agreement questionnaire was distributed among ward nurses, including nurses' comments about the ICNC service.

Findings and Data analysis: Completed questionnaires were entered manually into a 'Survey Monkey'™ pro-format to permit automatic report generation and a results summary. Additional comments were grouped into several themes. Each theme was summarized by paraphrasing one or more of the respondent's comments.

The number of comments in each category was subsequently collated.

The findings strongly demonstrated clinical support & benefits of an ICNC service, which adds knowledge and understanding towards critical care outreach services.

Conclusion: This survey suggests that the ICNC service is valued, and is perceived to prevent the development of adverse events. It has been considered as a clinical resource for ward nurses in regard to the identification of the deteriorating patient.

(1) Burns, C. (2006). Intensive Care Unit (ICU) Outreach Nurse: A literature review. Nursing Monograph,p.43-47

(2) Wilson, R.M., Runciman, W.B., Gibberd, R.W., Harrison, B.T., Newby, L., Hamilton, J.D. The Quality in Australian Health Care Study. Med J Aust, 1995. 163(9): p. 458-71.

(3) Chan KS, Tan CK, Fang CS, Tsai CL, Hou CC, Cheng KC, Lee MC. Re-admission to the of morbidity and mortality of surgical patients in the intensive care unit. Surg. Today.2009;39(4):295-299.

(4) Ball, C., Kirby, M. & Williams, S. (2003). Effect of critical care outreach team on patient survival from hospital and re-admission to critical care BMJ, 327, (1) Nov 1-4

(5) Eliott, S., Doric, A., Ernest, D., Chaboyer, W., Worrall-Carter, L., The ICU Liaison Nurse Service improves patient outcomes Aust. CritCare 21 (1) Feb.p57-58

(6) Eliott, S., Doric, A., Ernest, D. (2008). Mapping the ICU Liaison Nurse Services in Victoria Aust.CritCare 21,(1), Feb 73-74.

(7) Chaboyer, W., Gillespie, B., Foster, M., & Kendall, M. (2005). The impact of an ICU Liaison Nurse: a case study of ward nurses' perceptions Journal of Clinical nursing 14(6), 766-775.

(8) Richardson, A., Burnard, V., Colley, H., & Coulter, C. (2004). Ward nurses' evaluation of critical care outreach. Nursing in Critical Care 9(1), 28-33.

(9) Jones, D., Story, D., Clavisi, O., Jones, R., & Peyton, P.(2006). An introduction guide to survey research in anesthesia. Anaesthesia & Intensive Care 34, 245-253.

(10) Valentine, J. & Skirton, H. (2006). Critical Care Outreach- a meaningful evaluation Nursing in Critical Care 11,(1)p.288-296.

010

COMPLEMENTARY AND ALTERNATIVE MEDICINE USE IS ASSOCIATED WITH IMPORTANT CLINICAL ADVERSE EVENTS AMONG PATIENTS ON WARFARIN

M. Khan[1,2], D. Taylor[1,2], S. E. Taylor[1], A. Harding[1], E. Mitchell[1]

[1]Emergency, Austin Health, Heidelberg, Australia

[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

Introduction: Among patients taking warfarin, we determined CAM (complementary and alternative medicine) knowledge and use, and the association between CAM use and INR stability and adverse events.

Methods and Materials: We conducted an observational study in a large metropolitan ED. Cases were taking warfarin and controls (matched for age and gender) were not. A researcher-administered questionnaire was used to collect data on CAM knowledge and use, bleeding/clotting events and INR levels during the previous six months.

Results: 246 cases and 246 controls were enrolled. Approximately 50% in each group knew the potential for CAM/prescription drug interaction. Only 60 (24.4%) cases reported having been warned about CAM use while on warfarin. Fewer cases than controls reported CAM use (28.9% versus 37.0%, p=0.07) and significantly fewer cases used a CAM known to interact with warfarin (11.4% versus 18.7%, p=0.03). Cases reported significantly more bleeding (p<0.01) and clotting events (p=0.03). Among cases, CAM users and non-users had similar numbers of events below and above their therapeutic INR range and where the INR exceeded 5.0. However, case CAM users had more abnormal bleeding events (42.3% versus 29.7%, p=0.06) but similar clotting events (8.5% versus 9.7%, p=0.76) compared to non-users. Cases who used CAM known to interact with warfarin had significantly more clotting events than cases who used CAM not known to interact (17.9% versus 2.3%, p=0.02).

Conclusions: Among patients taking warfarin, CAM knowledge is poor. Also, CAM use (including interacting CAM) is common and is associated with increased rates of important clinical adverse events.

011

PHARMACOECONOMIC EVALUATION OF MIDAZOLAM VERSUS DROPERIDOL IN THE MANAGEMENT OF ACUTE AGITATION IN THE EMERGENCY DEPARTMENT

E. Chan[1,2], J. Knott[3], D. Liew[4], D. Taylor[2], D. Kong[1]

[1]Department of Pharmacy Practice, Monash University, Parkville, VIC, Australia

[2]Emergency, Austin Health, Heidelberg, VIC, Australia

[3]Emergency, Melbourne Health, Parkville, VIC, Australia

[4]Clinical Pharmacology, St Vincent Health, Fitzroy, VIC, Australia

Aim: To conduct a pharmacoeconomic evaluation of intravenous midazolam versus droperidol for the management of acute agitation in emergency department (ED).

Methods: A decision-analytic model was developed with the clinical outcomes portrayed as successful and unsuccessful sedation at specific time points to sedation, and the redosing required after initial sedation was achieved. Adopting the perspective of the Australian hospital system , the main endpoint measure in this study was the median cost of the management of acute agitation in the ED. Clinical and probability inputs were prospectively derived from patients enrolled in a double-blind, randomised, controlled trial conducted at a major Australian metropolitan ED with approximately 50,000 emergency attendances annually. Cost inputs were obtained from the latest Australian sources. Sensitivity and uncertainty analyses were conducted.

Results: Seventy-nine and 74 patients were evaluated in the droperidol and midazolam groups, respectively. Higher sedation drug costs were incurred in the droperidol group (median AU$21.10 versus AU$3.05), however the midazolam group had marginally higher hospitalisation costs (AU$1370 versus AU$1361). Midazolam was associated with an overall median cost saving of AU$60 per patient (4.0%) over droperidol (AU$1555 versus AU$1496). The study results were robust against variations in all costs and probabilities data inputs. Uncertainty analysis indicated a 100% probability of midazolam having an economic advantage over droperidol.

Conclusions: Whilst there were no differences in the measured clinical effectiveness between droperidol and midazolam in the randomised clinical trial setting, from a cost perspective, midazolam has economic advantage over droperidol when used in the management of acute agitation in ED setting.

012

MULTI-SOURCED FEEDBACK IS ACCEPTABLE TO AND IMPROVES THE PERFORMANCE OF EMERGENCY PHYSICIANS

F. Kerr[1], V. Lee[1], D. Taylor[1,2]

[1]Emergency, Austin Health, Heidelberg, VIC, Australia
[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

Introduction: Credible, useful feedback is a corner-stone of professional development. We report our experience with a multi-sourced feedback tool used for appraisal and performance management of our ED consultants.

Methods: The project was undertaken in a large, tertiary referral ED. Survey Monkey was used to develop a web-based, anonymous tool that assessed approachability, teaching, professionalism, floor management, communication and clinical skills. Consultants were assessed by their peers, assistant nurse unit managers (ANUMs) and registrars. The ED Director reported to each consultant their assessment, in comparison with their peers. This report and number/mean length of stay of patients seen comprised part of a formal yearly appraisal. Consultants were also surveyed regarding their opinions on the multi-sourced feedback.

Results: Twenty four consultants were assessed three times over a two-year period. Their mean 'overall performance' scores increased significantly over time (3.46, 3.57 and 3.61, respectively, p=0.03). Consultants scored highest in clinical skills and approachability and lowest in floor management and teaching. Eighteen (90%) of 20 consultants responded to the survey. Fifteen (83.3%) agreed that multi-sourced feedback is a useful tool. All (100%) agreed that consultants, registrars and ANUMs should be providing feedback. After experience with the assessment, 15 (83.3%) reported feeling less or not at all uncomfortable with it. Fourteen (77.8%) agreed that the feedback led to a better awareness of their practice and eight (44.4%) agreed that it had changed their practice.

Conclusion: Web-based multi-sourced feedback is a constructive tool that is acceptable, increases performance and identifies areas for improvement.

013

MANAGEMENT OF ACUTE AGITATION IN AUSTRALASIAN EMERGENCY DEPARTMENTS: VARIATIONS IN PRACTICE

E. Chan[1,2], D. Taylor[2], J. Knott[3], D. Kong[1]

[1]Department of Pharmacy Practice, Monash University, Parkville, VIC, Australia
[2]Emergency, Austin Health, Heidelberg, VIC, Australia
[3]Emergency, Melbourne Health, Parkville, VIC, Australia

Aim: We aimed to describe current prescribing practice of emergency medicine clinicians in the management of highly agitated patients and to identify perceived barriers and gaps in training.

Methods: We undertook an anonymous cross-sectional mail survey of ACEM members between June and September, 2009. A questionnaire including a case vignette of a hypothetical patient and three clinical scenarios was employed.

Results: Of 2045 members surveyed 786 (38.4%) responses were received. Where no history was available, midazolam was the preferred monotherapy for 80.0% of respondents, followed by haloperidol (5.7%) and olanzapine (4.9%). Most respondents (500, 63.9%) would also administer another sedative (combination therapy). The most common additional agents were haloperidol (238, 47.6%) olanzapine (100, 20.0%) and droperidol (82, 16.4%). The IV route was preferred for most drugs. The main reasons for using a combination were the differing mechanisms of action (437, 87.4%) and lower dosage requirements (289, 57.8%).

Reported barriers to management included a lack training (352, 44.9%) and national clinical guideline (313, 40.0%). Respondents were generally confident in all aspects of management, though relatively fewer trainees were confident in determining dosing. Institutional guidelines were considered most useful for 415 (415/783, 53.0%) respondents. If an ACEM endorsed guideline were to be developed in the future, 634 (81.0%) respondents would consider this useful.

Conclusions: There is considerable variation in the management of acute agitation in Australasian ED. Benzodiazepines and antipsychotics, either alone or in combination are commonly used. An ACEM endorsed guideline is recommended.

014

NATURE AND OUTCOMES OF CHEMICAL EYE EXPOSURES REPORTED TO THE VICTORIAN POISONS INFORMATION CENTRE

L. Sher[1], D. Taylor[1,3], J. Robinson[2], J. Browning[2], M. Colbridge[2], D. MacLeod[2], H. McCracken[2], C. McKenzie[2]

[1]Emergency, Austin Health, Heidelberg, Australia
[2]Emergency, Victorian Poisons Information Centre, Heidelberg, VIC, Australia
[3]Medicine, University of Melbourne, Melbourne, VIC, Australia

Introduction: We aimed to determine the nature, cause, management, outcomes and advice compliance of chemical eye exposures.

Methods: This was a prospective case-series comprising consecutive calls to the Victorian Poisons Information Centre relating to chemical eye exposures (January 2009 - January 2010). Data included demographics, place and cause of exposure, nature of the chemical, symptoms and advice given. 48 hours later, patients were telephoned to determine outcome and whether the advice had been taken.

Results: 1480 patients were enrolled (45.7% aged ≤15 years) with 937 (63.3%) followed up. Cleaning agents (32.6%), topical personal products (25.4%), industrial agents (11.8%), herbicides/pesticides (5.7%), petroleum products (4.2%) and miscellaneous agents (20.3%) comprised the exposure groups. Adult males and females were exposed to significantly more industrial agents (74.8% versus 25.2%) and topical personal agents (31.3% versus 68.7%), respectively ($p<0.001$). Children and adults were exposed to significantly more topical personal agents (65.2% versus 34.8%) and industrial agents (28.7% versus 71.3%), respectively ($p<0.001$). The median time between exposure and the call for advice was significantly shorter for children ($p<0.001$). 810 (54.7%) patients were advised that medical care was not required. The remainder were advised to seek care or were already receiving care. At follow up, only 63 (6.7%) patients were symptomatic. 850 (90.8%) had complied with the advice given. There were no compliance differences between adult males/females and children/adults ($p>0.05$).

Conclusions: Most exposures are of little consequence. However, there are clear epidemiological differences between gender and age groups. These findings will help inform prevention strategies.

015

TROPISETRON IS SUPERIOR TO METOCLOPRAMIDE FOR TREATMENT OF NAUSEA AND VOMITING IN THE EMERGENCY DEPARTMENT

J. Chae[1,2], D. Taylor[1,2], A. Frauman[1,2]

[1]Emergency, Austin Health, Heidelberg, VIC, Australia
[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

Introduction: The relative efficacy of tropisetron and metoclopramide in treating nausea/vomiting in the ED setting has not been reported. We compared these agents among undifferentiated ED patients.

Methods and Materials: We undertook a double-blind, randomised, controlled trial in a single ED. Adult patients requiring treatment for nausea/vomiting were enrolled. Each was randomly assigned to either tropisetron (5mg) or metoclopramide (10mg), intravenously. Primary endpoints were incidence of vomiting and decrease in nausea score from baseline (0-100 visual analogue scale) . Secondary endpoints were the requirement of 'rescue' anti-emetics, ongoing nausea over 48 hours and side effects.

Results: Fifty patients were enrolled in each group. The demographic variables, presenting complaint and nausea scores at baseline did not differ ($p>0.05$). By 180 minutes from baseline, there had been two and 20 episodes of vomiting in the tropisetron and metoclopramide groups, respectively (rate difference 0.14 episodes/person-hour [95%CI 0.07, 0.21], $p<0.001$). At every 30-minute interval, the decrease in nausea score from baseline was greater (and increasing) in the tropisetron group. At 120 minutes, the decreases were 44.4mm and 33.7mm, respectively (difference 10.7mm [95%CI -0.3, 21.7], $p=0.07$). Five (10%) and 13 (26%) patients required a rescue anti-emetic, respectively (difference 16% [95%CI -0.7, 32.7], $p=0.07$). Of patients followed up, 13/47 (27.7%) and 20/49 (40.8%) had ongoing nausea, respectively (difference 13.2% [95%CI -7.7, 34.0], $p=0.25$). The tropisetron group had significantly less akathisia ($p<0.05$).

Conclusions: All endpoints trend towards tropisetron as the superior agent. Further research, including cost-effectiveness studies, should clarify its place in the ED.

016

HOW CRITICAL CARE NURSES REPORT THEY ADMINISTER, MONITOR AND MANAGE OXYGEN THERAPY: A SURVEY

G. M. Eastwood[1], M. C. Reade[1], L. Peck[1], I. Baldwin[1], J. Considine[2], R. Bellomo[1]

[1]Intensive Care Unit, Austin Health, Heidelberg, VIC, Australia

[2]School of Nursing, Deakin University-Northern Health Clinical Partnership, Burwood, VIC, Australia

Background: Critical care nurses frequently and independently manage oxygen therapy. Despite the importance of oxygen therapy, there is limited evidence to inform critical care nurses' oxygen therapy practices.

Aim: We sought to establish if there is variability in self-reported oxygen therapy practices of critical care nurses and if so, examine the degree of variability.

Method: An on-line questionnaire of ACCCN members between April and June 2010 was conducted.

Results: The response rate was 36% (542/1523 critical care nurses). Overall, 378 (70%) respondents practiced in metropolitan critical care units; 278 (51%) had≥14 years of specialty practice. In response to falling SpO_2, 8.9% of nurses would never escalate oxygen therapy without a doctor's request, and 51% of nurses would not routinely escalate oxygen therapy in the absence of medical orders. Only 56% of nurses reported always increasing FiO_2 prior to endotracheal suctioning. In mechanically ventilated patients, 33% of nurses believed oxygen toxicity was a greater threat to lung injury than barotrauma. Independent of FiO_2, a stable SpO_2 of 90% would not raise a concern for 61% of respondents. Nurses in rural critical care units were less likely to independently titrate oxygen to their own target SpO_2, but more likely to independently treat a falling SpO_2 with higher FiO_2.

Conclusion: Critical care nurses varied in their attitude toward the risks and management of oxygen therapy. Identifying factors that influence critical care nurses' practice is a necessary prelude to observational and interventional studies aimed at improving oxygen therapy for critically ill patients.

017

OXYGEN ADMINISTRATION AND MONITORING FOR WARD PATIENTS IN A TEACHING HOSPITAL

G. M. Eastwood, L. Peck, H. Young, J. Prowle, D. Jones, R. Bellomo

Intensive Care Unit, Austin Health, Heidelberg, VIC, Australia

Background: Oxygen is commonly administered to hospitalised patients with cardio-respiratory disease, as well as during the peri-operative period. Despite widespread oxygen delivery to hospitalised patients, relatively little is known about how oxygen is routinely delivered and monitored.

Aim: To describe oxygen administration and respiratory monitoring of ward patients in a tertiary teaching hospital. To assess differences in characteristics and outcomes between patients who are versus are not-receiving oxygen.

Method: Prospective clinical audit of all non-ventilated adult ward patients in a tertiary teaching hospital in Melbourne, Victoria on 26th August 2009.

Results: All 323 eligible patients were audited (medical 218, surgical 105). At assessment, 76 patients (24%) were on oxygen therapy and of these, 57 patients (74%) received oxygen by nasal prongs. Overall, oxygen saturation was documented in 301 (93.2%) patients and RR documented in 283 patients (87.6%). Patients receiving oxygen had a lower median SpO_2 (94% vs. 96%, $p < 0.0001$), higher median RR (20/min vs. 18/min, $p < 0.0005$); and were older (68.8 v 63.1 years, $p = 0.0094$). The in-hospital mortality of patients receiving oxygen therapy was 15.8% compared with 5.3% for those not on oxygen ($p < 0.0056$).

Conclusion: Oxygen is administered to one-quarter of ward patients in our hospital. Oxygen saturation and RR are not documented in approximately 10% of patients. Oxygen therapy in ward patients identifies individuals with increased mortality. Continuing educational interventions to increase awareness of the high risk status of these patients and strategies to detect patients at risk of hypoxaemia are needed.

018

THE SAFETY AND FEASIBILITY OF EXERCISE REHABILITATION IN ICU

S. Berney[1], K. Haines[1], S. Warrillow[2], L. Denehy[3]

[1]Physiotherapy, Austin Health, Melbourne, VIC, Australia
[2]Intensive Care, Austin Health, Melbourne, VIC, Australia
[3]Physiotherapy, The University of Melbourne, Melbourne, VIC, Australia

Background: A critical care admission particularly for prolonged periods can result in the development of clinically significant weakness that can last for up to 5 years following ICU discharge. There is increasing interest in the role of exercise in ICU and its role in attenuating weakness. The aim of this study was to report the safety and feasibility of providing a structured exercise programme in critically ill patients.

Method: As part of a prospective randomised controlled trial patients were screened at day 5 for suitability for inclusion. Participants in the exercise arm of the study, who were considered safe to exercise according to predefined criteria, commenced a standardised protocol. The intensity of the programme was based on the results of an initial exercise test so that the subject completed 15 minutes of exercise twice daily. Adverse events were defined *a priori*.

Results: 74 patients completed an exercise programme. Out of a total of 699 exercise sessions, intervention was delivered on 590 occasions (84%) with the remaining 109 (16%) of patients declining to participate. A further 286 sessions were not completed due to patients temporarily not meeting safety criteria to exercise. A further 81 sessions were missed due to patients being unavailable. No adverse events occurred.

Discussion: The results show that exercise is both safe and feasible in critically ill patients provided that safety criteria are defined.

019

IMPLEMENTATION AND EVALUATION OF A DIRECT OBSERVATION TOOL FOR EMERGENCY DEPARTMENT TRAINEES

V. Lee[1], J. Brown[1], D. Taylor[1,2]

[1]Emergency, Austin Health, Heidelberg, VIC, Australia
[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

Introduction: The use of direct observation tools that assess ED trainee workplace performance is infrequent. We aimed to implement and evaluate the impact of a direct observation tool for our trainees. The rationale for its implementation was to promote feedback and learning.

Methods: This was a descriptive study of a modified version of the mini-Clinical Evaluation Exercise (mini-CEX) tool undertaken in a large, metropolitan ED. It involved a consultant directly observing a trainee at work for 2-3 hours, rating clinical and non-clinical competencies then providing immediate feedback of their strengths and areas for improvement. To evaluate the tool, a quantitative and qualitative survey of trainees was conducted five months after commencement of the program, using 5-point Likert scale and open-ended responses. These were reported as numbers who agreed/disagreed and as common themes identified, respectively.

Results: Ten ED trainees participated in the direct observation program and all were surveyed (response rate 100%). Nine disagreed that it took too much time away from their clinical work and all agreed that it gave them insight into their workplace performance. Eight agreed the feedback received was consistent and all agreed it was excellent. The provision of feedback had a positive impact on their work and was a strength of the program. Feeling self-conscious had a negative impact on their work.

Conclusions: Our direct observation program had little impact on work, gave considerable insight into workplace performance and provided valuable feedback. It is recommended as a useful workplace learning tool.

020

DOES EXECUTIVE IMPAIRMENT DEFINE A FRONTAL VARIANT OF ALZHEIMER'S DIESASE?

M. Woodward, H. Brodaty, K. Boundy, D. Ames

Aged Care, Austin Health, Heidelberg West, VIC, Australia

Background : This study was performed to improve the differential diagnosis between AD and FTLD and to better characterize the AD subgroup with greater executive dysfunction.

Methods: We nominated a frontal variant of AD (FvAD) group as those AD subjects with the lowest quartile of scores on the Frontal Assessment Battery (FAB), indicating greatest executive dysfunction, and compared them with the rest of the AD cases and those with FTLD across several baseline and follow up variables including cognitive, functional and behavioural scales.

Results: The 114 FvAD subjects had a mean age of 78.1 and MMSE 16.6, and the (remaining) AD group had a mean age of 78.4 and MMSE 22.4. There were 30 FTLD subjects with a mean age at baseline of 70.9 and a mean baseline MMSE of 23.4. The FvAD group was significantly more severely impaired than the other two groups on most baseline assessments. In an analysis of subjects matched at baseline for functional impairment, the FvAD and FTLD groups were not significantly different on most assessment scales. All three unmatched and matched groups declined similarly over 12 months.

Conclusions: The FAB defined a group of AD subjects with greater executive dysfunction that were distinguished from both the remainder of the AD and FTLD subjects in almost all domains except behavioural disturbance and probably were just more severely affected AD subjects. The FAB is thus more useful as a marker of dementia severity than *as* a scale to detect a frontal variant of AD or to distinguish AD from FTLD, in the initial stages of patient assessment. It is proposed that subjects with dementia presenting with greater executive impairment but without prominent behavioural symptoms are likely to have AD rather than FTLD, especially if they are quite functionally impaired. With time FTLD subjects develop increasing executive dysfunction and increasingly resemble the more severely affected AD subjects.

021

DETERMINATION OF LOCAL FIBRE CONFIGURATION USING BAYESIAN NEIGHBOURHOOD TRACT MODELING

T. G. Close[1,2,4], J. D. Tournier[1,3], F. Calamante[1,3], L. A. Johnston[2,4,5], I. Mareels[2,4], A. Connelly[1,3]

[1]Brain Research Institute, Florey Neuroscience Institutes (Austin), Heidelberg West, VIC, Australia
[2]Electrical and Electronic Engineering, University of Melbourne, Parkville, VIC, Australia
[3]Medicine, Nursing and Dentistry, University of Melbourne, Parkville, VIC, Australia
[4]National ICT Australia, Parkville, VIC, Australia
[5]Howard Florey Institute, Florey Neuroscience Institutes (Parkville), Parkville, VIC, Australia

Current state of the art methods for tractography typically operate by propagating a track based on the local fibre orientation information at any given point, independently of any other generated tracks. Such methods do not make use of additional information that might be available from fitting all tracts concurrently, which would allow the probability of one tract to be affected by the presence of another. Recent studies have used this type of information to improve the estimate of the local fibre configuration. King et al. [1] used a Bayesian random effects modelling approach to improve fibre orientation estimates in areas of complex tract crossings, based on the estimates of adjoining voxels. In contrast, Savadjiev et al. [2] used helical curve approximations to decide between, single fibre, crossing, fanning or bending models in each imaging voxel.

In this study we introduce a richer local fibre configuration model, which represents multiple tract segments explicitly by 3D curves with associated volumes.

The resulting local neighbourhood representations incorporate not only fibre orientation estimates but also estimates of the tract segment curvature and sub-voxel positioning.

1. King, MD et al. 2009. NeuroImage, 44, 753.

2. Savadjiev, P et al.2008 NeuroImage, 41 58

022

VALIDATING A LOCAL AIF METHOD FOR IMPROVED PERFUSION QUANTIFICATION IN STROKE

L. Willats[1], S. Christensen[2], H. Ma[3], G. Donnan[4,5], A. Connelly[1,5], F. Calamante[1,5]

[1]Brain Research Institute, Florey Neuroscience Institutes (Austin), Australia
[2]Department of Radiology, Royal Melbourne Hospital, Australia
[3]National Stroke Research Institute, Florey Neuroscience Institutes (Austin), Australia
[4]Florey Neuroscience Institutes, Australia
[5]Department of Medicine, University of Melbourne, Australia

In bolus tracking MRI, temporal dispersion of the contrast bolus due to arterial abnormality presents a significant problem for accurate perfusion quantification in stroke. One means to reduce the associated perfusion errors is to deconvolve the bolus concentration time-course (CTC) data with local Arterial Input Functions (AIF) measured close to the capillary bed and down stream of the arterial abnormalities causing dispersion. Because the MRI voxel resolution precludes direct local AIF measurements, they must be extrapolated from the surrounding data.

To date there have been no published studies directly validating these assumed local AIFs, which are commonly located using heuristic criteria, with associated territories assigned using interpolation and smoothing methods. We perform a validation of a novel local AIF method by measuring the residual dispersion remaining in the deconvolved perfusion maps, using our previously presented MLEM deconvolution algorithm (Willats 2008, NMR Biomed. 21:1126).

The novel local AIF approach is based on a previously presented method (Christensen 2007, ISMRM Proc. p591) that automatically locates the local AIF voxels as local minima in a bolus arrival time map, and assigns their small branch arterial territories according the temporal and spatial similarities with the tissue CTCs. The novel method also accounts for variations in perfusion, which can cause erroneous local AIF voxels to be located in transition regions between normal and abnormally perfused tissue. Validations of the original (Christensen) and novel local AIF methods were compared with a validation of the standard global AIF perfusion analysis across 20 sub-acute stroke patients.

The novel local AIF method was the most effective in reducing dispersion suggesting more accurate perfusion quantification. Importantly, the validation identifies significant areas for perfusion underestimation using the global AIF. This is valuable information for the identification of at-risk tissue and management of stroke patients.

023

ASSESSMENT OF AB DEPOSITION IN MILD COGNITIVE IMPAIRMENT WITH 18F-FLORBETABEN

K. T. Ong[1,4], V. L. Villemagne[1,3,4], A. Bahar-Fuchs[1], N. Langdon[1], G. Holl[2], C. B. Reininger[2], B. Putz[2], G. Jones[1], R. S. Mulligan[1], S. Pejoska[1,4], B. Rohde[2], C. L. Masters[3,4], C. C. Rowe[1,4]

[1]Nuclear Medicine Department, Austin Health, Heidelberg, VIC, Australia
[2]Bayer Schering Pharma, Berlin, Germany
[3]The Mental Health Research Institute, Melbourne, VIC, Australia
[4]Department of Medicine, University of Melbourne, Melbourne, VIC, Australia

Background: In vivo ß-amyloid (Aß) imaging with PET allows earlier and more accurate diagnosis of Alzheimer's Disease (AD) and helps identify Aß neuropathology in subjects with mild cognitive impairment (MCI).[1-4] The 18F-Florbetaben has shown high affinity for Aß plaques in vitro [5] and was able to distinguish AD from healthy controls (HC) and frontotemporal dementia. [6]

Objectives: Determine the predictive accuracy of Florbetaben for progression of MCI to AD

Correlate the extent of Aß burden to the degree of cognitive impairment

Compare Florbetaben binding between HC, MCI and AD, as well as MCI subtypes

Compare visual reading versus quantitative assessment of Aß burden

Methods: 26 HC, 45 MCI and 26 AD subjects underwent 18F-Florbetaben PET studies. Aß burden was assessed using Standardized Uptake Value normalised to cerebellar cortex (SUVR) at 90-110 minutes post injection.[6] Cognitive scores were correlated to the PET results.

Results: Tracer retention was significantly higher in ADs than HCs (neocortical SUVR 1.95±0.3 vs 1.28±0.2 respectively). About 50% of the MCIs presented an "AD-like" retention pattern, whilst the rest resemble a "HC-like" retention pattern. The average neocortical SUVR for this group was 1.46±0.4. Neocortical SUVRs correlated with composite episodic memory score (r=-0.52, p=0.0003) and MMSE (r=-0.41, p=0.005) in MCI but not in HC or AD. Preliminary evaluation shows that the accuracy of Florbetaben to predict MCI conversion to AD is 71%.

Conclusions: 18F-Florbetaben PET appears valuable in assessing AD neuropathology in vivo, and has predictive value for conversion to AD in MCI. These results are similar to 11C-PiB PET studies. Histopathological verification of the extent of Aß will determine the sensitivity and specificity of visual and quantitative assessments of regional 18F-Florbetaben binding.

(1) Rowe, CC, Ng S, Ackerman U, et al. Imaging â-amyloid burden in aging and dementia. Neurology 2007;68:1718-1725.
(2) Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurology 2004,55:306-319.
(3) Pike KE, Savage G, Villemagne VL, et al. Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. Brain 2007;130:2837-2844.
(4) Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurology 2007;6:734-746.
(5) Zhang W, Oya S, Kung MP, et al. F-18 stillbenes as PET imaging agents for detecting beta-amyloid plaques in the brain. J Med Chem 2005;48:5980-5988.
(6) Rowe CC, Ackerman U, Browne W, et al. Imaging amyloid â in Alzheimer's disease with 18F-BAY94-9172, a novel PET tracer: proof of mechanism. Lancet Neurology 2008;7:129-35.

024

IMPROVED PROBABILISTIC STREAMLINES TRACTOGRAPHY BY 2ND ORDER INTEGRATION OVER FIBRE ORIENTATION DISTRIBUTIONS

J. Tournier, F. Calamante, A. Connelly

Brain Research Institute, Florey Neuroscience Institutes (Austin), VIC, Australia

The probabilistic streamlines [1] approach is one of the most promising methods to perform diffusion-weighted tractography. However, current implementations work by stepping along the locally estimated white matter orientation; such first order integration methods are known to 'overshoot' [2]. In this study, we propose a novel higher order probabilistic streamlines method, based on 2nd order integration over fibre orientation distributions (iFOD2).

Rather than taking straight-line steps, this algorithm steps along an arc of a circle, locally tangent to the current direction of tracking. As for current methods, the actual path selected for each step is obtained by sampling a probability density function (PDF), but rather than sampling from the local FOD only, in iFOD2 the probability of each path is calculated as the product of the probabilities of each infinitesimal step making up that path. This allows the algorithm to select the most appropriate path in curving or crossing regions, as its tangent needs to match the FOD at each point along its length, rather than at the first point only.

The iFOD2 algorithm was compared to the equivalent first-order algorithm (iFOD1) using simulations, and real data acquired from a volunteer, with FODs estimated using Constrained Spherical Deconvolution [3] as implemented in MRtrix [4]. Simulations demonstrate the ability of iFOD2 to track through highly curved regions with no bias, whereas iFOD1 demonstrates a clear overshoot, which is greater using large step sizes. iFOD2 also correctly tracks through crossing fibre regions, whereas iFOD1 tends to switch over to the crossing fibre direction, especially using small step sizes. In vivo results correspond well with known anatomy, with excellent delineation of the highly curved subcortical U-fibres and the extensive crossing fibres in the centrum semiovale.

(1) Behrens et al., MRM 50:1077-88 (2003)
(2) Tournier et al., MRM 47:701-8 (2002)
(3) Tournier et al., NeuroImage 35:1459-72 (2007)
(4) MRtrix, http://www.brain.org.au/software/

025

AN OBJECTIVE APPROACH TO ASSESSMENT OF LANGUAGE LATERALISATION USING FUNCTIONAL MAGNETIC RESONANCE IMAGING

D. F. Abbott[1,2], A. B. Waites[1,2], G. D. Jackson[1,2]

[1]Brain Research Institute, Florey Neuroscience Institutes, Heidelberg West, Australia
[2]Department of Medicine, The University of Melbourne, Melbourne, VIC, Australia

Background and Aim: Language lateralisation based on functional magnetic resonance imaging (fMRI) is often used in clinical neurological settings. With most conventional methods, the laterality determined can be dependent on the quality of a particular study and chosen statistical threshold. We aimed to develop an objective threshold-independent method of assessing when individual patients have statistically atypical language lateralisation.

Methods: The method examines images over a full range of statistical thresholds. We calculated for an individual the distribution of laterality index (LI) as a function of two threshold types: t-threshold and number of voxels above threshold. We calculated LI curves for thirty healthy controls and determined which of the threshold types provided curves of least variance across subjects. Using those curves, we then determined whether individuals had atypical laterality by statistically comparing their LI-distribution curve to the group of control LI-distributions. We illustrate the method using fMRI of verbal fluency in healthy controls and patients with epilepsy.

Results: Curves of laterality index plotted as a function of number of voxels above threshold had the least variance across subjects. The method was successfully able to indentify subjects with atypical language lateralisation.

Conclusions: The method provides a robust and objective indication of atypical lateralisation, displaying more comprehensive information than conventional methods. Combined with images displaying a consistent number of active voxels within language regions, interpretation of brain function in the presence of disease is more straightforward.

026

GAIT SYMMETRY IN SCHOOL-AGED CHILDREN AND YOUNG ADULTS WHILST WALKING AT SLOW, NORMAL AND FAST SPEEDS

N. Lythgo[1], C. Wilson[2], M. Galea[1]

[1]RSRC/Austin Health, Kew, VIC, Australia

[2]Australian Catholic University, VIC, Australia

Introduction: Limited knowledge exists about the gait symmetry of school-aged children. This investigation recorded normative gait symmetry data from a large sample of healthy school-aged children and young adults whilst walking with shoes at slow, preferred and fast speed and assessed whether gait symmetry was affected by age.

Methods: A sample of 737 healthy children (5 to 13 yrs) and 82 young adults (19.6 ± 1.6 yrs) participated. The children were tested in schools, whereas the young adults were tested in a gait laboratory. Participants completed 6 to 8 walks across a GAITRite mat (80 Hz) at self-selected slow, normal and fast speeds. Footwear worn was athletic-type shoes. Gait parameters extracted were step and stride length, single support, double support, step and swing time. Symmetry measures were calculated by; Symmetry = ÷ Right Measure - Left Measure ç . Temporal measures were expressed relative to the gait cycle (%). Pearson's product moment correlation was used to examine the effect of age (SPSS).

Results and discussion: Symmetry was found to be unaffected by age. On average (combining conditions), measures of step and stride symmetry were 1.2 cm and 0.9 cm respectively. Symmetry measures for step, swing, single and double support times fell below 0.9%.

Conclusions: Symmetry was found to be remarkably invariant across age. Step and stride differentials fell around 1.1 cm whereas temporal differentials fell around 0.9%. This shows that gait is highly symmetrical in healthy children and young adults.

Acknowledgements: Funding was granted by the Faculty of Medicine, Health Sciences and Dentistry, University of Melbourne.

027

STRUCTURAL DEFICIT IN CHILDREN WITH FOREARM FRACTURE

X. Xu, S. Iuliano-Burns, A. G.H. Zadeh, Q. Wang, E. Seeman

Department of Medicine, University of Melbourne, Heidelberg Repatriation Hospital, Australia

Forearm fractures are common in children and associated with reduced bone mineral density (BMD). Its highest incidence coincides with the pubertal growth spurt, when there is a transient reduction in volumetric BMD (vBMD) and cortical thickness at distal radius. These observations lead us to hypothesize that bone structure deficits, especially thin cortical thickness, present in children with forearm fracture.

We recruited 20 children with forearm fracture occurred during play or sports and scanned their distal metaphyses of radius and tibia using high resolution peripheral quantitative computed tomography within 2 months post-fracture. Each fracture case was matched by 2-4 control children of similar age and sex.

Compared to matched controls, total vBMD of distal radius was 14% lower in fracture cases (234 ∓ 31 vs. 272 ∓ 49 mg HA/cm^3, $p < 0.001$) due to their 50% thinner cortices (0.22 ∓ 0.09 vs. 0.45 ∓ 0.20 mm, $p < 0.001$). There was no difference in trabecular bone volume fraction (BV/TV) between groups. However, their trabecular architecture was fashioned differently – fracture cases had thicker (81 ∓ 7 vs. 69 ∓ 10 μm, $p < 0.001$) but less (1.71 ∓ 0.20 vs. 2.06 ∓ 0.26/mm, $p < 0.001$) trabeculae than controls.

In commensurate with the observation at the distal radius, fracture cases also had thinner cortices and differently fashioned trabecular architecture relative to controls. We conclude that deficit in cortical thickness partly contributes to the risk of forearm fractures during puberty, while the significance of trabecular architecture needs further investigation.

028

BIOMECHANICAL EVALUATION OF TRANS-TIBIAL AMPUTEE'S SOCKET FABRICATED USING THE PRESSURE CAST (PCAST) TECHNIQUE

P. Lee[1], N. Lythgo[2], H. Connor[3], J. Lavranos[4], D. Oetomo[1]

[1]Mech Eng, University of Melbourne, Melbourne, VIC, Australia
[2]RSRC, VIC, Australia
[3]Physiotherapy, Austin Health, VIC, Australia
[4]Prosthetics Orthotics, Royal Melbourne, VIC, Australia

Introduction: Current prosthetic socket fabrication is expensive, labour intensive and overly reliant on a prosthetist's skill level. A pressure cast technique (PCAST) has been developed by our group so as to reduce these factors. The method involves an amputee placing their stump in a cylindrical water pressure chamber. Water pressure is applied to the stump until the amputee stands in a full weight bearing position. This produces a socket shape with minimal rectification. We compared the pressure distribution at the stump socket interface in amputees wearing the PCAST socket and the traditional hand cast patellar tendon bearing (PTB) socket.

Method: Three unilateral trans-tibial amputees participated. The subjects were prescribed and fitted with a PCAST and PTB socket. After a 2 -week familiarization period, walking trials were performed at self-selected speed along a 10m walkway with the PCAST and PTB. In addition to straight walks, subjects performed 30^0 left/right turns and sudden stops. Stump-socket interface pressures were measured during the walking trials by a VersaTek (Tekscan Inc, USA) pressure measurement system. The VersaTek sensors are thin (< 0.2mm) and flexible which allows them to be easily inserted between the stump and the socket without any modification to the prosthesis.

Results: The pressure and counter pressure developed at the socket walls for the walking conditions (straight, turns, stops) were similar for the PCAST and PTB socket. A more evenly distributed pressure profile, associated with a reduction in localized pressure, was found with the PCAST. This could lead to more comfortable artificial limbs.

029

ODC1 AND TCEAL7 ARE POTENTIAL MEDIATORS OF ANDROGEN ACTIONS IN SKELETAL MUSCLE

N. K.L. Lee, J. D. Zajac, H. E. MacLean

Medicine, University of Melbourne, Heidelberg, VIC, Australia

We aim to identify target genes mediating androgen actions in muscle, using our global androgen receptor knockout (ARKO) mice, which have a 20%↓ in muscle mass[1]. Two genes with altered expression in ARKO (and muscle-specific ARKO) muscle are *Odc1* (2.6-fold↑) and *Tceal7* (2.4-fold ↓). *Odc1* encodes ornithine decarboxylase, a regulator of proliferation[2], and *Tceal7*, an ovarian tumor suppressor[3]. We are now investigating the expression patterns and function of these genes in muscle *in vivo* and *in vitro*.

We determined the expression of *Odc1* and *Tceal7* in muscle of pre-pubertal (4 weeks old) and adult (12 weeks old), male and female mice (n=5-12/group). *Odc1* expression was 4-fold higher in adult versus pre-pubertal males ($p<0.05$) and higher in adult males than females ($p<0.05$). *Tceal7* expression was 5-fold lower in adult versus pre-pubertal males ($p<0.01$). In a human skeletal muscle cell line (n=6-10/group), *Odc1* expression was high in myoblasts, and decreased ~90% ($p<0.05$) in myotubes. *Tceal7* expression was low in myoblasts and high in myotubes. A similar result was observed in C2C12 mouse myoblasts and myotubes. To investigate *Odc1* function, C2C12 myoblasts were stably transfected with an *Odc1* overexpression vector or treated with an Odc1 inhibitor, *α* -difluoromethylornithine (DFMO). Cell proliferation was prolonged in transfected myoblasts ($p<0.05$). DFMO treatment inhibited myoblast proliferation by ~60% at 72hr ($p<0.001$).

Our data demonstrate that *Odc1* and *Tceal7* are regulated by androgens and the AR in adult muscle. *Odc1* is essential for muscle cell proliferation, suggesting androgens may act in part to regulate cell proliferation to increase muscle mass.

(1) HE MacLean et al. FASEB J. 22: 2676, 2008
(2) JA Nilsson et al. Cancer Cell 7: 433, 2005.
(3) J Chien et al. Oncogene 24: 5089, 2005

030

REDUCED MECHANICAL LOADING RESULTS IN DEFICITS IN CORTICAL BUT NOT TRABECULAR BONE STRUCTURE: A STUDY OF CHILDREN WITH LEGG-CALVE PERTHES DISEASE

S. Iuliano-Burns[1], S. Macleod[1], K. King[1], A. Ghasem Zadeh[1], R. Zebaze[1], I. Torode[2], E. Seeman[1]

[1]Endocrinology, University of Melbourne / Austin Health, West Heidelberg, Australia
[2]Orthopaedics, Royal Children's Hospital, Parkville, VIC, Australia

Bone size and cortical area is greater in the playing, than non-playing arm of tennis players, especially in those who commenced playing before puberty. There is little data assessing the structural basis underlying altered weight bearing in children.

We compared the side-to-side differences in tibial bone structure in children with unilateral Legg-Calve Perthes Disease (limited unilateral weight bearing) to test the hypothesis that relatively increased loading on the unaffected side increases total bone CSA and cortical area, while the affected side will have a smaller total CSA and cortical area relative to controls.

We compared distal tibiae architecture using HR-pQCT in 27 cases (n = 21 male, 11.1 ± 0.5yrs, 68% pre-pubertal, mean disease duration 1.4 ± 0.3yrs) and 27 controls (n = 21 males, 11.6 ± 0.6yrs). In cases, cortical area (8 ± 4%, $p<0.05$) and CSMI (4 ± 3%, $p<0.05$) were lower on the affected than unaffected side. The side-to-side difference for cortical area in cases was greater than controls (8 ± 4% v –2 ± 2%, $p<0.05$). In pre-pubertal cases, there was a trend for reduced cortical area of the affected limb compared to the same side in controls after adjusting for multiple comparisons (53.9 ± 5.1 v 64.6 ± 1.8 mm^2, $p<0.05$).

Unilateral loading did not enhance bone size or cortical area but unloading was associated with reduced cortical area but not total cross-sectional area. Reduced loading on weight bearing bones compromises bone strength and increases fracture risk.

031

UTILISATION OF THE INTERNET AMONG PATIENTS WITH RHEUMATOID ARTHRITIS

J. M. Luong, R. R.C. Buchanan, S. K. Lam, L. Schachna

Rheumatology, Austin Health, Heidelberg, VIC, Australia

Aims: A recent roundtable discussion by Arthritis Victoria considered novel strategies to disseminate information via the internet. We examined utilisation of the internet among rheumatoid arthritis patients attending either a public or private rheumatology practice.

Methods: A self-reported questionnaire was completed by 124 patients with rheumatoid arthritis, about half from a public outpatient clinic and half from a neighbouring private rheumatology practice.

Results: The mean age of study participants was 62.4 ± 13.5 with disease duration 12.8 ± 13.0 years; 100 were female and 39 were in regular employment. Overall, 48.4% accessed the internet for any purpose comprising 56.9% of private and 39.0% of public patients ($p=0.046$). Patients using the internet were more likely to be younger (difference 12.4, 95CI, 8.1-16.8 years), report English as their first spoken language ($p=0.013$), have completed high school ($p=0.008$) and be in regular employment ($p<0.001$). In multivariate analysis, regular employment (OR 5.0, 95CI, 1.7-14.8), English as a first language (OR 3.9, 95CI, 1.0-15.0) and younger age (OR (per 10y) 1.7, 95CI, 1.2-11.2) were associated with internet use. Among respondents using the internet, 71.6% accessed health information; websites were universally identified using search engines. Although half would like their rheumatologist to recommend internet sites, only 13.5% were provided a list of sites by a health professional. Preferred formats of health information were text/articles (91%) followed by video/multimedia (13%) and discussion forums (9%). While 81.3% found information easy to obtain, only 30.9% reported greater sense of control over management of their disorder from internet access.

Conclusion: Younger and employed rheumatoid arthritis patients, in particular, seek increased medical information from the internet. Rheumatologists should to be ready to respond to this need.

032

COMPLETENESS OF REFERRAL DETAILS TO RHEUMATOLOGISTS FROM GENERAL PRACTICE

D. Apostolopoulos, J. M. Luong, S. K. Lam, R. R.C. Buchanan, L. Schachna

Rheumatology, Austin Health, Heidelberg, VIC, Australia

Aim: Increasing workload and waiting times compel more rheumatologists to rely on referral letters from general practitioners to prioritise the urgency for initial consultation. In this cross-sectional study, we examine the completeness of referral letters to public outpatient and private rheumatology practice.

Methods: New referral letters for rheumatology consultation to a public hospital clinic (n=90) and neighbouring private rheumatology practice (n=147) were evaluated over a three-month period. A priori, 19 essential clinical details for a satisfactory rheumatology referral were identified.

Results: Data most frequently appearing in referral letters were chief complaint (98.1%), regional symptoms (83.8%), medication list (81.4%), past medical history (80.5%) and management-to-date (56.7%). Physical findings, both regional (26.2%) and general (9.1%), were uncommonly provided. Referrals to private practice were more likely to provide past medical history (p=0.05), family history (p=0.01), list of allergies (p<0.001) and regional examination findings (p=0.02). Of the 19 clinical details examined, a mean of 10.0 ± 2.6 appeared in private compared with 9.2 ± 2.7 in public referrals (p=0.03). In multivariate analysis, private referrals were more likely to provide regional examination findings (odds ratio (OR) 2.2, 95CI, 1.0-4.8), family history (OR 3.8, 95CI, 0.8-17.6) and list of allergies (OR 4.1, 95CI, 2.0-8.4). Among referrals to public rheumatology practice, use of a pro forma hospital referral did not increase the number of clinical details provided (9.5 ± 2.8 versus 8.8 ± 2.5, p=0.28).

Conclusions: Referral letters to private rheumatology practice were more detailed than to public outpatient clinics. Pro forma hospital referral forms did not increase completeness of referral details.

033

LOWER LIMB JOINT POWERS ARE MODIFIED IN AGING GAIT

L. Cofré, N. Lythgo, D. Morgan, M. Galea

RSRC/Austin Health, VIC, Australia

Introduction: Why does gait change in apparently healthy older adults? Why do steps shorten? Is it due to a loss of joint power? There is some agreement that age reduces ankle power during walking but it is unclear as to how this affects hip and knee function. This study investigated the effect of aging on lower limb joint power during gait by controlling walking speed.

Method: The gait of six healthy OLD (66.8 ± 5.4 yr) and 6 healthy YG (26.0 ± 3.3 yr) adults was recorded at 1.0 ms^{-1}, 1.3 ms^{-1} and 1.6 ms^{-1}. Five trials were recorded for each speed (speed tolerance ± 5%). Exclusion criteria were any medical conditions that affected gait or failure to achieve a maximum score on the Short Physical Performance Battery. Gait was recorded by an 8-camera VICON Motion System and outcome measures were extracted by an "in-house" software program.

Results: Ankle joint power generation (A2) reduced with aging (23% less), whereas knee joint power generation (K3) and hip joint power generation by the hip extensors (H1) and flexors (H3) increased with aging; the OLD increased H3 by 12%. The results support the hypothesis of a shift in the "locus of function" or a redistribution of joint power in OLD. It shows that the redistribution of joint powers is not related to speed but is an effect of aging. These findings show that the hip flexors play an important role in propelling the leg when ankle plantar-flexor function is compromised by aging.

034

KNEE FLEXION PRECEDES INITIAL CONTACT IN HUMAN GAIT

D. McKenzie, N. Lythgo, M. Galea

RSRC/Austin Health, Australia

Introduction: Initial foot-ground contact (IC) is an important event in human gait. Studies, however, have largely ignored the role of knee flexion (KF) prior to IC. This study investigated KF around IC to ascertain its role in preparing the foot for contact with the ground.

Methods: Sixteen healthy young adults participated. A VICON system recorded gait data for 6 walking trials at slow, preferred and fast walking speed. Data extracted were: the timing of KF prior to IC, the amount of KF prior to IC and at IC, and foot speed (FS) at IC. Data were assessed for normality. Friedman's x^2r statistic and RM MANOVAs were used to investigate the effect of speed.

Results and discussion: KF data distributions were found to be non-normal. At slow, preferred and fast gait speeds, 2.2%, 2.6% and 3.5% of stride was taken up with KF prior to IC respectively. The amount of KF prior to IC for these speeds was 0.8 °, 1.4 ° and 2.3 ° respectively. FS data distributions were found to be normal. FS at IC was 391.2 mm $\cdot s^{-1}$ at slow speed, 559.7 mm s^{-1} at preferred speed and 841.3 mm $\cdot s^{-1}$ at fast speed. Significant increases in KF and FS were found with increasing gait speed ($p < 0.001$).

Conclusion: These findings show that KF precedes IC in human gait with the amount and duration dependent upon walking speed. The hypothesis that KF prior to IC prepares the foot for ground contact by reducing FS to near zero magnitude was not supported.

035

GAIT FUNCTION IN INCOMPLETE SPINAL CORD INJURY PATIENTS USING THE C.A.M.P BLUE-ROCKER, BLUE-ROCKER COMBO AND BIONESS L300 FES SYSTEM

C. Tsikos[1], N. Lythgo[2], L. Cofré[2]

[1]Prosthetics Orthotics, Austin Health, Kew, VIC, Australia
[2]RSRC/Austin Health, VIC, Australia

Introduction: Limited gait research has compared the function of the Carbon-fibre dynamic Ankle Foot Orthoses (AFOs) with the new generation of Functional Electrical Stimulation (FES) systems. Hausdorff and Ring (year) compared the Bioness L300 for footdrop with 'standard' AFOs (described as being plastic; solid ankle or hinged) and found improved gait function. This study was a pilot investigation to examine the effects of the C.A.M.P Blue-Rocker, Blue-Rocker Combo and the Bioness L300 foot drop system on the gait characteristics of patients with incomplete spinal cord injury.

Methods: Three patients were fitted. All patients had an incomplete spinal cord injury. Reflective markers were placed on the body. The patients completed five walks across a 10 m level walkway in a gait laboratory wearing no device (CONTROL), the Bioness L300 and the C.A.M.P Blue-Rocker or Blue-Rocker Combo. A GAITRite walkway and an 8-camera Motion Measurement System (VICON, Oxford Metrics, England) sampling at 200Hz recorded temporospatial gait characteristics. These included gait speed, step length, stride length, stance time (%GC), single support time (%GC), and peak joint angular motion of the pelvis, hip, knee and ankle. Tests were conducted at baseline, 4 and 8 weeks.

Results and discussion: Preliminary data analysis of two patients shows the Bioness L300, compared to the other devices, improved gait speed, cadence, stride length and stance time. The Blue-Rocker Combo and Blue-Rocker, however, provided superior knee stability and step length symmetry. These systems prevented knee hyperextension in mid- to late-stance.

036

EARLY-ONSET ABSENCE EPILEPSY: 10% OF CASES ARE CAUSED BY GLUT1 DEFICIENCY

K. M. Lawrence[1], T. Arsov[1], S. Mullen[1], J. Damiano[1], M. Nolan[3], M. A. McShane[2], S. F. Berkovic[1], I. E. Scheffer[1,4]

[1]Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Heidelberg West, VIC, Australia
[2]Paediatric Neurology, The John Radcliffe Children's Hospital, Oxford, United Kingdom
[3]Neurology Department, Starship Children's Hospital, Auckland, New Zealand
[4]Department of Paediatrics, Royal Children's Hospital, Parkville, VIC, Australia

GLUT1 (Glucose transporter 1, encoded by *SLC2A1* gene) is the primary glucose transporter across the blood brain barrier. Mutations in *SLC2A1* cause a spectrum of phenotypes including classical GLUT1 encephalopathy, paroxysmal exertional dyskinesia, haemolytic anaemia, hemiplegia of childhood and absence epilepsy beginning from early childhood to young adult life. Low fasting cerebrospinal fluid (CSF) glucose (<2.2 mmol/L) together with an abnormal fasting CSF/plasma glucose ratio (<0.45) have traditionally been considered the diagnostic hallmark of GLUT1 deficiency.

Our recent study showed that *SLC2A1* mutations are responsible for 10% (4/34) cases of early-onset absence epilepsy (EOAE) – a genetic generalised epilepsy syndrome characterised by typical absence seizures and onset under the age of 4 years.

Here, we present a replication study of a new cohort of 40 patients with EOAE screened for mutations in the coding region of *SLC2A1* by direct sequencing. We found 4 EOAE patients with *SLC2A1* mutations, confirming the 10% *SLC2A1* mutation rate in EOAE. Two of the mutations were *"de novo"* and two were inherited. They either affected a conserved amino acid residue or resulted in a significant amino acid change. The four patients with GLUT1 EOAE were clinically indistinguishable from typical EOAE and all but one had normal CSF glucose or CSF/plasma glucose ratio.

In conclusion, we confirm that 10% of EOAE is caused by *SLC2A1* mutations and we show that the traditional tests for GLUT1 deficiency are not reliable in detecting children with GLUT1 EOAE. These findings indicate that mutational analysis should now become the principal test for diagnosis of GLUT1 deficiency syndrome.

037

A NOVEL GENETIC OCCIPITAL EPILEPSY SYNDROME IN SIBLINGS SHOWING CLINICAL AND EEG-FMRI CONCORDANCE.

P. W. Carney[1,2,4], S. Harvey[3], S. F. Berkovic[1,4], G. D. Jackson[2,4], I. E. Scheffer[1]

[1]Epilepsy Research Centre, Department of Medicine, University of Melbourne, Heidelberg West, VIC, Australia

[2]Brain Research Institute, Florey Neuroscience Institutes, Heidelberg West, VIC, Australia

[3]Children's Neuroscience Centre, Royal Children's Hospital, Flemington, WA, Australia

[4]Neurosciences, Austin Health, Heidelberg, VIC, Australia

Purpose: We describe siblings with a novel occipital epilepsy syndrome and localize their interictal epileptiform activity using EEG with functional MRI (EEG-fMRI).

Methods: A sister and brother presenting with refractory occipital seizures were identified and detailed electro-clinical information obtained. Functional imaging was performed at 3 Tesla with EEG recorded during continuous acquisition of gradient-recalled echoplanar images

Results: Seizure began at 12 years in the girl and 10 years in her brother. Seizure semiology involved elementary visual phenomena or visual loss. In longer seizures, head and eye deviation and post-ictal headache may occur. Neither sibling experienced autonomic symptoms. They developed frequent refractory seizures (10 or more daily) despite multiple anti-epileptic drugs. The ketogenic diet substantially reduced seizure frequency in the 17 year old sister and has recently been commenced in her 11 year old brother. They were the only children to unrelated parents with no family history.

EEGs demonstrated a normal posterior dominant rhythm with episodic occipital slowing. Occipital fast activity and sharp and slow discharges occurred bilaterally, with left-sided predominance in the boy. Seizures had a localized occipital onset. There was no fixation-off sensitivity. Structural imaging was normal. Siblings had normal intellect without regression.

Both subjects demonstrated BOLD signal change in the occipital cortex during fMRI. The sister showed bilateral negative BOLD in the lingual gyrus while her brother showed positive BOLD change in the left middle occipital gyrus. The girl also demonstrated bilateral anterior thalamic positive BOLD.

Conclusion: This novel childhood onset genetic occipital epilepsy syndrome is characterized by frequent, brief, refractory occipital seizures with onset at around 10 years of age. The striking electro-clinical concordance in siblings provides strong evidence for a genetic basis, but it is unclear if the etiology is monogenic or polygenic. Functional imaging supports an occipital focus for seizure generation.

038

PREDICTING SEIZURE CONTROL WITH ANTI-EPILEPTIC MEDICATION: VALUE OF AN EARLY CORTICAL EXCITABILITY STUDY

R. A.B. Badawy[1,2,3], R. A.L. Macdonell[2,3], S. F. Berkovic[2,3], M. R. Newton[2,3], G. D. Jackson[1,2,3]

[1]Brain Research Institute, Florey Neurosciences Institute, Heidelberg West, VIC, Australia
[2]Neurology, Austin Health, Heidelberg, VIC, Australia
[3]Medicine, The University of Melbourne, Melbourne, VIC, Australia

Background: Approximately 30% of patients with new-onset epilepsy do not respond to antiepileptic drugs (AED) but this is not predictable. We used transcranial magnetic stimulation (TMS) to determine whether it can be used to predict responsiveness to anti-epileptic medication.

Methods: TMS studies were performed on 99 patients with epilepsy (55 idiopathic generalized epilepsy [IGE], 44 focal epilepsy) within 2 weeks of presenting to the First Seizure Clinic. A second TMS study was performed 4-16 weeks on each patient following initiation of anti-epileptic medication. The amount of reduction in cortical excitability at the 250 ms interstimulus interval between the two studies (presentation – post medication) was measured. ROC curves were plotted. The optimal cut-off point was defined as the highest amount of reduction in cortical excitability that achieved ≥ 80% specificity for prediction of seizure freedom. This was found to be 93%. A TMS result consistent with seizure freedom was taken as any reduction in cortical excitability in each patient above this value following medication. Sensitivity and predictive values for seizure freedom were then calculated and compared to clinical seizure control in each patient assessed after 12 months of therapy.

Findings: Following AED use, 52% of the patients (65% in IGE) who became seizure free on medication had an over than 93% reduction in cortical excitability and thus their TMS results were predictive of good seizure control. Only 10% of the patients with ongoing seizures had a similar reduction in cortical excitability.

Interpretation: Seizure freedom is marked by a reduction in TMS measures of cortical excitability, evident shortly after beginning therapy. Failure to show this response to AED treatment may be valuable as an early predictor of pharmacoresistance in individual patients.

039

HEAD-NECK MOTION, TREMOR AND MUSCLE VOLUME ASYMMETRY IN CERVICAL DYSTONIA

N. Lythgo[1], K. Kotschet[2]

[1]RSRC/Austin Health, Kew, Australia
[2]St Vincent's Hospital, VIC, Australia

Introduction: Cervical dystonia (CD) is a neurological condition causing involuntary contractions of neck musculature that leads to abnormal postures (dystonia) and tremor. This study investigated the utility of 3D motion analysis (VICON), in conjunction with MRI, to objectively measure head-neck motion and tremor in CD.

Methods: Nineteen CD patients participated. Reflective markers were placed on the head, shoulders and trunk. The following movements were recorded by VICON: relaxed posture, neck range of motion in the sagittal (Flex/Ext), frontal (lateral Flex/Ext) and transverse planes (rotation). Tremor and head-neck motion relative to the thorax were extracted by "in-house" software. MRI imaging of the Sternocleidomastoid, Splenius Capitus, Semispinalis Capitus and Levator Scapulae muscles (right and left) were captured and used to estimate muscle volumes to calculate muscle volume asymmetry.

Results: At this stage, participants have been clinically assessed, completed MRI and 3D motion analysis. Only preliminary data is reported here. Data demonstrates that 3D motion analysis can record CD tremor. The tremor is in the order of 1 to 2 mm with a dominant frequency at 4 Hz (sagittal and transverse planes). Preliminary data analysis shows muscle volume asymmetry is not associated or correlated with head-neck posture. Pearson Product Moment correlations ranged from -0.23 to 0.5.

Conclusions: 3D motion analysis can identify movement abnormality and tremor in CD and may prove valuable in identifying neck musculature for botulinum toxin treatment. There appears, however, to be no relationship between muscle volume asymmetry and head-neck posture adopted by patients with CD.

040

FES-ASSISTED HAND EXERCISES INCREASE THE MOTOR CORTEX EXCITABILITY IN TETRAPLEGIC PATIENTS.

M. Zoghi, M. Galea

Rehabilitation Sciences Research Centre, The University of Melbourne, Kew, VIC, Australia

Aim: this study aimed to assess the effects of FES-assisted vs. non FES-assisted hand exercises on the excitability of the motor cortex in tetraplegic patients.

Background: The loss of hand function in people with tetraplegia (C5/6 level) after spinal cord injury has a severe impact on their daily activities and subsequent dependence on others. It has been shown that the number of patients with anatomically incomplete injuries with some axonal sparing across the lesion site is much higher than is apparent based on functional testing. Repetitive activation of these fibres using functional electrical stimulation (FES) could facilitate the reorganization of the cortical motor areas that control the function of these muscles. These changes can be assessed by Transcranial Magnetic Stimulation (TMS). Maximizing the function of these affected muscles through their spared corticospinal fibres might also promote axonal sprouting of collateral branches onto adjacent motor neurons thereby improving their functional connectivity. This could increase the strength of the affected muscles which can be invaluable for hand use in daily activities.

Method: We conducted a prospective assessor-blinded crossover study, comparing FES-assisted vs. non FES-assisted hand exercises (6 weeks) with a 4 week washout period between the two sets of training. The training sessions were monitored by a therapist over the internet. The excitability of the motor cortex was examined by obtaining the recruitment curves of motor evoked potential responses (MEPs) from the superficial finger flexor muscles with TMS.

Results: The results of one patient (complete lesion, C5/6) are presented. After 6 weeks of FES assisted exercises, MEP responses were more consistent and almost doubled in size, with an intensity of 1.3 x resting threshold. Conclusion: FES-assisted exercise for the hand has the potential to improve the functional connectivity of spared corticospinal axons after spinal cord injury.

041

SPINAL CORD COMPRESSION INJURY – THE ROLES OF EARLY DECOMPRESSION AND HYPOTHERMIA IN FUNCTIONAL RECOVERY.

N. F. Kerr[1], A. M. Gatt[1], A. Ghasem-Zadeh[2], E. Aleksoska[1], S. F. Cox[1], T. E. Wills[1], D. W. Howells[3], P. E. Batchelor[1]

[1]Department of Medicine, Melbourne University, Heidelberg, VIC, Australia
[2]Endocrinology Centre of Excellence, Melbourne University, Heidelberg, VIC, Australia
[3]National Stroke Research Institute, Heidelberg, VIC, Australia

Introduction: Spinal cord compression occurs in the majority of traumatic spinal cord injuries (SCI). Currently, patient stabilisation and difficulties organising early surgery means that decompressive surgery is performed relatively late. Persistent compression is not routinely modelled in animal SCI, however decompression is reported to provide functional recovery (Dimar et al, 1999). In this study we investigated the impact of hypothermia and early decompression on functional motor and tissue outcomes following traumatic SCI.

Methods: 12-16 week female F344 rats (n = 72) were subject to a moderate spinal cord contusion (150Kdyne) at T7-9. Epoxy spacers were inserted immediately after injury to compress the spinal cord by 45%. Decompression was performed either immediately, 2hrs or 8hrs post-injury. Half were treated with hypothermia (33^{o}C) commencing 30mins post-injury, maintained for 7.5hrs, with the other half remaining normothermic (37.4^{o}C) for the same period. Functional motor recovery was assessed over 8 weeks by the BBB score (Basso et al, 1996) and ladder stepping test.

Results: Overall tissue damage was assessed on H&E-stained sections. Hypothermia significantly improved behavioural and histological outcomes in the 8hr compression group. The hypothermics regained weight-supported locomotion while the normothermics remained paraparetic. Trends in favour of hypothermia were seen in behavioural and histological outcomes of the immediate and 2hrs decompression cohorts.

Conclusion: The data demonstrates increasing benefit of hypothermia with increasing duration of compression. In a model of SCI that replicates severe compression following initial trauma, hypothermia is of significant benefit. The data indicate that hypothermia would be a useful therapy to prevent neurological decline prior to decompressive surgery.

042

SCN1A MUTATION DETECTION IN MYOCLONIC ASTATIC EPILEPSY (MAE) AND INFANTILE SPASMS (IS)

I. Luk, S. F. Berkovic, I. E. Scheffer, S. Bellows, J. McMahon, J. A. Damiano, T. Arsov

Department of Medicine, University of Melbourne, Heidelberg, VIC, Australia

Purpose: *SCN1A* encodes for the voltage gated sodium channel alpha subunit (or $Na_v1.1$) necessary for generation and propagation of action potentials. Mutations in this gene cause some forms of epilepsy, such as GEFS+ and Dravet syndrome (70-80%). MAE is a rare generalized epilepsy (1-2% of all childhood epilepsies) with genetic determinants suggested by family studies and sporadic cases with *SCN1A* mutations. The aim of this study was to screen for *SCN1A* mutations in a larger cohort of MAE patients and to confirm our previous findings that the phenotypic spectrum of *SCN1A* mutations includes infantile spasms.

Method: A cohort of 92 patients diagnosed with MAE (46) and spasms (46) was screened for mutations in *SCN1A*. All 26 exons and the exon-intron boundaries were amplified with PCR and the PCR products were sequenced bi-directionally.

Results: Missense mutations in *SCN1A* were identified in 1/46 (2%) patients with MAE (Pro11His) and 2/46 (4%) patients with IS (Glu524Asp and Pro1345Ser). The phenotype of the MAE patient included febrile convulsions and normal development. One of the patients with infantile spasms had delayed onset of atonic seizures (7 years), moderate developmental delay and autism and the other had early onset infantile spasms (48 hours), regression, cerebal palsy and a movement disorder.

The Grantham scores (GS) for the identified mutations were medium for Pro11His (GS77) and Pro1345Ser (GS74) and low for Glu524Asp (GS45) and all three affected amino acid residues were highly evolutionary conserved suggesting the identified *SCN1A* mutations are likely pathogenic.

Conclusion: Our findings support the idea that the phenotypic spectrum of *SCN1A* mutations includes MAE and infantile spasms.

043

EEG-FMRI IDENTIFIES FOCAL CORTICAL BOLD SIGNAL CHANGE IN SOME INDIVIDUALS WITH CHILDHOOD ABSENCE EPILEPSY.

P. W. Carney[1,2], D. Flanagan[2], I. E. Scheffer[1], S. F. Berkovic[1], G. D. Jackson[2]

[1]Epilepsy research Centre, University of Melbourne, Heidelberg West, Australia
[2]Brain Research Institute, Florey Neuroscience Institutes, Heidelberg West, Australia

Purpose: We have performed EEG with functional MRI (fMRI) on over 40 subjects with an electro-clinical history of absence seizures (AS). Here we describe four subjects with asymmetric or unilateral focal cortical Blood Oxygen Level Dependent (BOLD) signal change.

Methods: Patients were recruited from local EEG departments and electro-clinical information was obtained. EEG was recorded during continuous acquisition of gradient-recalled echo planar images at 3 Tesla. In scanner EEG was reviewed off-line and epileptiform activity (generalised spike and wave) was marked as events of interest as part of an event related analysis using spm8.

Results: All patients had a typical electro-clinical picture for childhood absence epilepsy (CAE) at diagnosis. Two subjects were studied prior to treatment, and became seizure free on medication. The other 2 subjects were refractory to therapy at the time of study, one with generalised convulsions.

In all subjects negative BOLD change was seen in the parietal cortex, caudate nucleus and brainstem and positive BOLD in the thalamus; findings which are seen consistently in patients with CAE. These subjects also demonstrated increased BOLD in frontal association cortex, particularly in the region of the middle and superior frontal gyrus. In the new onset cases this was seen bilaterally but asymmetrically, while in the chronic refractory cases this was seen unilaterally only. Findings were reproduced in two subjects.

Conclusion: Our data suggests that focal cortical regions, as detected by functional imaging, may be important in the generation of AS in some subjects with an electro-clinical diagnosis of CAE.

044

BLUNTED SPLANCHNIC SYMPATHOINHIBITORY EFFECTS OF GASTRIC HORMONES IN OBESITY MAY CONTRIBUTE TO HYPERTENSION

J. M.Y. How, A. J.M. Verberne, B. C. Fam, D. M. Sartor

Medicine, University of Melbourne, Heidelberg, VIC, Australia

Sustained sympathoactivation is thought to contribute to obesity-related hypertension. Gastrointestinal hormones act synergistically at vagal afferents to induce splanchnic sympathoinhibition and a gastrointestinal vasodilator response. Our aim was to observe whether the splanchnic sympathoinhibitory effects induced by gastric leptin and cholecystokinin (CCK) are blunted in an animal model of obesity.

Sprague-Dawley rats were fed a medium high fat diet (MHFD; *n*=24) or low fat diet (LFD; *n*=8) for 13 weeks. At the end of this period, animals were anaesthetised and artificially ventilated. Arterial pressure (AP) was measured and plasma collected for analysis. Splanchnic sympathetic nerve discharge (SSND) responses to CCK (2 μg/kg) and leptin (15 μg/kg) administered close to the coeliac artery were evaluated. MHFD fed animals were grouped as obesity prone (OP; *n*=8) or obesity resistant (OR; *n*=8) and analysed according to both weight gain and diet.

OP animals showed increased AP, fat pad mass, adiposity, leptin levels and lower CCK levels compared to LFD and OR rats ($P<0.05$ for all). The sympathoinhibitory effects of CCK on SSND were attenuated in MHFD (-10±2 units) animals compared to the LFD (-21±2 units; $P<0.05$) animals. Inhibitory responses to leptin on SSND were reversed in MHFD (4±1 units) animals compared to LFD (-6±2 units; $P<0.001$) rats.

The results obtained from this study suggest that obesity induced by a MHFD decreases the splanchnic sympathoinhibitory effects of gastric hormones. Reduced sympathoinhibition may cause a shift in cardiovascular homeostasis and contribute to increased sympathoactivation in obesity.

045

AUTOMATED CLUSTERING OF DIFFUSION MRI TRACTOGRAPHY FIBERS USING GLOBAL INFORMATION

R. E. Smith[1,2], D. Tournier[1,2], F. Calamante[1,2], A. Connelly[1,2]

[1]Austin campus, Florey Neuroscience Institutes, Heidelberg West, VIC, Australia
[2]Brain Research Institute, Heidelberg West, VIC, Australia

Introduction: Diffusion Magnetic Resonance Imaging is used to investigate the brain white matter structure *in vivo* non-invasively. Plausible long-range neuronal connections may be inferred from these images through the process known as Tractography. These data sets are very large, particularly when the whole brain is studied; therefore interpretation of results is difficult. We report on the development of a novel clustering algorithm, which produces a global map of fiber structure and identifies regions of interest in an automated fashion.

Method: The tractography fibers are mapped onto a five-dimensional image, with both the positions and local orientations of each fiber contributing to the image. The edges of coherent bundles are determined volumetrically. Bound coherent regions are identified by traversing these edges, searching for loops orthogonal to the local density direction. Finally, tractography fibers are mapped to the identified regions through which they traverse.

Results: The algorithm identifies a wide range of interesting white matter structures, from the major fasciculi of the brain to cranial nerves and coherent gyral bundles, using data sets significantly larger than could be handled by any previously published technique. The degree of connectivity between each identified region is calculated as part of the clustering process, providing an intrinsic quantification of whole-brain structural connectivity.

Conclusion: The entire analysis procedure, from diffusion image reconstruction and modeling to whole-brain tractography and clustering, may now be fully automated. This framework will therefore permit detailed analysis of the structural connectivity of the brain *in vivo* without any *a priori* information, providing a new methodology by which to compare white matter connectivity in a range of neurological patient cohorts.

046

SEVERE INFANTILE MULTI-FOCAL EPILEPSY: A FOCAL EPILEPTIC ENCEPHALOPATHY DUE TO SCN1A MUTATIONS

S. T. Bellows[1], T. Arsov[1], J. M. McMahon[1], D. Gill[2], J. Damiano[1], S. M. Zuberi[3], J. C. Mulley[4,5], S. F. Berkovic[1], I. E. Scheffer[1,6]

[1]Epilepsy Research Centre, Department of Medicine (Neurology), The University of Melbourne, Austin Health, Melbourne, VIC, Australia
[2]The Children's Hospital at Westmead, Sydney, NSW, Australia
[3]Fraser of Allander Neurosciences Uni, Royal Hospital for Sick Children, Glasgow, United Kingdom
[4]Epilepsy Research Program, SA Pathology at Women's and Children's Hospital, Adelaide, SA, Australia
[5]School of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, SA, Australia
[6]Department of Paediatrics, Royal Children's Hospital, The University of Melbourne, Melbourne, VIC, Australia

Aim: To confirm that severe infantile multifocal epilepsy (SIMFE) is a recognizable electroclinical syndrome due to sodium channel gene *SCN1A* mutations in a significant proportion of cases.

Background: In our large study of infantile-onset epileptic encephalopathies, we identified a new genetic focal epileptic encephalopathy SIMFE (Harkin *et al*., 2007). SIMFE is characterised by multifocal seizures, multifocal epileptiform discharges and later cognitive slowing. 3/5 cases had *SCN1A* mutations.

Methods: Electroclinical characterization of patients with SIMFE. All *SCN1A* exons and exon-intron junctions were amplified from genomic DNA by PCR and sequenced in both directions using the ABI3730XL sequencing platform.

Results: 4 new patients with SIMFE were identified. All had multiple focal seizure types with varying semiology. Mean age of seizure onset was 9.5 months (5-19 months). Developmental regression or plateau occurred later than seizure onset (1.5-8 years). Cognitive ability ranged from severe intellectual disability to low average intellect. EEG studies typically showed multifocal epileptiform activity with no (or exceptional) generalised spike wave discharges. Brain imaging findings were normal (3) or mild ventriculomegaly (1). 3/4 (75%) had SCN1A mutations: 1 de novo missense, 1 truncation and 1 splice site mutation. Parental DNA was unavailable in 2 cases.

Conclusion: 6/9 (67%) patients with SIMFE have *SCN1A* mutations. These findings confirm the distinctive phenotype of SIMFE is due to *SCN1A* mutations in a high proportion of cases. *SCN1A* mutations are associated with focal seizures of mild type (temporal and occipital seizures) and also with a focal epileptic encephalopathy, SIMFE.

(1) Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, et al. The Spectrum of SCN1A-related infantile epileptic encephalopathies. Brain 2007;130: 843-852.

047

A SIMPLE CHANGE TO PHOTIC STIMULATION MARKEDLY IMPROVES EEG DIAGNOSIS OF FIRST SEIZURES

S. Mullen, I. E. Scheffer, S. F. Berkovic

Dept. of Medicine, Austin Health, Heidelberg, VIC, Australia

Aim – To evaluate a simply implemented change in EEG protocol for the diagnosis of idiopathic generalised epilepsy (IGE) in a first seizure cohort.

Background – The epileptiform response to intermittent photic stimulation (IPS), the photoparoxysmal response (PPR), in adults is associated with IGE. Patterned flash and repetition increase the sensitivity of this test in an individual. The diagnostic utility of these has not been assessed.

Method – In June 2006 we implemented an Intensified IPS protocol, replacing a plain glass protocol based on the gold-standard, European Consensus statement. This involved re-ordering frequencies to repeat the critical 10-20Hz band and adding a fine, metal mesh. A small LED strobe was used. Results of EEGs done for first seizure 30 months prior to and 30 months following implementation were audited. Spontaneous focal and generalised epileptiform changes were noted as well as the presence or absence of a PPR.

Results – There were 466 studies studied prior to the change in IPS and 526 after. A PPR was present in 3.2% of EEGs prior to intensified IPS and 6.2% post ($P<0.05$). EEGs in which a PPR was the only abnormality went from 5% of all IGE related abnormalities to 20% after introduction of the intensified IPS ($P<0.05$). Plain glass IPS thus detects 6% more IGE cases to resting and hyperventilated EEG while intensified IPS detects 25% more.

Conclusion – A simple change to IPS can improve detection of IGE related EEG changes by around 20% as compared to the current gold-standard protocol.

048

EPILEPSY-MIGRAINE SYNDROME DUE TO A MISSENSE MUTATION IN *SCN1A*

K. L. Oliver[1], T. Arsov[1], S. Kivity[2], Z. Afawi[3], J. Damiano[1], A. D. Korczyn[4], S. F. Berkovic[1]

[1]Epilepsy Research Centre and Department of Medicine, University of Melbourne, Austin Health, Heidelberg West, Australia

[2]Epilepsy Unit, Schneider Children's Medical Center of Israel, Petach Tikvah, Israel

[3]Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel

[4]Sieratzki Chair of Neurology, Tel-Aviv University, Tel-Aviv, Israel

Objective: *SCN1A* is a well-known epilepsy gene, especially within the genetic epilepsy with febrile seizures plus (GEFS+) spectrum. More recently *SCN1A* has been identified as the third familial hemiplegic migraine (FHM) gene. Here we describe an *SCN1A* mutation positive family with a unique epilepsy-migraine syndrome.

Method: This Ashkenazi Jewish family was ascertained as part of an ongoing genetic study into familial epilepsy. Detailed clinical histories were taken from family members. Genomic DNA was extracted from blood samples and all *SCN1A* exons and exon-intron junctions were amplified by PCR. PCR products were sequenced in both directions using the ABI3730XL sequencing platform and analysed for sequence variation using mutation detection software (Codon Code).

Results: The pedigree was consistent with autosomal dominant inheritance. Affected family members had epilepsy (n=1), migraine (n=1) or both (n=5). Seizures began with a numb or tingling feeling in the hand evolving to a tonic-clonic seizure. Onset age ranged from 8 - 15 years. No febrile convulsions were reported and interictal EEG recordings were all normal. Six family members described separate classical migraine attacks; none fulfilled the International Headache Classification (ICHD – II) criteria for FHM.

Sequencing identified a novel heterozygous missense *SCN1A* mutation, 5674C>G (Arg1892Gly), in the proband and subsequently in her two affected sons.

Conclusion: Previously one *SCN1A* mutation positive family with co-occurring FHM and epilepsy has been reported. The migraine described in this family does not meet FHM criteria and the focal epilepsy reported is unlike those usually associated with *SCN1A*. This family broadens the phenotypes associated with *SCN1A* and further supports the idea of a molecular link between epilepsy and migraine.

049

EXPLORING STRATEGIES TO SAFEGUARD THE FUTURE OF CLINICAL RESEARCH IN AUSTRALIA

B. G. Bardsley

N-CRESS, Dept of Neurology, Austin Health, Heidelberg, Australia

Clinical research plays an important role in multiple sclerosis (MS). Treatment options can be limited, particularly in clinically isolated syndrome and primary and secondary progressive MS. Even in relapsing-remitting MS, existing treatments do not yet provide certainty of clear-cut benefit or outcome because of the unpredictable nature of MS. Clinical trials provide an alternative to standard treatment options and importantly, hope for people who are grappling with the challenges and uncertainties of MS. Furthermore, the involvement of Australian centers in clinical trials ensures access to and expertise in cutting-edge treatments for the future benefit of people with MS, along with indirect benefits such as funding for staff and resources. Unfortunately, the future of clinical research is under threat in Australia. The massive cost of drug development, combined with the recent global financial crisis, has forced pharmaceutical companies to carefully select clinical trial centers on the basis of timeliness (of regulatory approval and study start), recruitment capacity, cost and quality. Australia has a reputation for producing high-quality data, but otherwise struggles to compete, particularly with countries such as India and China. In recognition of the increasing global competition for investment in clinical research, this project explores the threats to Australia's competitiveness and identifies strategies to improve on our timeliness, capacity and cost while maintaining quality. Examples include actively supporting Australia's move to a streamlined ethical review process; collaborating more effectively with sponsors to improve efficiencies; enabling strong early recruitment by identifying potential patients prior to study; allocating specific roles to study staff to enhance efficiencies; and allowing flexibility in staffing to match resources to project requirements. Adoption of these strategies will assist in safeguarding Australia's ongoing involvement and expertise in translational research, and patient access to new and better treatments for MS.

050

PCDH19 MUTATIONS CAUSE EPILEPSY AND MENTAL RETARDATION IN FEMALES (EFMR)

T. Desai[1], L. M. Dibbens[2,3], M. A. Bayly[2], T. Arsov[1], R. Kneen[4], J. Gibbs[5], S. Sisodiya[6], J. Gecz[2,3], F. McKenzie[7], S. F. Berkovic[1], J. C. Mulley[3,8], S. Von Spiczak[9], A. Ronan[7], I. E. Scheffer[1,10]

[1]Epilepsy Research Centre, Department Medicine, Austin Health, The University of Melbourne, Heidelberg West, VIC, Australia

[2]Epilepsy Research Program (SA Pathology), The Women's and Children's Hospital, North Adelaide, SA, Australia

[3]School of Paediatric and Reproductive Health, University of Adelaide, Adelaide, SA, Australia

[4]Department of Neurology, Littlewood's Neuroscience Unit, Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom

[5]The Countess of Chester Health Park, Chester, United Kingdom

[6]Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, United Kingdom

[7]Centre for Clinical Epidemiology and Biostatistics, Faculty of Health, NSW GOLD Service, Hunter Genetics, Newcastle, NSW, Australia

[8]School of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, SA, Australia

[9]Pediatric Epilepsy Genetics Research Group, Department of Neuropediatrics, University Medical Center, Kiel, Schleswig-Holstein, Germany

[10]Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, Melbourne, VIC, Australia

Aim: Epilepsy and mental retardation limited to females (EFMR) is a disorder that occurs in both familial and sporadic cases and is caused by mutations in the protocadherin 19 gene (PCDH19). We sought to determine the frequency of PCDH19 mutations in girls presenting with seizures before 3 years of age who also had developmental delay and/or intellectual disability.

Methods: We studied 119 unrelated female patients. We analysed PCDH19 gene variation by high resolution melt curve analysis and variants were confirmed by sequencing. Where available, we analysed the inheritance of the mutation in relatives.

Results: We identified PCDH19 mutations in 3/119 unrelated females: 2 missense mutations and 1 frameshift mutation. Mean age of seizure onset in the girls with EFMR was 9.3 months (3-17 months). The predominant pattern was of clusters of seizures, often triggered by fever. Seizure types included febrile, tonic, tonic-clonic seizures. Status epilepticus occurred in one girl at 4 years. Cognitive ability ranged from mild to moderate intellectual disability. Autistic features and behavioural problems were present in 2 patients. The two patients with missense mutations both have severely affected sisters who share their PCDH19 mutations.

Conclusion: Approximately 2.5% of females who experience seizures in infancy with a history of intellectual disability have a mutation in the PCDH19 gene. The presentation is characteristic and can be distinguished from Dravet syndrome. The finding of 2 sister pairs highlights the importance of PCDH19 mutational analysis when sisters present with early onset seizures and developmental delay.

051

DIAGNOSIS OF EPILEPSY FOLLOWING A FIRST 'SEIZURE' EVENT: VALUE OF AN EARLY CORTICAL EXCITABILITY STUDY

R. A.B. Badawy[1,2,3], G. D. Jackson[1,2,3], S. F. Berkovic[2,3], M. R. Newton[2,3], R. A.L. Macdonell[2,3]

[1]Brain Research Institute, Florey Neurosciences Institute, Heidelberg West, VIC, Australia
[2]Neurology, Austin Health, Heidelberg, VIC, Australia
[3]Medicine, The University of Melbourne, Melbourne, VIC, Australia

Background: We recruited patients presenting to the First Seizure Clinic with a first seizure or syncope to investigate the clinical utility of paired pulse transcranial magnetic stimulation (TMS) as an aid to early diagnosis of epilepsy.

Methods: Cortical excitability at the 250 ms interstimulus interval was measured in 221 patients within 2 weeks of presentation. ROC curves were plotted and t he optimal cut-off point was defined as the highest value that achieved≥ 80% specificity for the diagnosis of epilepsy . Thi s was found to be 176% in the baseline state and 194% after sleep deprivation. A TMS result consistent with epilepsy was taken as a measurement above these values in either hemisphere. Sensitivity, and positive and negative predictive values were calculated. The TMS findings (measured at presentation) were compared to the electro-clinical diagnosis (epilepsy, isolated seizure or syncope) after 36 months of follow-up.

Findings: Epilepsy was correctly categorized using TMS in 77 of 166 patients with a definite electro-clinical diagnosis (sensitivity: 46%). Sleep deprivation improved these results. Based on inter-hemispheric differences, focal epilepsy was correctly diagnosed in 19 of 59 patients (sensitivity: 32%). Of the 79 patients with an undetermined diagnosis initially, 46 patients were later diagnosed with epilepsy. A TMS study performed at presentation correctly identified 23 of these with a test sensitivity of 50%.

Interpretation: A positive TMS result is predictive of subsequent seizures in patients where the diagnosis is undetermined at initial presentation but a negative result is of limited value as it does not exclude epilepsy.

052

FILTERING FMRI USING A SOCK

K. Bhaganagarapu[1,2,3], D. F. Abbott[1,2,3], G. D. Jackson[1,2,3,4]

[1]Florey Neuroscience Institutes (Austin), Heidelberg West, Australia
[2]Department of Medicine, The University of Melbourne, Melbourne, VIC, Australia
[3]Brain Research Institute, Melbourne, VIC, Australia
[4]Department of Radiology, The University of Melbourne, Melbourne, VIC, Australia

It is well known that blood oxygenation level dependent (BOLD) function magnetic resonance imaging (fMRI) is restricted by low signal to noise and various artifacts. Sources of these artifacts vary from motion to physiological noise. Independent components analysis (ICA) is a data-driven analysis approach that is increasingly being used to filter fMRI of such noise [1-3]. However, one of the problems with ICA remains the interpretation of the results. Recently, we developed an automatic classifier that we call a Spatially Organised Component Klassifikator (SOCK) [4], which uses spatial criteria to help distinguish plausible biological phenomena from likely instrument and physiological noise. SOCK was shown to successfully remove artifactual components, without rejecting biological components in resting state data. Here, we utilize SOCK to automatically filter a conventional fMRI block-design language study. We assess the performance of the filter by comparing the significance of activation obtained in a conventional general linear model (GLM) analysis of the data with and without the use of SOCK. The use of the SOCK algorithm to filter fMRI substantially increased the significance and extent of activation in all subjects in this fMRI language study. We conclude that the use of automatic ICA-based denoising approaches like SOCK, offers a potentially useful approach to improving the quality of fMRI data.

(1) Perlbarg et al., Magnetic Resonance Imaging, 2007, 25, 35-46.
(2) Sui et al., NeuroImage, 2009, 46(1), 73-86.
(3) Tohka et al., NeuroImage, 2008, 39, 1227-1245.
(4) Bhaganagarapu et al., NeuroImage, 2009, 47(S1), S39-S41.

053

AUTOSOMAL DOMINANT VASOVAGAL SYNCOPE: CLINICAL AND LINKAGE RESULTS

K. Klein[1,2], C. Bromhead[3], K. Smith[3], C. J. O'Callaghan[2], I. E. Scheffer[1,2], J. M. McMahon[1,2], K. M. Lawrence[1,2], L. M. Dibbens[4,5], M. Bahlo[3], S. F. Berkovic[1,2]

[1]Epilepsy Research Centre, The University of Melbourne, Heidelberg West, VIC, Australia
[2]Department of Medicine, Austin Health and Northern Health, Heidelberg, VIC, Australia
[3]The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia
[4]Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, SA, Australia
[5]School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA, Australia

Purpose: Syncope is the most common differential diagnosis of epilepsy. The most frequent type is vasovagal syncope (VVS) for which the aetiology remains elusive. Here we provide evidence for a genetic aetiology by describing six families with autosomal dominant VVS and the linkage results in the largest family.

Methods: Patients with VVS and a family history of syncope were recruited. A questionnaire addressing features differentiating syncope from epilepsy was administered to all available family members. Medical records were obtained and additional diagnostic tests performed in selected individuals. Linkage analysis was performed in the largest family, containing 23 affected and 12 unaffected (including 9 married-in) family members.

Results: Six families with VVS, consistent with autosomal dominant inheritance, were recruited in addition to several smaller families. The largest comprised 30 affected individuals over three generations with a median onset at 8-9 years. Typical triggers for VVS were heat, sight of blood, injury, medical procedures, prolonged standing, pain and frightening thoughts. The triggers varied considerably within the family. The second largest family included 10 affected subjects over three generations with a median onset at 10-11 years and varying triggers. The other four families were smaller with 4-5 affecteds over 2-3 generations.

Linkage analysis in the largest family revealed a significant maximum LOD score of 3.28 on chromosome 15q.

Conclusion: Autosomal dominant VVS is not a rare phenomenon. Linkage to chromosome 15q was demonstrated in a large family. Identification of the genetic cause will help to further understanding of pathophysiology.

054

RECEIVING GENETIC RESULTS: EXPLORATION OF THIS EXPERIENCE IN FAMILIAL EPILEPSY

D. F. Vears[1], K. Dunn[2], S. A. Wake[2,3], I. E. Scheffer[1,2]

[1]Department of Medicine, University of Melbourne, Epilepsy Research Centre, West Heidelberg, VIC, Australia

[2]Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville, VIC, Australia

[3]Genetic Health Services Victoria, Royal Children's Hospital, Parkville, VIC, Australia

Statement of purpose: Rapid advances in genetic technology have lead to gene identification for many inherited disorders. However, there is little information on the impact of gene identification for people with genetic conditions other than hereditary cancers. This study explored the experience of receiving a genetic result in people with familial epilepsy in whom a genetic cause was identified.

Methods: Using a targeted sampling strategy, a wide selection of family members who had received a positive genetic test were invited to participate. In-depth, semi-structured interviews were conducted, audio-taped and transcribed. The data underwent thematic analysis.

Results: 20 individuals from 3 families with different epilepsy syndromes and different causative genes were interviewed. The mean time of receiving genetic results prior to the study was 10.9 years (range 5-14 years). Three major themes were identified: (i) living with epilepsy; (ii) clinical utility of the test; and (iii) 'talking about the family genes'. How epilepsy affected the individual influences the impact of receiving genetic results and the use of genetic information for reproductive decision-making. While altruism can influence decisions to participate in studies of gene identification, people also want to know what this means for them and how this may help them and their families. It cannot be assumed that family members communicate about their epilepsy. Information that may benefit others in the family may not be shared and understanding of genetic inheritance may be limited.

Conclusions: While this study focused on familial epilepsy, the findings are relevant to other genetic disorders. The results can inform the development of guidelines for genetic result disclosure, in the context of the research setting, and more broadly. They also reveal important insights into the family experience of genetic conditions and communication within families, and can be used to improve genetic counselling for individuals and families.

055

ACETYLCHOLINE RECEPTOR GENE ALPHA 7 (CHRNA7) IN IDIOPATHIC GENERALISED EPILEPSY (IGE): MUTATION DETECTION

J. A. Damiano, S. F. Berkovic, K. Lawrence, S. A. Mullen, I. E. Scheffer, T. Arsov

Medicine AH/NH, University Of Melbourne, Heidelberg, VIC, Australia

Purpose: Alpha-7-nicotinic-acetylcholine receptor (CHRNA7) is one of five acetylcholine receptors expressed in the brain. Given that two of these receptors have previously been implicated in epilepsy , and that CHRNA7 is deleted in 1% of patients with IGE (carrying the 15q13.3 microdeletion), this gene is considered to be a strong candidate gene for IGE. Previous studies have reported CHRNA7 variants in Juvenile Myoclonic Epilepsy and schizophrenia. Here we present results from our screen of patients with IGE for mutations in CHRNA7 and its partial duplication CHRFAM7A.

Method: 92 patients with IGE and 92 control blood bank DNA samples were screened. CHRNA7 exon 1-4 were bi-directionally sequenced and exons 5 -10 were initially screened for mutations with DHPLC and samples with variant denaturing profiles were subsequently sequenced. Long range PCR specific for CHRNA7 exons 5-10 was used to assign the mutations to either CHRNA7 or CHRFAM7A.

Results: We identified one missense mutation (Gly234Ser) in CHRNA7 in a patient with generalised tonic-clonic seizures alone with seizure onset at 23 years and schizophrenia . The second mutation (Val161Leu), assigned to CHRFAM7A, was found in a patient with IGE with absence seizures from 13 and generalized tonic-clonic seizures from 18 years. Both mutations affect highly conserved amino acids and were not found in 184 control chromosomes

Conclusion: Our findings support the view that mutations in CHRNA7 and CHRFAM7A may be relevant to IGE and could be relevant to the mechanism in cases with 15q13.3 microdeletion. Further studies are necessary to confirm the importance these findings and their functional significance.

056

EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITION ON DORSAL VAGAL STIMULATION-INDUCED INSULIN SECRETION

A. J. Verberne, B. M. Mussa, C. Rantzau, D. M. Sartor

Medicine, University of Melbourne, Heidelberg, VIC, Australia

We have previously shown that the dorsal motor nucleus of the vagus (DMV) regulates pancreatic exocrine secretion. Pancreatic islets receive vagal innervation and so the present study examined whether chemical activation of the DMV modulates pancreatic endocrine secretion and whether this process involves nitric oxide (NO). All experiments were conducted in isoflurane/urethane-anaesthetised mae Spargue-Dawley rats after over-night fast. Chemical activation of the DMV using bicuculline (BIM; $GABA_A$ antagonist, 100 pmol/25 nl) microinjections resulted in significant and rapid increases in glucose-mediated insulin secretion (9.1 ± 0.1 ng/ml peak) compared to saline infusion (5.5 ± 2.0 ng/ml, $P < 0.05$). A repeat microinjection of BIM made 40 min later did not increase insulin secretion significantly. Microinjection of BIM outside the DMV or microinjection of vehicle into the DMV did not affect insulin secretion. Blockade of muscarinic receptors (atropine methonitrate; 100 µg/kg/min, i.v. for 20 min) reduced the activation of glucose-mediated insulin secretion produced by stimulation of the DMV from 9.2 ± 0.9 ng/ml to 1.6 ± 0.1 ng/ml ($P < 0.05$). On the other hand, L-nitroarginine methyl ester (NO synthase inhibitor; 30 mg/kg, i.v.) significantly increased the excitatory effect of DMV stimulation on glucose-mediated insulin secretion (from 8.9 ± 1.2 ng/ml to 15.3 ± 3.0 ng/ml; $P < 0.05$). This suggests that NO may play an inhibitory role in the parasympathetic vagal regulation of pancreatic endocrine secretion.

057

EEG-FMRI IN FOCAL EPILEPSY: CORTICAL AND SUBCORTICAL INVOLVEMENT CORRELATES WITH DISTRIBUTION OF INTERICTAL DISCHARGES

R. A.B. Badawy[1,2,3], D. Flanagan[1], G. D. Jackson[1,2,3]

[1]Brain Research Institute, Florey Neurosciences Institute, Heidelberg West, VIC, Australia
[2]Neurology, Austin Health, Heidelberg, VIC, Australia
[3]Medicine, The University of Melbourne, Melbourne, VIC, Australia

Background: The structures involved in the generation of focal interictal discharges are not well characterized in humans. In this study we looked for patterns of BOLD signal change associated with focal interictal epileptiform discharges to identify some of the cortical and subcortical networks involved in their generation and how well they correlated with the spatial distribution of interictal discharges.

Methods: 27 patients with well defined focal epilepsy syndromes were studied using structural imaging and EEG-fMRI at 3 Tesla.

Results: 16 patients had structural abnormalities on MRI. A total of 39 interictal discharge (IED) datasets were available for analysis as nine patients had more than one type of IED or independent foci arising from more than one region. On EEG-fMRI, cortical activation was the common theme, frequently with clear localization either as a single focus or multiple foci, usually concordant with electro-clinical localization. The extent and lateralization of distant cortical and subcortical structure (thalamus, cerebellum and brainstem) involvement was highly dependent on the spatial distribution of IEDs recorded, although, in some subjects discrete, multi-focal areas of BOLD signal change were associated with discrete IEDs.

Conclusion: As a general rule, discharges with a discrete focal field are associated with more localized BOLD signal changes. Discharges with a broader field show more wide spread patterns. EEG-fMRI provides a useful tool in the study of patients with focal epilepsy, providing more information than is typically obtained from EEG alone.

058

EPILEPSY WITH OCCIPITAL FEATURES DUE TO *SCN1A* MISSENSE MUTATIONS

T. Arsov[1], S. Kivity[3], J. Damiano[1], Z. Afawi[2], K. Oliver[1], J. McMahon[1], K. Lawrence[1], I. E. Scheffer[1], S. F. Berkovic[1]

[1]Department of Medicine, University of Melbourne, Heidelberg, VIC, Australia
[2]Department of Neurology, Tel Aviv Sourasky Medical Centre, Tel Aviv, Israel
[3]Schneider Children's Medical Center, Schneider Children's Medical Center, Petaq Tikva, Israel

Objective: Mutations in *SCN1A* are an important cause of Dravet syndrome and genetic (generalized) epilepsy with febrile seizures plus (GEFS+). With increasing recognition that *SCN1A* may cause focal seizures and association with rare cases of benign occipital epilepsy of childhood, we studied a cohort of patients with occipital seizures .

Method: We screened 40 unrelated patients with epilepsy with occipital features. Genomic DNA was extracted from blood and all *SCN1A* exons and exon-intron junctions were amplified by PCR and sequenced in both directions using the ABI3730XL sequencing platform.

Results: *SCN1A* mutations were found in 2/40 cases. A *de novo* missense mutation Ala1429Val occurred in a patient with febrile seizures (onset 6 months) and focal seizures (onset 13 years) characterized by head and eye deviation before secondarily generalizing, triggered by flashing lights. Definitive epileptiform EEG abnormalities were not found. The second case was an inherited Lys876Thr mutation in a large Arab-Israeli family with GEFS+. The screened individual had a history of FS from age 12 months, with a pattern of benign partial epilepsy of childhood from 6 years and EEG showing occipital-parietal slowing. Of the 15 other affected family members with Lys876Thr mutation, two had additional occipital features including nausea and vomiting.

Conclusion: We confirm that *SCN1A* can cause epilepsy with occipital features. Both our cases and earlier reports had febrile seizures, highlighting the link between GEFS+ and benign occipital epilepsies, both in sporadic and familial cases. This observation is of diagnostic and counselling importance and highlights the overlap of generalized and focal epilepsies that have traditionally been separated.

059

^{18}F-AV-133 VMAT2 IMAGING IN THE DIFFERENTIAL DIAGNOSIS OF DEMENTIA WITH LEWY BODIES FROM ALZHEIMER'S DISEASE

S. Pejoska[1], C. C. Rowe[1], J. Drago[2], N. Okamura[3], R. Mulligan[1], H. F. Kung[5], M. Pontecovo[4], C. L. Masters[6], D. Skovronsky[4], V. L. Villemagne[1,6]

[1]Centre for PET, Austin Health, Heidelberg, VIC, Australia
[2]Exp Physiology, Howard Florey Institute, Melbourne, VIC, Australia
[3]Pharmacology, Tohoku University, Sendai, Japan
[4]Avid Radiopharmaceuticals, Inc., Philadelphia, PA, United States
[5]Radiology, University of Pennsylvania, Philadelphia, Australia
[6]Mental Health Research Institute, Melbourne, VIC, Australia

Objectives: Presynaptic monoaminergic terminal integrity can be assessed non-invasively by Positron Emission Tomography (PET).

Methods: The 18F-labeled tetrabenazine derivative, [18F]AV-133, was used for the noninvasive assessment of the vesicular monoamine transporters type 2 (VMAT2) in 10 patients with dementia with Lewy Bodies (DLB, aged 68±8 yrs), 10 patients with Alzheimer's disease (AD, aged 68±7 yrs), 20 patients with Parkinson's disease (PD, aged 67±9 yrs), and 10 healthy age-matched control subjects (HC, aged 70 ±14 yrs). Normalized tissue uptake value ratios (RT) at 90-110 min post injection were calculated using the primary visual cortex as the reference region.

Results: Compared to HC, VMAT2 RT were significantly lower by 87% and 74% in the posterior putamen, 64% and 63% in the anterior putamen, and 52% and 51% in the caudate nucleus of PD and DLB patients, respectively. In contrast to PD and DLB, no significant reductions in VMAT2 were observed in AD patients. Significant reductions in VMAT2 were observed in DLB when compared to AD in the anterior (2.17 ±0.3 vs. 3.63±0.4, P=0.02, effect size=4.1) and posterior putamen (1.87±0.3 vs. 3.50±0.4, P=0.005, effect size=4.6).

Conclusions: Studies with [18F]AV-133, allow identification of individuals with disorders characterized by degeneration of dopaminergic nigrostriatal afferents. These findings indicate that [18F]AV-133 can sensitively detect reductions of dopaminergic nigrostriatal afferents in PD and DLB patients and assist in the differentiation from AD.

060

CORRELATION OF ^{11}C-PIB TO POST-MORTEM AB PLAQUE LOAD

V. L. Villemagne[1,2], C. McLean[3], M. T. Fodero-Tavoletti[4,5], G. Jones[1], A. Rooney[2], F. Hinton[2], K. Ong[1], L. Leone[5], G. O'Keefe[1], C. L. Masters[2], C. C. Rowe[1]

[1]Centre for PET, Austin Health, Heidelberg, Australia
[2]Mental Health Research Institute, Melbourne, VIC, Australia
[3]Anatomical Pathology, Alfred Hospital, Melbourne, VIC, Australia
[4]Bio21 Institute, University of Melbourne, Melbourne, VIC, Australia
[5]Pathology, University of Melbourne, Melbourne, VIC, Australia

Background: It is postulated that the pattern of 11C-PiB PET retention reflects the regional distribution and density of aggregated beta-amyloid (Aß) in the brain. Objectives: The purpose of this study was to correlate the regional PiB retention values obtained from PET during life with the Aß plaque load measured by immunohistochemistry (IHC).

Methods: Comparison between region-matched PiB binding and 1E8 IHC plaque measurement in frontal, parietal, posterior cingulate, medial temporal and cerebellar cortex was carried out in 4 subjects with autopsy-confirmed neurodegenerative disease, two with sporadic Alzheimer's disease (AD), one with familial AD due to a presenilin-1 mutation and one with dementia with Lewy bodies (DLB). PiB-PET was performed 1-5 years (median 2.5 years) prior to death. Plaque load in brain slides was expressed as the ratio of the area of semiautomatically selected IHC-positive pixels to total pixels in field of view. PiB retention was assessed using Standardized Uptake Values (SUV) at 40-70 minutes post injection from ROI drawn onto the PiB images in the area of the brain samples.

Results: Regional PiB SUV correlated well with IHC plaque volume ($r = 0.74$, $p<0.0001$) when all cases and samples were pooled and separately, the 2 sporadic AD and the DLB cases showed excellent correlations ($r=0.96$; $r=0.85$; $r=0.89$, respectively). The PSEN1 AD case had poor correlation between PiB and IHC plaque volume ($r=0.29$, $p=0.57$).

Conclusions: While PiB PET might underestimate cortical plaque load in familial AD cases, possibly due to a lower affinity to the cotton wool plaques seen in this condition, these results indicate that PiB PET accurately reflects brain plaque load in sporadic dementias.

061

THE RENAL SYMPATHOINHIBITORY EFFECTS OF GASTRIC HORMONES ARE ATTENUATED IN OBESITY.

T. J. Pumpa, J. M.Y. How, A. J.M. Verberne, D. M. Sartor

Department of Medicine, University of Melbourne, Melbourne, VIC, Australia

Evidence suggests that gastric leptin acts synergistically with cholecystokinin on vagal afferents to reduce sympathetic nerve discharge (SND), and modulate blood flow. Our aim was to determine whether these responses, along with resting arterial pressure (AP) are altered in an animal model of obesity and contribute to obesity-related hypertension.

Sprague Dawley rats were fed either a medium high fat diet (MHFD; n=24), or a low fat diet (LFD, control; n=8) for 13 weeks. After this time MHFD rats were grouped into obesity prone (OP; n=8), or obesity resistant (OR; n=8) depending on weight gain. Electrophysiological techniques were used to record renal SND in isoflurane-anaesthetised animals. SND was measured in response to infusion of cholecystokinin (0.5, 1, 2, 4 and 8 µg/kg) and leptin (15 µg/kg) into the gastrointestinal circulation.

OP animals had significantly higher baseline AP and total fat pad weight (116 ±3 mmHg; 87±6 g; n=8) compared with OR (102 ±2 mmHg; 63±6 g; n=8; $P<0.05$) and control animals (100±4 mmHg; 62±3 g; n=8; $P<0.01$). SND responses to cholecystokinin were blunted at all doses in OP compared with OR and control animals ($P<0.05$). The sympathoinhibitory response to leptin was reversed in both OP and OR (5±3% and 4±2%) compared with control animals (-10±2%, $P<0.001$), while the hypotensive response to leptin in control animals was significantly attenuated in both OP and OR animals ($P<0.05$).

These results suggest that the sympathoinhibitory and vasodilatory effects of leptin and cholecystokinin are attenuated in obesity and may contribute to obesity-related hypertension

062

FACILITATING MS CLINICAL RESEARCH: THE NCRESS PERSPECTIVE

E. Cinc, M. McMurtrie, B. Bardsley, E. Heriot

NCRESS, Austin Health, Heidelberg, VIC, Australia

From patient recruitment, using a team approach, to a dedicated research clinic, N-CRESS provide a uniquely structured neuro-immunology service based at Austin Health. We provide support to people affected by Multiple Sclerosis and similar neurological disorders. Our service is multi faceted, encompassing clinical research, patient education and immunotherapy support. We are well equipped and resourced to conduct clinical trials. Having a dedicated Research Team consisting of a Manager, to oversee the finance and contracts; a research assistant responsible for ethics submissions; a secretary; and several multi skilled research nurses who coordinate their own trials, ensures all patients are cared for holistically using a primary nursing model. This model of care has a significant impact on treatment adherence and subsequently a high retention rate of patients to clinical trials being conducted here at N-CRESS. In our poster we intend to further demonstrate our unique model of research nursing, which is imperative in ensuring success in clinical trials and we will also explore the challenges and future of MS Research Nursing.

063

WHAT IS THE GENERATION Y NURSES ATTITUDE TOWARDS THE BED-SIDE NURSING ROLE AT AUSTIN HEALTH?

E. J. Dicello (née Wadeson)

7North Renal, Austin Health, Doreen, VIC, Australia

The purpose of this research project was to explore the generation Y nurses attitude towards the bed-side nursing role at Austin Health, and with that information create a program which promotes clinical excellence, and encourages the generation Y nurse to continue, and be future leaders in the bed-side nursing role at Austin Health.

The investigation was conducted using 3 focus groups. Each group included 6 participants. Participants were all generation Y (born after 1980) and over the age of 21 years. Participants must have graduated from nursing between 2005-2009, and they must be currently employed in a grade 2 nursing position on any of the wards at Austin Health including 8East, 8North, 7East, 7West, 6East or 6West.

The preliminary results reveal that amongst the generation Y nurses that were interviewed the bed-side nursing role is generally undesirable if considered to be done on a long-term basis. Reasons include, but are not limited to shift work, impact on work/life balance, difficulties with challenging patients/ families, burn-out, and lack of appreciation.

A conclusion has yet to be determined, as the results have not been finalised. It appears that amongst the generation Y nursing group at Austin Health, the bed-side nursing role is undesirable on a long-term basis. This group of nurses are high achievers, who are already thinking about what their next move is in their nursing careers. In addition to this, Austin Health strongly promotes self-development and upward career pathways, therefore this group of nurses are encouraged to start looking at their nursing career path soon after completing their graduate year.

064

TITLE: MENTAL HEALTH PRESENTATIONS IN THE EMERGENCY DEPARTMENT

N. J. Gaynor[1], G. Farrell[2], T. Shafiei[2]

[1]Emergency Psychiatry, Austin Health, Heidelberg, VIC, Australia
[2]Nursing, LaTrobe University, Melbourne, VIC, Australia

Introduction: Although the number of mental health presentations to emergency departments is increasing due to the integration of psychiatric services with general services, few studies have explored the characteristics of mental health patients presenting to emergency departments in Australia. Aim: this study investigated the characteristics of, and outcomes in relation to, people presenting with a mental health problem to the Austin Health emergency department.

Methods: A retrospective study was undertaken using data from the emergency department's electronic records system (Medtrak) for all adult patients aged 18-65 years old with an ICD-10 discharge diagnosis for mental health disorder over two months. Data on mental health presentation were compared with non-mental health presentations for arrival time, tirage category and length of stay in ED.

Results. Mental health patients made up 5.3% (n=290) of adult presentations to the emergency department, although the true figure may be higher as many patients left before being seen by a clinician or had a discharge diagnosis 'unknown'. Overall, mental health patients were more likely to stay longer than 8 hours in the ED compared to non-mental health patients. Mental health patients who were intoxicated, those who arrived after hours, or patients admitted to a mental health ward were more likely to stay longer than 8 hours in the emergency department.

Conclusions: The study findings are broadly in line with that reported for other Australian studies, although the present findings suggest that patients had significantly more routine investigations.

065

DOES THE RATE OF WEIGHT LOSS IMPACT ON ACHIEVING TARGET WEIGHT?

K. L. Purcell, C. J. Bouniu, E. Delbridge, P. Sumithran, L. Prendergast, J. Proietto

Department of Medicine AH/NH, Melbourne University, Heidelberg Heights, VIC, Australia

Introduction: Very low energy diets (VLEDs) have been used by obese individuals to achieve weight loss for more than 30 years. Despite the positive literature regarding the success of VLEDs, 98.5% of Australian dietitians do not recommend a weight loss of <1kg a week. This project aims to scientifically investigate this perception.

Methods: This was a randomised dietary intervention trial. Baseline testing was performed. Participants were randomised to either:

A. Rapid Weight Loss Group (RG) - participants consumed a commercially available VLCD preparation (Optifast®) for three months and had bi-weekly contact with a dietitian (approx. 6 appointments).

B. Gradual Weight Loss Group (GG) - participants were instructed bi-weekly by a dietitian to achieve a daily negative energy balance of 2000-2500 kilojoules a day over 9 month period replacing 1 meal with a VLCD (approx. 18 appointments).

Following the weight-loss intervention (3 or 9 months) participants underwent measurements as per baseline visit. Participants were deemed successful if they lost 15% of their body weight in their allocated time frame.

Results: Of the 120 participants (34 male and 86 female), average age 50±0.9 years, and initial BMI 35.3 ± 0.3 (/kg/m^2), 60 participants were randomised to the RG, and 60 to the GG. Following intervention, 83.4% of the RG achieved the goal of 15% weight loss compared to 50% in the GG ($p<0.05$). Retention rate was greater in the RG (98%) compared to the GG (85%) ($p<0.05$). Weight loss was associated with a reduction in fat free mass in both groups but was only significantly lower in the RG ($p=0.04$).

Conclusion: This study has shown that substantial weight loss (15% of initial weight) is more easily achieved if undertaken rapidly. Possible reasons will be discussed. More frequent consultations with a dietitian did not equate with greater weight loss.

066

EFFECT OF AN ARGININE-CONTAINING NUTRITIONAL SUPPLEMENT ON PRESSURE ULCER HEALING IN COMMUNITY SPINAL PATIENTS

S. Brewer[2], K. Desneves[1], L. Pearce[1], K. Mills[1], L. Dunn[3], D. Brown[3], T. Crowe[2]

[1]Nutrition and Dietetics Department, Austin Health, Heidelberg, VIC, Australia
[2]School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC, Australia
[3]Victorian Spinal Cord Service, Austin Health, Heidelberg, VIC, Australia

Background: Pressure ulcers (PU) are the most common secondary condition in people with spinal cord injuries. Emerging evidence suggests that the dietary intake of the essential amino acid arginine plays a significant role in healing of PUs.

Objective: To determine whether the use of an arginine-containing nutritional supplement could result in significantly shorter PU healing times in people with spinal cord injuries living in the community, compared with a matched historical control group.

Method: Eighteen spinal-cord-injured patients from the Austin Health Spinal Outreach Team received 9g of a commercial powdered arginine supplement per day until full PU healing occurred. Healing rates were compared against 17 historical control patients, as assessed by medical history audit.

Results: Baseline characteristics (age, gender, injury level and time) were similar between groups. Mean ulcer healing times were 10.5 ± 1.3 weeks versus 21 ± 3.7 weeks ($p<0.05$) in the intervention and control groups respectively. Comparison of healing rates in the intervention group against expected healing rates derived from the medical literature showed that intervention patients had a significantly shorter mean healing time (category 2 PU: 5.5±1.3 weeks versus 13.4 weeks; category 3 PU: 12.5 ± 1.9 weeks versus 18.2 weeks; category 4 PU: 14.4 ± 4.8 weeks versus 22.1 weeks). A diagnosis of diabetes did not significantly alter healing rates in either group.

Conclusion: Results from this observational study show a promising benefit of arginine-containing supplementation on PU healing for individuals with spinal cord injury living in the community.

(1) Stechmiller, J.K., Childress, B., Cowan, L. Arginine supplementation and wound healing. Nutr Clin Pract 2005; 20: 1, 52–61.
(2) Bennett, G., Dealey, C.,Posnett, J. The cost of pressure ulcers in the UK. Age Ageing 2004; 33: 3,230–235.

067

DOES ORAL BRANCHED CHAIN AMINO ACID SUPPLEMENTATION IN PATIENTS AWAITING LIVER TRANSPLANTATION IMPROVE NUTRITIONAL OUTCOMES?

B. Chapman[1], K. Hamilton[1], C. Brockbank[2], L. Pearce[1], P. Gow[3], T. Crowe[2]

[1]Nutrition & Dietetics, Austin Health, Heidelberg, VIC, Australia
[2]School of Exercise and Nutrition Science, Deakin University, Melbourne, VIC, Australia
[3]Liver Transplant Unit, Austin Health, Heidelberg, VIC, Australia

Aim / Background: Malnutrition is a poor prognostic indicator in liver transplantation. We investigated whether oral branched chain amino acid (BCAA) supplementation improves nutrition and clinical outcomes in patients awaiting liver transplant at Austin Health (AH).

Methodology: Forty-eight AH patients assessed for liver transplant in 2009 were approached to participate. Patients were prescribed 10g BCAA daily in addition to usual diet for 12 weeks. Dietary intake, biochemistry, handgrip strength (HGS), nutritional status and BCAA compliance were monitored. The intervention group was compared to 29 historical controls.

Results: Twenty-eight patients enrolled, with 10 (36%) successfully completing the intervention. No significant change in proportion of malnourished patients in either controls (55%) or BCAA group (80%) during the intervention was found. Both groups were unable to meet energy and protein requirements at baseline or at 3-months. A small non-significant decline in HGS for the BCAA group was shown (65.5+/-8.2 to 61.5+/-5.9), while the control group demonstrated a positive trend (69.6+/-4.6 to 72.1+/-4.9). No significant differences were found in albumin levels, dry weight or MELD scores during this study.

Discussion: Patients awaiting liver transplant have a high level of malnutrition at baseline and are unable to meet their nutrition requirements, however the addition of BCAA did not improve nutritional status or functional outcomes.

Conclusion: This pilot study showed that BCAA supplementation was not effective in improving clinical or nutritional outcomes in patients awaiting liver transplant when energy and protein intake is inadequate. The high level of attrition in the intervention group and inability of all patients to meet nutrition requirements warrants further investigation.

068

IDENTIFICATION OF MALNUTRITION ON THE ACUTE ONCOLOGY WARDS AT AUSTIN HEALTH AND DETERMINING THE IMPACT OF DOCUMENTING MALNUTRITION ON HOSPITAL FUNDING.

J. Passador, K. Kaegi, J. Rafferty, E. Adithama

Nutrition and Dietetics, Austin Health, Heidelberg, VIC, Australia

The prevalence of malnutrition among oncology patients ranges from 40-80%. Coding classifications have recently changed to enable a malnutrition diagnosis, as determined by a dietitian, to be included in a patient's Diagnosis Related Group (DRG). Documenting malnutrition in the patient's medical history may therefore impact on hospital financial reimbursement.

The aims of this study were to determine the prevalence of malnutrition in patients referred to the dietitian, assess if documentation of malnutrition was being recognised by coders and to determine if identification and inclusion of malnutrition in DRG's contributed to increased hospital funding.

Thirty-one acute oncology patients were referred for nutritional assessment during October 2009. Patients were assessed for malnutrition using the scored Patient Generated-Subjective Global Assessment (PG-SGA). PG-SGA malnutrition diagnoses were documented in the medical history. DRG's were reviewed to assess if malnutrition was recognised by the coders. A cost analysis was completed to look at changes in financial reimbursement with inclusion of malnutrition in DRG's.

For patients seen by the dietitian the prevalence of malnutrition was 61%. Malnutrition was documented in medical histories in 100% of cases, but only identified in the DRG in 28% of cases. Identification of malnutrition increased DRG codes for 11% cases, which would have resulted in additional hospital funding.

In the acute oncology wards there is a high prevalence of malnutrition. This small study demonstrated the potential impact of accurately coding for malnutrition. Further work is required across all areas at Austin Health to determine the true impact of this on hospital reimbursement.

069

INCREASED EXPRESSION OF TISSUE INHIBITOR OF METALLOPROTEINASES-1 (TIMP-1) IN CERVICO-VAGINAL FLUID PRECEDES SPONTANEOUS HUMAN LABOUR.

S. Liong[1,2], Y. J. Heng[1], M. Permezel[1,2], M. K.W. Di Quinzio[1,2], H. M. Georgiou[1,2]

[1]Obstetrics & Gynaecology, The University of Melbourne, Parkville, VIC, Australia
[2]Mercy Perinatal Research Centre, Mercy Hospital for Women, Heidelberg, VIC, Australia

Background: Matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitor of metalloproteinases; TIMPs) have been extensively studied in human gestational tissues during pregnancy and parturition. A number of MMPs are involved in matrix remodelling of the uterus, cervix and fetal membranes; a process that is critical in the preparation and progression of labour. It is postulated that biochemical changes occurring in the cervix and overlying fetal membranes may be reflected in the cervico-vaginal fluid (CVF). Previous analyses of CVF by our laboratory and others demonstrate the presence of several MMPs. However, there have been no published studies investigating the temporal expression of TIMPs in the CVF prior to and during labour.

Aim: To investigate the temporal expression of TIMP-1 in the CVF with impending labour.

Subjects & Methods: CVF samples were collected weekly from healthy, multiparous pregnant women from 36 weeks' gestation until the onset of spontaneous labour. TIMP-1 concentration in the CVF was quantified using ELISA. The Mann-Whitney U test was used for statistical analysis and significance was assumed when $p<0.05$.

Results: The concentration of TIMP-1 was significantly increased during labour compared to samples collected at 0-3,4-7, 8-14, 15-21, 22-28 and >28 days from labour ($n=184$, M-W test, $p<0.05$) TIMP-1 expression was also significantly elevated at 0-3 days before labour onset compared to samples collected at 8-14, 15-21 and 22-28 day from labour (M-W test, $p<0.05$).

Conclusion: It is well recognised that the expression of various MMPs is elevated in gestational tissues, particularly the cervix, in preparation for labour. It might be expected that an increase in MMP levels as labour approaches would correlate with a concomitant decrease in TIMP levels. However, TIMP-1 expression in the CVF was elevated with impending labour, thus demonstrating a delicate balance of MMPs and TIMPs in matrix remodelling.

070

EXERCISE REHABILITATION FOR PATIENTS FOLLOWING LUNG RESECTION SURGERY: A PILOT FEASIBILITY RANDOMISED CONTROLLED TRIAL.

C. L. Granger[1,2], C. Chao[1], L. Denehy[2], C. McDonald[3], S. Berney[1]

[1]Physiotherapy, Austin Health, Heidelberg, VIC, Australia
[2]School of Physiotherapy, The University of Melbourne, Parkville, VIC, Australia
[3]Department of Respiratory and Sleep Medicine, Austin Health, Heidelberg, VIC, Australia

Purpose: International pilot data suggest that rehabilitation following lung cancer surgery is associated with improvements in exercise capacity and health related quality of life (HRQoL). This study aimed to determine the feasibility of a rehabilitation program for patients following lung resection surgery; and describe HRQoL and physical function outcomes.

Methods: Patients with suspected lung cancer were recruited preoperatively and randomised to a rehabilitation or standard care group, at day one post-operative. The intervention group participated in a standardised exercise program; in an inpatient and subsequent outpatient setting. HRQoL and physical function were measured at pre-surgery, 2 and 12 weeks following surgery.

Results: Eighteen participants were recruited; ten (8 male) were diagnosed with lung cancer. Of these, three patients underwent rehabilitation and 7 received standard care. Domains of global quality of life and fatigue demonstrated clinically important improvements in the intervention group; compared to worse fatigue, physical function and pain in the control group. Both groups showed a decline in physical function (6minute walk test) at 2 weeks post-operatively, which recovered to baseline at 3 months. Submaximal exercise testing, measuring peak oxygen consumption, declined in both groups from baseline over time. Patients attended rehabilitation on 64% of occasions. No adverse events occurred during exercise training.

Conclusions: The results of this pilot study suggest that rehabilitation is safe, beneficial and well accepted by patients. Further research is required to establish sensitive and feasible outcome measures for exercise testing; and to determine optimal service delivery models and training programmes in the Australian setting.

071

VALIDITY OF THE VICON TO MEASURE 3D SCAPULAR MOTION

J. Blennerhassett[1], N. Lythgo[2], L. Wielders[3]

[1]Physiotherapy, Royal Talbot Rehab Centre-Austin Health, Kew, Australia
[2]RSRC-Austin Health, Kew, Australia
[3]Radboud University, Nijmegen, Netherlands

Introduction: Altered scapular motion is associated with shoulder pain, but is difficult to quantify. The VICON system is a non-invasive instrument that uses skin-markers to record 3D motion in vivo. Skin-markers lead to error when quantifying scapular motion as the scapula moves under the skin. This study investigated the validity of VICON to record scapular motion.

Method: Eight young adults without shoulder pathology performed shoulder flexion and abduction to 0 ° , 45 ° , 90 ° , 120 ° and 150 ° (3 trials per condition). Three skin-markers were placed on the right scapula over the acromion, spine (medial aspect) and inferior angle. While the arm was held in position, each scapular landmark was located and pointed to using a set-square (3 markers attached to it). The VICON simultaneously recorded the skin and set-square markers. Scapular orientation was then extracted from (1) the skin markers and (2) the set-square markers (criterion measure). The difference between these orientations was used to quantify measurement error in 3 movement planes; antero-posterior (AP), frontal (F), transverse (T).

Results: Mean (range) errors for flexion were: AP = -1.1 ° (-11.2 ° to 8.8 °), F = -1.0 ° (-7.2 ° to 9.7 °), T = -0.2 ° (-4.9 ° to 7.6 °). For abduction, errors were: AP = -2.0 ° (-8.9 ° to 5.8 °), F = -5.0 ° (-17.3 ° to 6.8 °), T = 1.1 ° (-4.6 ° to 8.5 °). The greatest errors were found for movements above 90 degrees.

Conclusion: There is some error when using skin-markers to measure scapular motion. For clinical purposes, the VICON shows potential to assess scapular motion up to 90 degrees of shoulder elevation.

072

AN EXERCISE AND EDUCATION PROGRAM IMPROVES WELL-BEING OF NEW MOTHERS: A RANDOMISED CONTROLLED TRIAL.

M. P. Galea[1], E. Norman[2], M. J. Sherburn[1], R. H. Osborne[3]

[1]Rehabilitation Sciences Research Centre, The University of Melbourne, Kew, VIC, Australia
[2]Physiotherapy, The Angliss Hospital, Ferntree Gully, VIC, Australia
[3]School of Health & Social Development, Deakin University, Burwood, VIC, Australia

The objective of this study was to evaluate the effect on new mothers' psychological well-being of a physiotherapy exercise and allied health education program in a randomised controlled trial.

Participants were primiparous and multiparous English-speaking women ready for discharge from The Angliss Hospital postnatal ward were eligible for this study. Women who were receiving psychiatric care were excluded. One hundred and sixty-one women were randomized into the trial.

The experimental group (n=62) received an eight week "Mother and Baby" (M&B) program, including specialised exercise provided by a women's health physiotherapist combined with mothercraft education. The other group (Education Only, EO; n=73) received only the same educational material as the experimental group. The main outcome measures included measures of psychological well-being (Positive Affect Balance Scale), depressive symptoms (Edinburgh Postnatal Depression Scale) and physical activity levels collected at baseline, after eight weeks (post-program) and then four weeks later.

The results showed a significant improvement in well-being scores and depressive symptoms of the M&B group compared with the EO group over the three time periods. More specifically, there was a significant positive effect on well-being scores and depressive symptoms at eight weeks [M&B: 11.82 (2.08), 95% CI 11.24 - 12.37; EO: 10.47 (2.26), 95% CI 9.96 - 11.01], and this effect was maintained four weeks after completion of the program. The number of women identified as "at risk" of postnatal depression pre-intervention was reduced by 50% by the end of the intervention.

Conclusion: A physiotherapy exercise and health education program is effective in improving postnatal well-being. Routine use of this program may reduce longer term problems such as postnatal depression.

Trial Registry: National Institutes of Health. Trial register number: NCT00361478.

073

THE ASTEX®: A NEW DEVICE FOR ASSESSMENT OF TEXTURE DISCRIMINATION IN THE HAND

K. J. Miller[1], B. A. Phillips[2], C. L. Martin[1], H. E. Wheat[3], A. W. Goodwin[3], M. P. Galea[1]

[1]Rehabilitation Sciences Research Centre, Department of Physiotherapy, The University of Melbourne, Kew, VIC, Australia
[2]Faculty of Health Sciences, LaTrobe University, Bundoora, VIC, Australia
[3]Anatomy and Cell Biology, The University of Melbourne, Parkville, VIC, Australia

Purpose: The ability to appreciate the surface texture of objects is important for successful grasping and manipulation of objects. In this study the clinimetric properties of the AsTex®, a new instrument developed to assess texture discrimination in the hand, were investigated.

Method: Test-retest and inter-rater reliability, and age- and gender-stratified normative values for texture discrimination indices (TDIs) were established for the index finger in neurologically normal participants aged between 18 and 80 years (n=142). Test-retest reliability was also examined in participants who had experienced a stroke a minimum of one-year prior to participating in the trial (n=22).

Results: The AsTex® required less than 5 minutes to administer and had good clinical utility. Test-retest (ICC=0.98, 95% CI=0.97-0.99) and inter-rater reliability (ICC=0.81, 95% CI=0.73-0.87) were found to be excellent for the index finger in neurologically normal participants. Test-retest reliability of the AsTex® in individuals with stroke was also excellent (ICC=0.86, 95%CI=0.68-0.94). The standard error of measurement was 0.14 mm. In neurologically normal participants there was a trend for TDIs in the index finger to rise (indicating poorer sensory discrimination) after the fourth decade. Subjects aged 70 years or older had significantly higher TDIs than younger subjects ($p < 0.001$).
Conclusion: The AsTex® is a reliable and clinically useful instrument for quantifying hand sensation capabilities. The age- and gender-stratified normative data will facilitate the interpretation of AsTex® assessment findings in clinical populations.

074

WALKING ON LEVEL AND SLOPED SURFACES FOLLOWING STROKE.

P. Phan[1], J. Blennerhassett[1], N. Lythgo[2], M. Morris[3]

[1]Physiotherapy, RTRC, Austin Health, Melbourne, VIC, Australia
[2]RSRC, Austin Health, Melbourne, VIC, Australia
[3]Physiotherapy, The University of Melbourne, Melbourne, VIC, Australia

Background and Aim: The ability to negotiate slopes is an important aspect of community mobility, yet has not been studied in people with stroke. This study investigated the spatiotemporal gait patterns whilst walking on uphill, downhill and level surfaces post-stroke.

Method: Fifteen people with stroke and 15 healthy controls matched for sex, age and height participated. Gait was recorded by a GAITRite system on three conditions: level, uphill and downhill (slope gradient was 1:12). Examples of measures investigated were speed, step length and asymmetry, cadence and base of support (BOS). Between and within group comparisons were performed using MANOVA.

Results: The stroke differed significantly to healthy controls for all gait measures ($p < 0.05$), consistent with other studies post-stroke. The controls maintained walking speed across all conditions (mean ±SD cm/s speeds: level =143.8 ±17.1; up =145.2 ±17.5; down =143.3 ±19.4). In contrast, the stroke group walked slower downhill (70.2 ±32.2 cm/s), compared to level (77.2 ±35.6 cm/s) and uphill (76.8 ±35 cm/s)($p < 0.01$). Slope condition did not affect cadence for either group. However step length varied, with the stroke group exhibiting shorter steps when walking downhill compared to level and uphill ($p < 0.01$).

Conclusion: When walking on level and sloped surfaces, people with stroke are slower, take shorter and more asymmetrical steps and have a wider BOS relative to matched healthy controls. People with stroke make further changes when walking downhill (relative to level and uphill) by decreasing speed and taking shorter steps without changing degree of asymmetry or cadence.

075

EARLY SENSITIVITY TRAINING FOR PARENTS OF PRETERM INFANTS: IMPACT OF *PREMIESTART* ON THE DEVELOPING BRAIN

C. Newnham[1,2], J. Milgrom[1], A. Gemmill[1], P. Anderson[2], T. Inder[3]

[1]Parent-Infant Research Institute, Austin Health, Heidelberg West, VIC, Australia
[2]Victorian Infant Brain Studies, Melbourne, VIC, Australia
[3]St Louis Children's Hospital, St Louis, United States

Background: Preterm infants are at increased risk of persistent developmental difficulties. Perinatal medical complications only explain about 30% of the variance in outcomes. There is growing evidence that environmental factors also play a role. Previous environmental interventions that aim to reduce stress in hospitalised preterm infants have shown promising results. The *PremieStart*[1] intervention trialed here focuses on sensitivity training of the carers (parents) of preterm infants.

Aims: We aimed to assess, in a randomised controlled trial, if intervention was associated with (i) structural brain changes at term equivalent and (ii) short-term medical benefits. We hypothesized that our intervention (adapted from a previous program) would buffer preterm infants from the harmful effects of stress, resulting in enhanced brain development compared to control infants.

Methods: Quantitative MRI analyses included volumes of tissue types (total tissue, cerebrospinal fluid, cortical grey matter, deep nuclear grey matter, myelinated and unmyelinated white matter) for 16 brain areas. Diffusion measures included apparent diffusion coefficients (ADC), Fractional anistropy (FA) and axial and radial diffusivity, in 12 manually selected regions of interest.

Results: Forty-five mothers of 52 infants born < 30 weeks gestation were randomised to intervention (n=22) and control (n=23) groups. Of 48 MRI scans successfully conducted, diffusion data were acquired in 27 cases (infants were often unable to remain still). Maturation and connectivity of white matter were significantly enhanced in the intervention group, who displayed greater restriction in ADC (p=.0006) with some suggestion that the effect varied by region (p=.10) with the strongest effect in the superior region. A similar trend of improved while matter maturation was suggested by FA values.

Conclusions: Parental sensitivity training is a promising approach, with short-term benefits for brain development that could contribute to later advantages in cognitive function. Our results are consistent with evidence that early experience influences cerebral development.

(1) Milgrom, J., Newnham, C., Anderson, P., Doyle, L.W., Gemmill, A.W. Lee, K., Hunt, R., Bear, M. & Inder, T. (2010). Early Sensitivity Training for Parents of Preterm Children: Impact on the Developing

076

PRELIMINARY RESULTS: AUSTIN HEALTH-DIABETES LIFESTYLE CHANGE PROGRAM

N. Gazis[1], A. McCubbin[1], B. Gordon[2], R. J. MacIsaac[3]

[1]HARP, Austin Health, Heidelberg West, Australia
[2]Physiotherapy, Austin Health, Heidelberg, Australia
[3]Endocrinology, Austin Health, Heidelberg West, Australia

Introduction/Background: The Austin Health – Diabetes Lifestyle Change Program (AH-DLCP) is a 16-week multidisciplinary group program aiming to lower lifestyle risk in overweight type 2 diabetic out patients. Reducing lifestyle risk factors can prevent diabetes complications and increase physical and mental well being.1,2 A modest weight loss of 5-10% can improve glycaemic control3 and cholesterol4 and lower blood pressure.5 The aims of the program include a) assisting patients achieve a 5-10% reduction in body weight, b) maintaining the loss for a year and b) reducing diabetes related distress. The present study examined preliminary weight loss and diabetes distress outcomes following completion of three group programs. High levels of distress have been strongly linked with depression and can lead to poorer self care in diabetic patients.6

Method: Three cohorts of type 2 diabetes out patients (n= 23; 65% male; mean weight = 101.8kg , *SD* = 16.9*;* mean body mass index = 35.4) completed the AH-DLCP. The main outcome measure was weight (kg) at baseline and after treatment. Diabetes distress pre and post treatment was measured with the Problem Areas In Diabetes (PAID) scale. High scores on the PAID have been found to be strongly associated with depression.7 The mean baseline PAID score was 24.4 (*SD* = 1.4)

Results: The mean weight loss after 16 weeks was 5kgs (*SD* = 4.7) and represented a significant reduction of 4.9% of baseline body weight (t = 5.6, $p<.001$). A significant reduction of 55% was found in the mean PAID score 10.9 (*SD* = 3.7) compared with the baseline (F = 3.14, df = 19, $p<.001$).

Conclusions: Preliminary results of the first three cohorts of the AH-DLCP have demonstrated that the 16 week group lifestyle change program was successful in reducing weight and lowering distress associated with the management of diabetes. Modest weight loss and better emotional coping with diabetes has been linked to better health outcomes in overweight patients and supports the continuation of the program.

(1) De Groot et al, Psychosom Med, 2001; 63: 619-630
(2) Lustman et al, Diabetes Care, 2000; 23: 924-933
(3) Wing RR et al. Arch Intern Med. 1987;147:1749-1753
(4) Blackburn G. Obes Res. 1995;3 (Suppl 2):211S-216S
(5) Mertens IL, Van Gaal LF. Obes Res. 2000;8:270-278
(6) Lin et al, Diabetes Care, 2004; 27:2154–2160
(7) Herman et al, Diabetologia, 2006; 49: 469–477

077

THE DCAS ASSESSMENT DATABASE – AN INNOVATIVE USE OF IT TO PROVIDE TIME-EFFICIENT, PATIENT-CENTRED DIABETES CARE

A. J. McCubbin

HARP Diabetes, Austin Health, Heidelberg, VIC, Australia

Introduction/Background: The Diabetes Complications and Assessment Service (DCAS) provides a comprehensive assessment of a client's diabetes, with a care plan compiled and mailed to the referring physician and client. This model however was time-consuming for staff, and documentation not available across clinic sites.

Aim: To automate administration processes in DCAS, and improve the level of client-centred care

Methods: The existing, paper-based assessment template was replaced with an SQL database with Microsoft Access user interface. Correspondence was automated through the database. Staff at community health sites could login remotely to the network to access the system.

The latest version added validated screening tools for psychosocial issues, depression and sleep apnoea. A separate letter to clients was also instigated, to explain results and recommendations in easy-to-understand language.

Results: The DCAS Assessment Database has many advantages over the previous, paper-based system:

Five hours per week of admin staff time saved

Medications, referrals and physicians' details are stored and retrieved

Assessments are accessible across sites, allowing clients to move seamlessly between them

Easy data extraction for quality improvement, KPI tracking and research

Continuous feedback from clinicians has been the key driver for change. Version 3.0 is easier to navigate compared to Version 1.0, and is more client-centred by including a patient letter, assessment of self-management behaviours and psychosocial issues.

Conclusion: The development of the DCAS Assessment Database has delivered large time savings, improved quality of care, an increased client-centred approach and a powerful evaluation tool.

078

IMPAIRED CORTICAL RESPONSE TO A RESPIRATORY STIMULUS IN UNTREATED SEVERE OBSTRUCTIVESLEEP APNOEA

W. R. Ruehland[1,2], P. D. Rochford[1], J. A. Trinder[2], K. E. Webster[3], F. J. O'Donoghue[1]

[1]Institute for Breathing and Sleep, Austin Health, Heidelberg, VIC, Australia

[2]The University of Melbourne, Parkville, VIC, Australia

[3]La Trobe University, Bundoora, VIC, Australia

Introduction: Obstructive sleep apnoea (OSA) pathogenesis is thought to be multifactorial and a deficit in sensory processing of respiratory stimuli has been proposed as a possible contributor. Respiratory Related Evoked Potentials (RREP) are the averaged cortical response to a respiratory stimulus; the early components (Nf, P1) are thought to reflect arrival of afferent information at the somatosensory cortex. Although previous studies have examined RREPs in OSA, this study is unique in that it examines the RREP in response to inspiratory resistive loads spanning the threshold of conscious detection. This may be important as impaired detection and response to minor upper airway threat may lead to worsening collapse, which is difficult to remedy.

Methods: Seven untreated severe OSA patients and 5 age and gender matched healthy controls participated. Participants were awake, seated and had EEG and EOG recorded (Fz, Cz, Pz referenced to linked ears). They wore a nasal mask connected via a non-rebreathing valve and tubing to a resistive loading manifold. Various resistive loads (approx. 0, 1.2, 2.2, 3.0, 6.2 $cmH_2O/L/sec$), as well as manifold occlusion were presented approximately 90 times each during mid-inspiration, every 2-4 breaths and in a random block design. Participants were cued prior to stimulus presentation and signalled conscious detection (yes/no) with a button press.

Results: The early positive RREP peak (P1@Cz) increased in amplitude with increasing load magnitude ($p=0.018$). There was a trend for reduced P1 amplitude in OSA subjects vs. controls. Also, for the lower resistive loads (1.2, 2.2, $3.0cmH_2O/L/sec$) mean P1 amplitude appeared to increase with load magnitude for control but not OSA participants.

Conclusion: These preliminary results suggest impaired sensory processing of respiratory stimuli in OSA, such that OSA patients may have difficulty distinguishing low intensity respiratory loads. Further data collection is underway, including examination of whether treatment eliminates any impairment.

079

MISPERCEPTION OF SLEEP LATENCY AND SLEEP DURATION IN PREGNANCY

D. L. Wilson[1], S. P. Walker[2], A. Fung[2], M. Barnes[1]

[1]Respiratory and Sleep Medicine, Austin Health, Heidelberg, VIC, Australia

[2]Perinatal Medicine, Mercy Hospital for Women, Heidelberg, VIC, Australia

Introduction: Although it is accepted that sleep disturbance is common during pregnancy, there is a lack of objective sleep data due to the difficulty in persuading these women to spend a night in a sleep laboratory. It is likely that in this group, as found in the general population, self-report sleep quality is unreliable. This study compares self-reported sleep quality with objective measures on polysomnography (PSG) during pregnancy.

Methods: Twenty-one women in the third trimester (T3) and 17 women in the first trimester (T1) of pregnancy and 10 non-pregnant controls underwent overnight PSG and reported their perceived sleep onset latency (SOL), total sleep time (TST) and sleep quality.

Results: Table 1. Objective vs. Subjective SOL and TST in the T3, T1 and Control Groups

Sleep Parameter	T3	T1	Control	p
SOL (mins)	36.0 ± 42.9	19.8 ± 17.7	29.8 ± 56.3	.46
TST (mins)	343.6 ± 83.6	409.0 ± 37.7	427.0 ± 57.3	.002**
SOL_s - SOL_o	10.8 ± 29.4	12.4 ± 13.1	9.0 ± 22.6	.93
Abs. SOL_s - SOL_o	22.7 ± 21.0	14.8 ± 10.1	17.1 ± 16.6	.34
TST_s - TST_o	11.9 ± 57.8	-49.0 ± 61.4	-30.5 ± 36.7	.005**
Abs. TST_s - TST_o	46.4 ± 43.5	50.6 ± 59.9	39.3 ± 25.8	.81

Note: $_s$ = subjective $_o$ = objective Abs. = Absolute value ** $p < .01$

On average, all groups overestimated their SOL. The T3 group tended to overestimate TST, which differed significantly from the T1 and control groups who underestimated TST on average. Absolute values however show that all groups incorrectly reported TST by 40-50 minutes with substantial variability. The accuracy of sleep perception did not differ between those who reported their sleep quality as the same as usual or worse than usual.

Discussion: Sleep perception during pregnancy tends to be unreliable, but not significantly more so than in healthy controls. Negative perceptions of sleep quality regardless of objective measures have been shown to result in impaired daytime functioning. Misperception of sleep also has important implications for the vast number of studies based on self-report in the pregnant population.

080

INTER-RATER RELIABILITY IN THE INTERPRETATION OF LUNG FUNCTION TESTS

P. D. Rochford[1], P. A. Guy[2], P. Liakakos[1], D. Brazzale[1], C. F. McDonald[1], G. Hamilton[2], J. J. Pretto[3]

[1]Institute for Breathing and Sleep, Melbourne, VIC, Australia

[2]Respiratory Medicine, Monash Medical Centre, Melbourne, VIC, Australia

[3]Respiratory & Sleep Medicine, John Hunter Hospital, Newcastle, NSW, Australia

Rationale: Lung function tests (LFT) are integral in the diagnosis and treatment of respiratory disease. Although substantial efforts have been made to standardise methods and instrumentation, there are very few data on the reliability of interpretation of test results, despite the potential to affect patient outcomes.

This study describes the degree of variability in interpretation of LFTs in a cross-section of Australian and New Zealand respiratory laboratories.

Methods: 10 LFTs (spirometry±BD and TLCO) covering a range of values and presented in a standard format were distributed for interpretative comment. Participants also completed a questionnaire to document their profession, reporting experience, qualifications and public/private lab status.

Results: 84 reporters participated (61% physicians, 21% scientists, 14% registrars). Wide variation in agreement for major reporting categories was found (table). There was also substantial variation seen in the interpretation of volume effects on TLCO.

	% Raw Agreement median (range)	
Spiro: Normal v Abnormal	100	(65-100)
Spiro: Pattern of Abnormality (Obs/Res/Mixed)	89	(44-100)
Spiro: Severity rating	56	(36-100)
Spiro: BD reversibility	90	(70-100)
TLCO: Normal v Abnormal	86	(43-100)
TLCO: Severity rating	83	(45-94)

Conclusion: Significant inter-rater variation in all areas of LFT interpretation was observed, although the degree of variation depended upon the pattern of LFT values. These results provide a reference against which future efforts to improve LFT reporting reliability may be compared.

081

PSG SCORING RELIABILITY IN A LARGE COHORT OF SCORERS IN AUSTRALASIAN SLEEP LABORATORIES: INITIAL RESULTS FROM A NOVEL INTER-LABORATORY PROFICIENCY TESTING PROGRAM

P. Rochford

IBAS, Heidelberg, VIC, Australia

Study	1	2	3	4	5	6	7	8
AHI CV%	27%	30%	31%	13%	26%	53%	42%	10%
ArI CV%	43%	35%	38%	35%	39%	43%	47%	25%
SEff CV%	5%	5%	5%	7%	24%	17%	11%	30%
Mean K Resp	0.64	0.68	0.65	0.73	0.72	0.6	0.62	0.78
Mean K Ars	0.48	0.53	0.51	0.56	0.49	0.62	0.49	0.56
Mean K Sleep	0.69	0.65	0.69	0.67	0.62	0.68	0.75	0.41
n Scorers	69	60	67	62	73	68	86	83

Inter-scorer variation is a major source of error in the diagnosis of sleep disorders. Techniques aiming to improve PSG measurement reliability are lacking and this report describes early results from a novel proficiency testing program.

Methods: Australian and New Zealand sleep laboratories using Compumedics® software were invited to participate. A web-based interface was developed to provide scorers with test PSGs (8 over 12 months). PSGs were scored for sleep, arousals and respiratory events. Scoring reliability was evaluated using a novel analysis tool that derives a target "maximum likelihood score" from all submitted PSGs. Agreement between individual scorers and the target was assessed with Kappa (K) and coefficient of variation (CV%).

Results: CV% of apnea-hypopnea index (AHI), arousal index (ArI) and sleep efficiency (SEff) and mean K values for each of the 8 studies are shown.

Conclusion: Kappa values indicate substantial agreement on all but arousal scoring. Nevertheless, there were large inter-scorer differences with a mean CV% across all studies of 13% for SEff, 29% for AHI and 38% for ArI. This degree of variability must be considered when using these results for diagnostic purposes. This novel proficiency testing program provides a mechanism for achieving improvement in scoring reliability through a quality assurance program conducted on a large scale.

082

ELEVATED SLOPE PHASE III FROM UPG CORRELATES WITH INTRA-BREATH CHANGES IN LOW FREQUENCY REACTANCE (X5HZ) IN PATIENTS WITH COPD.

G. Nolan, K. A. Southwell, P. D. Rochford, C. F. McDonald

Respiratory Medicine, IBAS/Austin Health, Heidelberg, VIC, Australia

Background: Spirometry is insensitive to small airway function in COPD, yet Spirometry remains the means of confirmation of diagnosis in all current guidelines. Small airway function can be assessed by FOT and also quantifying ventilation homogeneity.

Aim: To examine correlation between dynamic expiratory flow limitation and a simple index of inhomogeneity of tidal ventilation from hand held Ultrasonic Pneumography (UPG).

Method: 11 patients (8:3, M:F) with a diagnosis of COPD confirmed by post bronchodilator Spirometry (FER<LLN) underwent FOT and tidal breathing measures using UPG. The FOT data were analysed for mean resistance and reactance at 5Hz (Xrs5) and peak to peak within breath changes in Xrs5 over 60 seconds of tidal breathing. A further 30 seconds of tidal breathing was recorded by UPG. RR, TV, and Vd, slope of Phase II (PII) & Phase III (PIII), and KPLv were calculated. Spearman's correlation was applied. ROC curves constructed for both FOT and UPG variables referenced to FER from Spirometry.

Results: There was a significant correlation between peak to peak change in X5Hz within breath and PIII and KPLv ($P<0.001$). Area under the ROC for FER, X5Hz, peak to peak X5Hz, PIII, and KPLv were 93%, 62%, 86%, 85% and 91% respectively.

Conclusion: We have demonstrated that a UPG in a desktop tidal breath test is as sensitive to changes in ventilatory capacity in patients with COPD as low frequency FOT and correlates with FOT variables of small airway disease. The specificity and sensitivity of this tidal breathing test in small airway disease is at least as good as a forced Spirometry.

083

THE PREVALENCE AND SEVERITY OF SLEEP APNOEA IN OLDER ADULTS WITH SELF-REPORTED INSOMNIA.

R. Schembri[1,2], A. Huther[2], J. Spong[1], K. Greenwood[2], F. O'Donoghue[1], M. Barnes[1]

[1]Institute for Breathing and Sleep, Austin Health, Melbourne, VIC, Australia

[2]School of Health Sciences, RMIT University, Melbourne, VIC, Australia

Sleep apnoea and insomnia are prevalent conditions in the older population. While the co-morbidity of sleep apnoea and insomnia is known, the relationship is often reported in terms of sleep apnoea patients also exhibiting symptoms of insomnia. As insomnia is commonly diagnosed by self-report, little is known about the prevalence of sleep apnoea in patients presenting with insomnia.

This study examines the prevalence and severity of sleep apnoea in older adults whose primary sleep complaint is insomnia.

31 participants (14 male) aged 57–89years (*M*= 69.7, *SD*=8.6), with a primary complaint of insomnia were recruited from the community. Participants were screened via clinical interview by an experienced sleep physician and enrolled if they met the DSM-IV criteria for primary insomnia, and were considered unlikely to have sleep apnoea. Participants completed subjective and objective measures of sleep, including Polysomnography.

Significant sleep apnoea with an Apnoea Hypopnoea Index (AHI) of 15 or greater was found in 51.6% of participants. These participants had a mean(*SD*) AHI of 30.7(12.1) and a mean(*SD*) nadir oxygen saturation of 81.5(8.2)%. 16.1% of participants had an AHI of 40 or greater, and these participants had a mean(*SD*) AHI of 45.6(4.0), and a mean(*SD*) nadir oxygen saturation of 78.8(11.2)%.

Identifying sleep apnoea in the insomnia population can be difficult in older adults, where both conditions are common and can exhibit similar symptoms. While insomnia is the most common sleep complaint in older adults, these preliminary data suggest that even after screening for sleep apnoea, unsuspected sleep apnoea is present in around half of presenting cases.

In clinical practice, where the diagnosis of insomnia is commonly based on self-report, the presence of sleep apnoea may go undiagnosed without objective diagnostic screening. This may have a major impact on the response to treatment and the health and wellbeing of patients.

084

MANUAL VERSUS AUTOMATED DETECTION OF OXYGEN DESATURATION INDEX DURING SLEEP.

T. J. Churchward[1,2], J. Miles[1], I. Sparks[1], W. R. Ruehland[1,2], F. O'Donoghue[1,2], P. D. Rochford[1,2]

[1]Department of Respiratory and Sleep Medicine, Austin Health, Heidelberg, VIC, Australia

[2]Institute for Breathing and Sleep, Heidelberg, VIC, Australia

Introduction: Obstructive Sleep Apnoea (OSA) is common and current resources for OSA diagnosis are inadequate, so there is increased interest in simplified monitoring. Oxygen desaturation has shown promise as a measurement in abbreviated investigations. Oxygen Desaturation Index (ODI) is increasingly reported, but there is no generally accepted desaturation definition.

Aims: (1) To develop a clearly defined, precise and robust oxygen desaturation definition and (2) Compare this definition to an automatically generated ODI (using Compumedics Profusion3.2 software).

Methods: Three scientists experienced in analysis scored desaturations on a sleep study (Compumedics E-Series, Nellcor N595 oximeter set to shortest averaging (2-4 seconds)) and results were discussed, on an event-by-event basis until a revised rule set was devised to improve alignment with consensus. This process was repeated until no further alignment was achieved. This rule set was then tested on 2 new studies (2 scorers) and results compared between manual and automated analyses.

Results: Rule summary: Baseline: maximum SpO2 value in 15 second window; Event start: start of decline to >=4% SpO2 reduction; Event end: start of increase of >= 2% within 5 seconds. ODIs (events/hr) and pairwise proportion of specific agreement (PSA) for manual scorers (S1,S2) and automatic analysis (P3) were, respectively, Study#2: [ODIs: 130,132,119, PSAs: 0.99 (S1-S2), 0.95 (S1-P3)], Study#3 [ODIs 90,86,80, PSAs 0.96 (S1-S2) , 0.94(S1-P3)].

Discussion: This rule set is simple and shows promise as a highly repeatable manual desaturation measure. Accuracy of the automated ODI measure compares reasonably, although with tendency to underscore, in comparison, in this data set.

085

COMMUNICATION IMPAIRMENT IN CHILDREN WITH ANXIETY DISORDERS: A PILOT STUDY.

E. Morkham, R. Stargatt

Austin Child and Adolescent Mental Health Service, Austin Hospital, Heidelberg, Australia

There are few studies examining communication skills in children with anxiety disorders.

This pilot study aimed to identify the nature and frequency of communication impairment in children with anxiety disorders attending the Victorian State Wide Child Inpatient Unit (VSCIP)

Method: A retrospective review of 28 case records of children referred between Sept 1, 2005 and July 1, 2006 at the VSCIP was conducted. Children (4 to 12 yrs) with anxiety disorders were compared to children with other psychiatric diagnoses on results of the Children's Communication Checklist –Two.

Results: There was no statistical difference between the two groups on the incidence of communication impairment. 75% of the anxiety group scored <45 (mean 38.25std dev 22.94) while 60% of the other psychiatric disorders group scored <45 (mean 48.20 std dev 20.70). Effect size analyses were applied to the subscales using Cohen's (1988) guidelines. Medium effects were found in the anxiety disorders group in semantics, coherence, use of context, social relations and interests compared with the other psychiatric diagnoses group.

Conclusions: Results from this study suggest that children with anxiety disorders have a high incidence of communication impairment similar to that of other psychiatric disorders in children. They may however have particular difficulties with some aspects of communication.

086

IDENTIFYING BARRIERS TO COMMUNICATION: USING THE INPATIENT FUNCTIONAL COMMUNICATION INTERVIEW (IFCI) IN A SUBACUTE SETTING

S. M. Poole

Speech Pathology, Austin Health, West Heidelberg, VIC, Australia

Introduction and aims of the study: The Inpatient Functional Communication Interview (O'Halloran et al, 2004) was used to conduct interviews with a series of patients on two aged care rehabilitation wards, who were not referred for speech pathology assessment. The aims of the study were to identify any existing barriers to effective communication between patients & staff, to provide intervention if specific barriers to communication were identified and to assess the collated data and implement strategies to improve communication between staff and patients on the wards.

Methods: During 1 month 39 patients were interviewed by a ward speech pathologist on average 8 days after admission.

Results: 64% patients required immediate intervention related to breaking down communication barriers. 7 barriers to effective communication were identified: confusion about discharge plan (31%), hearing impairment (28%), lack of understanding about rehabilitation (28%), vision impairment (20%), poor use of interpreters (15%), isolation and loneliness (15%) and disorientation to time and place (8%).

Conclusions: Several new processes were needed to eliminate and manage the identified communication barriers. The following initiatives were implemented to address the issues and have been sustained: communication screening for all patients admitted to both wards; hearing and vision impairment signs, hearing aid management training for nursing staff, hearing aid management plans for all appropriate patients, hearing aid education offered to all patients with aids; a weekly information session about rehabilitation for all new patients and carers; multidisciplinary assessments with interpreters for patients speaking languages other than English; 2 weekly social communication groups to combat isolation; orientation boards in each patient's room. Patients who were not referred for speech pathology assessment benefited from input from a speech pathologist to assist in breaking down barriers to communication.

(1) O'Halloran R, Worrall L, Toffolo D, Code C & Hickson L (2004) The Inpatient Functional Communication Interview. UK: Speechmark Publishing Ltd

087

REFLUX AFTER OESOPHAGECTOMY - CAN IT BE PREVENTED? : A RANDOMISED CONTROLLED TRIAL

A. Aly[1], G. G. Jamieson[2], D. I. Watson[2], P. G. Devitt[2], R. Ackroyd[3], C. J. Stoddard[3]

[1]Surgery, Austin Hospital, Heidelberg, VIC, Australia

[2]Surgery, Royal Adelaide Hospital, Adelaide, VIC, Australia

[3]Surgery, Royal Hallamshire Hospital, Sheffield, United Kingdom

Background: Oesophagectomy for oesophageal carcinoma is a major undertaking with a definite morbidity and mortality. Long term survival rates are modest and post operative quality of life becomes increasingly important. Reflux of duodeno-gastric fluid is a significant problem after oesophagectomy. Symptoms may be severe and impact considerably upon the quality of life. Previous uncontrolled studies have suggested that a fundoplication type anastomosis may limit post oesophagectomy reflux.

Aim: To determine whether a modified fundoplication at the gastro-oesophageal anastomosis prevents reflux after oesophagectomy.

Methods: Prospective multicentre randomised controlled trial to compare a conventional end of oesophagus to side of gastric conduit anastomosis with a modified fundoplication anastomosis in patients undergoing oesophagectomy with intrathoracic anastomosis. Major outcomes were reflux symptoms, symptoms of dysphagia and complications.

Results: Fifty six patients were enrolled. The fundoplication anastomosis was associated with significantly lower incidence of reflux (40% vs 70%), as well as a reduced incidence of severe reflux (8% vs 30%). Disturbance of sleep due to reflux was significantly reduced in the fundoplication group (18% vs 47%), as was the incidence of respiratory symptoms. The fundoplication anastomosis was not associated with an increase in dysphagia and there was no difference in complications between the 2 groups.

Conclusions: Fundoplication anastomosis during oesophagectomy is effective in protecting patients from reflux symptoms after oesophagectomy, and improves quality of life, particularly with regard to sleep disturbance.

088

OFF PUMP CORONARY ARTERY SURGERY IS RELATIVELY KIND TO KIDNEYS: EVIDENCE FROM A NOVEL BIOMARKER OF ACUTE KIDNEY INJURY

P. Hayward[1], M. Haase[2], Y. Zhu[3], T. Nguyen[3], G. Eastwood[4], L. Peck[4], G. Matalanis[1], R. Bellomo[4]

[1]Department of Cardiac Surgery, Austin Health, Melbourne, VIC, Australia

[2]Department of Nephrology, Charite Hospital, Berlin, Germany

[3]School of Medicine, University of Melbourne, Parkville, VIC, Australia

[4]Department of Intensive Care Medicine, Austin Health, Melbourne, VIC, Australia

Background: Controversy persists regarding the benefits of off-pump coronary bypass surgery (OPCAB) in reducing acute kidney injury (AKI) in patients undergoing surgical coronary revascularisation. The limitations of existing studies include the use of relatively crude indices of AKI, focusing on post-operative elevation of serum creatinine or requirement for haemofiltration. We sought to characterise subclinical renal damage during OPCAB using neutrophil-gelatinase-associated lipocalin (NGAL), a novel biomarker for AKI whose reference ranges* have been well established.

Methods: 40 consecutive patients (aged 60-80, median age 68.5) undergoing OPCAB were enrolled in this retrospective, non-randomised study. Serum NGAL and creatinine were measured pre-operatively, upon arrival at ICU and 24 hours post surgery. Data was compared against that derived from a parallel cohort of on-pump (ONCAB) coronary cases (n=40).

Results: Baseline preoperative renal function (serum creatinine) or injury (NGAL) did not differ between groups. Intro-operative release of NGAL was less overall during OPCAB surgery ($p=0.01$, Mann Whitney U Test) with markedly lower mean concentration than after ONCAB surgery (70ng/mL vs. 145ng/mL, $p<0.001$). The proportion of patients reaching the threshold marker for AKI was significantly lower in the OPCAB cohort compared with its ONCAB counterpart (2/40 vs. 17/40, $p<0.001$). There was no difference in serum NGAL concentrations by 24 hours (t 1/2 20 minutes. Serum creatinine at 24 hours was significantly higher after ONCAB cases ($p=0.004$).

Conclusion: Using a novel biomarker for AKI, these results suggest that there is a significant reduction in subclinical renal damage during OPCAB compared with on-pump surgery. However, larger studies with randomised cohorts are required to assess whether this biochemical evidence can be correlated with a clinically relevant difference in risk of renal failure following coronary surgery.

* NGAL reference range

Normal: < 60ng/mL

Low risk of AKI: 60 - 150ng/mL

Threshold for AKI: > 150ng/mL

089

EFFECT OF LIPID EXPOSURE ON GRAFT PATENCY AND CLINICAL OUTCOMES: ARTERIES AND VEINS ARE DIFFERENT

Y. Zhu[1], P. A. Hayward[1], A. Stewart[2], D. L. Hare[2], B. F. Buxton[1]

[1]Cardiac Surgery, Austin Health, Heidelberg, VIC, Australia

[2]Cardiology, Austin Health, Heidelberg, VIC, Australia

Objective: Lipid management after CABG aims to stabilise coronary disease and maintain graft patency. We evaluated the influence of lipids upon conduit patency and clinical outcomes up to 13 years after primary CABG surgery.

Methods: From a prospective database, we identified 1207 grafts amongst 413 patients who underwent CABG 3-13 years previously (mean 9.4 years) with clinical events (mortality, revascularization, myocardial infarction) recorded and surveillance angiography performed as part of a radial artery trial. All available lipid assays during follow-up were collected from pathology laboratories. Annualised mean lipid exposure was calculated from 6077 lipid measurements (total cholesterol, HDL, LDL, triglycerides). Clinical, angiographic and demographic data were analysed against these. Graft failure was defined as occlusion, string sign or >80% stenosis.

Results: Absolute events included 154 failed grafts, 56 deaths, 16 MIs and 62 patients requiring revascularization. Clinically, elevated total cholesterol was associated with MI (p=0.007) and revascularization (p=0.028) and increased HDL positively influenced survival (p=0.010). Overall, only HDL differed significantly between failed and patent grafts. In multivariate analysis, venous graft failure was associated with increased total cholesterol (p=0.048), increased triglycerides (p=0.025) and trended towards decreased HDL (p=0.059). Surprisingly, increased LDL was favourably correlated with arterial graft patency in the diabetic subset (HR=0.38, p=0.005) with a weaker but significant association in arterial grafts overall (HR=0.73, p=0.047).

Conclusion: Lipid abnormalities known to be risk factors for native vessel disease appear to similarly influence vein grafts, but arterial grafts seem at least more resistant to the effects of lipid exposure, which may be beneficial.

090

LIVER TRANSPLANTATION USING GRAFTS FROM CARBON MONOXIDE POISONED DONORS

B. Wang, M. Fink, G. Starkey, R. Jones

LTU, Austin Hospital, Heidelberg, Australia

Background: The relative lack of organ donors has stimulated the need to consider extended criteria donors. Carbon Monoxide (CO) poisoning can cause brain death and multi organ damage. Liver transplantation (LT) from donors who have died from CO poisoning has been rarely performed in the past. A search of the UNOS database revealed that LT from such donors has occurred only twice in the ten year period from 2000 to 2009.

Method: The Australian and New Zealand experience of LT from CO poisoned donors was analysed using the ANZLTR.

Results: From 1989 to 2009 20 patients have been transplanted in Australia using 19 liver grafts from CO poisoned donors (including a pair who underwent split LT). Among the 20 LT, 3 were carried out in Victoria and 2 Victorian Co liver grafts were successfully transplanted interstate. Seventeen of the 19 CO poisoned organ donors were less than 40 years old (mean=30; median=28) and pre organ donation ALT ranged from 18 to 413 U/L (mean=125; median=85). Most of the recipients in this group were very sick pre-LT (FHF=2; Re-LT=2; HCC=2; HCV=7; ETOH=5; PBC=1; PSC=1). All 20 grafts functioned well post LT, with peak ALT ranging from 131 to 1430 U/L (mean=817). Primary non-function and vascular complications did not occur. Four of the 20 recipients died of complications that were not the result of poor graft function between 1 and 3644 post LTx days (fungal infection=1; myocardial infarction=1; intracranial haemorrhage=1; congestive cardiac failure=1). The surviving 16 patients have a functioning liver graft (patients/graft survival: mean=2553 days). The 1-, 5- and 10-year patient and graft survival rates were all 85%, which is not significantly different from the TSANZ survival rates for all LT over an equivalent period (88%, 80% and 71%, despite the fact that the recipients of grafts from CO poisoned donors were relatively high risk.

Conclusion: LT can be successfully performed using grafts from CO poisoned donors.

091

PULMONARY VEIN ISOLATION FOR ATRIAL FIBRILLATION USING THE ARGON-BASED CRYOABLATION PROBE: A REPORT OF EARLY EXPERIENCE FROM AN AUSTRALIAN CENTRE.

W. Y. Shi, G. Matalanis

Cardiac Surgery, Austin Hospital, University of Melbourne, Melbourne, VIC, Australia

Introduction: Cryoablation using an argon-based probe for atrial fibrillation (AF) as a concomitant cardiac surgical procedure is a safe and promising technique. We aimed to review our results using pulmonary vein isolation lesions.

Methods: Since October 2007, 23 patients have undergone cryoablation for AF, in combination with mitral valve surgery (n=14), coronary artery surgery (n=4) and other procedures. There were 16 males and mean age was 65±12 years. Preoperatively, paroxysmal AF was present in 6 patients, atrial flutter in 1, and permanent AF in the remaining. A lateral mini-thoracotomy was performed in 1 and sternotomy in the remaining. In 18, cryoablation lines were applied to the left atrium producing a pulmonary vein isolation circle. A line was then extended to the P2-P3 segment of the mitral annulus with a corresponding external line extending to the coronary sinus. In 4 patients, lesions were extended to include the right atrium.

There were no early or late mortalities. There were no strokes. At 6 weeks, 17 of the 21 patients available for follow-up were clinically in sinus rhythm while at 6 months, clinical sinus rhythm was present in 14 out of 17 patients. So far, 16 patients have undergone 24-hour Holter monitoring at a mean of 8.2± 6.2 months after surgery, all showing no evidence of recurrent atrial fibrillation.

Conclusions: Concomitant cryoablation for atrial fibrillation using the described lesion set during cardiac surgery has yielded encouraging outcomes. Greater patient numbers and long-term follow up will reveal the full potential of this approach.

092

FOAM ECHOSCLEROTHERAPY – RESULTS OF NON-SURGICAL VARICOSE VEIN TREATMENT AT THE AUSTIN HOSPITAL.

P. Chu, M. Hua, J. Chuen, D. Duong, G. Fell

Surgery, Austin Hospital, Heidelberg, VIC, Australia

Varicose veins are a prevalent problem in the general community with up to 27% of adults experiencing some manifestations of venous insufficiency. Up to 6% of patients with long term varicose veins may develop ulceration which becomes a chronic debilitating problem, with significant burdens on the health system.

Traditional treatment of venous insufficiency with compression therapy and surgery have been shown to be effective in achieving ulcer healing but definitive treatment of varicose veins with surgery, significantly reduces ulcer recurrence compared to long term compression therapy. Surgery is associated with potential wound complications, small risk of injury to adjacent neurovascular structures, and the need for anaesthesia, which may be an issue in some medically frail patients. Surgery has also recurrence rates of up to 20% or more, and re-operation can prove even more hazardous.

The Austin Vascular Unit in conjunction with the Austin Vascular Laboratory, has provided an alternative, non-surgical treatment of varicose veins the past 4 years using ultrasound-guided foam sclerotherapy. This minimally-invasive technique is performed on an ambulatory basis without the need for anaesthesia or hospitalisation, and without the associated risks of surgery. We present a retrospective review of our results for this procedure at the Austin Hospital, and compare it to the published results.

We conclude that this technique provides a safe, cheap and reliable treatment of varicose veins, and a useful alternative to surgery in the public sector.

093

IMPACT OF PATIENT-PROSTHESIS MISMATCH AFTER MITRAL VALVE REPLACEMENT: AN AUSTRALIAN MULTICENTRE ANALYSIS OF EARLY OUTCOMES AND MID-TERM SURVIVAL.

W. Y. Shi[1], P. A.R. Hayward[1], C. H. Yap[2,3], D. T. Dinh[3], C. M. Reid[3], G. C. Shardey[4], J. A. Smith[4]

[1]Department of Cardiac Surgery, Austin Health, University of Melbourne, Melbourne, VIC, Australia
[2]Department of Thoracic Surgery, Austin Health, Melbourne, VIC, Australia
[3]Department of Epidemiology and Preventative Medicine, Monash University, Melbourne, VIC, Australia
[4]Department of Cardiothoracic Surgery, Monash Medical Centre, Department of Surgery, Monash University, Melbourne, VIC, Australia

Background: Patient-prosthesis mismatch (PPM) is a theoretical entity characterized by cardiac impairment due to inadequate prosthesis size relative to body surface area (BSA). It is uncertain whether PPM after mitral valve replacement indeed produces detrimental effects. We examined its impact in an Australian population.

Methods: From 2001 to 2009, 1,006 mechanical and bioprosthetic mitral valves were implanted across 10 institutions. Effective orifice areas (EOA) were obtained from a literature review of in-vivo echocardiographic data. Non-significant, moderate and severe PPM was defined as an indexed EOA (EOA÷BSA) of >1.20cm^2/m^2, ≤1.20cm^2/m^2 and ≤0.9cm^2/m^2 respectively. Early outcomes and 7-year survival were compared between these 3 grades.

Results: Non-significant, moderate and severe PPM was observed in 34%, 53% and 13% respectively. Patients with PPM were more likely male (42% vs. 52% vs. 62%, p<0.0001) and obese (14% vs. 20% vs. 56%, p<0.0001). At 30-days, there was similar mortality (5% vs. 5% vs. 6%, p=0.83) and mortality/any morbidity (24% vs. 27% vs. 29%, p=0.40). Seven-year survival was comparable (72±4.1% vs. 76±3.2% vs. 69±10.3%, p=0.76). Among congestive heart failure (CHF) patients, there was a trend towards superior survival in moderate PPM compared to severe PPM after Kaplan-Meier analysis (75±5.3% vs. 65±11.3%, p=0.09), but not confirmed by Cox regression (p=0.21). PPM was not a predictor of events after logistic and Cox regressions with and without propensity-score adjustment.

Conclusions: Although common, PPM was not associated with early or mid-term adverse outcomes. In CHF patients, techniques may be used to implant prostheses with larger EOAs to avoid physiological disturbance.

094

TUMOURS SURVIVE VASCULAR DISRUPTING AGENT OXI4503 TREATMENT BY UNDERGOING EPITHELIAL TO MESENCHYMAL TRANSITION (EMT)

L. M. Nguyen, T. Fifis, C. Malcontenti-Wilson, V. Muralidharan, C. Christophi

Surgery, The University of Melbourne, Heidelberg, VIC, Australia

Background and aims: Vascular disrupting agents (VDA) such as Oxi4503 target tumour vasculature leading to massive tumour necrosis. However, a thin rim of viable tumour cells persists at the tumour periphery, leading to regrowth. Tumour cells which survive treatment appear to have undergone epithelial to mesenchymal transition (EMT), a process characterized by loss of cell-cell adhesion and increased cell mobility, both necessary for tumour metastasis. We investigated EMT in tumours following Oxi4503, by examining expression of several key factors involved including E-cadherin, ß-catenin, and ZEB1.

Methods: An established mouse model of colorectal liver metastases was used. Oxi4503 was administered as a single intraperitoneal injection (100mg/mL) at 16 days post tumour induction. Tumours were examined at one hour, 24 hours and 5 days post treatment. Immunohistochemistry and/or Western blotting were used to identify changes in EMT markers following treatment.

Results: Oxi4503 resulted in dramatic down regulation of E-cadherin at the tumour periphery, and a redistribution of ß-catenin in the tumour cells from the cell junctions to cytoplasm and nucleus. Treatment caused upregulation of ZEB1, a transcription inhibitor of the E-cadherin gene, accounting for the observed reduction in E-cadherin. ZEB1 also stabilizes nuclear localization of ß-catenin, which activates the wnt signalling pathway leading to EMT.

Conclusion: These results strongly support our hypothesis that following VDA treatment, surviving tumour cells undergo EMT. This may result in resistance to treatment and promote tumour metastasis. A clear understanding of the mechanisms involved may lead to improved treatment with lower incidence of tumour recurrence and metastasis.

095

THE EFFECTS OF CAPTOPRIL IN A MOUSE MODEL OF LIVER REGENERATION

S. Koh, E. I. Ager, C. Malcontenti-Wilson, C. Christophi

Surgery, The University of Melbourne, Heidelberg, VIC, Australia

Background: Liver resection is the best treatment option for colorectal cancer (CRC) liver metastasis patients. However, tumour recurrence is high. Blockade of the renin-angiotensin system (RAS) with angiotensin-converting enzyme inhibitor (ACEi), captopril, inhibits tumour growth , while lisinopril, another ACEi, promotes liver regeneration after resection. Captopril, with a sulfhydryl group, has unique characteristics among ACE inhibitors. This study investigated the effects of captopril on mouse liver regeneration.

Methods: Male CBA mice were randomly assigned to sham resection or 70% liver resection groups. Liver tissues were collected on days 2, 4 and 8 after operation. The effects of captopril (750mg/kg) were measured by liver to body weight ratio (LBW, measure of liver regeneration), immunohistochemistry to quantify hepatocyte proliferation (anti-Ki67) and apoptosis (anti-active caspase-3), and hepatocyte size using image analysis.

Results: Captopril did not affect LBW ratio on day 1. By day 2, captopril increased LBW ratio (0.49 ± 0.02, 0.57 ± 0.02, p = 0.027) compared to untreated controls. By day 8, captopril had retarded regeneration (0.74 ± 0.00, 0.62 ± 0.02, p = 0.011). Interestingly, despite enhanced liver regeneration on day 2, hepatocyte proliferation (53.7 ± 7.9, 3.4 ± 0.9%, p = 0.002) and size (406.3 ± 18.8, 303.1 ± 12.7 m m^2, p < 0.001) were decreased following treatment.

Conclusion: Captopril may promote the early stages of liver regeneration independent of hepatocyte proliferation or hyperplasia, but may instead result from decreased hepatocyte apoptosis. Characterizing the mechanisms of captopril on liver regeneration is important in targeting the RAS to improve CRC patient outcomes.

096

SINGLE DOSE CUSTODIOL VERSUS MULTIDOSE NORMOTHERMIC BLOOD CARDIOPLEGIA FOR MYOCARDIAL PROTECTION: AUSTRALIAN SINGLE-CENTRE ANALYSIS OF 134 MATCHED PATIENTS UNDERGOING COMPLEX CARDIAC OPERATIONS

F. F. Viana, P. Hayward, W. Shi, M. Shaw, I. Harley, B. Marino, R. Bellomo, S. Seevanayagam, G. Matalanis

Cardiac Surgery, Austin Health, Heidelberg, VIC, Australia

Objectives: A single-dose strategy for cardioplegia is attractive in long operations. We hypothesized that a single administration of Bretschneider histidine-tryptophan-ketoglutarate crystalloid solution (Custodiol) offers myocardial protection comparable to repeated warm blood cardioplegia.

Methods: We analysed a single-centre prospectively-compiled database, identifying 67 moderate to high risk patients who received Custodiol. Sixty seven patients who received blood cardioplegia were matched for type of operation, mortality risk, left ventricular function, ischaemic time and surgeon. Preoperative characteristics, intraoperative and 30-day outcomes were compared between groups and between the pairs.

Results: There were no statistically significant differences in major preoperative variables, except for a higher number of type A aortic dissection in the Custodiol group (9 versus 1, p=0.02). There were no differences in the intraoperative variables. There were no differences in postoperative cardiogenic shock, inotrope use, cardiac arrhythmias, ventilation time, intensive care unit stay or readmission rates. There was a higher number of returns to operating theatre in the blood cardioplegia group (19 vs 6, p=0.007), with a trend towards re-operation for bleeding in the blood cardioplegia group (9 vs 2, p=0.055). Postoperative sodium serum levels were lower in the Custodiol group (138.04 +/- 3.95 vs 140.79 +/- 3.16, p<0.0001). At 30 days, mortality was similar (2 vs. 5, p=0.44).

Conclusions: The use of Custodiol in complex cardiac operations is convenient, simple and safe. Postoperative serum sodium concentrations should be monitored closely.

097

LIPID MANAGEMENT AFTER CORONARY SURGERY: HOW EFFECTIVE ARE WE AT SECONDARY PREVENTION?

Y. Zhu[1], P. A. Hayward[1], A. G. Stewart[2], D. L. Hare[2], B. F. Buxton[1]

[1]Cardiac Surgery, Austin Health, Heidelberg, Australia

[2]Cardiology, Austin Health, Heidelberg, VIC, Australia

Objective: To assess the proportion of CABG patients in Victoria who achieve and maintain target lipid levels up to 13 years after surgery.

Methods: From a prospectively compiled database, we identified 440 CABG patients followed for up to 13 years as part of a radial artery study. All available lipid assays conducted during the follow up period were collected from pathology databases. 6077 lipid studies (total cholesterol, LDL, HDL and triglycerides) in total were obtained. These were used to calculate annualised mean lipid exposures for each patient. Based upon National Heart Foundation guidelines, we determined the proportion of patients whose mean lipid exposure attained target levels (total cholesterol<4.0mmol/L, LDL<2.0mmol/L, HDL>1.0mmol/L and triglycerides<1.5mmol/L). This was compared against the baseline proportion who had achieved these targets pre-operatively.

Results: In those who had pre-operative data available, baseline target levels for total cholesterol, HDL, LDL and triglycerides were attained by 16%, 64%, 14% and 39% of these patients respectively. Annualised mean lipid exposures up to 13 years of follow up for all patients revealed somewhat improved but still suboptimal figures of 24%, 83%, 20% and 53%. Of 141 diabetic patients, target attainment was significantly higher for total cholesterol (31%; p=0.038) and LDL (28%; p=0.006) but lower for HDL (75%; p=0.002) and triglycerides (40%; p<0.001).

Conclusion: Despite some improvements seen over careful follow up, only HDL targets appear attainable for the majority of CABG patients. Over half still do not achieve non-HDL national lipid targets. The influence of this on outcome is under review.

098

INITIAL OUTCOMES OF LOCALISED PROSTATE CANCER TREATED WITH HIGH-INTENSITY FOCUSED ULTRASOUND (HIFU)

P. Royce[2], K. W.K. Ong[1], H. Woo[4], A. Troy[1], S. Appu[3], T. Dean[4], D. M. Bolton[1]

[1]Urology, Austin Health, Heidelberg, VIC, Australia

[2]Urology, Alfred Health, Prahran, VIC, Australia

[3]Urology, Southern Health, Clayton, VIC, Australia

[4]Urology, Sydney Adventist Hospital, NSW, Australia

Introduction: High-Intensity Focused Ultrasound (HIFU) is a minimally invasive ablative therapy for the treatment of localised prostate cancer.

Methods: We retrospectively reviewed the outcomes of 87 patients from Victoria and New South Wales with localised prostate cancer who were treated primarily with HIFU. Patients had the following clinical staging: T1a= 1 patient, T1b= 2 patients, T1c= 34 patients, T2a= 17 patients, T2b= 20 patients, T2c= 8 patients, T3a= 4 patients, T3b= 1 patient. 15 patients had a Gleason score between 8 and 10; 71 patients had a Gleason score between 5 and 7; 1 patient's Gleason score was less than 5.

Results: 87 patients with a mean age of 71.9 years were systematically reviewed up until this point. The mean baseline PSA was 8.37 (range: 0.98 – 26.7). In all, 64.7% achieved a PSA nadir of ≤1.0 $ngml^{-1}$ at 12 months, with 51.5% achieving ≤0.5 $ngml^{-1}$. In the first 3 months post-HIFU, 8% of patients were treated for urinary tract infection. In addition, 6.9% developed proctitis with one of them subsequently found to have a recto-urethral fistula. Mild stress urinary incontinence (no pads) was reported in 42.5% of patients at 3 months. 14 out of 36 patients were retreated with HIFU, 6 underwent salvage radical prostatectomy, 4 had salvage external beam radiotherapy; 10 were commenced on androgen deprivation therapy and 2 were offered active surveillance.

Conclusion: HIFU potentially offers an alternative to the conventional active surveillance and radical therapy in certain instances of localised prostate cancer. However, long-term outcome data needs to be collected and further evaluation is required.

099

IMPACT OF RADIAL ARTERY HARVEST ON FOREARM FUNCTION AND SYMPTOMS: A LONG-TERM ANALYSIS FROM A RANDOMISED TRIAL

Y. Zhu[1], I. E. Hadinata[1], P. A. Hayward[1], B. F. Buxton[1], D. L. Hare[2]

[1]Cardiac Surgery, Austin Health, Heidelberg, Australia

[2]Cardiology, Austin Health, Heidelberg, VIC, Australia

Introduction: The radial artery (RA) has gained popularity as a conduit for coronary artery bypass surgery (CABG) in spite of a paucity of patient-centred analysis of long-term quality of life after its removal. We sought to characterise this further.

Methods: Patients from a radial artery trial completed a questionnaire up to 14 years after primary CABG (mean 9.3 years, range 4-14), which included 9 statements concerning hand and forearm symptoms or limitations in daily life. Response rate was 82% (404 of 491 survivors): 230 of these had received a RA graft (RA group). Responses were graded in order of severity from 0 to 7, > 3 (mild concern) being regarded as a significant symptom. Mean response to each question was compared with the non-RA harvest group (n=174).

Results: In the RA group, 92% to 99% reported no significant symptoms, with the most frequent symptoms relating to pain and numbness (8% each). Mean scores ranged from 0.9 to 1.6 on eight questions with 89% responding positively to a question regarding overall functionality. Compared with the non-RA group, this was not significantly higher for pain or numbness. Among the RA group, mean scores for scar appearance and discomfort were 0.95 (1= no concern) and 0.93 respectively, suggesting satisfactory cosmesis and no impact on function.

Conclusion: Radial artery harvesting is associated with high patient satisfaction. A small proportion of patients appear to experience forearm pain and numbness, however, this is not different to those without artery removal and may therefore be unrelated to surgery.

100

ANGIOTENSIN II INDUCED CHANGES IN EPITHELIAL AND MESENCYMAL MARKERS IN COLORECTAL CANCER CELLS.

E. I. Ager, C. Christophi

The University of Melbourne, Australia

Background: Epithelial to mesenchymal transition (EMT) is strongly implicated in tumour progression.

Aim: To determine if the renin angiotensin system (RAS), which has been targeted to reduce tumour growth, can induce EMT in human colorectal cancer (CRC) cells.

Method: Human DLD-1 cells were cultured in control medium or medium supplemented with the RAS peptide ANG II alone or with specific blockers of the functionally antagonistic ANG II type 1 and 2 receptors (AT1R and AT2R). Cell migration was assessed by wound scratch assay and cytoskeletal structure by phalloidin staining. Flow cytometry was used to determine the level of epithelial (E-cadherin) and mesenchymal (ZEB1) markers as well as inducible nitric oxide synthase (iNOS), the overexpression of which can facilitate cancer cell migration and invasion.

Results: ANG II significantly increased cancer cell migration and this effect was inhibited by AT1R blockade. Low concentrations of the AT2R blocker also inhibited ANG II-induced migration but high concentrations increased migration. ANG II treatment induced cytoskeletal structures associated with migration (lamellipodia) and decreased E-cadherin expression, while AT1R blockade was associated with a decrease in ZEB1. ANG II treatment increased iNOS expression in a subpopulation of DLD-1s; while blockade of the AT2R significantly decreased iNOS expression in the entire population.

Conclusion: The RAS induced changes typical of EMT. The significant role of the AT2R in regulating iNOS expression might be important in determining the anti-cancer efficacy of RAS blockers which target either or both of the ANG II receptors.

101

THE INFLUENCE OF THE RENIN-ANGIOTENSIN SYSTEM ON MACROPHAGE PLASTICITY

S. Wen, E. I. Ager, C. Christophi

Surgery, University of Melbourne, Heidelberg, VIC, Australia

Macrophages can be classically or alternatively activated to either inhibit or promote tumour progression, respectively. However, the role of resident liver macrophages in the progression of colorectal liver metastases and the influence of the renin-angiotensin system (RAS), which has immunomodulatory effects and can be targeted to inhibit tumour growth, have yet to be defined.

This study aimed to determine if the RAS could affect macrophage activation status and if liver macrophages are altered during growth of liver metastases.

Here we showed expression of key RAS receptors, AT1R and AT2R, by a mouse macrophage cell line (P338D1). Flow cytometry indicated an increase in CD14 (a marker of classical activation) expression when P338D1s were treated with the RAS peptide Ang II compared to the alternative RAS peptide Ang-(1-7). This effect was mitigated by AT1R blockade. In contrast, no change in the alternative activation marker CD206 was observed.

Using a mouse model of colorectal liver metastases, macrophage depletion by gadolinium chloride (20 mg/kg) during the late stages of tumour growth was shown to significantly decrease tumour load compared to controls. This effect was not observed when macrophages were depleted during the early stages of metastatic growth. The protein levels of VEGF and TNF- α (key growth factors produced by tumour associated macrophages) also differed according to tumour growth stage.

These results suggest that macrophages have divergent effects on tumour growth depending on the stage of tumour progression and that changes in macrophage function might be, at least in part, regulated by the RAS.

102

INCREASED AT1R EXPRESSION ON CANCER CELLS CAN REDUCE THE EFFICACY OF AT1R-TARGETED ANTI-CANCER TREATMENTS

J. Chan, E. I. Ager, J. Neo, C. Malcontenti-Wilson, C. Christophi

Surgery, Austin Health, Heidelberg, VIC, Australia

Background: Blockade of the angiotensin (ANG) II type 1 receptor (AT1R) or angiotensin converting enzyme (ACE) inhibition reduces tumour growth in a mouse model of colorectal cancer liver metastases (CRLM). ANG-(1-7), an antagonistically acting peptide, has also been identified as a potential anti-cancer agent.

Aim: To establish if combined AT1R blockade by irbesartan and ANG-(1-7) infusion could result in greater inhibition of CRLM than either treatment alone.

Methods: Treatments (irbesartan at 50 mg/kg s.c. and ANG-(1-7) at 24 μg/kg/hr by i.p pump) were administered daily for 21 days in a mouse model of CRLM. Tumour burden was assessed by liver-to-body weight ratio and stereology. Immunohistochemistry was used to assess AT1R levels.

Results: Combined ANG-(1-7) and irbesartan did not improve on single treatment strategies ($P=0.667$). However, while irbesartan previously inhibited tumour growth in this model, in the current experiments irbesartan failed to affect tumour burden. Analysis of AT1R expression showed increased expression in tumour cells from irbesartan-insensitive (current study) compared to irbesartan-sensitive tumours (previous study) ($P<0.0001$). Given the apparent change in cancer cell phenotype, the efficacy of ACE inhibition (captopril) was also assessed. In contrast to irbesartan, captopril retained its anti-tumour activity.

Conclusions: Although the results do not support increased efficacy with combined treatment, potential benefits may have been masked by changes in tumour phenotype. The results also suggest that upregulation of tumour cell AT1R could render them insensitive to AT1R-blockade. However, the effectiveness of ACE inhibition was not altered, suggesting that these treatment strategies have different mechanisms of action.

103

TRAINING IN MITRAL VALVE SURGERY NEED NOT AFFECT EARLY OUTCOMES AND MID-TERM SURVIVAL: A MULTICENTRE ANALYSIS.

W. Y. Shi[1], P. A.R. Hayward[1], C. H. Yap[2], D. T. Dinh[2], C. M. Reid[2], G. C. Shardey[3], J. A. Smith[3]

[1]Department of Cardiac Surgery, Austin Hospital, University of Melbourne, Melbourne, VIC, Australia
[2]Department of Epidemiology and Preventative Medicine, Monash University, Melbourne, VIC, Australia
[3]Department of Surgery, Monash Medical Centre, Monash University, Melbourne, VIC, Australia

Objectives: Mitral valve surgery may be regarded as less favourable for training, due to greater mortality risk, technical complexity, and difficulty in supervision. We examined this perception by review of a multicentre experience.

Methods: We analysed a multicentre database over a 7-year period containing 2,216 isolated and combined mitral procedures. Of these, 2,048 were performed by consultants and 168 by trainees (92% vs. 8%). Preoperative characteristics, 30-day postoperative outcomes, and 6-year survival were compared between groups. Propensity-score matching was performed to correct for group differences.

Results: There were no statistically significant differences in major preoperative variables such as urgency, endocarditis and left ventricular dysfunction. There were similar rates of mitral repair (52% vs. 49%, p=0.48) and concomitant procedures. At 30 days, mortality was comparable (5.3% vs. 4.2%, p=0.56) with a trend towards higher readmission (15% vs. 10%, p=0.075) and mortality/any morbidity in consultant procedures (33% vs. 26%, p=0.059). Among 142 propensity-matched patient pairs, there was similar mortality (4.9% vs. 4.2%, p>0.99) and mortality/any morbidity (28% vs. 24%, p=0.52). At 6 years, survival was similar in both the entire group (79±1.4% vs. 78±4.0%, p=0.73) and the matched pairs (74±7.2% vs. 80±4.4%, p=0.64). Trainee status did not predict early or late adverse events after multivariate analysis. In the institution with the highest proportion of training cases (31%), analysis showed no association between trainee cases and adverse outcomes.

Conclusions: Trainee outcomes are not inferior even when corrected for risk. This suggests that excellent training and supervision can be achieved in mitral valve surgery.

104

COMPARABLE PATENCIES OF THE RADIAL ARTERY AND RIGHT INTERNAL THORACIC ARTERY OR SAPHENOUS VEIN BEYOND 8 YEARS: RESULTS FROM THE RADIAL ARTERY PATENCY AND CLINICAL OUTCOMES TRIAL

P. A.R. Hayward[2], D. L. Hare[1,3], I. E. Hadinata[1], M. Shaw[2], G. Matalanis[2], B. F. Buxton[1]

[1]The University of Melbourne, Parkville, VIC, Australia
[2]Cardiac Surgery, Austin Hospital, Heidelberg, VIC, Australia
[3]Cardiology, Austin Hospital, Heidelberg, VIC, Australia

Objective: To investigate the optimum conduit for coronary targets other than the left anterior descending artery, we are evaluating long-term patencies and clinical outcomes of the radial artery, right internal thoracic artery, and saphenous vein through the Radial Artery Patency and Clinical Outcomes trial. This is a progress report on an ongoing clinical trial.

Methods: As part of a 10-year prospective, randomized, single-center trial, patients undergoing primary coronary surgery were allocated to the radial artery (n=198) or free right internal thoracic artery (n =196) if aged less than 70 years (group 1), or radial artery (n = 113) or saphenous vein (n = 112) if aged at least 70 years (group 2). All patients received a left internal thoracic artery to the left anterior descending, and the randomized conduit was used to graft the second largest target. Protocol-directed angiography has been performed at randomly assigned intervals, weighted toward the end of the study period. Grafts are defined as failed if there was occlusion, string sign, or greater than 80% stenosis, independently reported by 3 assessors. Analysis is by intention to treat.

Results: At mean follow up of 8.74 years, protocol angiography has been performed in groups 1 and 2 in 258 and 133 patients, respectively. There are no significant differences within each group in preoperative comorbidity, age, or urgency. The overall free arterial graft patency in group 1 is 88.0%, (RA 91.3% vs RITA 84.6%, p=0.027). In group 2, there was no significant difference between the radial artery and the saphenous vein (RA 93.9% vs SV 86.6%, p=0.170).

Conclusion: At mean 5-year angiography in largely asymptomatic patients, the selection of arterial or venous conduit for the second graft has not significantly affected patency. This finding offers surgeons, for now, enhanced flexibility in planning revascularisation.

PREDICTORS OF STOKE FOLLOWING PERCUTANEOUS CORONARY INTERVENTION (PCI): FINDINGS FROM A LARGE MULTICENTRE AUSTRALIAN PCI REGISTRY

T. Lancefield[1], S. J. Duffy[2], D. J. Clark[1], A. E. Ajani[3], G. New[4], D. Stub[2], M. Sebastian[5], W. Chan[2], A. Finlay[6], A. L. Brennan[6], N. Andrianopoulos[6], A. M. Dart[2], C. Reid[6]

[1]Cardiology, Austin Health, Heidelberg, VIC, Australia
[2]Cardiology, The Alfred Hospital, Melbourne, VIC, Australia
[3]Cardiology, Royal Melbourne Hospital, Melbourne, VIC, Australia
[4]Cardiology, Eastern Health, Box Hill, VIC, Australia
[5]Cardiology, Barwon Health, Geelong, VIC, Australia
[6]CCRE Therapeutics, Monash University, Melbourne, VIC, Australia

Background: Although infrequent (between 0.07-0.6% of cases), stroke is a devastating complication following percutaneous coronary intervention (PCI). Knowledge of predisposing risk factors is limited. In particular, the contribution of arrhythmia has not been assessed in previous large registry studies.

Aims: We sought to determine the stroke rate in a large multicentre Australian PCI registry and to identify and compare the clinical and procedural risk factors associated with in-patient and out-of-hospital stroke following PCI.

Methods: We evaluated data from 12,702 patient procedures between April 2004 and November 2009, enrolled in the Melbourne Intervention Group registry. We assessed clinical characteristics and procedural details in patients that had either an in-patient or an out-of-hospital stroke, compared to patients without stroke. Out-of-hospital stroke was up to 12 months following PCI, and excluded patients with in-patient stroke. Multivariate analysis was used to identify independent predictors.

Results: Thirty-one patients (0.2%) had an in-patient stroke and 57 (0.4%) had an out-of-hospital stroke within 12 months. Common features associated with stroke in both cohorts were older age, renal impairment, history of cerebrovascular disease and atrial fibrillation during PCI (all $p<0.05$). In-patient strokes were also more likely to occur in patients with diabetes, prior heart failure, reduced ejection fraction, urgent procedures, STEMI as the indication for PCI and cardiogenic shock (all $p<0.05$). Out-of-hospital strokes were also more likely to occur in female patients and in patients with prior CABG, rheumatoid arthritis and peripheral arterial disease. Diabetes was the only independent predictor of in-patient stroke. Independent predictors of out-of-hospital stroke were age, female gender and post-procedure arrhythmia.

Conclusions: Risk factors for peri-procedural stroke differ from longitudinal risk. Long-term anticoagulation in patients with post-procedure arrhythmia should be considered.

106

ANAEMIA IS A SIGNIFICANT PREDICTOR OF INCREASED MORTALITY IN SUBJECTS WITH HEART FAILURE WITH REDUCED EJECTION FRACTION: MEDIUM TERM FOLLOW UP

K. J. Lu[1], D. L. Hare[1,2], L. G. Kearney[1,2], M. Ord[1,2], D. Toia[1], B. Wai[1,2], L. M. Burrell[2], P. M. Srivastava[1,2]

[1]Cardiology, Austin Health, Heidelberg, VIC, Australia
[2]Medicine, Austin Health, Heidelberg, VIC, Australia

Background: Anaemia is common in patients with heart failure with reduced ejection fraction (HFREF) and is associated with adverse outcomes. Recent trials focusing upon improving anaemia through use of iron supplements and erythropoietin have delivered mixed results. We assessed the impact of anaemia in subjects with SHF managed in a multidisciplinary heart failure clinic (HFC).

Methods: Consecutive Patients with HFREF between 2000-2005 were prospectively enrolled. Baseline clinical characteristics, pathology tests and medication use (ACEi/ARB, B blocker, spironolactone) were compared. The primary endpoint of all cause mortality was determined via clinical follow-up and reference to the National Death Index.

Results: Six hundred and twenty-six patients participated with a mean follow up of 2.5 ± 1.6 years. Patients with anaemia (male <13g/dl, female <12g/dl; 43%) had higher all cause mortality 47% vs. 27% ($p<0.01$). Patients with anaemia were: older (mean age 72.8 ± 11 vs 66.7 ± 16 years $p<0.05$), more likely male (73% vs 67% $p<0.05$), had higher rates of diabetes (32.4% vs. 22.9% $p<0.01$), renal impairment (Cr >1.2mg/dl; 23.3% vs 10.6% $p<0.01$) and ischaemic aetiology (70% vs 56% $p<0.01$). Adherence to medications was similar: ACEi/ARB (91%, p=NS), B Blockers (67% p=NS) and spironolactone (43% p=NS). Independent predictors of mortality were: anaemia (HR 1.7 [1.2-2.4] $p<0.01$), LV systolic function (HR 1.6 [1.3-2.1] $p<0.01$), absence of B blocker therapy (HR 1.6 [1.1-2.3] $p<0.01$), NYHA class (HR 1.4 [1.1-1.8] $p<0.01$), and increasing age (HR 1.1 [1.0-1.1] $p<0.01$)

Conclusions: Despite good adherence to guideline based therapy in HFC, HFREF mortality remains high. Anaemia is independently associated with mortality and screening for reversible causes and subsequent treatment if identified is advisable.

107

PLASMA NT-BNP IS ASSOCIATED WITH SYSTOLIC BUT NOT DIASTOLIC DYSFUNCTION IN TYPE 2 DIABETES

S. K. Patel[1], P. M. Srivastava[1,2], L. M. Burrell[1]

[1]Medicine (Austin Health), University of Melbourne, Melbourne, VIC, Australia
[2]Department of Cardiology, Austin Health, Melbourne, VIC, Australia

Background: Type 2 diabetes (T2DM) is associated with increased cardiovascular (CV) morbidity and mortality. Circulating amino-terminal pro-brain natriuretic peptide (NT-BNP) is elevated in CV disease and associated with adverse outcomes. Few studies have assessed NT-BNP in diabetes. We examined the relationship between plasma NT-BNP and cardiac abnormalities.

Methods: Subjects with T2DM attending a complications surveillance programme at Austin Health were studied. Assessment for anthropometric and metabolic variables, including measurement of plasma NT-BNP was performed. Cardiac function was assessed by transthoracic echocardiography (ECHO) and classified into normal or abnormal (presence of left ventricular hypertrophy, diastolic (DD) and/or systolic dysfunction (SD)). NT-BNP levels were normalised by log transformation and the geometric means presented.

Results: The study consisted of 172 (109 male, 63 female) T2DM subjects aged (mean±SE) 62±1 y. Compared to a normal ECHO (27%), those with an abnormal study (73%) were significantly ($P < 0.05$) older, had increased systolic BP, reduced estimated glomerular filtration rate (eGFR) and NT-BNP (89.3 vs. 28.7 pg/ml, $P < 0.0001$). Those with DD (66%) and SD (22%) had higher plasma NT-BNP compared to those with a normal study (DD 76.6; SD 161.3 vs. 28.7 pg/ml, $P < 0.0001$). This difference remained significant in the SD group, after adjusting for age, eGFR and systolic BP ($P=0.001$). In those with DD, the difference in NT-BNP was not significant after accounting for the same covariates.

Conclusion: Plasma NT-BNP is useful for the detection of SD in T2DM, but is not associated with the more prevalent DD after accounting for renal function and age.

IS PATENT FORAMEN OVALE A CAUSE OF CRYPTOGENIC ACUTE CORONARY SYNDROME?

A. H. Al-Fiadh, K. Profitis, O. Farouque, E. Jones

Medicine / Cardiology, University of Melbourne / Austin Health, Heidelberg, VIC, Australia

Background: Acute coronary syndromes (ACS) are usually caused by plaque rupture and thrombosis in an atherosclerotic coronary artery. Nine to 14% of ACS patients have angiographically normal coronary arteries. Patent foramen ovale (PFO) is associated with paradoxical systemic embolism and is found in 25-30% of individuals at autopsy. We hypothesise that PFO may be a cause of coronary artery embolism. We sought to determine the prevalence of PFO in ACS patients with angiographically normal coronary arteries.

Methods: Coronary angiography, transthoracic (TTE) and transoesophageal (TOE) echocardiography were performed with antecubital and femoral vein injection of agitated saline contrast. Coronary flow reserve (CFR) was measured in nine patients using an intracoronary guidewire.

Results: Twenty-nine patients were recruited (16 female), mean age 53±13 years. Risk profile included smoking (40%); hypertension (32%); dyslipidaemia (43%); family history of premature coronary disease (40%). No patient was diabetic. Median troponin I was 1.57mcg/L (IQR 0.48 -7.00). Seven patients (6 female) had classical apical ballooning syndrome. PFO was detected in 14 patients (48%), and no vegetations or intracardiac thrombus were identified. There was no difference in PFO diagnosis between TTE and TOE, or between antecubital and femoral saline contrast injections. CFR was normal (3.8±1.1).

Conclusions: Prevalence of PFO may be higher among patients with ACS and angiographically normal coronary arteries compared to the general population. TTE with agitated saline contrast via the antecubital vein is a reliable diagnostic tool to detect PFO. Paradoxical coronary embolisation may be a cause of ACS and warrants further investigation.

EVALUATION OF LEFT VENTRICULAR LEAD OUTPUT IN CARDIAC RESYNCHRONISATION THERAPY (CRT): MINIMUM OUTPUTS FOR MAXIMUM GAINS

M. Wong, L. Bittinger, S. Letcher, G. Chiavaroli, M. Swale, D. O'Donnell

Cardiology, Austin Health, Heidelberg, VIC, Australia

Objective: To evaluate effect of programming output minimally above capture threshold for LV lead outputs on

LV capture percentages.

Background: Standard pacing protocols commonly set programmed lead outputs at twice the chronic threshold. In

CRT devices, high left ventricular (LV) threshold may be encountered leading to high programmed

outputs, increased battery usage and possible phrenic nerve pacing. Programming twice the

threshold may not be necessary to achieve optimal Biventricular (BiV) pacing for CRT patients.

Method: Patients with LV leads implanted for >4 weeks with stable threshold had their LV output programmed

to a single increment above capture threshold:

- 0.1mV with Medtronic devices &
- 0.25A with St Jude devices

Each patient then underwent 24 hour Holter monitoring. QRS

morphologies were established for LV, RV and BiV pacing at the commencement of the Holter. The

remaining 24 hours was analysed by 12 lead Holter using established morphologies.

Results: N=43

Male Gender: 28 (65%)

Age (years): 71±10

Duration of implant (months): 15±20

Mean % LV Capture: 98.4%

No. of patients ≥96% LV capture: 35 (91%)

Device company:

- St Jude Medical: 13 (30%)
- Medtronic: 30 (70%)

Conclusion: Programming lead outputs minimally above threshold produced successful LV capture 98.4% of beats

on a 24 hour Holter monitor. In 35 (91%) patients, LV capture was ≥96% of beats. There was no

difference in percentage of LV capture between pacemaker companies.

In a chronic lead, programming the LV lead output at the minimum possible setting above threshold,

as opposed to a standard “twice threshold” output allows reliable LV capture. This facilitates successful

CRT, minimises risk of phrenic nerve stimulation and further optimises maximal battery longevity.

110

RETINAL MICROVASCULAR ENDOTHELIAL FUNCTION IS ATTENUATED IN PATIENTS WITH CHRONIC AND ACUTE CARDIOVASCULAR DISEASE

A. H. Al-Fiadh[1], S. Ristevski[1], J. XIE[2], M. Freeman[1], S. K. Patel[1], F. Islam[2], T. Nguyen[2], A. Wilson[3], D. Clark[1], L. Burrell[1], T. Wong[2], O. Farouque[1]

[1]Medicine / Cardiology, Austin Health / University of Melbourne, Heidelberg, VIC, Australia
[2]Centre for Eye Research Australia / University of Melbourne, East Melbourne, VIC, Australia
[3]St. Vincent's Health / University of Melbourne, Fitzroy, VIC, Australia

Background: Advances in imaging technology have enabled real time assessment of retinal microvascular dilatation to flicker light; a nitric oxide dependent phenomenon. We sought to determine if retinal microvascular endothelial function was impaired in patients with chronic and acute cardiovascular disease by comparing dynamic retinal changes in healthy volunteers, stable patients with vascular risk factors (VRF) and patients with a recent acute coronary syndrome (ACS).

Methods: After pupil dilatation, retinal arteriolar and venular segments between half and two disc diameters from the margin of the optic disc were selected for measurement. Retinal vessel diameter changes in response to diffuse luminance flicker light were measured using the Digital Vessel Analyzer, and expressed as percentage increase over baseline diameter. Data are expressed as adjusted mean±SE.

Results: There were 137 subjects recruited. Stable patients with VRF and ACS were older than healthy volunteers (59±9 vs 59±9 vs 36±9 years). After adjustment for age and gender, there was a trend to reduction in retinal venular dilatation in patients with VRF and ACS. Table: 1

	Healthy (n=27)	VRF (n=84)	ACS (n=25)	P value
Women, n (%)	15 (56%)	32 (38%)	6 (24%)	0.06
Risk factor score, mean±SD	N/A	3.0±0.8	2.4±1.4	0.01
Retinal arteriolar dilation %	2.84±0.56	2.62±0.26	1.93±0.46	0.34
Retinal venular dilation %	5.24±0.52	3.85±0.24	4.04±0.42	0.08

Conclusion: Patients with VRF and ACS have a trend to attenuated retinal venular dilatation compared to healthy subjects. Further studies are needed to determine if retinal microvascular dysfunction in these patient groups may be an indicator of generalised vascular disease.

111

PRECIPITANTS OF ACUTE DECOMPENSATED HEART FAILURE: A COMPARISON BETWEEN OUTPATIENTS AND INPATIENTS

D. Taylor[1,2], M. Ng Tang Fui[1], A. Chung[1], L. Gani[1], J. Zajac[1,2], L. Burrell[1,2]

[1]General Medicine, Austin Health, Heidelberg, VIC, Australia
[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

Aim: We aimed to compare the precipitants of acute decompensated heart failure (ADHF) among patients admitted with diagnoses inclusive of ADHF (outpatients) and patients admitted without ADHF but who developed it during their stay (inpatients).

Methods: This was a prospective, analytical, observational study undertaken in the Austin Hospital (September 2008-February 2010). Consecutive patients admitted to a General Medicine unit, and diagnosed and treated for ADHF were enrolled. The unit medical staff completed a specifically designed data collection document.

Results : 359 patients were enrolled (42.7% male, mean age 81.9 years). The outpatient (n=315) and inpatient (n=44) groups did not differ in gender, age, risk variables (living alone, cognitive impairment, multiple medications, compliance), medication use (diuretics, spironolactone, ACEI) or cause of known heart failure (ischaemia, hypertension, valve dysfunction, 'other'). The ADHF precipitants ($p=0.05$) comprised infection (39.8% patients), ischaemia (17.3%), tachyarrhythmia (16.2%), fluid/salt restriction non-compliance (9.2%), medication non-compliance (6.7%), renal failure (5.8%), medication reduction (5.0%), intravenous fluid complication (3.9%) and 'other' (13.9%). Significantly more inpatients had their ADHF precipitated by tachyarrhythmia (27.3% versus 14.6%, p=0.03) and intravenous fluid complications (20.5% versus 1.6%, p<0.001). Inpatients also had a significantly greater death rate (25.0% versus 9.5%, p<0.01). Among all patients with an intravenous fluid complication there was also an increased death rate (28.6% versus 10.7%, difference [95%CI] = 17.9 [-9.8, 45.5], p=0.10).

Conclusion: Inpatient ADHF is a dangerous condition. While infection and ischaemia are the common precipitants, the contribution of intravenous fluid complications, an iatrogenic condition, is considerable and associated with an increased death rate.

112

TRANS-RADIAL VERSUS FEMORAL ARTERY ACCESS FOR PERCUTANEOUS CORONARY INTERVENTION (PCI) - HAS IT IMPROVED CLINICAL OUTCOMES?

M. Mok[1], A. Black[3], N. Andrianopoulos[2], D. Clark[1]

[1]Cardiology, Austin Health, Heidelberg, VIC, Australia
[2]Melbourne Interventional Group, Melbourne, VIC, Australia
[3]Cardiology, Geelong Hospital, Geelong, VIC, Australia

Background: Previous studies have found that trans-radial access (TRA) for percutaneous coronary intervention (PCI) is associated with a low risk of bleeding complication and improved patient comfort. The aim of the study is to compare the outcomes of TRA versus femoral access (FA) in a large, multi-centre registry.

Methods: The Melbourne Interventional Group (MIG) database is composed of prospectively collected detailed patient & procedural data on consecutive patients undergoing PCI.

Prospectively collected data from 12,086 PCI performed between April 2004 and December 2009 was obtained from the MIG registry. TRA (469; 3.9%) was compared to FA (11,617; 96.1%) in regards to patient factors, procedural factors and clinical outcomes (in-hospital, 30 days and 1 year).

Brachial access cases and patients requiring an intra-aortic balloon pump were excluded in this analysis.

Results: TRA patients had higher BMI (29.1 versus 28.1; p<0.001), smoking rate (72.6% versus 67.1%; p=0.012), family history of ischaemic heart disease (49.0% versus 41.5%; p=0.002), more likely received thrombolysis (7.0% versus 4.9%; p=0.047) and suffered a NSTEMI (31.1% versus 25.4%; p=0.005). FA patients had more prior PCI (25.3% versus 19.6%; p=0.006) and CABG (9.2% versus 6.0%; p=0.02). No significant differences numbers of STEMI, unstable angina, elective or rescue PCI. More Type C lesions were performed FA (16.2% versus 12.2%; p=0.013) and more drug eluting stents used (47.0% versus 32.0%; p<0.001). There was no difference in hospital length of stay, bleeding complications, 30 day or 1 year death, MI, TVR and MACE

Conclusions: TRA patients had higher BMI, more NSTEMI and a higher proportion had thrombolysis. More FA patients had previous PCI or CABG, and had more Type C lesions treated and DES used. In hospital bleeding, and 30 day and 1 year outcomes were not significantly different between TRA and FA. This data suggests that it is reasonable to employ either TRA or FA approach for PCI.

113

DEPRESSION & AUTONOMIC NERVOUS SYSTEM FUNCTION IN HEART FAILURE

S. J. Lonie[1,2], A. Stewart[1], D. Toia[1], D. Hare[1,2]

[1]Departmen of Cardiology, Austin Health, Heidelberg, VIC, Australia
[2]Department of Medicine, The University of Melbourne, VIC, Australia

Background: Depression is associated with increased morbidity and mortality in chronic heart failure (CHF) patients. Dysregulated autonomic activity as measured by heart rate variability (HRV) is found in patients with depression and in CHF patients. Autonomic dysregulation may mediate the relationship between depression and CHF outcomes; however, the association between depression and HRV in CHF patients is unknown.

Aims: This study aimed to examine depression and HRV in stable systolic CHF patients.

Method: Participants completed a 30-minute electrocardiogram for HRV analysis, a clinical interview for depression, self-report questionnaires (mood, social support, anxiety & hopelessness) and had blood taken. Frequency and time domain HRV indices were extracted from recordings. Differences in and prediction of HRV were examined as a function of depression category and severity, adjusting for possible confounders (e.g. age, gender, LVEF etc.) and psychosocial covariates, using ANOVA, multiple regression and path analysis.

Results: The sample comprised 45 participants, 35 males (78%), 10 females (22%), mean age 58.84 years (S.D. ± 12.43), NYHA Class I, N=14 (31%), II, N=26 (58%) and III, N=5 (11%), with mean LVEF 41.22 (S.D. ± 11.08). Significant differences were found between patients with no depression and depression in all HRV frequency domain measures, with depression significantly associated with decreased HF (nu) measures and increased LF (nu) and LF/HF measures. Regression analyses demonstrated depression had a direct effect on HRV and social support had direct and indirect effects on HRV via depression.

Conclusion: In CHF patients, both minor and major depression appear to be associated with altered autonomic activity. Differences in HRV measures in patients with depression highlights predominant sympathetic over parasympathetic cardiac modulation, a potential explanation for worse outcomes in depressed CHF patients. The apparent strong influence of social support on cardiac autonomic activity in CHF patients warrants further research.

114

NATIONAL SURVEY OF AUSTRALIAN CARDIOLOGISTS' BELIEFS AND PRACTICE REGARDING SCREENING, DIAGNOSIS AND MANAGEMENT OF DEPRESSION

A. G.O. Stewart[1], A. Driscoll[2], D. L. Hare[1,3]

[1]Department of Cardiology, Austin Health, Heidelberg, VIC, Australia
[2]Department of Epidemiology & Preventive Medicine, Monash University, Melbourne, VIC, Australia
[3]Department of Medicine, The University of Melbourne, Parkville, VIC, Australia

Over one third of cardiac patients are depressed. It is unclear whether depression is thought important by cardiologists.

Purpose: (1) Examine cardiologists' beliefs and practice in relation to depression screening, diagnosis, treatment and referral

Method: A national survey of all Australian cardiology members of the Cardiac Society of Australia & New Zealand (CSANZ) was conducted with a single page questionnaire.

Results: Sixty percent of members responded (511 of 822 eligible). The majority screened for depression "sometimes", 49.8%, and 32.4%, "often" or "always". While 96.8% reported using no standard screening tool, 71.3% reported they would use a short screening questionnaire if available. For responsibility of depression identification, General Practitioners (GPs) were highest ranked, followed by cardiologists and cardiac rehabilitation. For treatment of depression, GPs were ranked highest, followed closely by psychiatrists, then cardiologists and psychologists. Most referred patients "sometimes" (40%) to GPs, psychiatrists and psychologists, and 56% of all surveyed "very much" wanted feedback on patient progress. Most reported never treating depression with antidepressant medication (40%), and the strongest predictor treatment was confidence in treating depression ($r=0.616$, $p<0.0001$), followed by, nomination of cardiologists for treatment (0.387, $p<0.0001$), belief in antidepressant efficacy ($r=0.358$, $p<0.0001$), and depression having an etiological role in CVD ($r=0.158$, $p<0.0001$). Most believed depression was only "slightly" related to CVD development (41.2%), but "moderately" related to cardiac events and death, 53% and 49% respectively.

Conclusions: Australian cardiologists are interested in depression but don't use diagnostic screening tools. These results will assist the development of clinical pathways.

115

PREDICTORS OF MORTALITY IN INFECTIVE ENDOCARDITIS IN CONTEMPORARY AUSTRALIAN PRACTICE OVER 13 YEARS.

K. J. Lu, R. Reddy, L. G. Kearney, B. Wai, M. Ord, P. M. Srivastava

Cardiology, Austin Health, Heidelberg, VIC, Australia

Background: Infective endocarditis (IE) is a disease associated with high morbidity and mortality. We analysed the prognostic significance of epidemiological, clinical and microbiological factors in adult patients with IE at an Australian tertiary teaching hospital over a 13 year period.

Methods: Consecutive patients managed at the Austin hospital between 1996 and 2009 with definite IE by modified Duke criteria were retrospectively enrolled. Baseline clinical, biochemical, microbiological characteristics were analyzed. Primary outcome was all cause mortality.

Results: One hundred and forty eight patients were enrolled with a mean follow up of 3.8 +/- 3 years. The median age was 58 ± 17 years, 66% were male, 22% were diabetic, 16% had renal impairment (Cr >0.12 mmol/L), 14% had preexisting heart failure (HF), 16% had prosthetic valves and *Staphylococcus aureus* was the most common pathogen (52%). Vegetations on echocardiography were most frequently found on the mitral valve (36%) and then aortic valve (27%). Surgery was undertaken in 46% of patients. In-hospital and long-term mortality was 24% and 47% respectively. On logistic regression analyses; new onset heart failure complicating index admission (HR 2.73 [1.54-4.83] $p<0.001$), S *aureus* infections (HR1.96 [1.18-3.22] $p<0.01$), increasing age (HR 1.05 [1.03-1.07] $p<0.001$), and low haemoglobin (Hb<12g/dl) (HR 1.02 [1.01-1.03] $p<0.001$) were independently associated with increased mortality. However, surgical treatment was protective (HR 0.46 [0.24-0.87] $p<0.02$) for mortality.

Conclusions: IE remains associated with high mortality despite improvements in diagnosis and management. New onset heart failure, *S. aureus* infections and anaemia should alert clinicians to increased risk of adverse events.

116

CORRELATION AND CHARACTERISTICS OF ELECTROCARDIOGRAPHIC CHANGES WITH CORONARY ANATOMICAL FINDINGS IN PATIENTS WITH ACUTE CIRCUMFLEX CORONARY ARTERY OCCLUSION.

F. Chahadi

Cardiology, Austin Health, Heidelberg, VIC, Australia

Background: Use of the electrocardiogram (ECG) is a vital assessment tool for the management of acute coronary syndromes. The study aim was to determine the characteristics of ECG ST-segment changes in acute circumflex coronary artery occlusion and to correlate these changes with coronary artery dominance and likely lesion location.

Methods: A retrospective analysis was performed in a major tertiary referral centre with consecutive recruitment of 62 patients (51 male) presenting with acute circumflex coronary artery occlusion who underwent emergency percutaneous coronary intervention. Coronary artery dominance, lesion location and initial 12-lead ECG ST-segment changes prior to PCI were recorded and analysed.

Results: The inferior leads (II,III, aVF) had the highest mean values for ST-segment elevation 1.46+/-0.37 mm, 1.73+/-0.44 mm and 1.52+/-0.43 mm respectively, while the precordial leads V_5 & V_6 showed the highest mean values of 1.32+/-0.48 mm & 1.28+/-0.39 mm respectively ($p<0.05$). ST-segment depression was greatest in precordial leads V_{1-4} (mean=1.56+/-0.42mm) and inferior leads for proximal lesions only ($p<0.05$). Proximal lesions of the circumflex coronary artery were associated with a trend to greater ST-segment elevation load ($p=0.07$). There was no difference in ST-segment changes that allowed differentiation between a dominant and non-dominant circumflex.

Conclusions: In patients with acute circumflex coronary artery occlusion, there was a progressive increase in ST-segment elevation across the precordial leads from V1 to V6, and a progressive decline in ST-segment depression. More proximal occlusions were associated with a greater ischaemic burden on ECG.

117

CHRONIC RENAL IMPAIRMENT IS A SIGNIFICANT PREDICTOR OF INCREASED MORTALITY IN SUBJECTS WITH HEART FAILURE AND NORMAL EJECTION FRACTION WITH MEDIUM TERM FOLLOW UP

K. J. Lu[1], H. L. D[1,2], L. G. Kearney[1,2], M. Ord[1,2], D. Toia[1], B. Wai[1,2], L. M. Burrell[2], P. M. Srivastava[1,2]

[1]Cardiology, Austin Health, Heidelberg, VIC, Australia

[2]Medicine, Austin Health, Heidelberg, VIC, Australia

Background: Heart failure(HF) with normal ejection fraction (HFNEF) is increasing in prevalence. We compared the characteristics and medium-term prognosis in patients with HFNEF managed in a multidisciplinary heart failure clinic (HFC) of a tertiary teaching university hospital over a 5 year period.

Methods: Patients with HF presenting to the HFC between 2000-2005 were prospectively enrolled. Baseline clinical characteristics, pathology tests and medication use (ACEi/ARB, B blocker, spironolactone) were recorded. The primary endpoint of all cause mortality was determined via clinical follow up and reference to the National Death Index.

Results: Seven hundred and eighty-eight patients were enrolled with a mean follow up of 2.6 ± 1.6 years. Patients with HFNEF (N=160: 20%) had lower all cause mortality compared to those with heart failure with reduced ejection fraction (HFREF), (21% vs 35% $p<0.001$). Compared to those with HFREF, they were: older (mean age 71±14 vs 69±14 years $p<0.05$), more likely female (50% vs 31% $p<0.01$), more likely to be hypertensive (24% vs 5% $p<0.01$) , less likely to have an ischaemic aetiology, (4% vs 51% $p<0.01$) and had less renal impairment (Cr>1.2g/dl: 9.4% vs 15.8% $p<0.04$). They also had lower prescription of: ACEi/ARB (65% vs 92% $p<0.01$), Beta blockers (31% vs 68% $p<0.01$) and spironolactone (11% vs 43% $p<0.01$).

On logistic regression analyses in patients with HFNEF, chronic renal impairment (HR 5.2 [2.1-13.2] $p<0.01$) and increasing age (HR 1.1 [1.0-1.1] $p<0.05$) were independently associated with increased mortality..

Conclusion: HFPEF is associated with high all cause mortality rates. Renal impairment and age are important predictors of death in this cohort.

118

ANGIOTENSIN CONVERTING ENZYME 2: A NOVEL MARKER OF CORONARY ARTERY DISEASE

M. Freeman[1,2], S. K. Patel[1], E. Velkoska[1], K. Griggs[1], R. G. Dean[1], A. Al-Fiadh[1,2], M. Horrigan[2], O. Farouque[2], L. M. Burrell[1]

[1]Department of Medicine (Austin Health), University of Melbourne, Heidelberg, VIC, Australia
[2]Department of Cardiology, Austin Health, Heidelberg, VIC, Australia

Background: Angiotensin converting enzyme (ACE) 2 is a newly discovered member of the renin angiotensin system localised to endothelial cells of intramyocardial vessels and smooth muscle cells. Cardiac ACE2 is elevated in ischemia, and its expression is increased in atherosclerotic plaques. To date the effect of coronary artery disease (CAD) on plasma ACE2 levels is unknown. We hypothesised that plasma ACE2 levels are increased in CAD, and elevated across the coronary circulation.

Methods: Subjects requiring coronary angiography for suspected CAD were recruited between 2008-2009. Blood samples were taken from the femoral artery, coronary sinus and coronary artery ostium. ACE2 activity was measured using an ACE2 quenched fluorescent substrate and compared in those with angiographically normal coronary arteries (NCA, defined as < 50% stenosis), and significant CAD (>50% stenosis in ≥ 1 major epicardial vessel).

Results: 139 subjects completed the study. The mean±SD age was 65.0±10.4 years, BMI 29.9±5.7 kg/m^2 and 70% were male. ACE2 activity was not significantly different between the femoral artery, coronary sinus and coronary ostium sites (p=0.24). There was a significant effect with ACE2 activity and CAD (p<0.0001). ACE2 (geometric mean [25th, 75th interquartile range]) was significantly higher in CAD compared to NCA subjects at the femoral (p=0.016), coronary sinus (22.6 [14.1, 37.9] vs. 15.6 [10.2, 23.6] pmol/min/ml, p=0.007) and coronary artery ostium (21.5 [13.5, 33.7] vs. 15.1 [10.9, 22.7] pmol/min/ml, p=0.009). These differences remained significant after adjusting for age and gender.

Conclusion: Elevated plasma ACE2 activity may reflect the burden of coronary atherosclerosis in human CAD.

119

EFFICACY OF VERY-LOW-CALORIE DIETS AS A TREATMENT FOR OBESITY IN INDIVIDUALS WITH TYPE 2 DIABETES COMPARED TO NON-DIABETIC CONTROLS.

S. T. Baker[1], G. Jerums[1], L. A. Prendergast[2], S. Panagiotopoulos[1], B. J. Strauss[3], T. Vu[1], G. E. Thomas[1], J. Proietto[1]

[1]Endocrine Centre, Austin Health, Heidelberg West, Australia
[2]Department of Mathematics and Statistics, La Trobe University, Bundoora, VIC, Australia
[3]Body Composition Laboratory, Monash University, Clayton, VIC, Australia

A previous study (n = 15) has suggested that obese people with type 2 diabetes (T2DM) on a very-low-calorie diet (VLCD) lose less weight than people without T2DM. Our aim was to investigate the effect of VLCDs on weight and body composition in obese people with and without T2DM, including those treated with insulin and sulfonylurea therapy.

We recruited 70 participants, 37 with T2DM and 33 with normal fasting glucose (NG), into a 24-week VLCD program. A 12-week intensive phase incorporating three Optifast® meal replacements daily was followed by a 12-week transition to a low carbohydrate, calorie-reduced diet. Anthropometric data were collected, and 38 participants (21 NG, 17 T2DM) also underwent abdominal MRI of visceral (VAT) and subcutaneous adipose tissue (SAT) at 0 and 24 weeks.

While BMI and abdominal SAT were similar at baseline, the T2DM group had greater mean waist circumference and VAT (1675cm^3 vs. 1103cm^3, p <0.01). Weight loss at 24 weeks was less in the T2DM vs. NG group (-5.9 ± 1.0% vs. -10.0 ± 1.5%, p = 0.029). Changes in body weight correlated with changes in VAT (r = 0.57, p <0.02) and SAT (r = 0.68, p <0.01) in the T2DM and similarly the NG group. Treatment of T2DM with insulin or sulfonylureas attenuated weight loss and improvement in VAT in this subgroup compared to the NG group and T2DM subgroup on diet or metformin alone. Higher baseline plasma insulin levels and a trend towards reduced ketosis was seen in the T2DM group on insulin or sulfonylureas, however ketosis did not correlate with measures of hunger/satiety used in this study.

In conclusion, obese individuals with T2DM following a VLCD program may not achieve weight loss equivalent to non-diabetics. Sulfonylurea and insulin therapy appears to attenuate weight loss and reduction in VAT.

120

PATHWAY SELECTIVE INSULIN RESISTANCE: A POTENTIAL LINK BETWEEN OBESITY, INFLAMMATION AND ELEVATED TRIGLYCERIDES IN TYPE 2 DIABETES

S. P. Mangiafico[1], J. Wentworth[2], B. C. Fam[1], S. Andrikopoulos[1], J. Proietto[1]

[1]Medicine AH/NH, University of Melbourne, Heidelberg Heights, VIC, Australia

[2]Autoimmunity and Transplantation, Walter and Elisa Hall Institute, Parkville, VIC, Australia

Hypertriglyceridaemia, in the face of low HDL-cholesterol, hyperinsulinaemia and chronic low-grade inflammation is considered a pro-atherogenic cocktail for patients with Type 2 diabetes. Our hypothesis is that hyperinsulinaemia raises the production of proinflammatory cytokines in insulin resistant tissues and leads to mildly elevated plasma levels of cytokines, which cause hypertriglyceridaemia.

We investigated this hypothesis using human peripheral blood mononuclear cells (PBMCS) and PEPCK transgenic rats, a model of obesity-induced insulin resistance. Lean PVG control rats served as controls. Fasting plasma triglycerides, glucose, insulin, whole body glucose turnover and insulin sensitivity were assessed under baseline conditions and following hyperinsulinaemic clamps. Insulin signalling through PI-3 kinase and MAPKinase were assessed via western blotting. Cytokine mRNA for IL-6, TNF-α and IL-1ß was measured by Real Time PCR. Human PBMCS were incubated in the presence or absence of insulin and/or inhibitors of insulin signalling. Cytokine production was assessed by multiplex ELISA. Hepatic triglyceride secretion rate was estimated following intravenous administration of Triton-WR 1339, in the absence or presence of LPS, which induces cytokine production.

Adult PEPCK rats exhibit fasting hyperinsulinaemia, mildly elevated blood glucose and triglycerides. Fasting hyperinsulinaemia in PEPCK rats was associated with increased expression of the cytokines IL-6, TNF-α and IL-1ß and impaired insulin signalling through PI-3 Kinase, but not MAPKinase. Insulin stimulation induced cytokine production in cultured PBMCS, which was prevented by preincubation with a MAP Kinase inhibitor. Hypertriglyceridaemia in obese rats was associated with increased hepatic triglyceride secretion, an effect which was reproduced following LPS injection to lean controls.

The current study provides further evidence that hyperinsulinaemia induces proinflammatory cytokine production in insulin resistant tissue, supporting the hypothesis that hyperinsulinaemia-induced proinflammatory cytokine production is a possible cause of hypertriglyceridaemia in Type 2 Diabetes.

121

PARTIAL, BUT NOT TOTAL DELETION OF SEPS1 LEADS TO IMPROVED INSULIN SECRETION.

S. Weng, T. Samarasekera, B. C. Fam, J. Proietto, S. Andrikopoulos

Department of Medicine (AH/NH), The University of Melbourne, Heidelberg Heights, VIC, Australia

Selenoprotein S (SEPS1) is an antioxidant enzyme that has been shown to protect against oxidative stress (H_2O_2) induced cell death in a pancreatic ß-cell line. We previously showed that ß-cell specific over-expression of SEPS1 were protected against alloxan-induced diabetes. Interestingly these SEPS1 transgenic mice displayed reduced insulin secretion *in vivo*, associated with mild hyperglycaemia.

Based on these findings, we hypothesised that the deletion of SEPS1 will predispose mice to oxidative stress induced diabetes. The aim of this study was to generate and characterise SEPS1 global deleted mice.

SEPS1 global deleted mice were generated by PGK-Cre and fed a standard chow diet. At eight weeks of age, plasma glucose and insulin responses were examined following an intravenous glucose tolerance test (IVGTT) on total (-/-) and partial (+/-) deleted mice and negative littermates (+/+).

Plasma glucose levels during the IVGTT were not significantly different amongst three groups of mice. The result showed that SEPS1 mice (+/-) had better insulin secretory response compared with totally deleted (-/-) and negative littermates (+/+). No significant difference in insulin secretion was observed between totally deleted SEPS1 mice (-/-) and negative littermate.

In conclusion, insulin secretion was surprisingly enhanced in partially deleted SEPS1 mice and this may be due to a modestly increased reactive oxygen species level. In total SEPS1-deleted mice, the capacity for insulin secretion may have been completely compensated for by the upregulation of another antioxidant protein. These results suggest that the capacity for insulin secretion is strongly associated with the level of antioxidants.

122

INDUCIBLE MUSCLE-SPECIFIC UBL5 KNOCKOUT HAS NO EFFECT ON GLUCOSE METABOLISM

W. Pong, J. Favaloro, B. C. Fam, S. Andrikopoulos, J. Proietto

Department of Medicine (AH/NH), University of Melbourne, Heidelberg Heights, VIC, Australia

Disruption of the mitochondrial unfolded protein response is thought to contribute to skeletal muscle mitochondrial dysfunction in Type 2 Diabetes. UBL5, a small ubiquitin-like protein highly conserved among eukaryotes, is required for the activation of the mitochondrial unfolded protein response in *C. elegans*. We previously reported that homozygous global deletion of UBL5 is embryonic lethal, highlighting the importance of this gene in embryonic development. However, mice with a 50% global reduction in UBL5 mRNA levels displayed no phenotypic differences compared to littermates.

This study sought to investigate the role of UBL5 in muscle glucose metabolism.

Skeletal muscle-specific homozygous UBL5 knockout mice were generated by crossing UBL5-floxed mice (on the C57BL/6 background) with either MCK-Cre mice, or with mice expressing an inducible Cre recombinase (MerCreMer) in skeletal muscle. Gene deletion was induced in 8-week old mice by tamoxifen gavage (4mg/mouse/day) for 2 weeks and assessed by Real Time PCR. Phenotypic parameters such as body weight, food intake and glucose tolerance was measured.

Complete deletion of UBL5 (with MCK-Cre) in both skeletal muscle and heart also resulted in embryonic lethality. In contrast, inducible skeletal muscle homozygous knockout (MCM-UBL5-/-) mice were viable and were shown to have a 50% reduction of UBL5 mRNA in white quadriceps. At 10 weeks of age, there were no differences in body weight and food intake between MCM-UBL5-/- mice and littermates. Glucose tolerance was assessed following a 2g/kg body weight oral glucose load. Preliminary results showed there were no significant differences in plasma glucose and insulin levels.

These results suggest that UBL5 is vital for cardiac development. Furthermore, a 50% reduction of UBL5 mRNA in skeletal muscle is not sufficient to induce any changes in glucose metabolism in these mice.

123

IDENTIFICATION OF TARGET GENES IN OSTEOBLASTS THAT ARE REGULATED DIRECTLY VIA THE ANDROGEN RECEPTOR

P. Russell, C. Chiang, M. Chiu, M. V. Clarke, J. D. Zajac, R. A. Davey

Medicine, University of Melbourne, Heidelberg, VIC, Australia

Background and aim: Androgens are important for bone growth and maintenance in males. Our aim was to identify target genes that are regulated by the AR in osteoblast (OBLs), the bone forming cells. To achieve this we generated a mouse model in which the AR is deleted in mineralising OBLs (mOBL-ARKOs).

Methods: Microarray was performed on RNA from bones of mOBL-ARKOs and controls at 6 and 12 weeks of age (n=3/group). Genes identified to be regulated by microarray were confirmed by quantitative real time PCR (n=19-20/group).

Results: Microarray analysis identified 25 and 366 genes regulated by more than 1.5 fold ($P<0.05$) in mOBL-ARKOs at 6 and 12 weeks of age respectively. Of these, 13 genes were regulated at both 6 and 12 weeks of age. We confirmed a number of these genes to be regulated in the bones of mOBL-ARKOs compared to controls including genes involved in: OBL development (type 1a1 collagen (2 fold increase at 6wks ($P<0.05$), osteocalcin (1.85 fold increase at 12wks ($P<0.05$)); growth and development (growth hormone (3.5 fold increase at 12wks ($P<0.01$), Wnt4 (2.3 fold increase at 12wks ($P<0.05$)), Tgfß2 (2 fold increase at 12wks ($P<0.05$)), ltbbp2 (3 fold increase at 6 and 12wks ($P<0.01$)); and regulatory genes (Dpp4 (2.7 fold increase at 6wks ($P<0.05$), adiponectin (2.5 fold increase at 12wks ($P<0.01$)).

Conclusion: Androgens act directly via the AR in mineralizing OBLs to regulate a number of target genes associated with the development and regulation of OBLs and skeletal growth.

124

DETECTION AND ASSESSMENT OF PROGRP-DERIVED PEPTIDES IN HUMAN COLORECTAL CANCER CELLS AND THE ROLE THEY PLAY IN CELLULAR PROLIFERATION.

O. Patel[1], M. Chang[1], M. Nordlund[2], A. Shulkes[1], G. Baldwin[1]

[1]Department of Surgery, University of Melbourne, Heidelberg, VIC, Australia

[2]Department of Medical Biochemistry, Radiumhospitalet, Rikshospitalet University Hospital, Oslo, Norway

Background and aim: Amidated gastrin-releasing peptide (GRP) is the prototypical autocrine growth factor. We have previously demonstrated that non-amidated peptides derived from the C-terminus of proGRP are also biologically active in colorectal cancer (CRC) cell lines *in vitro*, via a receptor distinct from the GRP receptor [1]. This study investigates the quantities of proGRP-derived peptides present in a panel of 5 CRC cell lines as well as the effect of such peptides on proliferation *in vitro.*

Methods: proGRP-derived peptides obtained from boiling water extracts of the human CRC cell lines DLD-1, SW1222, HCT 15, HT-29 and HCT 116 were quantitated by region-specific ELISA. Proliferation of DLD-1 cells after reduction of proGRP-derived peptide concentrations by transfection with proGRP shRNA was measured by [^{3}H]-thymidine incorporation.

Results: In CRC cell extracts ELISA assays for proGRP-derived peptides containing residues 48-61, 56-88 or 48-88 detected 3-15, 10-60 and 20-152 fmol/10^6 cells, respectively. Little or no GRP18-27amide or GRP1-27amide was detected. Stable transfection of DLD-1 cells with proGRP shRNA significantly reduced proliferation.

Conclusions: These results indicate that non-amidated peptides derived from the C-terminus of proGRP are present in CRC cells and stimulate the proliferation of CRC cells *in vitro*. Such peptides are attractive targets for novel CRC therapies.

(1) Patel O., Dumesny C., Shulkes A. and Baldwin G.S. (2007) C-terminal fragments of the gastrin-releasing peptide precursor stimulate cell proliferation via a novel receptor. Endocrinology. 148:1330-1339

125

SLOWING DOWN B-CELL METABOLISM AND INSULIN SECRETION PROTECTS B-CELL FUNCTION UNDER CONDITIONS OF CHRONIC NUTRIENT OVERLOAD

M. Stathopoulos, A. Blair, B. C. Fam, J. Proietto, S. Andrikopoulos

Medicine (AH/NH), University of Melbourne, Mentone, VIC, Australia

We have previously presented at this meeting that islet ß-cell specific FBPase transgenic mice display reduced glucose-stimulated insulin secretion (GSIS) as a result of reduced glycolysis. We further showed that ß-cell specific FBPase transgenic mice displayed better glucose tolerance and insulin secretion following a chronic high fat diet. We therefore suggested that deceleration of ß-cell function protected against high fat induced impairments in glucose tolerance and insulin release. Interestingly, studies have shown that obesity and insulin resistance may be present well before the development of type 2 diabetes (T2D) and it is not until the decline in ß-cell function that an individual will transition from impaired glucose tolerance to T2D. For this reason we conducted *in vitro* studies to investigate the mechanism by which ß-cell specific FBPase transgenic mice are protected from high fat diet induced ß-cell dysfunction.

We hypothesised that islets isolated from ß-cell specific FBPase transgenic mice would maintain better GSIS following chronic exposure to high fat compared to negative littermates due to reduced oxidative and/or endoplasmic reticulum stress. Islets were isolated from transgenic mice and negative littermates and incubated with or without 1mM palmitate for 48 hours. Insulin secretion was assessed following exposure to either 2.8 mM or 20 mM glucose for 60 minutes and the data presented as fold increase of basal (2.8 mM) glucose.

The results highlight that under control conditions ß-cell specific transgenic mice show a significant impairment in secretory capacity compared to negative littermates. In contrast, following chronic palmitate exposure ß-cell specific FBPase transgenic mice showed a significantly better secretory response in comparison with negative littermates.

This data supports our hypothesis that ß-cell specific FBPase transgenic mice can maintain better GSIS following chronic exposure to high fat due to a reduction in glycolytic flux.

126

ARCHITECTURAL DECAY OF THE SKELETON AND ACCUMULATION OF VISCERAL ABDOMINAL FAT FOLLOWING ANDROGEN DEPRIVATION THERAPY FOR PROSTATE CANCER

E. Hamilton[1], E. Gianatti[1], B. Strauss[3], J. Wentworth[2], A. Ghasem Zadi[1], R. Zebase[1], D. Lim Joon[1], D. Bolton[1], E. Seeman[1], J. D. Zajac[1], M. Grossmann[1]

[1]University of Melbourne Austin Health, Heidelberg, Australia
[2]WEHI, Australia
[3]Monash University, Australia

Introduction: Androgen deprivation therapy (ADT) for treatment of prostate cancer reduces bone mineral density (BMD), but the structural basis of the BMD deficit has not been determined. ADT also increases fat mass, but the relationship between sex steroid deficiency and abdominal fat distribution is controversial.

Aim: We hypothesized that ADT reduces cortical and trabecular volumetric BMD (vBMD) and increases visceral abdominal fat following ADT.

Methods: 26 males, mean age 70.6 (6.8) years, with non-metastatic prostate cancer were studied at baseline, 6 and 12 months after commencing ADT. Bone microarchitecture was assessed using high resolution peripheral quantitative CT (HR-pQCT), and abdominal fat distribution was assessed using Slice-O-Matic software analysis of L4-5 computed tomography images.

Results: ADT decreased total testosterone (12.8 to 0.7nmol/L), and estradiol (103.7 to 29.6pmol/L). After 12 months of ADT, total vBMD at the distal radius decreased by 5.2%, due to a decrease in cortical vBMD (-11.3%) and trabecular vBMD (-3.5%). Changes at the tibia were similar, with a decrease in total vBMD (-4.2%,), cortical vBMD (-6.0%,) and trabecular vBMD (-1.5%). Osteocalcin (15.40 to 28.33ng/mL), P1NP (44.19 to 81.38g/L), and C-telopeptide (0.357 to 0.702ng/mL), increased significantly. Subcutaneous fat (240.7 107.5 to 271.27 92.8cm^2) and visceral fat area (160.8 61.7 to 195.9 69.7cm^2) increased, as did total cholesterol (4.7 + 1.0 to 5.3, + 1.1 mmol/L) and triglycerides (1.1 + 1.5 to 1.6 + 1.1). 12 months

Conclusions: ADT-induced sex steroid deficiency is associated with decay of both cortical and trabecular bone, as well as accumulation of visceral abdominal fat. Since fractures, insulin resistance and cardiovascular disease are major causes of morbidity and mortality in men with non-metastatic prostate cancer, a better understanding of physiological changes associated with ADT should improve the identification of those at highest risk of these complications in the future.

127

QUANTIFYING INTRACORTICAL POROSITY PRODUCING CORTICAL REMNANTS IDENTIFIES INDIVIDUALS AT RISK FOR FRACTURE BEYOND EXISTING PARAMETERS

R. Zebaze, A. Ghasem-Zadeh, S. Iuliano-Burns, K. King, E. Seeman

Endocrinology/Medicine, University of Melbourne, West Heidelberg, VIC, Australia

Background Most age-related bone loss occurs by intracortical remodelling of cortex adjacent to the marrow which produces coalescence of pores leaving a transitional zone (TZ) of trabecular-appearing cortical remnants. We therefore hypothesized that quantifying this TZ improves identification of individuals with fracture more effectively than current methods.

Methods We studied 20 women with prevalent forearm fracture and 47 age, height, and weight matched controls. Images were collected at the distal radius using HRpQCT (Scanco). Total, cortical and trabecular volumetric density (Ct.D, Tr.D, and vBMD) were measured. Thicknesses of the compact-appearing cortex (Th.CC) and TZ (Th.TZ) and the proportion of the total (CC + TZ) cortical thickness occupied by the CC and the TZ (%CC and %TZ) were measured. Porosity (Po) was measured in each zone.

Results The %CC was lower in cases than controls (70.91±1.03 vs 72.85±0.40; p=0.03) mostly due to an increase in the %TZ in cases (15.59±1.1 vs 12.82±0.43 %; p=0.01). While Th.CC was reduced in absolute terms (664.1±39 vs 710.3±28 mm) in fracture cases compared to controls, this was not significant. Porosity in the TZ (48.32±2.93 vs 42.02±1.48%; p=0.03) not CC (2.4±0.52 vs 2.89±0.43%, NS) was higher in cases than controls

Differences in Ct.D, vBMD and Tr.D between cases and controls were smaller and/or non-significant respectively 262.5±12.79 vs 288.54±9.5 (p=0.12), 853.6±14.59 vs 858.94 (NS) and 111.8 ±7.84 vs 132.2±5.55 (p=0.04)

Conclusion Assessment of the cortical decay of the transitional zone composed of cortical remnants may allow identification of individuals at risk for fracture beyond currently existing parameters.

INSULIN RESISTANCE CAUSES A PROGRESSIVE IMPAIRMENT IN SECOND-PHASE BUT NOT FIRST-PHASE INSULIN SECRETION.

C. N. Joannides, S. P. Mangiafico, S. H. Lim, B. C. Fam, J. Proietto, S. Andrikopoulos

Medicine, University of Melbourne, Heidelberg Heights, VIC, Australia

An impairment in glucose mediated insulin secretion has repeatedly been recognised as an early sign of beta-cell dysfunction, which contributes to glucose intolerance and Type II Diabetes. However the mechanisms underlying progressive beta-cell dysfunction in insulin resistance remain unclear.

Accordingly, we utilised the PEPCK transgenic rat, a model of primary insulin resistance, to determine whether or not insulin resistance leads to defects in insulin secretion.

Four and twelve week-old overnight fasted PEPCK transgenic and PVG/c control rats were used in this study. An intravenous tolerance test to glucose (1g/kg bodyweight) and the sulfonylurea, tolbutamide (4mg/kg) was performed to assess insulin secretory function. Islet architecture and beta-cell number were determined using immunohistochemistry.

Insulin secretion to both glucose and tolbutamide were comparable in 4 week-old rats and histologically, no differences in islet architecture could be detected. A progressive reduction in insulin secretion to a glucose bolus was observed in the 12 week-old transgenic rats compared to the controls. Interestingly, the insulin secretion defect was selective for second-phase whilst first-phase was preserved. Tolbutamide-induced insulin secretion was similarly suppressed in the PEPCK transgenic rats suggesting that the defect may lie at the level of the potassium channel.

The results suggest that a primary defect in insulin resistance leads to a progressive reduction in second-phase insulin secretion and that impaired proximal pathways may be the mechanism by which this occurs.

	Plasma Insulin (ng/mL)			
	Intravenous Glucose Tolerance Test		Intravenous Arginine Tolerance Test	Intravenous Tolbutamide Tolerance Test
Area under the curve	1st phase (ng/mL x 5min)	2nd phase (ng/mL x 40min)	1st phase (ng/mL x 5min)	1st phase (ng/mL x 5min)
4w PVG/c	6.6 (0.8)	41.3 (3.6)	10.2 (1.3)	5.1 (1.0)
PEPCK	5.9 (1.1)	34.2 (5.7)	20.7 (4.5)	5.1 (1.1)
8w PVG/c	29.3 (2.8)	143.7 (16.8)	___	___
PEPCK	19.9 (2.4)*	86.9 (5.8)**		
12w PVG/c	28.5 (3.7)	174.9 (22.1)	11.9 (3.8)	13.1 (1.9)
PEPCK	25.8 (3.7)	81.4 (8.0)**	19.6 (2.5)	7.4 (1.4)*

Data presented as mean (SEM); n = 7-21, * $p<0.05$ vs. PVG/c, ** $p<0.005$ vs. PVG/c

129

DEFINING THE SURFACE/VOLUME RATIO OF BONE IDENTIFIES THE SUSCEPTIBILITY OF BONE TO BEING REMODELLED AND LOST AND INDIVIDUALS AT RISK FOR FRACTURE BETTER THAN BONE DENSITY MEASUREMENTS

A. Ghasem-Zadeh, R. Zebaze, S. Iuliano-Burns, K. King, E. Seeman

Endocrinology/Medicine, University of Melbourne, West Heidelberg, VIC, Australia

Background: Bone densitometry is used to estimate fracture risk. However, the measurement is neither sensitive or specific. Fracture is structural failure so that specific structural changes need to be quantified. More specifically, bones surface/volume ratio determines susceptibility of matrix to being remodeled so that an in vivo estimate of this geometric property may better predict fracture risk than bone 'density' studies. We tested this hypothesis by quantifying bone structure using Strax 1.0, image analysis method allow estimation of structural decay *in vivo*.

Material & Methods: We studied women with forearm fracture and their age, height and weight matched controls using HR-pQCT images of the distal radius (in 23 cases and 50 controls), tibia (in 32 cases and 53 controls) and fibula (32 cases and 52 controls) measuring total volumetric bone mineral density (TvBMD), cortical vBMD (CtvBMD), and trabecular vBMD (TrvBMD) using the Scanco software and processed using a new software (Strax1.0) to estimate the fraction of the bone within the subperiosteal envelope accessible to remodeling (FBAR %); this is an estimate of surface/volume as bone requires a surface to be remodeled.

Results: At the distal radius, FBAR (%), correlated weakly with CtvBMD (r = 0.18, NS) but strongly with TvBMD (r= 0.67 p <0.0001) and TrvBMD (r = 0.87 p<0.0001). Similar correlations were obtained at the tibia .At the distal radius, FBAR was 18.5% lower in cases than controls. Whilst densities were lower in cases than controls, differences were relatively modest. The odds ratios for fracture were 5.68 (95% CI: 1.49 - 21.57; p <0.05) for FBAR, 1.54 (95% CI: 0.39-6.1; NS) for Ct vBMD, 2.9 (95% CI: 0.92-9.14; NS) for TvBMD. Similar results were obtained at the tibia and at the fibula.

Conclusion: Bone fragility is not captured by 'density'; *in vivo* identification the structural configuration of cortical and trabecular bone accessible to being remodeled, and so decayed, is likely to improve identification of individuals at risk for fracture.

130

PROPERTIES OF TRABECULAR ARCHITECTURE AT METAPHYSEAL REGION ARE ESTABLISHED IN EARLY LIFE AND DECAYED BEFORE MENOPAUSE

Q. Wang, S. Iuliano-Burns, X. Wang, A. Ghasem-Zadeh, E. Seeman

Endocrine centre, Austin Health, Heidelberg West, Australia

Trabecular bone dominates metaphaseal regions of long bones where fragility fractures commonly occur in later life. The property of trabecular architecture established at the cessation of growth, strong or weak, is important determinant of the emergence of skeletal fragility in following decades.

Previously we reported that there was no change in trabecular architecture at the metaphyses during adolescence. However, it is unclear whether individual's trabecular architecture at this region is different or not from adult women.

Using high-resolution peripheral quantitative computed tomography, we measured distal metaphyses of radius and tibia in 72 daughter-premenopausal mother pairs and compared their bone structural traits.

The average age (SD) of mother and daughter were 45.6 (5.0) and 12.7 (3.3) years old, respectively. Bone size, cortical thickness and cortical volumetric bone mineral density in both radius and tibia were significantly greater in mothers than daughters. However, girls had greater trabecular BV/TV at radius (0.134 ± 0.024 vs. 0.124 ± 0.033, p = 0.03) and tibia (0.145 ± 0.021 vs. 0.135 ± 0.032, p < 0.01) than mothers due to their greater trabecular number but similar trabecular thickness. Familial similarity (mother-daughter correlation) in trabecular thickness and number was present (r = 0.4-0.5, p <0.01) at both radius and tibia (Figure).

These results indicate that trabecular structure is established before puberty and there is substantial trabecular bone loss during premenopausal period. We concluded that measuring trabecular properties during childhood may early identify these individual of high risk of skeletal fragility in later life.

131

BONE MICROARCHITECTURE DURING AND AFTER LACTATION

M. Gomez[1], A. Bjornerem[2], A. Ghasem-Zadeh[1], T. Vu[1], E. Seeman[1]

[1]Endocrine Centre, Austin Health, University of Melbourne, VIC, Australia

[2]Institute of Community Medicine, University of Tromso, Norway

Oestrogen deficiency increases bone remodelling and bone loss. Lactation is an oestrogen deficient state in which it is believed that bone is lost by endocortical resorption to provide calcium for skeletogenesis.

We hypothesized that lactation is associated with increased intracortical porosity (rather than or in addition to endocortical resorption) and trabecular thinning without loss of connectivity. We also proposed these changes would reverse after cessation of lactation.

Volumetric bone mineral density (vBMD) and microarchitecture at the distal tibia (DT) and distal radius (DR) were assessed using high-resolution 3-D peripheral quantitative computed tomography (HR-3D-pQCT XtremeCT; Scanco Medical, Switzerland) in 100 women aged 20-42 years around 14 days after delivery, at 5.2 months of breast feeding exclusively and then 6 or more months after breast feeding had stopped.

During lactation, (n=82), total vBMD decreased (DT 0.6%, DR 0.1%) and cortical vBMD decreased (DT 0.3%, DR 0.2%) ($p<0.05$). Reductions were observed in cortical area (DT 1.8%, DR 1.4%), cortical thickness (DT 1.8%, DR 1.3%) and trabecular number (DT 4.4%, DR 2.1%) while trabecular thickness increased (DT 5.9%, DR 4.5%) and trabecular CSA (marrow) increased (DT 0.2%, DR 0.3%) (all $p<0.05$). Post-weaning, (n=26) cortical vBMD decreased further (DT 0.6%, DR 0.7%) while trabecular vBMD increased (DT 4%, DR 6.3%).

We infer that intracortical and endocortical bone loss occurs during lactation producing lower cortical vBMD and thinner cortices. Cortical remnants produced by intracortical remodelling look like trabeculae falsely increasing ‘trabecular' thickness. After lactation, cortical vBMD was not restored, at least within the first six months.

132

PREDICTIVE VALUE OF LEFT VENTRICULAR MASS AND GEOMETRY IN TYPE 2 DIABETES MELLITUS FOR ADVERSE CARDIOVASCULAR EVENTS

B. Wai[1,2], S. K. Patel[1], M. Ord[2], S. Grant[1], P. M. Srivastava[1,2], L. M. Burrell[1,2]

[1]Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC, Australia

[2]Department of Cardiology, Austin Health, Heidelberg, VIC, Australia

Background: Diabetes mellitus (T2DM) is associated with left ventricular hypertrophy (LVH) and concentric remodelling (CR) in the absence of LVH. The clinical significance of LVH and CR in T2DM remains controversial. We hypothesized that LVH and CR are common in subjects with T2DM and predict major adverse cardiovascular events (MACE).

Methods: We studied 554 consecutive subjects with T2DM attending a complications surveillance program at Austin Health, Melbourne, Australia . LVH was defined as left ventricular mass indexed to height$^{2.7}$ ($LVMH^{2.7}$); >49g/$m^{2.7}$ in males and >45g/$m^{2.7}$ in females, and CR as a relative wall thickness >0.42 without LVH. MACE was defined as all cause mortality, stroke, heart failure admission, myocardial infarction or acute coronary syndrome and coronary and peripheral vessel revascularization. Follow-up was censored at first MACE at a mean of 4.9 ± 2.1 years. Cox regression analyses were performed with LVH and CR as independent variables to identify the predictors of MACE.

Results: The mean ± SD age of subjects was 62 ± 13 years, mean body mass index (BMI) was 31.0±5.9kg/m^2 and 59% were male. During the follow up period, 150 MACE occurred. LVH was present on echocardiography in 56% of subjects, and CR in 17%. Significantly higher MACE are seen in LVH with Kaplan Meier analysis (Log Rank Test: Chi Square 19.2, $P < 0.001$). LVH predicted MACE independent of age, sex, BMI, blood pressure, duration of T2DM and history of macrovacular disease (HR 1.59; CI 1.08-2.35; P=0.02). CR did not independently predict MACE after correcting for the same covariates.

Conclusions: LVH is common in T2DM and predicts MACE, but CR does not independently predict MACE. This is independent of pre-existing macrovascular complications. More aggressive or novel therapy specifically targetting LVH in T2DM to minimise MACE require further investigation.

133

THE SKELETAL RESPONSE TO VITAMIN D SUPPLEMENTATION DURING SUNLIGHT DEPRIVATION: A RANDOMISED TRIAL IN ANTARCTIC EXPEDITIONERS

S. Iuliano-Burns[1], K. King[1], J. Ayton[2], S. Hillam[2], G. Jones[3], E. Seeman[1]

[1]Endocrinology, University of Melbourne / Austin Health, West Heidelberg, VIC, Australia
[2]Polar Medicine, Australian Antarctic Division, Kingston, TAS, Australia
[3]Menzies Research Institute, Hobart, TAS, Australia

Antarctic expeditioners experience prolonged sunlight deprivation that results in vitamin D insufficiency (25(OH)D <50 nmo/l) within 4 months unless baseline values are greater than 100 nmol/L.

We randomly assigned 90 expeditioners (mean age 44yrs) to 3 regimens of vitamin D3; 50 000IU once at departure, monthly for 12 months or every 2 months for 12 months to test the hypothesis that the maintenance of vitamin D sufficiency (>50 nmol/L) required repeat dosing.

Participants provided serum samples prior to departure, at 6 months and on return at 12 months for assay of vitamin D (25(OH)D), parathyroid hormone (PTH) and bone markers (Osteocalcin). Differences were assessed using repeated measures ANOVA.

At baseline, mean 25(OH)D levels were 64 ± 2 nmol/L. After 12 months, serum 25(OH)D decreased by 20 ± 6% ($p < 0.01$) in the group receiving a single dose at baseline, increased by 26 ± 6%, ($p < 0.01$) in the monthly regimen, and was maintained in the two monthly regimen (2 ± 7%, NS). Bone turnover was unchanged using the monthly regimen but was elevated in the 2-monthly (16 ± 5%, $p < 0.01$) and the single dose (21 ± 8%, $p < 0.05$) groups. No group differences were detected for PTH.

During an expedition, 50 000IU vitamin D every 2 months was sufficient to maintain serum 25(OH)D levels, but a monthly dose is required to improve vitamin D status so should be considered for those with vitamin D insufficiency prior to departure.

134

METAL IONS UPREGULATE GASTRIN GENE EXPRESSION IN GASTRIC CANCER CELLS

L. Xiao, S. Kovac, A. Shulkes, G. Baldwin, O. Patel

The Department of Surgery, Austin Health, The University of Melbourne, Heidelberg, VIC, Australia

Background and Aim: We have shown that gastrin is elevated in gastrointestinal cancers and that hypoxia, which is a characteristic of many tumours, upregulates gastrin gene expression. The transition metal ion cobalt (II), a hypoxia mimetic, exerts a much stronger stimulatory effect than hypoxia on the gastrin gene in AGS cells (a gastric cancer cell line). In this study we examined the transcriptional regulation of the gastrin gene by other metal ions.

Methods: After exposure to different metal ions, the gastrin mRNA levels in AGS cells were measured by real-time PCR. The gastrin promoter activity was examined using the luciferase assay.

Results: Cobalt (II) or zinc increased gastrin mRNA expression in a dose-dependent manner, with the latter being a more potent inducer. The gastrin mRNA concentration was increased 55 ± 14 and 50 ± 6 fold by zinc at concentrations of 100μM and 150μM respectively. Cadmium (II), which is a xenobiotic metal ion, was the most sensitive stimulant and increased gastrin mRNA expression by 4.2 ± 0.1 fold at the low concentration of 5μM. Other metals including calcium, copper and nickel did not increase gastrin mRNA. The gastrin promoter was stimulated in a similar manner by these metals in AGS cells.

Conclusion: Gastrin gene expression is increased by zinc, cobalt or cadmium but not calcium, copper or nickel. Whether these metals are responsible for the increase in gastrin gene expression in gastrointestinal cancers will be investigated in animal models of tumourogenesis.

135

E/E' IS AN INDEPENDENT PREDICTOR OF HEART FAILURE ADMISSION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

B. Wai[1,2], S. K. Patel[1], M. Ord[2], S. Grant[1], R. MacIsaac[3], G. Jerums[3], P. M. Srivastava[1,2], L. M. Burrell[1,2]

[1]Medicine, University of Melbourne, Austin Health, Heidelberg Heights, VIC, Australia

[2]Cardiology, Austin Health, Heidelberg, VIC, Australia

[3]Endocrinology, Austin Health, Heidelberg, VIC, Australia

Background: Type 2 diabetes mellitus (T2DM) is a strong and independent risk factor for the development of heart failure (HF). Modern echocardiographic techniques with tissue Doppler allow non-invasive assessment of left atrial (LA) pressure. The ratio of peak early diastolic mitral inflow and septal annular velocities (E/e') can be used to assess LA pressure; an E/e'>15 was shown to be associated with an elevated LA pressure measured invasively. We hypothesised that an increased E/e' in T2DM subjects would be associated with increased risk of HF hospitalisation.

Methods: We studied 782 consecutive subjects with T2DM attending a complications surveillance program at Austin Health, Melbourne, Australia. An echocardiogram was performed at baseline and subjects were followed for 5.5±1.8 years. The primary outcome was decompensated HF leading to hospitalisation. Cox regression analyses were performed to identify the predictors of HF.

Results: The mean±SD age of subjects was 62.8±12.8y, body mass index (BMI) of 30.9±6.1kg/m^2, HbA1c of 7.8±1.85% and 59% were male. Over the follow-up period, 63 subjects developed decompensated HF. The hazard ratio of HF admissions in subjects with an E/e'>15 was 5.5 (3.0-9.9; P<0.001). A unit increase in E/e' was associated with a 9% excess risk of HF admission after correcting for age, gender, BMI, blood pressure, history of macrovascular disease, LV mass and LV ejection fraction (HR 1.09 95% CI:1.06-1.12; p< 0.001).

Conclusions: Tissue Doppler E/e' independently predicts HF admissions in subjects with T2DM and out-performed traditional clinical and echocardiographic parameters. E/e' represents an effective tool in assessing HF risk in T2DM. Further studies are needed to determine if more intensive therapy in T2DM subjects with elevated E/e' would improve clinical outcomes.

136

DIETARY SALT INTAKE AND MORTALITY IN PATIENTS WITH TYPE 2 DIABETES

R. J. MacIsaac[1], E. I. Ekinci[1], S. Clarke[2], M. C. Thomas[3], J. Moran[4], K. Cheong[1], G. Jerums[1]

[1]Endocrine Centre, Austin Health, West Heidelberg, VIC, Australia

[2]Endocrinology Department, Mater Hospital, South Brisbane, QLD, Australia

[3]Diabetes Complications Department, Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia

[4]Intensive Care Unit, The Queen Elizabeth Hospital & Health Service, Woodville, SA, Australia

Introduction: High dietary salt intake is associated with elevated blood pressure levels but it is unclear if it is also associated with increased mortality in patients with type 2 diabetes. The aim of this study was to determine whether baseline 24-h urinary sodium excretion can predict all-cause and cardiovascular mortality in patients with diabetes.

Methods: A prospective, cohort follow up study of 638 patients with type 2 diabetes attending a single clinic followed for a median of 9.9 years. The main outcome measures were predictors of all-cause and cardiovascular mortality as determined by Cox regression and competing causes modelling, respectively.

Results: During 5475 patient-years of follow-up, there were 175 deaths (28%), 43% of which had cardiovascular disease listed as a major contributing cause (n=75). We found that 24-h sodium excretion was inversely associated with all-cause mortality, after adjusting for other factors independently associated with all-cause mortality (including age, gender, duration of diabetes, atrial fibrillation, the presence and severity of kidney disease, i.e., eGFR and log AER). For every 100 mmol rise in 24-h sodium excretion, all-cause mortality was reduced by 28% (95% CI, 6-45%; p=0.02). There was also a significant relationship between 24-h sodium excretion and cardiovascular mortality (sub hazard ratio, 0.65; 95% CI, 0.44 – 0.95; p=0.03), after adjusting for the competing risk of non-cardiovascular death, and other predictors. Overall, 24-h sodium excretion was stronger predictor than conventional? modifiable risk factors, including HbA1c, lipid and blood pressure levels.

Conclusion: In patients with type 2 diabetes, a low dietary intake of sodium was associated with increased all-cause and cardiovascular mortality. Although seemingly paradoxical, these findings may possibly be explained by activation of the renin-angiotensin system, the sympathetic nervous system and other metabolic pathways that is observed when salt intake is reduced. Further trials, specifically in patients with diabetes, are necessary to confirm whether this association is causal.

137

FRUCTOSE FEEDING PROTECTS AGAINST FAT-INDUCED OBESITY.

N. Wong, W. Pong, J. Proietto, S. Andrikopoulos

Medicine (AH/NH), University of Melbourne, Heidelberg Heights, VIC, Australia

The consumption of fructose not only correlates with the rise in obesity but is also linked to insulin resistance, glucose intolerance, hypertriglyceridaemia and hypertension. Fructose is a key ingredient found in many sweetened beverages and for this reason excess consumption has been blamed for the alarming rate of obesity and Type 2 diabetes (T2D). In recent decades, diet sodas have gained widespread popularity as they are seen as healthier alternatives, yet the rate of obesity and T2D are still increasing. Interestingly, most studies focus on fructose exposure alone but in reality fructose is often consume with fat. Little is known about the interaction of fat and fructose on body weight and glucose regulation. This study was aimed at determining the effect of fructose, high fat and the combination of fat and fructose on body weight and glucose metabolism in mice. At 8 weeks of age, the animals were fed a chow, 15% fructose, 60% high fat or the combination diet for 20 weeks, followed by an IVGTT or OGTT to measure insulin secretion and glucose tolerance. Body weights and food intake was monitored weekly. As expected, both fructose and high fat alone increased adiposity. Unexpectedly, the combination diet restored body weights to similar levels of the chow fed control. Despite the improvement in body weight, glucose metabolism was still dysregulated, showing a step-wise impairment from fructose to high fat to high fat + fructose. Taken together, these data suggest that when fructose and high fat were combined, they had a synergistic effect to improve body weight but not glucose tolerance. The implication of this data is that diet sodas devoid of fructose together with high fat may contribute to the increased incidence of obesity.

138

INVESTIGATING DNA BINDING-INDEPENDENT SIGNALLING PATHWAYS OF THE ANDROGEN RECEPTOR

T. P.S. Pang, R. A. Davey, M. V. Clarke, J. P.J. Skinner, H. E. MacLean

Medicine (Austin Health), University of Melbourne, Heidelberg, VIC, Australia

Androgen-bound androgen receptor (AR) translocates to the nucleus, binds DNA and regulates gene expression. However, it may also have DNA binding-independent actions, including indirect gene repression [1] and activation of ERK/CREB signalling [2]. We are using our AR knockout (ARKO) mouse model, which has deletion of AR DNA binding activity, to investigate DNA binding-independent AR actions.

We demonstrated AR protein and mRNA levels are normal in genital skin fibroblasts (GSFs) from ARKO males. Using ARKO GSFs, we showed that the mutant AR has reduced nuclear translocation following androgen-binding. Both wildtype and ARKO GSFs have increased ERK phosphorylation following 1 min 100 nM androgen treatment ($p<0.05$) and this is abolished by the AR antagonist bicalutamide ($p<0.001$). Preliminary data show that the genomic AR target gene, *ApoD*, is regulated by androgens in wildtype but not ARKO GSFs, and has lower expression in ARKO cells. The non-genomic AR target gene, *Ngfr*, is down-regulated by androgens in wildtype GSFs and we are currently investigating its expression in ARKO cells. We are also investigating the effect of orchidectomy ± androgen replacement, including examining non-genomic target gene expression and phosphorylation of second messenger pathways in wildtype and ARKO mice. Preliminary data show that the expression of the genomic AR target gene *Odc1*, is up-regulated by androgens in wildtype but not ARKO kidney.

This study has characterised the function of mutant AR expressed in ARKO mice. Further characterisation of androgen effects in orchidectomised ARKO males will determine the physiological relevance of non-DNA binding-dependent actions of the AR.

(1) Kallio PJ et al. J. Mol. Endo. 9: 1017-1028, 1995

(2) Unni E et al. Cancer Research 64:7156-7168, 2004

139

IDENTIFICATION OF PHYSIOLOGICAL ROLE OF CALCITONIN IN PREGNANCY USING CALCITONIN RECEPTOR KNOCKOUT MICE

J. P.J. Skinner, S. A. Sastra, M. V. Clarke, J. D. Zajac, R. A. Davey

Medicine, University of Melbourne, Heidelberg, VIC, Australia

The role of the calcitonin (CT) and its receptor (CTR) as a regulator of bone cell metabolism has been well documented. We have previously shown that during times of calcium stress, such as hypercalcemia, CT, acting via the CTR, acts as a potent inhibitor of osteoclast action, the cells responsible for bone resorption [1].

We hypothesise that this inhibitory role of calcitonin via the CTR is important to protect the maternal skeleton against excessive resorption during pregnancy and lactation. Studies in mice have shown that during pregnancy and lactation there is a reduction of 20-25% of bone mineral density (BMD) [2]. Therefore the aim of the following study was to determine if CT acts via the CTR to limit bone loss during pregnancy.

To achieve this we have generated two CTR knock-out (CTRKO) mouse models, a global CTRKO and an osteoclast-specific CTRKO (OCL-CTRKO). Female global-CTRKO, OCL-CTRKO and control mice were time mated at 8 weeks of age and checked on a daily basis for the presence of vaginal plugs. Female breeders were sacrificed at 18 and 39 days post-mating to represent pregnancy and lactation groups respectively.

Preliminary analysis of cortical bone in control (n=4) vs OCL-specific CTRKO (n=7) pregnant females, and control (n=2) vs Global CTRKO (n=2) pregnant females showed no difference suggesting that cortical bone may be preserved in CTRKO mice during pregnancy.

We are currently analysing the trabecular bone, which is more metabolically active and is therefore, a more likely source of calcium mobilisation from bone.

(1) Davey et al. The calcitonin receptor plays a physiological role to protect against hypercalcemia in mice JBMR 23(8):1182-93, 2008.

(2) Kovacs C.S, Kronenberg H.M. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation Endocr Rev 18(6):832-72, 1997.

140

ANGIOTENSIN-(1-7) REDUCES PORTAL PRESSURE VIA GUANYLATE CYCLASE-DEPENDENT NITRIC OXIDE BUT ANGIOTENSIN II RECEPTOR INDEPENDENT PATHWAY IN CIRRHOTIC RATS

C. B. Herath[1], K. Mak[1], Z. Jia[1], L. M. Burrell[1], P. W. Angus[2]

[1]Department of Medicine, The University of Melbourne, Heidelberg Heights, VIC, Australia
[2]Department of Gastroenterology and Hepatology, Austin Health, Heidelberg, VIC, Australia

Background: The 'alternate arm' of the renin angiotensin system consisting of angiotensin converting enzyme 2 (ACE2) which generates angiotensin-(1-7) (Ang-(1-7)), and the putative Ang-(1-7) receptor MAS, has been shown to counter-regulate the deleterious effects of angiotensin II (Ang II) in several vascular beds but its role in portal hypertension is unknown. We therefore investigated the effects of Ang-(1-7) on portal pressure response to Ang II and methoxamine (alpha agonist).

Methods: 4-week bile duct ligated (BDL) rat livers (n=40) were perfused *in-situ* via the portal vein with carbogenated standard Krebs Henseleit solution. Ang II (60 pmoles) or methoxamine (300 nmoles) bolus was injected before and after preincubation with Ang-(1-7) [1 μM] with or without various blockers. Perfused livers were analysed for endothelial nitric oxide synthase (eNOS) protein expression.

Results: The maximal pressure response to both Ang II and methoxamine and area under the pressure response curve were reduced by more than one-third ($P<0.01$) in livers pre-treated with Ang-(1-7). This effect of Ang-(1-7) was not blocked by MAS receptor antagonist A779 [10 μM], or blockade of AT1 [1 μM] or AT2 [1 μM] receptors and was not reproduced using the MAS agonist AVE0991 [1 μM]. Bradykinin B2 receptor and cyclooxygenase blockers were also without effect. However, it was abolished by both NOS inhibitor L-NAME [100 μM] and guanylate cyclase inhibitor ODQ [10 μM] and this was associated with a significant ($P<0.01$) reduction in phosphorylated eNOS levels.

Conclusions: In the cirrhotic rat liver, Ang-(1-7) ameliorates both Ang II- and methoxamine-induced portal pressure via a guanylate cyclase-dependent NO pathway that is not mediated by the MAS or angiotensin receptors. This pathway presents a potential novel target for therapeutic intervention in portal hypertension.

141

GASTRIN INCREASES ITS OWN SYNTHESIS IN GASTROINTESTINAL CANCER CELLS VIA THE CCK2 RECEPTOR.

S. Kovac, L. Xiao, A. Shulkes, O. Patel, G. S. Baldwin

Surgery (Austin), University of Melbourne, Heidelberg, VIC, Australia

Introduction: Gastrin expression is increased in the gastrointestinal (GI) tumours and circulation of patients with GI cancers and may accelerate the growth of the cancers. The mechanism for the increase in gastrin is not known. We investigated whether gastrin could increase its own expression via a positive feedback loop.

Methods: Quantitative PCR was used to measure human gastrin mRNA in GI cancer cells. Radioimmunoassay was used to measure the gastrin precursor Gastrin-gly. To begin to understand how gastrin gene expression was regulated, proximal 1300bp fragment of the human gastrin promoter and promoter deletion fragments were cloned into pGL3 luciferase vector and transfected into gastric cancer cells (AGS) expressing the gastrin receptor (CCK2R).

Results: Gastrin mRNA levels were increased 44-fold in AGSCCK2R cells, 4.4-fold in colorectal cancer cells (Colo320CCK2R) and 4.4 fold in T-lymphocytes (JURKAT), expressing endogenous CCK2R. Treatment of CCK2R-expressing cells with Gamide led to significantly increased expression of gastrin peptides as measured by Ggly assay in the cell medium. The AGSCCK2R cells stimulated with Gamide increased gastrin mRNA (90-fold) and promoter activity (4-fold) in a time-dependent manner which was completely abolished with a CCK2R antagonist. The gastrin responsiveness was still present within the proximal 365bp fragment of the gastrin promoter, and did not involve Sp1 regulatory elements as mutation of either Sp1 site did not reduce the response. The MAP kinase pathway is involved in the gastrin positive-feedback loop as promoter activity was completely abolished following treatment with MAPK inhibitors.

Conclusion: We have demonstrated the presence of a positive-gastrin feedback loop which may contribute to the sustained increase in circulating gastrins observed in patients with GI cancers.

142

ABSENCE OF RAGE (RECEPTOR FOR ADVANCED GLYCATION ENDPRODUCTS) ATTENUATES LIVER FIBROSIS IN MICE

M. Goodwin[1], C. B. Herath[2], Z. Jia[2], M. T. Coughlan[3], J. M. Forbes[3], P. W. Angus[1]

[1]Gastroenterology, Austin Health, Heidelberg, VIC, Australia

[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

[3]Baker Heart and Diabetes Institute, Melbourne, VIC, Australia

Background: Advanced glycation endproducts (AGEs) are compounds formed through the non-enzymatic reaction of proteins with sugars. They contribute to the pathogenesis of diabetic renal and vascular disease via a number of mechanisms, including activation of receptors, induction of epithelial-to-mesenchymal transition and oxidative stress. The best characterised AGE receptor is RAGE (receptor for advanced glycation endproducts). This study sought to assess the role of the AGE-RAGE axis in chronic liver injury, specifically the development of hepatic fibrosis.

Methods: The model of liver fibrosis used was bile duct ligation (BDL). 10 week old wild-type (WT) C57BL/6 mice and mice with a genetic deletion of RAGE (RAGE KOs) were randomised to either BDL or sham surgery. The impact of RAGE deletion on the evolution of fibrosis was studied by sacrificing the animals at 4 time points post surgery – 3, 7, 14 and 21 days. Liver biochemistry was assessed at the time of sacrifice; assessment of liver fibrosis was performed by hydroxyproline assay and picro sirius red staining. Real-time quantitative PCR was used to determine expression of genes associated with fibrosis and RAGE.

Results: KO mice post BDL had significantly less cholestasis (assessed though serum bilirubin concentration) at days 3, 7 and 14 compared with WT animals ($P<0.05$). At day 14, the KO animals had significantly less fibrosis, with reduced hydroxyproline content and picrosirius red staining ($P<0.05$). These findings were substantiated by a reduction in Collagen I gene expression at this time point ($P<0.05$). At day 3, the KO animals had reduced expression of pro-inflammatory genes, including IL-6 and MCP-1 ($P<0.05$).

Conclusions: These results suggest that RAGE contributes to the development of hepatic fibrosis, perhaps in part by activation of inflammatory processes early post injury. Strategies to interrupt the AGE-RAGE axis may offer therapeutic benefits for preventing the development of hepatic fibrosis.

143

ROLE OF P21-ACTIVATED KINASE 1 IN SURVIVAL OF COLORECTAL CANCER CELLS

H. Liu, N. Huynh, G. Baldwin, H. He

Department of Surgery, Austin and Northern Health, Heidelberg, VIC, Australia

Background and Aim: P21-activated kinase 1 (PAK1) functions as a key node in various signalling pathways leading to cell survival, migration and growth. PAK1 is overexpressed and activated in several human tumors including colorectal cancer (CRC), which is the second most common cause of cancer death. PAK1 is also required for vascular endothelial growth factor (VEGF) expression and consequently for angiogenesis, which in turn promotes tumor growth and metastasis. VEGF is a downstream target gene of hypoxia-inducible factor 1α (HIF-1α) which is involved in cellular adaptation to hypoxia and important for cell survival. The aim of this study was to investigate the importance of PAK1 in CRC cell survival.

Methods: PAK1 knock-down (KD) clones of the human CRC cell line DLD1 were obtained by stable transfection with shRNA targeting PAK1 . Hypoxia was mimicked by treatment with $CoCl_2$ and cell survival was measured by [^{3}H]-Thymidine incorporation. Protein expression was determined by Western Blot and VEGF production was measured by ELISA.

Results: After treatment with 150 m M $CoCl_2$, cell survival was significantly reduced by 40% in PAK1 KD cells ($P<0.05$). The expression of HIF-1α and the production of VEGF were both significant lower in PAK1 KD cells compared to control cells. Conclusion: This study demonstrates that PAK1 is required for cell survival and VEGF production of CRC cells. PAK1 may regulate these cellular processes through a HIF-1α - dependent pathway.

144

ANGIOTENSIN-(1-7) IS A MESENTERIC VASODILATOR IN EXPERIMENTAL CIRRHOSIS

J. A. Grace[1,2], Z. Jia[1], C. B. Herath[1], L. M. Burrell[1], P. W. Angus[1,2]

[1]Department of Medicine, University of Melbourne, Heidelberg Heights, VIC, Australia
[2]Department of Gastroenterology, Austin Health, Heidelberg, VIC, Australia

Background: Splanchnic vasodilatation associated with hyporesponsiveness to endogenous vasoconstrictors plays a central role in the pathogenesis of portal hypertension. Cirrhosis causes upregulation of hepatic angiotensin converting enzyme 2 (ACE-2), increased circulating levels of its peptide product, the vasodilator angiotensin-(1-7) (Ang1-7), and upregulation of hepatic Mas receptor, the Ang1-7 receptor (MasR). This 'alternate arm' of the renin-angiotensin system (RAS) is thought to oppose the profibrotic and vasoconstrictive effects of angiotensin II but whether it contributes to mesenteric vasodilatation in cirrhosis is unknown.

Aim: To determine mesenteric vascular expression of the RAS and the effects of Ang1-7 on mesenteric vasculature tone in healthy rats and those with cirrhosis and portal hypertension induced by bile duct ligation.

Methods: Dose response curves to methoxamine were performed in isolated perfused mesenteric arterial beds, with and without preincubation with Ang1-7, and in the presence of the nitric oxide synthase inhibitor, L-NAME, or A779, a MasR antagonist. Arterial beds were harvested and real time QPCR performed to determine gene expression of ACE, ACE2, angiotensin II type I receptor and MasR.

Results: Perfusion with Ang1-7 significantly blunted the vasoconstrictor response to methoxamine in cirrhotic animals, but had no effect in healthy controls. Both L-NAME and A779 blocked the effect of Ang1-7 in cirrhotic animals. Mesenteric arterial ACE2 was upregulated 5 fold, providing a potential source for increased local Ang1-7 production.

Conclusions: In cirrhotic mesenteric vessels ACE2 expression is increased and its peptide product, Ang1-7, markedly impairs the vasoconstrictor response to methoxamine, via activation of MasR and release of nitric oxide. These findings support a role for ACE2, Ang1-7 and MasR in the pathogenesis of mesenteric vasodilatation in cirrhosis.

145

BIOLOGICAL ACTIVITIES OF PROGASTRIN PEPTIDES

K. M. Marshall, M. Yim, O. Patel, G. Bramante, G. S. Baldwin, A. Shulkes

Surgery, Austin Health, University of Melbourne, Heidelberg, VIC, Australia

Introduction: Progastrin (PG) is processed into a number of biologically active peptides. These peptides are produced by a variety of gastrointestinal cancers and may accelerate cancer development because of their effects on cell growth, migration and apoptosis. However it is not clear which of the various post-translationally processed PG molecules are biologically relevant . Our group has previously shown that the C-terminal flanking peptide (CTFP) is a major stored and circulating gastrin form in sheep and humans and is biologically active. Here we further explore the biological profile of CTFP and compare it to another non-amidated form of gastrin, glycine-extended gastrin (Ggly).

Aims: To compare the biological activities of two non-amidated products of PG: CTFP and Ggly.

Methods: Ggly and CTFP were compared using gastric (AGS) and colonic (DLD-1) cell lines in a series of *in vitro* assays including cell survival (MTT), proliferation (cell division), migration (transwell) and induction of signal transduction pathways. We also studied the effects of Ggly and CTFP on colonic proliferation (immunohistochemistry) in gastrin-deficient mice.

Results: CTFP and Ggly stimulated significant increases in cell survival, proliferation and migration *in vitro*. CTFP and Ggly activated procarcinogenic pathways by stimulating phosphorylation of Akt and beta-catenin/Tcf-4 transcription. Ggly and CTFP also significantly increased colonic proliferation *in vivo*.

Discussion: CTFP shares many biological activities with Ggly but is expressed at higher levels. Thus CTFP should be considered as a relevant form of PG in terms of its procarcinogenic potential.

146

INVESTIGATING THE RENIN-ANGIOTENSIN SYSTEM IN THE DEVELOPMENT OF NON-ALCOHOLIC STEATOHEPATITIS

K. Y. Mak[1], J. A. Grace[1,2], C. B. Herath[1], Z. Jia[1], P. W. Angus[1,2]

[1]Department of Medicine, The University of Melbourne, Melbourne, VIC, Australia
[2]Department of Gastroenterology, Austin Health, Heidelberg, VIC, Australia

Background: Non-alcoholic steatohepatitis (NASH) is an increasingly important cause of hepatic fibrosis. Recent studies have shown that the renin-angiotensin system (RAS) plays a key role in the pathogenesis of liver fibrosis and have stimulated major interest in the potential therapeutic role of RAS blockade in liver disease.

The aim of this study was to use methionine and choline deficient (MCD) diet fed rats to characterise and compare the components of both classical and alternative arms of the RAS in NASH.

Methods: Sprague-Dawley rats were fed MCD diet for 12 weeks with animals sacrificed at two time points (8 and 12 weeks). Hepatic fibrosis was evaluated by histological assessments (hematoxylin and eosin staining (H&E) and picrosirius red staining). Quantitative real time PCR (qRT-PCR) was also performed to characterise the hepatic gene expressions of the RAS.

Results: Picrosirius red staining of the liver sections demonstrated that MCD rats developed hepatic steatosis by week 8 compared with rats fed on normal diet. H&E staining also showed infiltration of mixed inflammatory cells in the hepatic parenchyma. Pericellular fibrosis was observed in liver at week 8, progressing to portal-portal and centro-portal bridging fibrosis by week 12. In addition, qRT-PCR studies showed markedly elevation of the RAS components at week 12, including angiotensin converting enzyme (ACE) 2 ($p<0.001$), angiotensin II type 1 receptor (AT1) receptor ($p<0.001$), ACE ($p<0.01$) and profibrotic marker alpha-smooth muscle actin (α-SMA) ($p<0.01$) compared with control rats.

Conclusion: MCD rats displayed hepatic steatosis and inflammation that resulted in pericellular and bridging fibrosis. This sequence of events is comparable to that which occurs in human NASH. Moreover, both the classical and alternate RAS were significantly upregulated at week 12. These findings suggest that the RAS is critically important in the development of NASH and is a potential therapeutic target.

(1) Paizis G, Cooper ME, Schembri JM, Tikellis C, Burrell LM, Angus PW. Up-regulation of components of the renin-angiotensin system in the bile duct-ligated rat liver. Gastroenterology. 2002;123:1667-1676
(2) Paizis G, Gilbert RE, Cooper ME, Murthi P, Schembri JM, Wu LL, Rumble JR, Kelly DJ, Tikellis C, Cox A, Smallwood RA, Angus PW. Effect of angiotensin ii type 1 receptor blockade on experimental hepatic
(3) Osterreicher CH, Taura K, De Minicis S, Seki E, Penz-Osterreicher M, Kodama Y, Kluwe J, Schuster M, Oudit GY, Penninger JM, Brenner DA. Angiotensin-converting-enzyme 2 inhibits liver fibrosis in mice.

147

TOLL-LIKE RECEPTOR 7/8-INDUCED TYPE 1 INTERFERON RESPONSES ARE IMPAIRED IN HEPATITIS C PATIENTS WITH RAPID FIBROSIS POST LIVER TRANSPLANT

J. Howell[1,2], R. Sawhney[1,2], N. Skinner[2], A. Testro[1], P. Angus[1], P. Gow[1], K. Visvanathan[2]

[1]Liver Transplant Unit, Austin Health, Heidelberg, Australia

[2]Medicine, Monash Medical Centre, Clayton, VIC, Australia

Background: Toll like receptors (TLRs) are critical to innate immune antiviral responses and hepatitis C (HCV) alters TLR function to evade immune clearance. The role of TLRs in HCV recurrence post liver transplantation has not been characterised and whether rapid progression of HCV fibrosis is related to altered TLR responses is unknown.

Methods: Peripheral blood mononuclear cells (PBMCs) from 55 HCV post liver transplant patients and 30 non-HCV post transplant matched controls were stimulated with TLR subclass-specific ligands LPS (TLR4), P3C (TLR2), PIC (TLR3), R848 (TLR7/8), CpGA (monocyte TLR9) and CpGB (plasmacytoid dendritic cell TLR9) for 24 hrs. Production of interleukin 6 (IL-6), tumour necrosis factor (TNF) and interferon alpha (IFNα) was then measured by analyzing cell culture supernatants using ELISA and compared between groups using Mann Whitney test. Rate of fibrosis progression was calculated using protocol liver biopsies graded by Metavir scoring (F0-4; R= fibrosis stage/ year post transplant). Rapid fibrosis was defined as >0.5 units/yr, dichotomized about the median observed rate (0.4).

Results: 11 of 55 HCV post liver transplant patients had rapid fibrosis progression (20%). PBMCs from HCV subjects produced greater amounts of IL-6 in response to TLR2 (p=0.009), TLR3 (p=0.02) and monocyte TLR9 stimulation (p=0.04) compared with controls. HCV subjects also produced greater levels of TNF in response to TLR3 (p=0.009) and TLR7/8 stimulation (p=0.02). Baseline levels of both IL-6 (p=0.014) and TNF (p=0.05, NS) were also higher in HCV subjects. IFNα production was not different between HCV subjects and controls. In contrast, PBMCs from HCV rapid-fibrosers produced less IFNα in response to TLR7/8 stimulation compared with HCV slow-fibrosers (p=0.014) and controls (p=0.02).

Conclusion: Our data demonstrate that post transplant TLR function is intact in HCV patients despite immunosuppression. However, TLR 7/8-induced IFNα production is reduced in HCV rapid-fibrosers, suggesting a novel immune mechanism for aggressive HCV recurrence post transplant.

148

DONOR AND RECIPIENT AGE AND HLA-A MISMATCH: MODIFIABLE RISK FACTORS FOR RAPID HCV RECURRENCE POST TRANSPLANT

J. Howell, R. Sawhney, R. Jones, M. Fink, B. Wang, P. Angus, P. Gow

Liver Transplant Unit, Austin Health, Heidelberg, Australia

Background: Hepatitis C (HCV) related cirrhosis is the commonest indication for liver transplantation worldwide. Recurrence post liver transplant is universal, with a subgroup having an aggressive course leading to rapid liver fibrosis and graft loss. This study examines risk factors for rapid progression of liver fibrosis in HCV infection post liver transplantation.

Methods: Retrospective data analysis was conducted on 103 adult patients with HCV who underwent liver transplantation at a single centre. Rate of fibrosis progression was calculated using protocol liver biopsies graded by Metavir scoring (F0-4; R= fibrosis stage/ year post transplant). Rapid fibrosis was defined as >0.5 units/yr, dichotomized about the median observed rate. Variables studied included demographic, cofactor, clinical, operative and post transplant event data and were analyzed using chi square, T test, Mann Whitney U test and Spearman correlation as appropriate.

Results: 15 of 103 patients developed post transplant cirrhosis (16%) and 25 of 103 (24%) had rapid fibrosis progression. Overall median fibrosis rate was 0.4 fibrosis units/yr. Mean fibrosis rate was 1.13 fibrosis units/ yr in rapid fibrosers, compared with 0.23 fibrosis units/ yr in slow fibrosers. Risk factors associated with rapid fibrosis progression were: HCV viral load >107 IU/ml within the first 12mths post transplant (p=0.04, OR 3.38), HCV viral load greater than 108 IU/ml post transplant (p=0.01, OR 13.0), recipient age at transplant (p=0.05), donor age (p=0.03) and HLA-A mismatch (p=0.04, OR 3.6). In addition, correlation statistics revealed a positive correlation between fibrosis rate and average HCV viral load within the first 12mths post transplant (p=0.02, r=0.47) and recipient age at transplant (p=0.02, r=0.23).

Conclusion: Our data supports previous work to suggest use of older donor livers should be minimized in HCV positive recipients. They also suggest that HLA-A matching and early viral load testing may be another strategy for reducing the risk of rapid post transplant disease progression.

149

EARLY-ONSET VERSUS LATE-ONSET NON-ANASTOMOTIC BILIARY STRICTURES: RISK FACTORS REFLECT DIFFERENT PATHOGENESIS

J. Howell, P. Gow, P. Angus, R. Jones, B. Wang, M. Fink

Liver Transplant Unit, Austin Health, Heidelberg, VIC, Australia

Background: Non-Anastomotic biliary strictures (NAS) cause significant morbidity and mortality post orthotopic liver transplantation. Early-onset and late-onset NAS may be separate entities with differing pathogenesis. Risk factors and pathogenesis of early-onset versus late-onset NAS have not been well established. This study examines the role of operative, demographic and immunological factors in the development of early-onset and late-onset NAS.

Methods: Retrospective analysis was conducted on 398 adult patients receiving orthotopic liver transplants performed between 1st Jan 1990 to 31st May 2008 at a single institution. Patient data was obtained from computerised and historical records. Diagnosis of NAS required visualisation on at least two radiological studies (MRCP, T tube cholangiogram, percutaneous transhepatic cholangiogram (PTC) or ERCP). Early NAS were defined as developing 1 year post transplant (minimum 10 years follow up).

Results: Complete data was available on 96 patients with NAS. Of these, 54 were early-onset (56%) and 42 late-onset (44%). Overall risk factors for NAS (univariate) were PSC (p=0.001), Roux loop anastomosis (p=0.001), Tacrolimus (p=0.02) and CMV IgG donor-recipient mismatch (p=0.029). Hepatitis C (p=0.018) and CMV IgG positive recipient (p=0.031) were protective. However, multivariate analysis revealed PSC was the only risk factor for NAS (p=0.001, OR 2.788). Early-onset NAS were associated with HTK perfusion solution (p<0.0001), MELD score (p=0.006), Tacrolimus (p=0.04), donor requiring CPR (p=0.046), age at transplant (p=0.033) and donor age (0.029). In contrast, late-onset strictures were associated with Tacrolimus (p=0.0001), PSC (p=0.0008) and Roux loop anastomosis (p=0.07).

Conclusion: PSC is an important risk factor for NAS. Early-onset strictures are associated with transplantation factors including donor age, age at transplant, HTK perfusion solution, MELD and grafts from donors requiring CPR. Late-onset NAS are associated with choice of immunosuppression and PSC/ Roux loop, suggesting an immunological pathogenesis.

150

ROLE OF NEUTROPHIL CD64 AS AN EARLY DIAGNOSTIC MARKER OF SEPSIS

R. Krishnan[1], S. Valentine[2], R. Dauer[2], C. Smith[2]

[1]Northern Pathology, Austin Health, Epping, VIC, Australia
[2]Haematology, Austin Health, Heidelberg, VIC, Australia

Introduction: Neutrophil CD64 also known as the Fc Gamma receptor I (FcgR1), is a transmembrane glycoprotein with high affinity for IgG (immunoglobulin G). It is constitutively expressed on neutrophils at very low levels and up regulated during a physiological response to bacterial components; complement products and pro-inflammatory cytokines like G-CSF (granulocyte –colony stimulating factor), etc. The significant elevation of this marker in response to pro-inflammatory processes makes it an attractive target for diagnosis of sepsis. Sepsis is one of the most common causes of mortality in hospitals. Vulnerable populations such as neonates, elderly patients etc. are most affected. Currently the diagnosis of sepsis is based on clinical features and combined results of FBE (full blood examination), film morphology, CRP (C- reactive protein), ESR (erythrocyte sedimentation rate) and blood gases. The confirmatory diagnosis of sepsis lies in positive blood culture results. These may not always be reliable and have high rates of false negatives and false positives.

Method: To aide quicker diagnosis of sepsis, we tested the usefulness of neutrophil CD64 index using the Leuko64 kit, provided by Trillium Diagnostics. Tests were performed on whole EDTA (ethylene diamine tetra acetic acid) blood using BD-FC500 flow cytometer for analysis.

Results: Our results have shown a positive outcome with a sensitivity of 93.7% and specificity of 64.8%. The effect of pro-inflammatory cytokine G-CSF was also observed in 5 patients who were administered G-CSF for peripheral stem cell mobilization. These patients showed high nCD64 index as expected.

Conclusion: Neutrophil CD64 analysis has shown promise as an early diagnostic indicator of sepsis. The low specificity of the test is a reflection of the crude design of the study. Further refinement of the study design is required after acquiring Ethics Committee approval, which will lead to incorporation of the test as part of the septic screen.

151

ICOS-IG PROLONGS CELLULAR XENOGRAFT SURVIVAL AND IS ASSOCIATED WITH CD4+CD25+FOXP3+ T REGULATORY CELLS AND IL10 EXPRESSION

R. Hodgson[1], A. Ziolkowski[2], D. Christiansen[1], E. Mouhtouris[1], C. Simeonovic[2], F. Ierino[1], M. Sandrin[1]

[1]Department of Surgery, University of Melbourne, Heidelberg, VIC, Australia

[2]John Curtin School for Medical Research, Australian National University, Canberra, ACT, Australia

Aim: To determine effect of local expression of ICOS-Ig on xenograft survival.

Methods: A porcine cell line (PIEC) secreting ICOS-Ig was generated and examined for efficacy to inhibit allogeneic and xenogeneic proliferation responses in vitro and to prolong xenograft survival in vivo. Graft infiltrating cells were characterised for the expression of lymphocyte markers including Foxp3. Intragraft cytokine levels were assessed by Q-PCR.

Results: Soluble ICOS-Ig markedly inhibited both allogeneic and xenogeneic T cell proliferation in vitro. The median graft survival time of PIEC-ICOS-Ig grafts was significantly prolonged compared to wild-type (34 vs 12 days, $p<0.05$), with 20% of grafts surviving >170 days. PIEC-ICOS-Ig graft showed an increase in Foxp3+ perigraft infiltrating cells compared to wild-type grafts at day 7 (5.2% vs 1.0%, $p<0.001$) and day 14 (4.7 vs 0.7%, $p<0.01$). Furthermore, the majority of Foxp3+ cells were CD4+CD25+ T cells. PIEC-ICOS-Ig grafts also significantly prolonged the median survival of wild-type grafts at a distal site (from 12 to 28 days, $p<0.05$) but not allografts, indicating specificity. This was associated with a similar increase in Foxp3+ cell numbers in both grafts (day 7: 5.3% vs 4.0%, NS; day 14: 2.9% vs 3.5%, NS). An increase in the level of mRNA for IL-10 but not TGFß or IFNγ was seen in the PIEC-ICOS-Ig grafts.

Conclusions: This study demonstrates prolonged xenograft survival by local expression of ICOS-Ig. We propose that the accumulation of CD4+CD25+Foxp3+ cells and secretion of IL-10 at the periphery of the graft is responsible for this observation.

152

ADCC, CDC AND BINDING ACTIVITIES OF ANTI-LE^Y ANTIBODY HU3S193 WITH MUTATED FC

Z. Liu[1], I. J.G. Burvenich[1], P. A. Ramsland[2], D. Makris[1], L. Allan[1], A. Perani[1], B. Gloria[1], R. Murphy[1], F. E. Smyth[1], A. M. Scott[1]

[1]Tumour Targeting Lab, Ludwig Institute for Cancer Research, Austin Branch, Heidelberg, VIC, Australia

[2]Structural Immunology Laboratory, Macfarlane Burnet Institute for Medical Research, Heidelberg, VIC, Australia

The Fc domain of an antibody plays a key role in antibody immune function including antibody dependant cellular cytotoxicity (ADCC) via Fc receptors on NK cells, and complement dependant cytotoxicity (CDC) through binding and activating complement. The Fc domain also influences the serum half-life of administered therapeutic antibodies through differential binding affinity to the Fc neonatal receptor (FcRn). Some key FcRn-Fc binding sites have been identified through crystallization studies. Specifically, amino acids I253, H310 and H435 affect FcRn binding and influence antibody half-life in the circulation. Residues of D270, K322, P329 and P331 have been suggested as binding sites for complement C1q and S298 and D265 have been explored in FcγR interactions (Idusogie et al., 2000; Shields et al., 2001).

However, the residues involved in both ADCC and CDC activity are not clear yet. The humanized monoclonal antibody 3S193 (hu3S193) specifically binds the Lewis-y (Le^y) antigen and has shown strong ADCC and CDC activities *in vitro* and a long serum half-life in Phase I first-in-man trials (Scott et al., 2007).

In this study we have generated 13 different mutants of hu3S193 at the area FcRn, FcγR or C1q binding sites.

The binding activities of these mutants to Le^y antigen, FcγR and FcRn were assessed by ELISA, BIAcore and FACS. ADCC and CDC activities were examined by ^{51}Chromium-release assays.

This data shows each mutant has different binding properties and immune functions.

These results can be used to select the antibodies with the preferred characteristics suited to different clinical purposes.

(1) Idusogie EE et al. J Immunol. 2000; 164(8):4178-84.
(2) Shields RL et al. J Biol Chem. 2001; 276(9):6591-604.
(3) Scott AM et al. Clin Cancer Res 2007; 13(11):3286-92

IMMUNO-MONITORING OF T REGULATORY CELLS IN PERIPHERAL BLOOD OF RENAL AND LIVER TRANSPLANT RECIPIENTS

W. Lin[1], D. Christiansen[1], L. Fu[5], M. A. Roberts[2,3], R. M. Jones[4], M. S. Sandrin[1], F. L. Ierino[2,3]

[1]Department of Surgery (AH/NH), The University of Melbourne, Heidelberg, VIC, Australia
[2]Department of Medicine, The University of Melbourne, Heidelberg, VIC, Australia
[3]Department of Nephrology, Austin Health, Heidelberg, VIC, Australia
[4]Austin Health, Liver Transplant Unit, Heidelberg, VIC, Australia
[5]Australian National University, School of Finance, Actuarial Studies and Applied Statistics, Canberra, ACT, Australia

Background: Regulatory T cells (Tregs) have a crucial role in many transplant models in facilitating acceptance of transplanted organs, inducing tolerance and influencing rejection and malignancy development. However, there is limited data on the role of these cells in clinical transplantation.

Aim and Method: To characterize putative Tregs by determining the expression of characteristic phenotypic markers (CD4, CD25, Foxp3, CD62L) by Flow Cytometry. The peripheral blood of 95 renal transplant recipients and 109 liver transplant recipients was analysed at different time points post-transplant and correlated with clinical parameters.

Results: In renal transplant recipients the level of $CD4^+CD25^+Foxp3^+$ remained stable over time post-transplant, however this was significantly lower compared to both dialysis patients with End Stage Renal Failure and normal controls ($p<0.001$ & $p<0.01$ respectively). Furthermore, significantly higher levels of $CD4^+CD25^+$ T cells were observed in patients with post-transplant malignancy compared to those without ($p=0.044$). In addition, the level of $CD4^+CD25^+Foxp3^+CD62L^+$ subset positively correlated with the patient's age at the time of analysis ($p=0.037$). In contrast to the stable level of $CD4^+CD25^+Foxp3^+$ cells seen in renal transplant recipients, analysis of the first 46 liver transplant recipients showed a significant negative correlation with time post-transplant.

Conclusion: The higher relative number of $CD4^+CD25^+$ cells may be a contributing factor in development of malignancy in transplant recipients. $CD4^+CD25^+Foxp3^+$ cells are regulated differently in renal and liver transplants. The correlation of Treg levels with clinical outcomes may lead to new clinical diagnostic tools.

AN UNUSUAL FINDING IN RENAL TRANSPLANT PATIENT.

E. Edelman[1], A. Mahony[2]

[1]Microbiology-Austin Pathology, Austin Health, Heidelberg, VIC, Australia
[2]Infectious Diseases, Austin Health, Heidelberg, VIC, Australia

We are presenting a case of a post renal transplant patient on combination of immuno-suppressive agents and co-trimoxazole prophylaxis, who developed symptoms of intermittent diarrhoea.

A faecal specimen was sent to the laboratory for routine examination. Long, oval oocysts measuring 30 um x 12.5 um were detected in direct wet preparation by light and phase-contrast microscopy suggestive of *Isospora belli*. The same organism was also seen in Ethyl-acetate concentration. Autofluorescense of oocyst was detected. Acid-fastness of *I.belli* was proved by Modified Kinyoun's and Modified Iron-Haematoxylin stains. A second specimen was received approximately a week later with the same microscopy result. Treatment with ciprofloxacin(14 day course) was started after the second finding. A third specimen received 2 weeks after commencement of treatment was negative for *I.belli*.

Diarrhoeal symptoms reoccurred after one week of cessation of treatment, and faeces specimen was sent to laboratory again. Very occasional oocysts were detected on this occasion.

Isospora belli is an intestinal coccidian infecting humans. Oocysts are found in faeces of humans, and diagnosis is based on demonstrating oocysts in stool specimens. Although it has been found in various parts of the world, certain tropical areas in Western Hemisphere appeared to have some well defined location of endemic infections. Most cases of infection in temperate areas involve foreign travel and homosexual contact.

I.belli can cause serious and sometimes fatal disease in immuno-competent humans. The disease is often chronic and recurrences are common. Clinical disease in *I.belli* infections is usually more severe in immunocompromised patients.

Clinical management of *I.belli* in immunocompromised patients is very difficult and some clinical details and diagnostic features will be discussed.

(1) Koru,O.,et al.Case Report:Isospora belli infection in renal transplant patient.Turkish Parasitology Journal,31(2):98-100.2007.
(2) Butler,T.,De Boer, W.G.R.M. Isospora belli infection in Australia.Pathology 1981:13:593-595.
(3) Garsia , L.S.,and Brucken ,D.A.1997.Diagnostic Medical Parasitology,3rd ed.,p.593-607 or p.937.ASM Press,Washington D.C.

DYSREGULATION OF INTRACELLULAR SIGNAL TRANSDUCTION IN HEPATITIS C AND ITS IMPLICATION IN HCV FIBROSIS

K. El Khobar[1,2,3], L. Earnest-Silveira[1,2,3], D. Johnson[1,2,3], R. Chin[1,2,3], C. Bock[4], P. Angus[1,5], J. Torresi[1,2,3]

[1]Department of Medicine, Austin Hospital, University of Melbourne, Heidelberg, VIC, Australia
[2]Department of Infectious Diseases, Austin Hospital, University of Melbourne, Heidelberg, VIC, Australia
[3]Austin Center for Infection Research, Heidelberg, VIC, Australia
[5]Department of Hepatology and Gastroenterology, Austin Hospital, University of Melbourne, Heidelberg, VIC, Australia

Background & Aims: Hepatitis C viral proteins have been shown to dysregulate critical cellular signalling pathways to promote liver fibrosis and oncogenesis. NS5A protein potentially blocks STAT1 and STAT3 activation and interacts with the p85 subunit of PI3K of the PI3K-AKT pathway. The core and E2 envelope proteins reportedly activate MAPK/ERK signalling. Meanwhile, increase STAT3 activation has been associated with up-regulation of TGFß expression, promotion of liver inflammation. In this study, we aimed to establish an in vitro model to determine the effects of HCV core-E1-E2 and NS3-NS5b protein expresssion on JAK/STAT signalling in hepatocytes and their contribution to liver fibrosis.

Methods: Recombinant adenoviruses expressing HCV (genotype 1a) structural (rAdHCV-CoreE1E2) and non-structural proteins (rAdHCV-NS3-5b) and control adeno-GFP were used to infect Huh7 cells. Western immunoblots were performed to determine the expression of total and phosphorylated STAT3, STAT1, AKT and ERK, and total SOCS3 and SOCS1 at 24, 48, 72, 96, and 120h pi.

Results and Conclusions: HCV viral proteins produced an initial up-regulation of pSTAT3 up to 72h pi followed by a decrease, while there were no changes in pSTAT1 expression. HCV proteins increased SOCS3 and SOCS1 expression, also the pAKT and pERK expression. Increased pSTAT3 expression correlates with increase cell survival, while SOCS3 expression as STAT3 negative regulator, matches with pSTAT3 expression. Meanwhile, increased ERK and AKT expression also correlates with cell survival and anti-apoptotic promotion. By modulating the JAK/STAT, ERK and AKT pathways, hepatitis C virus is able to promote persistent infection, cell survival and contribute to liver pathogenesis.

NOVEL ADJUVANTS TO INCREASE HUMORAL NEUTRALISING ANTIBODY AND CELLULAR IMMUNE RESPONSES TO RECOMBINANT MAMMALIAN CELL DERIVED HEPATITIS C VIRUS-LIKE-PARTICLES

D. F. Johnson[1,2,3], B. Chua[4], R. Chin[1,3], L. Earnest Silveira[1,3], T. Bock[5], W. Zeng[4], D. Jackson[4], J. Torresi[1,2,3]

[1]Department of Medicine, Austin Health, Melbourne, VIC, Australia
[2]Department of Infectious Diseases, Austin Health, Melbourne, VIC, Australia
[3]Austin Centre for Infection Research, Austin Health, Melbourne, VIC, Australia
[4]Department of Microbiology and Immunology, University of Melbourne, Melbourne, VIC, Australia
[5]Department of Virology, Robert Koch Institute, Berlin, Germany

Clearance of Hepatitis C virus (HCV) requires strong CD4+, CD8+ and neutralising antibody (NAb) responses. Virus like particles (VLPs) resemble mature virus inducing protective humoral and cellular responses against HCV.

HCV VLPs were isolated from Huh7 cells infected with adenoviruses expressing HCV genotype 1a core-E1-E2 proteins. HCV VLPs in adjuvants A or B stimulated the maturation of dendritic cells.

Mice immunised with HCV VLPs alone, alum or two novel adjuvants (A & B) were assessed for; (1) humoral responses against VLPs and a recombinant HCV E2 protein, (2) production of mouse Ab secreting cells in splenocytes (B cell ELISPOT assays) (3) Neutralising antibody (NAb) in a Huh 7 cell entry assay (4) core specific CD8 T cell responses (IFN γ ELISPOT assay).

High ab titres (VLP/PBS, 3.5Log10; VLP/Alum, 4.1 Log10; VLP/A, 4.4 Log10 and VLP/B, 4.8Log10) were produced in immunised mice and strong B cell responses were detected by ELISPOT (VLP/PBS, 22; VLP/Alum, 72; VLP/A, 307; and VLP/B, 413 Antibody Secreting Cells /million splenocytes).

Mouse anti-HCV VLP serum neutralized VLP entry into Huh7 cells (VLP/A + VLP/B neutralised binding by 80% and 84% respectively).

MHC Class I transgenic mice immunized with HCV VLPs produced strong T cell HCV Core specific IFN γ responses.

Mammalian cell derived HCV VLPs stimulate DC maturation, produce strong humoral and cellular immune responses and neutralising antibodies. The immunogenicity of the HCV VLPs was significantly improved by the addition of novel adjuvants A and B. HCV VLPs are a viable vaccine strategy for HCV.

VANCOMYCIN MINIMUM INHIBITORY CONCENTRATION (MIC) PREDICTS OUTCOME IN *STAPHYLOCOCCUS AUREUS* BACTERAEMIA INDEPENDENT OF METHICILLIN SUSCEPTIBILITY AND ANTIMICROBIAL THERAPY.

N. E. Holmes[1], J. D. Turnidge[4], W. J. Munckhof[5], O. Robinson[6], T. M. Korman[7], M. V.N. O'Sullivan[8], T. L. Anderson[9], S. A. Roberts[10], W. Gao[1], K. J. Christiansen[6], G. W. Coombs[9], P. D.R. Johnson[1,2,3], B. P. Howden[1,3]

[1]Infectious Diseases, Austin Health, Heidelberg, VIC, Australia
[2]Microbiology, Monash University, Clayton, VIC, Australia
[3]Microbiology and Immunology, University of Melbourne, Parkville, VIC, Australia
[4]Laboratory Medicine, Women's and Children's Hospital, Adelaide, SA, Australia
[5]Infectious Diseases and Microbiology, Princess Alexandra Hospital, Brisbane, QLD, Australia
[6]Infectious Diseases and Microbiology, Royal Perth Hospital, Perth, WA, Australia
[7]Infectious Diseases, Southern Health, Clayton, VIC, Australia
[8]Infectious Diseases and Microbiology, Westmead Hospital, Sydney, NSW, Australia
[9]Infectious Diseases and Microbiology, Royal Hobart Hospital, Hobart, TAS, Australia
[10]Microbiology, Auckland District Health Board, Auckland, New Zealand

Background. The management of patients with *Staphylococcus aureus* bacteraemia (SAB) remains a challenge, especially when the isolate is methicillin-resistant (MRSA) because of concerns about reduced efficacy of vancomycin compared with anti-staphylococcal penicillins.

Methods. We examined the relationship between antibiotic treatment, 30-day mortality and microbiological parameters of blood culture isolates in a cohort of patients with SAB. Basic clinical and demographic data were recorded and initial blood culture isolates underwent detailed susceptibility testing.

Results. 532 patients from 8 hospitals in Australia and New Zealand were included. All those with MRSA were treated with vancomycin, but patients with MSSA received either flucloxacillin (81.1%) or vancomycin (18.9%) at the discretion of treating clinicians. Thirty-day mortality was increased in patients with MRSA and in those who received vancomycin. However even in patients with MSSA treated with flucloxacillin, mortality was significantly higher if the vancomycin Etest MIC of their isolate was > 1.5 µg/mL, compared with those with lower MIC isolates (27.2% versus 12.0%, $p<0.001$). In a multivariate model age, place of onset of bacteraemia and vancomycin MIC remained independent predictors, but methicillin resistance and antibiotic choice were no longer significant.

Conclusions. A clear association between vancomycin MIC and 30-day mortality exists in patients with SAB, irrespective of methicillin susceptibility and antibiotic treatment received. This suggests that factors other than reduced vancomycin efficacy may be contributing to poor outcome.

158

A SUSTAINED HOSPITAL OUTBREAK OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM* BACTERAEMIA DUE TO THE EMERGENCE OF VANB *E. FAECIUM* SEQUENCE TYPE 203

P. D.R. Johnson[1], S. A. Ballard[3], E. A. Grabsch[3], T. P. Stinear[2], T. Seemann[2], H. Young[3], L. Grayson[1], B. P. Howden[1]

[1]Infectious Diseases, Austin Health, Australia
[2]Microbiology and Immunology, Melbourne University, VIC, Australia
[3]Microbiology, Austin Health, VIC, Australia

Background : There has been a significant rise in vancomycin-resistant *Enterococcus faecium* (VREfm) bacteremia at our health service since 2007, despite improved infection control and reduced rates of MRSA infection. We wondered whether a new more virulent strain of VRE could explain this paradox.

Methods : *Enterococcus faecium* bacteremia (including VRE) over a 12-year period (1998-2009) was analysed using multi-locus sequence typing (MLST), antibiotic and anti-septic susceptibility profiles and optical mapping and whole genome sequencing of historical and recent isolates.

Results : For 10 years, the bacteremia rate due to *vanB* VREfm remained stable and sequence type (ST) 17 was predominant. In 2005 ST203 vancomycin-susceptible *E. faecium* first appeared at our institution, and from March 2007, coinciding with the appearance of a *vanB* VREfm ST203, VRE bacteremia has increased exponentially. Although we found no difference in antiseptic susceptibility or presence of genes encoding putative virulence determinants (espEfm, hylEfm, and fms genes), comparative genomics revealed almost 500 kb of unique sequence between ST17 and ST203, suggesting that other genomic factors are responsible for the success of ST203 *E. faecium*.

Conclusions : The application of MLST has uncovered the emergence of an epidemic clone of *E. faecium* ST203 that acquired the *vanB* locus and is largely responsible for the current outbreak of VRE bacteremia. The reason for the apparent increase in virulence of ST203 compared with our older endemic ST17 strain, has yet to be determined.

ANTIGEN PROCESSING & PRESENTATION OF IMMUNODOMINANT EPITOPES FROM THE TUMOUR ANTIGEN NY-ESO-1; DISPARITY BETWEEN MELANOMA CELLS AND VACCINE INDUCED RESPONSES?

A. J. Knights, T. Lowen, W. Chen, J. Cebon

Austin branch, Ludwig Institute for Cancer Research Melbourne, Heidelberg, VIC, Australia

The Cancer-Testis antigen NY-ESO-1 is an attractive target for cancer vaccination based on its highly-restricted tissue expression profile and frequent immunogenicity. We have conducted several clinical trials targeting NY-ESO-1 in melanoma patients, resulting in broad immune responses to the NY-ESO-1 protein vaccine. However, robust clinical responses have not been observed. Many immunodominant and sub-dominant epitopes are identified and our published data indicate many of these are efficiently cross-presented by dendritic cells (DC). Whether the repertoire of epitopes cross-presented by DC corresponds to those efficiently presented on melanoma cells is not well characterized and disparities may arise due to differences in their use of antigen processing machinery.

In this study we have examined the recognition of early passage melanoma cell lines by cytotoxic T cells expanded from the PBMC of patients with vaccine-induced NY-ESO-1 responses, and epitope presentation by DC cross presenting the NY-ESO-1/ISCOMATRIX® vaccine. CTL clones specific for a number of the known NY-ESO-1 epitopes were employed and a panel of cell lines selected that were characterized for MHC expression, antigen expression/heterogeneity and induction of immunoproteasome components upon IFN-γ exposure.

T cell clones derived from NY-ESO-1 vaccinated patients demonstrate a moderate-to-high functional avidity and were capable of specifically killing cells pulsed exogenously with peptide. Three HLA-Cw3 restricted immunodominant and subdominant epitopes demonstrate differential presentation by melanoma cells, with the vaccine-induced immunodominant epitope 92–100 solely dependant on IFN-γ treatment, whereas recognition of the subdominant HLA-A2-restricted epitope 157–165 was variable; with both increased and decreased recognition observed upon IFN-γ treatment.

Due to the paucity in exposure of tumour cells to IFN-γ in vivo, novel strategies may be required alongside vaccines to modulate the tumour environment to favor increased levels IFN-γ or vaccine approaches modulated to promote presentation of a repertoire of epitopes better corresponding to that of the tumour cells.

SMAC MIMETICS PROMOTE CYTOTOXIC T LYMPHOCYTE-MEDIATED BYSTANDER KILLING OF MELANOMA CELLS AND ENHANCE THE EXPANSION AND FUNCTION OF ANTIGEN-SPECIFIC T CELLS

A. J. Knights, J. Fucikova, J. Cebon

Austin Branch, Ludwig Institute for Cancer Research Melbourne, Heidelberg, VIC, Australia

Inhibitor of apoptosis (IAP) proteins such as XIAP, cIAP1, and cIAP2 play critical roles in regulating apoptosis resistance and promoting survival in cancer cells. Second mitochondria-derived activator of caspases (Smac) is released from the mitochondria following apoptotic stimuli leading to the inhibition/degradation of IAPs, releasing their inhibitory effects and inducing apoptosis. Novel small molecule Smac mimetics have been developed to exploit this pathway and promote apoptosis in cancer. An alternative mechanism of action has been proposed involving activation of the NF-kappaB pathway leading to enhanced TNF-alpha production and autocrine apoptosis induction via TNF death receptors.

We tested a Smac mimetic currently in phase I trials in advanced solid tumours, LCL161 (Novartis Oncology), for its potential to synergise with cancer vaccines. Three levels of sensitivity to Smac mimetics have been described in solid tumours. 1) Direct apoptosis induction, 2) apoptosis induction only together with exogenous TNF-alpha and 3) resistance to LCL161. We confirm these findings in early passage melanoma cell lines. *In vitro* analysis with human cells was employed to assess suitability for combination with vaccination. Firstly, enhancement of T cell mediated lysis of melanoma cells, and secondly, assessments of immunomodulatory properties were addressed. We demonstrate that LCL161 does not directly enhance T cell killing of melanoma cells in antigen-specific cytotoxicity assays. However, the compound promotes bystander killing of antigen-negative neighbouring melanoma cells otherwise refractory to killing. This aspect is highly beneficial for targeting heterogeneously expressed antigens in cancer. Furthermore, we demonstrate that LCL161 has no detrimental effects on the survival or function of T lymphocytes; rather LCL161 enhances the expansion of antigen-specific cells and increases secretion of NF-kappaB controlled cytokines IL-2 and TNF-alpha.

In summary our pre-clinical data provide a rationale for the combination of Smac mimetics with therapeutic cancer vaccines targeting heterogeneously expressed tumour antigens.

161

UTILISING THE IMPROVED ACCURACY OF MONTE CARLO BASED CALCULATION ALGORITHMS TO GREATER UNDERSTAND DELIVERED DOSE IN HEAD AND NECK INTENSITY MODULATED RADIATION THERAPY (IMRT).

N. Anderson, C. Lawford, M. Rolfo, M. Wada

Austin Radiation Oncology, Heidelberg Heights, VIC, Australia

Purpose/Objective(s): Intensity modulated radiation therapy (IMRT) is a modality utilised frequently in the management of head and neck malignancies. Effective clinical outcomes correlate strongly with multiple dose parameters to both targets and organs at risk (OAR). As a consequence, dose calculation accuracy is imperative for a reliable and accountable dose delivery. TPS utilising Monte Carlo (MC) calculation algorithms provide a more realistic dose representation. The extent and significance of this discrepancy was quantified in this analysis of head and neck radiotherapy patients.

Materials/Methods: Ten patients were included in this planning study. Optimised IMRT plans were generated utilising CMS XiO (SP calculation algorithm), with a simultaneous integrated boost to GTV, PTV_1, PTV_2 and PTV_3. Each plan was recalculated utilising a MC calculation algorithm for dose comparison.

Results: As a consequence of MC calculation, dose to targets and OAR increased. GTV (2.1%, p = 0.023) and PTV_3 (3.3%, p = <0.001) presented significant deviations.

95% conformity indices (CI) discrepancy (PTV_1 (p = <0.001), PTV_2 (p= <0.001) and PTV_3 (p= <0.001)), and homogeneity indices (HI) (p = 0.004) increased significantly.

Spinal cord Dmax (2.0%, p = 0.042), left (3.0%, p= 0.005) and right parotid (6.8%, p = 0.008) mean, right parotid V30Gy (9.0%, p = 0.008) and larynx V50Gy (22.1%, p = 0.054) all presented significant dose escalation. Integral dose at V20% (of total dose), V15%, V10%, V5% and V3% was significantly higher (p = <0.001 at all levels).

Conclusion: Precision radiation therapy is dependent on multiple parameters. Calculation algorithms play an integral role in the correlation of treatment planning with ultimate delivery. While providing a greater representation of delivered dose, MC algorithms reduce perceived and actual dose discrepancy. IMRT and image guidance demands a precise dose calculation. This study has shown the discrepancy in using an alternate algorithm in head and neck radiotherapy, and how toxicity correlation may be potentially distorted as a consequence of dose representation inadequacies.

162

TKTL1- A PROMISING ANTI-TUMOUR TARGET IN MELANOMAS

P. Lo, A. Behren, A. Jayachandran, J. Browning, J. Cebon

Cancer Vaccine, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Enhanced glucose consumption is the most consistent hallmarks of cancers. Unlike normal differentiated cells, which generate the energy via mitochondrial oxidative phosphorylation, most cancer cells produce energy by fermenting glucose to lactate in the cytosol even in the presence of oxygen, a phenomena known as aerobic glycolysis or the Warburg Effect. The pentose phosphate pathway (PPP) allows glucose conversion to ribose for nucleic acid synthesis, glucose degradation to lactate, and regeneration of redox equivalents. The transketolase-like protein 1 (TKTL1) has been suggested as a key enzyme in PPP and its over-expression has been reported in a variety of human cancer tissues. The strong correlation between high TKTL1 expression and poor patients' survival as well as tumor progression has also been shown in different types of cancers.

In order to investigate the functional role of TKTL1 in melanoma, studies using small molecule TKTL1 inhibitors, siRNA and shRNA technology were performed on human melanoma cell lines. Over-expression of TKTL1 in melanoma and few normal tissues was confirmed by quantitative RT-PCR assays of normal tissues, melanomas and cell lines. TKTL1 protein levels in melanoma were evaluated by immunohistochemistry and suppression of growth by a small molecule TKTL1 inhibitor was observed in melanoma cell lines.

Using shRNA technology TKTL1-suppressed melanoma cell clones will be established and assessed in a murine model to investigate the role of TKTL1 in tumour growth and progression using different functional assays including the evaluation of tumour growth, migration and invasion.

This data provides new evidence for the role of TKTL1 in melanoma and indicates that TKTL1 is not only a tumor marker but also as a good target for anticancer therapy.

DUX STUDY: A PHASE II STUDY EVALUATING DUAL TARGETING OF THE EGFR USING THE COMBINATION OF CETUXIMAB AND ERLOTINIB IN PATIENTS WITH CHEMOTHERAPY REFRACTORY METASTATIC COLORECTAL CANCER

A. Weickhardt[1], T. Price[2], N. Pavlakis[3], E. Rioniotis[4], A. Dobrovic[5], R. Salemi[5], A. Scott[1], J. Mariadason[1], G. Chong[4], N. Tebbutt[4]

[1]Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia
[2]Queen Elizabeth Hospital, Adelaide, SA, Australia
[3]Royal North Shore Hospital, Sydney, NSW, Australia
[4]Austin Health, Melbourne, VIC, Australia
[5]Peter MacCallum Cancer Center, Melbourne, VIC, Australia

Background: Cetuximab has proven efficacy in advanced CRC. However , the activity of cetuximab monotherapy is modest, hence further approaches are required. The combination of cetuximab (C) and erlotinib (E) has shown synergistic activity in preclinical models. This phase II study evaluated the combination of C and E in patients with advanced CRC.

Methods: Eligibility included: metastatic CRC, PS 0-1, failure of prior fluoropyrimidine, oxaliplatin and irinotecan, but no previous EGFR targeted therapy, and measurable disease. Treatment was: C (400mg/m^2 iv wk1 then 250mg/m^2 iv qw and E (100mg/day po). Patients continued treatment until disease progression with CT scans q6w. Primary endpoint was response rate (RECIST) evaluated separately in *KRAS* wild-type versus *KRAS* mutant tumours. Secondary endpoints included toxicity, PFS and OS and correlation of tumour genotype (*KRAS/BRAF/PI3K*) with efficacy parameters. The planned sample size was 50 patients, with a 1 stage design.

Results : 50 pts were enrolled. Median age was 62.7 years. 48 patients were evaluable for response (2 withdrew from study due to rash before 6 week scan). The RR was 41% (95% CI 26 - 57%) in *KRAS* wt tumours. There were no responses in 11 patients with *KRAS* mutations, after which the TMC ceased recruitment of KRAS mutant tumours. No responses occurred in the 8 *BRAF* mutant tumours and patients who were both *KRAS/BRAF* wt had a RR of 52% (95% CI 34 – 69%). Frequent grade 3/4 toxicities were: rash 44%, hypomagnesemia 16%, fatigue 11%, and diarrhoea 4%. There were no toxic deaths and 60d all cause mortality was 4% (95 CI 0 – 9%). PFS and OS are encouraging.

Conclusions: The combination of C and E achieves a promising RR in patients with chemotherapy refractory CRC, which is confined to patients with *KRAS/BRAF* wild type tumours. This data suggests that EGFR targeted monotherapy may not completely inhibit EGFR signalling and that dual targeting of EGFR may be a valid therapeutic strategy in CRC

164

CORRELATION OF ENGRAFTMENT, MUTATION STATUS AND RESPONSE TO CHEMOTHERAPY IN PRIMARY TUMOR XENOGRAFT MODELS OF NSCLC

T. John[1,2], D. Kohler[2], N. Yanagawa[2], M. Li[2], D. Panchal[2], M. Pintilie[2], F. A. Shepherd[2], M. Tsao[2]

[1]Cancer Vaccine Laboratory, Ludwig Institute for Cancer Research, Heidelberg, Australia
[2]Ontario Institute for Cancer Research, University Health Network Princess Margaret Hospital, Toronto, Ontario, Canada

Background: Recapitulating the complexity of tumor growth in pre-clinical models is important for mechanistic and functional studies of cancer biology. Primary tumor xenografts may represent the histological complexity of NSCLC better than cell lines. However, not all tumors engraft when implanted into scid mice. We assessed whether histological and mutational profiles affect the ability for tumors to engraft and whether these predict response to chemotherapy.

Methods: Tumor fragments from patients undergoing curative surgery were placed subcutaneously into NOD-scid mice within 24 hours of surgery. Patient characteristics for tumors that engrafted (XG) and did not engraft (No-XG) were compared. Patient tumor DNA was analyzed using the OncoCarta™ Panel v1.0 (Sequenom®). XGs were treated with intraperitoneal cisplatin, vinorelbine, pemetrexed or saline (10 replicates/group). A tissue microarray containing all primary and XG tumors was developed for immuno-histochemical studies.

Results: Of 63/157 (40%) implanted NSCLCs that engrafted, histology was maintained in 97% XGs. Mutations in primary tumors were also detected in XGs. Histological factors significantly associated with engraftment included squamous histology, poor tumor differentiation and larger tumor size. Significantly fewer EGFR mutated tumors engrafted (p=0.012); conversely, more KRAS mutated tumors did engraft (p=0.062). In multivariate analysis including age, sex, stage and mutation, patients whose tumors formed XG had significantly shorter DFS compared to no-XG patients (HR 3.43 95%CI 1.21-9.74; p=0.021). Response to chemotherapy was seen in 5/21 (23%) cisplatin, 8/18 (44%) vinorelbine and 1/12 pemetrexed treated XGs. pS6RP H-score appeared predictive for response to cisplatin. Mutational status, ERCC1 H-score andP53 did not predict response to chemotherapy.

Conclusions: XGs closely mirror primary tumors histologically and mutationally. Response rates to chemotherapy were similar to that observed clinically. However, tumors that engraft are biologically more aggressive. This should be considered when interpreting results from pre-clinical studies.

165

ROLE OF THE C-MYC ONCOGENE IN TRANSCRIPTIONAL REGULATION OF HISTONE DEACETYLASE 3

T. Li[1,2], J. Mariadason[1]

[1]Oncogenic Transcription, Ludwig Institute of Cancer Research, Melbourne, VIC, Australia
[2]Austin Medicine Department, University of Melbourne, Melbourne, VIC, Australia

Introduction: Constitutive activation of the Wnt signalling pathway and the subsequent upregulation of the c-myc oncogene is a key event in the development of colon cancer. Histone deacetylase 3 (HDAC3) is a transcriptional co-repressor which has also been shown to be upregulated in colon cancer and to promote the proliferation and survival of colon cancer cells.

Aim: The aim of this study was to determine whether the upregulation of HDAC3 in colon cancer cells is mediated by c-myc.

Methods: We investigated the role of c-myc in the transcriptional regulation of HDAC3 using a combination of bioinformatic, molecular and immunohistochemical approaches.

Results: The overexpression of HDAC3 in human colon cancer was confirmed using immunohistochemistry. A bioinformatic search of the human HDAC3 promoter identified a consensus c-myc binding site (CACGTG) 1.5kb upstream of the HDAC3 transcription start site, which was conserved in the mouse HDAC3 promoter. Downregulation of c-myc expression using the inducibe LS174T dominant negative T-cell factor 4 (DN-TCF4) system and c-myc targeting siRNA resulted in decreases in both HDAC3 mRNA and protein levels. Conversely, overexpression of c-myc in the RKO colon cancer cell line significantly induced HDAC3 mRNA and protein expression. The direct effect of c-myc on HDAC3 promoter activity is currently being investigated using a HDAC3 promoter-luciferase construct, and by mutating the c-myc binding site through site-directed mutagenesis.

Conclusion: In summary, our findings implicate a role for c-myc in the regulation of HDAC3 expression in colon cancer cells.

AN INTENSIFIED CONDITIONING REGIMENT WITH INTRAVENOUS (IV) BUSULPHAN-MELPHALAN (BU-MEL) AND PHARMACOKINETIC MONITORING PRIOR TO AUTOGRAFTING FOR POOR PROGNOSTIC LYMPHOMA

P. W. Ho, T. Leung, J. Siderov, A. Grigg

Haematology, Austin Hospital, Heidelberg, Australia

Autografting can cure a proportion of patients with relapsed lymphoma, but those who have persistent PET positive disease demonstrable post-salvage chemotherapy pre-autograft have a poor prognosis. BEAM (carmustine, etoposide, cytarabine and melphalan) has been the standard conditioning regiment for autografts for lymphoma at the Austin Hospital; local experience with this regimen has demonstrated a median survival of approximately 19-months and 4 year event-free survival rate of 20%.

Oral busulphan- Iv melphalan has been a conditioning regiment used elsewhere in Australia for many years but variable gut absorption of busulphan (which may vary 10 fold between patients), results in efficacy and toxicity (particularly mucositis) which is difficult to predict in individual patients. IV busulphan has more predictable pharmacokinetics and drug levels can be monitored in real time allowing individualised dose adjustments to achieve an optimal drug exposure. Overseas experience suggests that this regime improves outcome in patients with chemorefractory lymphoma or myeloma.

We have initiated a pilot study of IV bu-mel in patients with high risk lymphoma, defined by failure to achieve complete remission with induction, early relapse or chemoresistant relapse.

We have compared the bu-mel group with a retrospective cohort (matched for disease status) treated with BEAM (see attached table). There has been no transplant related mortality and no difference in duration of neutropenia. However bu-mel has resulted in more severe mucositis (WHO Grade 4), requiring enteral/paraenteral nutrition and narcotic analgesia. One bu-mel recipient patient developed cryptogenic organising pneumonia which responded to steroids.

In summary, IV bu-mel is a new conditioning regiment which almost certainly increases risk of transplant related morbidity. Long-term follow up whether this is justified by improvement in long-term event free survival .

Median (range)	BEAM	IV bu-mel
Total	5	4
Duration (days) severe neutropenia (ANC< 0.2)	8 (6-8)	7 (6-8)
Mucositis	4.8 (2-8 days)	10 (7- 11 days)
Narcotic infusion	2.8 (0-7 days)	4 (0-7 days)
TPN/NG Feeds required	1 (20%)	4 (100%)
ICU Admissions	0 (0%)	1 (20%)
Significant infection	0 (0%)	1 (20%)
Mortality	0 (0%)	0 (0%)

167

TARGETING MUTANT K-RAS WITH NOVEL SIRNA-ANTIBODY CONJUGATES FOR THE TREATMENT OF COLORECTAL CANCER

V. Pillay[1], R. Jove[2], A. M. Scott[1]

[1]Tumour Targeting Laboratory, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia
[2]Department of Molecular Medicine, Beckman Research Institute at City of Hope, Duarte, CA, United States

Colorectal cancer is one of the most common cancers and one of the leading causes of cancer related deaths in Australia and worldwide despite significant improvements in surgery, radiotherapy and chemotherapy. More effective therapies for the treatment of this disease are clearly needed. K-Ras is an oncogenic protein that is mutated in ~50% of colorectal cancers and is a key driver of tumourigenesis. In colorectal cancer patients, K-Ras mutations also confer resistance to therapeutic agents that target the EGFR signalling pathway, specifically the monoclonal antibodies (mAbs) cetuximab and panitumumab. Mutant K-Ras is therefore a promising molecular target for novel colorectal cancer therapies, but has evaded all attempts at direct therapeutic targeting so far.

There is considerable interest and effort in the development of specific and potent inhibitors of mutant K-Ras activity. Small interfering RNAs (siRNAs) are one of the newest and most powerful tools of biomedical research. The high sequence specificity of siRNAs makes it an attractive tool for selectively inhibiting mutant K-Ras.

We have designed Dicer substrate siRNAs (DsiRNAs) against mutant K-Ras and were able to demonstrate knockdown in colorectal cancer cells containing G12V, G12C and G12D mutations. To further develop these siRNAs as a therapeutic for use in patients, we have directly conjugated DsiRNAs to the tumour-targeting mAb hu3S193 (a humanised monoclonal antibody to the Lewis-Y antigen) in order deliver siRNAs specifically to colorectal tumour cells and the correct location within the cell.

This will hopefully lead to a new class of the therapeutic agents for the treatment of colorectal cancer.

168

EVALUATION OF INFILTRATING T REGULATORY CELLS IN PROSTATE CANCER TISSUE

D. T.S. Woon[1], N. Rajarubendra[1], I. D. Davis[1], G. Whitty[1], M. Saxena[1], D. M. Bolton[2]

[1]Surgery/Uro-oncology, LICR, Heidelberg, VIC, Australia
[2]Surgery, Austin Health Urology, Heidelberg, VIC, Australia

Introduction: The role of immunity is not well defined for prostate cancer(PC). Regulatory T cells(Treg) are a subpopulation of T cells that can act to suppress the immune system. They express surface antigens CD4 and CD25; and intracellular FoxP3. Treg prevent overactivity of immune response by actively suppressing activation of the immune system and hence prevent pathological conditions such as autoimmune disease. Therefore, overactivity of the immune system (low Treg level) may play an important role in the pathogenesis of chronic abacterial prostatitis. In contrast, local immunosuppression (high Treg level) maybe an important factor in the development of PC .

We hypothesise that local immunomodulation is reflected by relative increased in the proportion of Treg levels in PC tissue.

Materials and methods: Fresh PC tissues from patients and their matching peripheral blood were collected. White blood cells (WBC) were separated from peripheral blood using Ficoll separation technique. PC lymphocytes were studied as single cell suspensions. WBC and prostate tissue lymphocytes were stained for surface antigens CD3, CD4, CD8, CD25, CD45 and intracellular antigen FoxP3. Using flowcytometry, Treg were identified (CD4, CD 25 and FoxP3 positive). The percentage of Tregs cells in prostate cancer tissues were identified and compared with matching blood.

Results: Our previous small pilot study had shown two patterns in patients with PC: (1) Clear evidence of tumour-infiltrating lymphocytes(TIL), or (2) No TIL. When TIL were present in PC, the proportion of Treg in the tissue was >2.8 times higher than in the matched peripheral blood samples. More samples are currently being collected and examined.

Conclusion: Base on the small study, we hypothesise that high levels of Treg in PC may lead to local immunosuppression.

169

HEREDITARY NON POLYPOSIS COLORECTAL CANCER AND FAMILIAL ADENOMATOUS POLYPOSIS: INFORMATION DAYS FOR FAMILIES

E. Lynch[1,2,3], M. Kentwell[2], F. Macrae[2,4], A. Boussioutas[5], L. Lipton[2], E. Sadek[6], K. Grant[6], S. Fitzpatrick[6]

[1]Genetics - Cancer Services, Austin Health, West Heidelberg, VIC, Australia
[2]Familial Cancer Centre, The Royal Melbourne Hospital, Melbourne, VIC, Australia
[3]Royal Children's Hospital, Genetic Health Services Victoria, Melbourne, VIC, Australia
[4]Department of Colorectal Medicine & Geneics, The Royal Melbourne Hospital, Melbourne, VIC, Australia
[5]Familial Cancer Centre, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
[6]Cancer Council Victoria, VIC, Australia

Individuals with Hereditary Non Polyposis Colorectal Cancer (HNPCC) and Familial Adenomatous Polyposis (FAP) require ongoing medical screening to reduce the high risk of developing cancer. It has been suggested longer-term psychosocial support may also be necessary to address the ongoing challenges faced by this group.

Inspired by a presentation at the 2005 InSIGHT meeting by St Mark's Hospital in the U.K, FCCs across Victoria and the Cancer Council of Victoria (CCV) jointly hosted information days for patients and their families with HNPCC and FAP in 2007 and 2009 respectively.

A planning committee consisting of genetic counsellors, a geneticist, an oncologist, a consumer and a gastroenterologist developed programs for the information days. The programs included patient stories, information on genetics and medical management, current and future research information, psychosocial presentations, and discussion groups amongst the attendees facilitated by genetic counsellors.

The four Victorian FCCs mailed invitations provided by the CCV to all of their patients recorded as being part of HNPCC or FAP families with known mutations. 32 people attended the FAP information day and 19 attended the HNPCC information day.

A detailed evaluation questionnaire conducted by the CCV showed that both days were well received by those who attended. In the FAP group, the attendees indicated the most valuable aspects of the day were meeting others with FAP, and information on diet and lifestyle following colectomy. In the HNPCC group, the attendees indicated the most valuable aspects of the day were meeting others with HNPCC and update on new information. The attendees also identified additional issues to discuss at future information sessions.

170

THE INFLUENCE OF THE MICROENVIRONMENT ON GENE-EXPRESSION IN MELANOMA CELLS

A. Behren[1], M. Anaka[1], C. Gedye[1], O. Caballero[2], J. Cebon[1]

[1]Cancer Vaccine, LICR Melbourne - Austin Branch, Heidelberg, VIC, Australia
[2]Branch of Human Cancer Immunology at Memorial Sloan-Kettering Cancer Center, Ludwig Institute for Cancer Research Ltd, New York, NY 10065, United States

Melanoma is a very heterogeneously malignancy with a high mortality for advanced stage disease. Current models to examine the underlying biology of melanoma are challenged by conflicting results resulting from this heterogeneity as well as the use of different cell culture systems and animal models.

We used two different types of media (one standard media and one “stem cell media”) and different culture methods (adherent vs. non-adherent) to measure differences in gene-expression and to compare these to signatures derived from corresponding xenografts and original patient tumours. Melanoma cell lines cultured in parallel in the different media were subcutaneously injected into animals (NOD/SCID mice) and the resulting tumours were applied to gene expression arrays. By cross comparison of the xenograft and cell line derived gene-signatures with the gene expression signature from the original patient tumours we could identify genes which are influenced heavily by the microenvironment as well as gene families which are not changing under different circumstances.

The large differences in gene expression observed in-vitro cultures were reduced in the xenografts. Tumours derived from cell lines grown in standard, serum-containing media show less change in their gene-expression than melanoma cells grown in stem cell media.

These investigations reinforce the requirement for in-vivo validation of initial in-vitro observations when testing novel melanoma therapies that target specific genes or pathways.

171

A 12 MONTH AUDIT OF BRCA MUTATIONS IDENTIFIED BY THE AUSTIN FAMILIAL CANCER CLINIC

M. J. Burgess[1], S. Saya[2], E. L. Lynch[1], A. Bankier[1], M. B. Delatycki[1,2]

[1]Department of Clinical Genetics, Austin Health, Heidelberg, VIC, Australia
[2]Murdoch Childrens' Research Institute, Parkville, VIC, Australia

The Austin Family Cancer Clinic, which forms part of the Department of Clinical Genetics at Austin Health is one of the four family cancer clinics (FCC) in Victoria. The Austin FCC services the Northern suburbs of Melbourne as well as the regional centres of Albury-Wodonga, Shepparton and Ballarat. This is a population of approximately 1 million. It became a stand alone clinical genetics service on 1 July 2009 following the outcome of the Victorian Cancer Action Plan.

An audit was conducted to ascertain the frequency and nature of BRCA1 and BRCA2 mutations identified in individuals seen by the FCC for the year from 1st July 2009 to the 30th June 2010. BRCA 1 and 2 mutation detection was offered to all individuals with a personal history of breast and/or ovarian cancer and a BRCApro score of ≥10%.

Forty-eight individuals were tested for BRCA1 and BRCA2 mutations.

There were six BRCA1 and six BRCA2 mutations identified. There was one unclassified variant whilst the remaining 35 tests did not identify a mutation. All twelve with BRCA mutations had breast cancer (six bilateral) and two also had ovarian cancer. Breast cancer pathology was available for nine individuals. Four women with BRCA1 mutations had grade 3, triple negative pathology. Of the five woman with BRCA2 mutations, four had grade 2, ER positive and one had grade 3, triple negative pathology.

172

SUSTAINED IE GENE INDUCTION AND AP-1 ACTIVITY IS LINKED TO HDACI-INDUCED APOPTOSIS IN MULTIPLE TUMOUR TYPES

A. C. Chueh, L. Tögel, J. M. Mariadason

Oncogenic Transcription Laboratory, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Histone deacetylase inhibitors (HDACi) are an emerging class of cancer therapeutics, currently approved for the treatment of cutaneous T-cell lymphoma (CTCL). Through screening a large panel of 30 colorectal cancer cell (CRC) lines, we have recently established that the HDACi-induced transcriptional response and associated apoptosis are linked to a unique gene expression signature consisting of multiple immediate-early (IE) and stress response genes, selectively induced by HDACi in sensitive lines.

To determine whether the findings made in colorectal cancer cells are universally applicable across multiple tumor types, we have screened a panel of 33 non-colon tumor cell lines (including that of CTCL, multiple myeloma, NSCLC, melanoma, breast and prostate cancer) for HDACi sensitivity by PI/FACS analysis.

Consistent with the findings in CRCs, quantitative RT-PCR analysis revealed that the expression of IE genes Fos, Jun and Atf3 are selectively induced by HDACi treatment in the 5 most sensitive cell lines identified when compared to the 5 most refractory lines.

To study kinetics of IE gene induction, we performed time-course experiments on cancer cell lines treated with various stimuli, including EGF, phorbol ester PMA, and HDACi. Whereas EGF and PMA induce IE gene expression in a rapid and transient manner, HDACi-induction of IE gene expression is sustained over 24 hours, suggesting that the prolonged expression of these genes may be responsible for apoptosis induction.

To test this hypothesis, we performed RNAi-mediated knockdown of AP-1 transcription factors (Fos, Jun, Atf3) and examined their contributions to the HDACi-induced apoptosis.

Preliminary data demonstrated that the down-regulation of Jun and Atf3 (but not Fos) could partially block HDACi-induced apoptosis. Furthermore, we demonstrated that AP-1 reporter activity is robustly and preferentially induced in sensitive CRCs following HDACi treatment.

We are currently pursuing the role of AP-1 activation in apoptosis induction upon HDACi treatment of tumour cells.

173

PERIPHERAL BLOOD BDCA-1 (CD1C) POSITIVE DENDRITIC CELLS PULSED WITH NY-ESO-1 ISCOMATRIX® MAY BE TOLEROGENIC IN PATIENTS WITH MINIMAL RESIDUAL CANCER AT HIGH RISK OF RELAPSE: A PILOT CLINICAL TRIAL

G. A. Whitty[1], J. Quirk[1], L. Morris[1], L. Seddon[1], T. Tai[1], T. Cavicchiolo[2], L. Ebert[1], H. M. Jackson[1], L. Miloradovic[3], E. Maraskovsky[3], W. Chen[1], J. Cebon[1,2], I. D. Davis[1,2]

[1]Ludwig Institute for Cancer Research, Heidelberg, Australia
[2]Joint Ludwig-Austin Oncology Unit, Austin Hospital, Heidelberg, VIC, Australia
[3]CSL Limited, Parkville, VIC, Australia

Background : Peripheral blood dendritic cells (PBDC) can prime naïve T cells in vivo. NY-ESO-1 (ESO) is a "cancer-testis" antigen expressed in many types of cancer. ISCOMATRIX® adjuvant (IMX) is a saponin-based immunomodulator. ESO/IMX induces strong antibody and T cell responses to ESO in patients with minimal disease but this is less apparent in advanced cancer. We performed a pilot clinical trial (LUD2003-013) using ESO/IMX pulsed onto PBDC and showed almost complete absence of antibody or T cell responses. We asked whether immune tolerance mediated through ESO-specific regulatory T cells ($Treg_{ESO}$) or myeloid-derived suppressor cells (MDSC) had been induced.

Methods: Leukapheresis was performed before each vaccination. Cells were processed in a dedicated facility using the CliniMACS$^{®plus}$ Instrument. CD1c+ B cells were depleted using anti-CD19 antibody-conjugated MicroBeads, followed by positive selection of CD1c+ PBDC, overnight pulsing with ESO/IMX, washing, and intradermal injection x3 at 4-week intervals.

Results: Eight patients were enrolled. PBDC production was feasible and treatment was well tolerated. Treg were identified on phenotypic (CD4+25+FoxP3+) and functional criteria (lack of IFN-γ production and suppression of stimulated $CD8^+$ T cells) but total Treg numbers did not change consistently during treatment. Regardless of whether patients showed an immune response, the presence of $Treg_{ESO}$ was not able to be confirmed and MDSC cell numbers were inconsistent.

Conclusions: ESO/IMX-pulsed PBDC were not immunogenic in this patient population. The hypothesis that ESO/IMX-pulsed PBDC might induce immune tolerance perhaps mediated by $Treg_{ESO}$ or MDSC could not be confirmed, perhaps due to low patient numbers.

174

VILLIN EXPRESSION IS FREQUENTLY LOST IN MSI COLON CANCER

S. Al-Obaidi, D. Williams, D. Arango, J. M. Mariadason

Oncogenic Transcription, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Colon cancers can be broadly classified as microsatellite stable (MSS) or having microsatellite instability (MSI). Significant differences exist in the mutational spectrum, histopathological presentation and prognosis of these tumour subtypes.

We identified the actin-binding cytoskeletal protein, villin, as significantly downregulated in MSI colon cancers by gene expression profiling of a panel of MSI and MSS colon cancer cell lines. This finding was confirmed by qPCR and at the protein level, and in a cohort of primary MSI colon cancers.

Investigation of the mechanism of villin loss failed to identify mutations in the coding sequence, or evidence of epigenetic inactivation. Point mutations in repeat elements within the villin promoter were identified in MSI cell lines, however this did not correlate with loss of villin expression. Conversely, villin promoter reporter activity strongly correlated with endogenous villin expression across the cell line panel, suggesting loss of villin expression is mediated by loss of a key transcriptional regulator.

Previous studies have demonstrated regulation of the villin gene by the Cdx family of homeobox transcription factors, which are important regulators of intestinal differentiation. Examination of Cdx-1 and Cdx-2 expression in a panel of 42 colon cancer cell lines revealed a strong correlation between Cdx-1 and villin expression. Furthermore, transient transfection of Cdx-1 in to villin negative RKO cells robustly induced villin promoter activity.

Given its role in actin binding and cytoskeletal structure, we postulate that loss of villin may contribute to loss of the differentiated phenotype of MSI colon cancers.

175

THE ALKALINE PHOSPHATASE GENE CLUSTER IS EPIGENETICALLY REGULATED IN COLON CANCER.

G. Corner[1], A. Azerbayjani[2], J. Shin[2], N. Ahmed[2]

[1]Oncogenic Transcription, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia
[2]Oncology, Montefiore Medical Center, New York, NY, United States

Treatment of colon cancer cell lines with the differentiation inducing agent, sodium butyrate, induces activity of the gut epithelial cell differentiation marker alkaline phosphatase (ALP). However, the contributions of the four highly homologous ALP isoforms to activity have not been clearly elucidated, and little is known about the transcriptional regulation of ALP expression.

Our work shows that mRNA expression of all 4 isoforms is co-ordinately induced upon butyrate treatment with the placental and placental-like 2 isoforms the most strongly induced and therefore likely to contribute most to alkaline phosphatase activity.

Furthermore, we also observed that all 4 isoforms were either co-ordinately induced by butyrate treatment, or not induced at all, in different colon cancer cell lines. Transient transfection of an iALP promoter reporter construct demonstrated induction in all cell lines indicating the necessary transcription factors are present in all lines, and suggesting epigenetic factors may be determining differential induction. Inhibition of DNA methyltransferase activity chemically or genetically in HCT116 cells where ALP activity cannot be induced, resulted in inducibility of all ALP isoforms, pointing to methylation as a likely regulator of ALP expression.

Furthermore, bisulfite treatment and sequencing of the ALPi promoter in 12 colon cancer cell lines demonstrated that the degree of promoter methylation correlates strongly with inducibility. Whether this is also the case for the other ALP isoforms is currently being tested.

These findings identify the ALP gene cluster as a family of co-ordinately regulated genes which are susceptible to epigenetic inactivation in colon cancer.

176

THE ROLE OF ZINC IN PROSTATE CANCER

W. Ranasinghe, M. Chang, L. Xiao, A. Shulkes, D. Bolton, G. Baldwin, O. Patel

Department of Urology, Austin Hospital/ University Of Melbourne, Heidelberg, VIC, Australia

Background: High concentration of Zinc in prostate tissue plays a vital role for prostate health. Zinc and cobalt chloride have been shown to induce Hypoxia Inducible Factor 1 alpha (HIF1 alpha) and its downstream products such as Vascular Endothelial Growth Factor (VEGF). However the relationship between zinc and HIF1alpha in prostate cancer cells is still unclear. We aimed to investigate the effect of zinc on HIF1alpha and its downstream products in three prostate cell lines (PC3, LNCaP and DU145).

Methods: Prostate cells were cultured in RPMI1680 medium containing 7.5% FBS. HIF1apha protein and VEGF expression were measured using Western-blot and ELISA, respectively.

Results: HIF1alpha levels in PC3 cells were significantly higher compared to LNCaP and ACHN cells (renal) under normoxic conditions. In PC3 cells, zinc inhibited HIF1alpha and VEGF in a dose dependent fashion. Although cobalt treatment lowered the HIF1alpha levels, it increased the VEGF expression. Interestingly, Zinc reversed the cobalt-induced VEGF expression. Similar to PC3 cells, zinc also reduced VEGF expression stimulated by cobalt chloride treatment in LnCap cancer cells.

Conclusions: Prostate cancer cell lines differentially expressed HIF1alpha. Zinc treatment of PC3 cells reduced HIF1aplha and VEGF levels however further studies are needed to determine the mechanism which in turn can reveal newer target sites for anti cancer therapy.

INVESTIGATION OF THE ROLE OF WNT PROTEIN SIGNALING IN CRC ANGIOGENESIS AND METASTASIS USING RNA INTERFERENCE (RNAI)

L. Low[1], H. Seow[1,2], T. Fifis[3]

[1]School of Biomedical Sciences, Victoria University, Melbourne, VIC, Australia
[2]School of Graduate Studies, University Putra of Malaysia, Selangor, Malaysia
[3]Department of Surgery, University of Melbourne, Austin Health, Melbourne, VIC, Australia

Activation of tumour angiogenesis by angiogenic promoters leads to tumour metastasis and is the main cause of treatment failure in many cancers including colorectal cancer (CRC). Approximately 70% of CRC mortality is due to liver metastasis. Recent work has shown that WNT proteins are associated with CRC angiogenesis and metastasis.

This study aims to identify WNT proteins that contribute to tumour angiogenesis by blocking their expression using RNAi.

Upregulated WNT expression was identified in a number of CRC cell lines in comparison to normal colon mucosa by RT-PCR and real time PCR. Active WNT signaling in CRC cell lines was confirmed by western blot which showed the accumulation of beta-catenin in the nucleus.

Interestingly, our results shown three out of ten WNTs, namely WNT7B, WNT5B and WNT10B, show higher expression in CRC cell lines in comparison to normal colon fibroblast. The involvement of WNT7B in vascular formation has been reported in two other studies (Lobov et al. 2005; Shu et al. 2002). Therefore, WNT7B appears to be the most promising candidate for further studies, in evaluating the of role WNT in CRC angiogenesis.

We are currently using invasion, migration and tube formation assays and RNA silencing to determine the role of upregulated WNTs in angiogenesis. The effect of WNTs silencing on the expression of WNT signaling target genes will be investigated via real time RT-Profiler.

The results so far show that certain WNT ligands are specifically upregulated in CRC tumour cell lines. Elucidating the role of these ligands in angiogenesis and tumour metastasis may lead to better cancer treatments.

(1) Lobov, IB, Rao, S, Carroll, TJ, Vallance, JE, Ito, M, Ondr, JK, Kurup, S, Glass, DA, Patel, MS, Shu, W, Morrisey, EE, McMahon, AP, Karsenty, G & Lang, RA 2005, 'WNT7b mediates macrophage-induced progr
(2) Shu, W, Jiang, YQ, Lu, MM & Morrisey, EE 2002, 'Wnt7b regulates mesenchymal proliferation and vascular development in the lung', Development, vol. 129, no. 20, pp. 4831-42.

178

SEQUENTIAL ALTERNATING DRUG TREATMENT: OVERCOMING RESISTANCE IN ADVANCED RENAL CELL CANCER

P. H.M. Tan[1,2], I. D. Davis[1,2], D. M. Bolton[2], A. M. Scott[1,2], P. Sluka[1]

[1]Ludwig Institute for Cancer Research, Uro-Oncology, Heidelberg, VIC, Australia

[2]Urology, Austin Health, Heidelberg, VIC, Australia

Introduction: Tyrosine kinase receptor and mTOR-inhibitors have doubled progression free survival of patients with metastatic renal cell cancer. However, at best these treatments cause stabilization of disease and not cure. Current approach to treating resistant disease is to switch to another drug as monotherapy. However, sequential use of single drugs often gives responses that are less frequent and shorter in duration. Drug combinations have been tested but toxicity is often prohibitive. RCC resistance to sunitinib and everolimus are not well understood. A recent animal study showed drug-resistant RCC cells regain drug sensitivity when transplanted to treatment-naive mouse. This indicates that mechanism of resistance is likely related to complex cancer/host adaptation and/or upregulation of alternate signaling pathways. Drug resistance could be delayed by planned alternating use of these drugs prior to the establishment of resistance. This is the rationale for ANZUP clinical trial, EVERSUN. This is the first in vivo study investigating the role of sequential alternating drug ("flip-flop") treatment in advanced RCC.

Methods: Xenografts of SKRC-52 (human metastatic-RCC line) were established in BALB/c mice. Mice were assigned to four groups: Control, Sunitinib, Everolimus and Flip Flop (week on/week off between sunitinib and everolimus). Endpoints are: tumour volume, animal weight, PET-FDG and FLT, molecular biology and immunohistochemistry.

Results: Control mice lost more weight than treatment groups. Everolimus-group recorded the highest increase in tumour volume. Flip Flop group recorded similar change in animal weight to monotherapy group.

Conclusion: Volumetric assessments are not reliable measures of response to treatment. Mice in the everolimus group maintained their weight despite a paradoxical increase in tumour volume. Sequential alternating treatment does not appear to reduce animal weight loss or tumour volume within a 4-week treatment period. An extended animal study is currently underway.

179

IDENTIFICATION OF MELANOMA SUB-POPULATIONS BASED ON FUNCTIONAL PROPERTIES

C. Hudson[1], A. Behren[1], M. Anaka[1], P. Lo[1], J. Browning[2], J. Cebon[1]

[1]Cancer Vaccine, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

[2]Anatomical Pathology, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Despite the fact that malignant melanoma accounts for just a small percentage of all skin cancers it is responsible for the majority of deaths. An increasing incidence and lack of successful therapeutic options in the management of advanced disease highlight the urgent need for new strategies to fight this devastating cancer. Over the past decades "cancer stem cells" have been identified in a variety of solid tumours. In melanoma, however, this has not been shown conclusively.

The cancer stem-cell theory proposes that not every cancer cell is of equal importance and that each tumour contains a small subpopulation of cells which is responsible for metastasis, recurrent disease and resistance against therapies. These cells are a promising target for cancer therapeutics.

Results from our and also other labs show that either the percentage of cancer stem cells is extremely high in melanoma or that melanoma cells show high plasticity, enabling phenotype switching when required. While most of the work in this field is based on the expression of certain surface molecules which could be associated with the presence of cancer stem cells, we have established a suite of assays including the identification of slow-cycling cells, colony-forming abilities, resistance to chemotherapy, aldehyde dehydrogenase activity and efflux of rhodamine. This allowed for the separation of functional sub-populations in early passage melanoma cell lines established directly from patient tumours.

By deep analysis of gene- and protein expression with subsequent analysis of underlying pathways and transcription factors we hope to identify and validate targets pivotal to these functions. Independent of the presence of cancer stem cells as a static target this strategy will allow us to interfere with critical melanoma functions.

180

INTRATUMOURAL FUNCTIONAL HETEROGENEITY OF MELANOMA CELLS WITH DISTINCT GENOMIC PROFILES

M. Anaka, A. Behren, C. Hudson, F. Lo, I. D. Davis, J. Cebon

Cancer Vaccine Lab, Ludwig Institute for Cancer Research Melbourne - Austin Branch, Heidelberg, VIC, Australia

Tumour cell heterogeneity is recognized as a key explanation for therapeutic failure.

Two theories of cancer development, the cancer stem cell and clonal evolution hypotheses, account for tumour cell heterogeneity. The cancer stem cell hypothesis posits that a cancer cell with stem-like properties of self-renewal and multi-lineage differentiation gives rise to the various cell types of a tumour. Clonal evolution predicts the existence of multiple cell types within a tumour resulting from sequential mutations and genomic aberrations leading to clonal outgrowths and cancer progression.

We asked whether an early passage melanoma cell line displays genomic heterogeneity, and how this heterogeneity is related to differences in gene expression and cellular function. Eleven single cell clones established from a single melanoma cell line, along with the parental cell population and a sample of the original tumour sample, were analyzed for genomic aberrations and gene expression profiles using Illumina 610 Quad SNP and Illumina HT-12 Expression BeadChips respectively. All clones were also analyzed for anchorage independent growth, clonogenicity, proliferation, migration, and resistance to chemotherapies.

Significant differences in regions of chromosomal copy number change were observed between the clones, with the majority of the chromosomal profile matching the original tumour sample, indicating maintenance of representative genomic heterogeneity in short term cell culture. The clones also differed markedly in all functional assays performed, and these differences are currently being related back to differences in gene expression.

These results indicate that clinically relevant functional heterogeneity can be in part explained by the existence and maintenance of clonal cell populations displaying distinct genomic aberrations.

181

CAUSATIVE ROLE OF EPITHELILAL-TO-MESENCHYMAL TRANSITION IN MELANOMA PROGRESSION

A. Jayachandran, A. Behren, P. Lo, J. Cebon

Cancer Vaccine Laboratory, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Melanoma, the most aggressive form of skin cancer is refractory to current treatment options and its acquisition of metastatic competence still remains to be resolved. Epithelial-to-mesenchymal transition (EMT), the phenotypic switching of cell states, is a crucial mechanism which may contribute to metastatic competence in melanoma.

The objective of this study is to initially identify whether cellular plasticity exists in human melanoma cell-lines and tumour samples. Next, to identify molecular elicitors, novel markers and signalling pathways regulating EMT, with particular emphasis on reversing EMT.

By performing a qPCR-based EMT gene-expression profiling we identified three subsets of melanoma cell-lines that resembled the various steps of EMT namely, epithelial-like (E-cadherin high, no N-cadherin), EMT-hybrid (intermediate E-cadherin and N-cadherin) and mesenchymal-like (N-cadherin high, no E-cadherin). These EMT phenotypes were also representative in patient tumour at RNA level. These distinct cellular subsets were compared with primary human melanocytes and exhibited differential expression patterns of EMT associated transcription factors Snail1, Snail2, Twist1, Zeb1 and Zeb2, indicative of the occurrence of EMT in melanoma. We observed that the functional heterogeneity in melanoma is a consequence of the cellular plasticity facilitated by EMT. Subsequently, epithelial-like cell-lines were sessile, mesenchymal-like were highly migratory and invasive and EMT-hybrids showed intermediate migratory and invasive abilities. Migration was quantified by combined MTT and Boyden chamber assays and invasion was assessed by cell movement through matrigel-coated trans-well and quantified by MTT assay. Furthermore, we identified TGF-beta1 as a potent inducer of EMT in epithelial-like subsets of melanoma cell-lines. These observations suggest that EMT is major event controlling melanoma progression and inhibition/reversal of EMT may be a valid therapeutic option in melanoma.

182

CD151 EXPRESSION: A BIOMARKER FOR PROSTATE CANCER PROGRESSION

S. Detchokul[1], B. Newell[1], J. Ang[1], M. W. Parker[2], E. D. Williams[3], A. G. Frauman[1]

[1]Medicine, University of Melbourne, Heidelberg, VIC, Australia
[2]Structural Biology Laboratory, St Vincent's institute of Medical Research, Melbourne, VIC, Australia
[3]Monash Institute of Medical Research, Monash University, Clayton, VIC, Australia

We have shown that the tetraspanin CD151 has prognostic value in prostate cancer (PCa); patients whose cancer has low expression of CD151 have better prognosis than those with high level[1]. We are now interested in CD151's role in PCa as a motility and metastasis promoter. Human PCa cell lines LNCaP and PC3 were used in cell migration and invasion assays. The motility and invasiveness of wild-type LNCaP (low endogenous level of CD151) vs. CD151 transfected LNCaP cells and PC3 (high endogenous CD151) vs. CD151 knock-down PC3 cells (KD PC3) was analyzed. LNCaPs transfected with CD151 showed increased motility and invasion compared to control LNCaPs ($P<0.05$), while KD PC-3 cells demonstrated reduced motility and invasion compared to control PC3s ($P<0.05$). Paired primary and secondary PCa generated using a SCID mouse model bearing implanted human PCa cell lines are being examined for expression of CD151 and vascular cells, by immunohistochemistry. Although the mechanism is unclear, CD151 appears to promote cell motility and imparts a worse prognosis in PCa. These findings suggest that CD151 could be a useful biomarker for the prognostication of PCa.

(1) Ang J et al. Cancer epidermiol Biomarkers & Prevention (2004) 13: 1717-21

183

THE RELATIVE UTILITY OF CYTOLOGY AND HISTOLOGY SPECIMENS IN ASSIGNING NSCLC PATHOLOGICAL SUBTYPES

A. Campbell[1], T. John[1,2,3], M. Newman[2], S. White[1], P. L.R. Mitchell[1]

[1]Medical Oncology, Austin Health, Heidelberg, VIC, Australia
[2]Pathology, Austin Health, Heidelberg, VIC, Australia
[3]Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Aims: Pathological subtypes of non-small cell lung cancer (NSCLC) have important implications for prognosis and treatment. Subtyping may prove difficult with cytology specimens, but may be the only tumour tissue available. We reviewed NSCLC cases to determine the rate of histological versus cytological diagnoses and the ability to determine a subtype.

Methods: Records of patients newly diagnosed with NSCLC during 2007-2009 were reviewed. Cases were identified as having definitive versus suspected histological or cytological subtypes or NSCLC not otherwise specified.

Results: Of 129 cases, 83 (64.3%) had histology compared to 41 cases (31.7%) who had cytology only. Five patients were treated expectantly without definitive histology. Immunohistochemistry was available for some cases to support the diagnosis. Patients with cytology only tended to be older (median age 70 years, range 52- 94) compared to patients who had histology (median age 68 years, range 38-87; $p=0.09$), had poorer performance status and more comorbidities. There were no significant differences in clinical stage between the groups. The majority were stage IV. A subtype was more likely to be assigned in patients who had histology compared to cytology only (46.9% vs 24.3%; $p=0.012$). In 45 cases both histology and cytology was available. In 20 cases histology provided additional diagnostic information, whilst in 7 cases cytology was superior to histology. In 6 cases cytology was diagnostic despite negative histology. There was a trend toward worse median overall survival in the cytology only group (6.18 months vs 7.6 months; $p=0.18$).

Conclusions: Cytology is a useful adjunct to the diagnosis of NSCLC, however histology more commonly provides definitive subtyping and every effort should be made to obtain histological samples where clinically appropriate.

No conflict of interest

184

A COMPARATIVE REVIEW OF 3D-CRT AND AN IMRT TECHNIQUE FOR ANAL CANCER USING CMS XIO AND CMS MONACO.

D. Smith, K. Churcher, D. Lim Joon

Radiation Oncology, Austin Health, Heidelberg, VIC, Australia

Introduction: Anal canal cancers account for a small proportion of our department's clinical radiotherapy workload, <1% of all patients annually. However, due to the complexity of target volumes which include loco-regional lymphatics and the need to avoid the adjacent bladder, small bowel, femoral heads and external genitalia, the relative clinical planning time and treatment workload for this patient cohort can be large. We propose to introduce an IMRT approach to the planning and treatment of anal canal tumours, so as to improve conformality of prescribed dose compared to our standard 3D-CRT technique, as well as improving critical tissue/organ sparing.

Methods: 5 patients recently planned and treated with a 3D conformal RT approach and stages T2N0M0 to T3N2M0 anal cancer were selected to establish an IMRT protocol for the planning and treatment of anal canal patients. Each patient had two IMRT plans created one using CMS XiO and one using CMS Monaco. The resultant plans were compared to the original 3D-CRT plan (previously delivered) and evaluated for dose conformality and OAR sparing using standard dose volume histogram (DVH) data, and Conformality Index.

Conclusion: We have shown an increase in the conformality of dose utilising an inversely planned IMRT approach to anal cancer radiotherapy treatment compared with our standard 3D-CRT approach. This increase in target dose conformality in conjunction with a dose reduction to organs at risk has the potential to reduce the impact of radiation-related toxicities. In addition, we believe Monaco/XiO to offer a more robust planning solution with improved efficiency/user control.

185

IDENTIFICATION OF DUSP5 AS A POTENTIAL MEDIATOR OF WNT/B-CATENIN - MAPK/ERK SIGNALLING CROSSTALK IN COLON CANCER

L. Tögel, A. C. Chueh, J. M. Mariadason

Oncogenic Transcription Laboratory, Ludwig Institute For Cancer Research, Heidelberg, VIC, Australia

The Wnt/ß-catenin and MAPK/ERK signalling pathways are key regulators of normal intestinal epithelial cell homeostasis and are simultaneously hyper-activated in the majority of colon cancers. Constitutive activation of Wnt /ß-catenin signalling by either loss of APC or activation of ß-catenin occurs in 90% of colorectal cancers, while constitutive activation of MAPK/ERK signalling due to KRAS or BRAF mutations occurs in approximately 60% of colon cancers.

Given the co-ordinate activation of these pathways in normal colon and colon cancer we hypothesized that crosstalk between these pathways is likely to be an important form of additional regulation.

To identify potential crosstalk regulators, we searched for genes involved in Wnt/ß-catenin and MAPK/ERK signalling that are co-ordinately or oppositely regulated in a series of colon cancer microarray databases and cell culture systems.

This analysis identified dual specificity phosphatase 5 (DUSP5) to be significantly reduced in colorectal tumours compared to adjacent normal tissue (1.6 fold change, $p<0.004$), and to be robustly induced upon sustained inhibition of Wnt/ß-catenin signalling in LS174T DNTCF colon cancer cells. These findings were confirmed by qPCR. DUSP5 is induced upon activation of MAPK/ERK signalling and serves as negative feedback regulator of this pathway by dephoshporylating ERK1/2.

To test this in colon cancer cells, we treated LIM1215 cells with EGF for 10 minutes to 8 hours. A robust increase in pERK1/2 was observed within 10 minutes of EGF treatment and strong induction of the MAPK/ERK target genes JUN and FOS was observed after 1h. EGF treatment also resulted in marked induction of DUSP5 expression which peaked 2h after treatment indicating this feedback pathway is intact in these colon cancer cells.

Collectively these results suggest that repression of DUSP5 expression by Wnt/ß-catenin signalling may facilitate coordinate activation of these two pathways in colon cancer cells.

^{177}LU-CHX-A"-DTPA HUA33 RADIOIMMUNOTHERAPY (RIT) IN COMBINATION WITH CHEMOTHERAPY AND CETUXIMAB IN A COLORECTAL CANCER XENOGRAFT MODEL

F. Lee[1], A. Yuen[1], A. Osborn[1], D. Wang[1], A. Rigopoulos[1], F. Smyth[1], M. Brechbiel[2], A. Scott[1]

[1]Tumour targeting, Ludwig institute for Cancer Research, Heidelberg, VIC, Australia

[2]2Radioimmune & Inorganic chemistry Section, ROB,, National Cancer Institute, Bethesda, Maryland, United States

RIT using radiolabelled monoclonal antibodies (mAb) to treat colorectal cancer (CRC) is a promising therapeutic strategy, especially when combined with other treatment modalities. Humanised mAb A33 (huA33) targets the glycoprotein antigen A33, expressed on 95% of colorectal cancers and normal colonic epithelium. ^{131}I-huA33 together with capecitabine was evaluated in a recent Phase I trial[3]. Of the 18 patients evaluable for tumour response, there was 1 partial response, 10 with stable disease, and 7 with progressive disease. The physical properties of the radio lanthanide Lutetium-177 (^{177}Lu) would indicate that this isotope may be better suited for RIT. Cetuximab has been shown to have anti-tumour activity in 10 to 20 % of unselected metastatic CRC patients, especially those without KRAS mutation.

Aims: This laboratory study evaluated the biodistribution and therapeutic properties of single dose ^{177}Lu-huA33 RIT alone and when combined with Cetuximab and 5-FU/leucovorin in mice bearing cetuximab sensitive CRC xenografts.

Results: The uptake of radioconjugate by LIM1215 xenografts reached a maximum of 94.0 + 42.3 %ID/g by 72 hrs p.i. and retention was prolonged for over two weeks. Xenograft growth was retarded by a single administration of 50 to 300 µCi ^{177}Lu-huA33 in a dose dependent manner with higher doses inducing durable disease stabilization.. Disease stabilization was also achieved using cetuximab alone. Combining RIT with subtherapeutic cetuximab resulted in greater tumour responses (including CRs). The addition of 5-FU to RIT and cetuximab also resulted in substantial durable anti-tumour effects compared to single agents.

Conclusions: Compared to ^{131}I- huA33, the lower abundance and energy of the g-emission of ^{177}Lu portend well for use its use with huA33 as a RIT agent based on dosimetric considerations. ^{177}Lu-huA33 RIT of CRC has potential as a single therapeutic agent, but can achieve increased efficacy when used in conjunction with approved therapies of cetuximab and 5FU.

[1]Herbertson et al J Clin Oncol, 25, No. 18S, 2007: 4078

ADEQUACY OF DATA PROVISION AND ANALYSIS IN ONCOLOGY PHASE III TRIALS OVER THE PAST FIVE YEARS

H. K. Gan[1], B. You[2], G. Pond[3], E. X. Chen[2]

[1]Austin Ludwig Cancer Unit, Austin Hospital, Heidelberg, VIC, Australia

[2]Department of Medical Oncology and Hematology, Princess Margaret Hospital, Toronto, Ontario, Canada

[3]Department of Medical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada

Aims: This study aimed to examine the adequacy of data provided in oncology randomised controlled trials (RCTS) published in selected journals (*Ann Oncol; Br J Cancer; Breast Cancer Res Treat; Cancer; Eur J Cancer; J Clin Oncol; J Natl Cancer Inst; Lancet; Lancet Oncol; N Engl J Med*) between 2005-2009 inclusive.

Methods: Eligible RCT's tested therapeutic drug regimens for adult patients with solid tumours. Two authors (HKG, BY) identified RCT's by searching electronic databases and manual review of the relevant journals. They independently assessed each publication for the adequacy of data provided against accepted standards as described in the CONSORT Statement (Altman *et al,* Annals of Internal Medicine 134: 663). In brief, the following parameters were assessed: background, participants, interventions, objectives, outcomes, sample size, randomization processes, blinding, statistical methods, participant flow, recruitment, baseline data, number analysed, outcomes data, ancillary analyses, toxicity, interpretation, generalisability and overall evidence.

Results: A total of 368 publications were examined. Problems were identified in the following areas:

Randomization procedures were inadequately described in > 95% of cases.

Use of interim analysis was inadequately described in 54% of cases.

The use of blinding was inadequately described in 44% of cases

Statistical methods were inadequately described in 15% of cases, including inadequate information about p-values, alpha values and/or beta values.

Outcomes data was inadequately described in 42% of cases, including failure of provide any confidence intervals and failure to explicitly state the p-value.

Conclusions: Despite the publication of the CONSORT statement and its endorsement by major medical journals, reporting of oncology RCT's still suffers from inadequate data provision. The CONSORT statement should be followed more closely by investigators and enforced more rigorously by journals as failure to do some may compromise the ability to interpret and use these results for patient care.

MANAGEMENT OF LUNG CANCER IN VICTORIA: HAVE WE MADE PROGRESS?

P. L.R. Mitchell[1], V. Thursfield[2], G. Wright[3], D. Ball[4], G. Richardson[5], L. Irving[6], G. Giles[2]

[1]Cancer Services, Austin Health, Heidelberg, VIC, Australia
[2]The Cancer Council Victoria, Melbourne, VIC, Australia
[3]St Vincent's Hospital, Melbourne, VIC, Australia
[4]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
[5]Cabrini Health, Melbourne, WA, Australia
[6]Melbourne Health, Melbourne, VIC, Australia

Aims and methods: We aimed to document lung cancer management and compare results to a 1993 survey. Patients (pts) diagnosed during 6 months of 2003 were identified from the Victorian Cancer Registry and data obtained from source documents.

Results: Data were available for 841 (82% of eligible pts): median age 72 yrs and 63% male. Most were ex-smokers (57%), with 8% never smokers (71% female). Diagnostic modality was: clinical only 10%; pathological - cytology alone 20%, histology 70% (NSCLC 86%, small cell (SCLC) 13%).

Of NSCLC (n=655) characteristics were: adenocarcinoma 40%, squamous 23%, large cell 13%; stage I/II 23%, III 26%; PS 0-1 63%; PET scan 43%; specialist referral 99%; discussed at MDM 33%. With curative intent, 191 pts (29%) received, as single modality or in combination: surgery 125 pts (65%); radiotherapy 91 pts (48%); chemotherapy 66 pts (35%). 326 pts (50%) had palliative treatment while 138 pts (21%) received no therapy. Considering all NSCLC pts, 258 (39%) received chemotherapy and 365 (56%) radiotherapy at some time during their illness.

Considering the 101 pts with SCLC, 67% had PS 0-1, 33% limited disease, 99% saw a specialist and 21% discussed at MDM. 9% received no treatment while 27 pts (27%) were treated with curative intent, including 16 (20%) with chemoradiation.

Median and 5-year survival were: NSCLC 6.9m (SE 0.42) and 11%; SCLC 7.2m (SE 0.96) and 3%, respectively. Considering all pts, better survival on multivariate analysis was associated with younger age, good PS, no weight loss, early stage, never smoking and better socio-economic status. Changes over 1993-2003 were: median 3yrs older; fewer males; less SCLC; more adenocarcinoma and less squamous. When adjusted for age and sex, the overall 5yr survival of has not changed in the last decade.

Conclusions: Despite increased use of PET scanning and more multi-modality therapy, lung cancer survival has not improved over the last 10 years. New strategies are required to improve patient outcomes.

The authors have no conflict of interest in relation to this study.

189

VALIDATION OF ^{11}C-CHOLINE PET SCANS WITH PATHOLOGY FOR RADIOTHERAPY TARGET DEFINITION IN PROSTATE CANCER

J. H. Chang[1,5], D. Lim Joon[1], I. D. Davis[3,4,5], A. M. Scott[2,3], S. T. Lee[2,3], D. Clouston[6], V. Khoo[7], S. Gong[2], L. Shanker[3], C. S. Hamilton[1]

[1]Radiation Oncology Centre, Austin Health, Melbourne, VIC, Australia
[2]Centre for PET, Austin Health, Melbourne, VIC, Australia
[3]Ludwig Institute for Cancer Research, Melbourne, VIC, Australia
[4]Ludwig-Austin Joint Medical Oncology Unit, Austin Health, Melbourne, Australia
[5]University of Melbourne, VIC, Australia
[6]Focus Pathology, Melbourne, VIC, Australia
[7]Department of Clinical Oncology, Royal Marsden Hospital Trust & Institute of Cancer Research, London, United Kingdom

Background and purpose: To validate the use of ^{11}C-choline PET scans in defining dominant intraprostatic lesions (DILs) for radiotherapy target definition.

Material and methods: Eight men with prostate cancer who had ^{11}C-choline PET scans prior to radical prostatectomy were studied. Several methods were used to contour the DIL on the PET scans: visual, PET edge, Region Grow, absolute standardised uptake value (SUV) thresholds, and percentage of maximum SUV thresholds. Prostatectomy specimens were sliced in the transverse plane, and a pathologist defined the DILs on each of these slices. The pathologist-defined DILs were used as the gold standard for correlation with the PET scan-defined DILs. The accuracy of correlation was assessed by Dice similarity coefficient (DSC) and Youden index.

Results: The contouring method resulting in both the highest DSC and the highest Youden index was 60% of the maximum SUV ($SUV_{60\%}$), with values of 0.64 and 0.51 respectively. However we could not show that $SUV_{60\%}$ was the best contouring method, as it was not statistically significantly better than the other methods by either measure. The degree of correlation shown using these methods is however consistent with previous studies that have justified using imaging for DIL radiotherapy target definition.

Conclusions: Although not statistically significant, $SUV_{60\%}$ resulted in the best correlation between ^{11}C-choline PET and pathology amongst all the methods studied. The degree of correlation shown using these methods is sufficient for guiding DIL radiotherapy dose escalation.

190

STIMULATION OF PROSTATE CANCER CELL PROLIFERATION BY BONE-DERIVED FACTORS

P. Sluka, G. Whitty, I. D. Davis

Uro-Oncology Laboratory, The Ludwig Institute for Cancer Research, Melbourne-Austin Branch, VIC, Australia

Introduction: Paracrine interactions that occur between cancer cells and the surrounding microenvironment are known to be important for the establishment and metastasis of cancer. The most common site of prostate cancer (PC) metastasis is the bone. Numerous studies have identified bone-derived factors that influence PC cell biology. We hypothesised that bone-derived factors are able to induce the proliferation of PC cells. This study aimed to examine the effect of selected factors known to be produced by bone stroma, namely EGF, acidic FGF, HGF, ß-NGF, TGF-ß, and TNFα on the proliferation of PC cells. The effect of cell culture medium conditioned by osteoblasts was also examined.

Method: The PC-derived cell lines PC3, LNCaP, and DU145 were used in this study. Expression of receptors for the above-mentioned cytokines was assessed using real-time RT-PCR. Proliferation was assessed by MTS conversion after 5 days of continuous cytokine treatment. Osteoblast-conditioned medium was generated using the HOS, MG63, and SaOs2 osteoblast cell lines. Conditioned medium from the HT1080 fibrosarcoma cell line was used as a non-bone control.

Results: The PC cell lines expressed receptors for all of the cytokines examined at the mRNA level. LNCaP cell proliferation was increased by acidic FGF and decreased by TGF-ß. Treatment with TNFα decreased proliferation of all PC cell lines. EGF, HGF, and ß-NGF did not effect proliferation of any PC cell line. Osteoblast-conditioned medium increased proliferation of PC3 and DU145 cells but not LNCaP cells, while HT1080 (fibrosarcoma)-conditioned medium did not effect proliferation of any PC cell line.

Conclusions: These data suggest that PC cells are able to respond to defined bone-derived factors and the nature of this response varies between individual cancers. Current studies are examining the effect of these cytokines on other functional parameters, namely cell survival, adhesion, and migration, and on primary PC epithelial cells.

191

PRODUCTION OF MONOCLONAL ANTIBODIES FOR CLINICAL TRIALS AND TRANSLATIONAL CANCER RESEARCH.

A. Perani, B. Gloria, D. Wang, F. E. Smyth, A. M. Scott

Cell Biology Group, Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

The Clinical Program of the Ludwig Institute for Cancer Research (LICR) aims to translate basic laboratory discoveries into early phase clinical trials in cancer patients. The LICR Antibody Program has developed six recombinant humanized antibodies into human trials, and has a pipeline of novel antibodies in progress. Through the combined efforts of the global LICR laboratories and GMP production facilities, LICR sponsored clinical trials have been conducted in sites in the US, Europe, Australia and Japan.

A key component of the LICR approach has been to focus on the identification of antibodies selectively targeting antigens preferentially expressed in tumour tissue, and the molecular engineering of chimeric or humanised antibodies to these targets. The development of high producing cell lines is crucial in the development of each antibody construct.

The Cell Biology group is located within the Biological Development Facility of LICR Melbourne-Austin Branch and is responsible for the production of monoclonal antibodies from hybridomas or from industrially relevant mammalian cell lines.

This poster summarise the history of the group and will also detail our current capabilities for cell line development and therapeutic antibody production.

192

MANIPULATION OF EPIGENETIC CONTROL OF CANCER-TESTIS ANTIGEN (CTAG) EXPRESSION IN BLADDER CANCER CELL LINES USING AZACYTIDINE

C. J. Pezaro[1], P. Sluka[1], J. Cebon[2], G. Whitty[1], I. D. Davis[1]

[1]Uro-Onc Lab, Ludwig Institute for Cancer Research, Heidelberg, Australia

[2]Cancer Vaccine Lab, Ludwig Institute for Cancer Research, Heidelberg, Australia

Background: CTAg are considered potential targets for the immunological targeting of cancers. During normal cellular differentiation, many genes including CTAg are silenced by a process of DNA methylation. Treatment with 5-Azacytidine (Aza), a DNA demethylating agent, can lead to increased expression of CTAg in both solid tumor and lymphoid cell lines in vitro. Our aim was to investigate the effect of Aza on CTAg expression in bladder cancer cell lines, and to correlate changes in CTAg expression with changes in cell biology.

Methods: Three commercial, high-passage bladder cancer cell lines and one primary early-passage cell line were treated with Aza in two treatment schedules. CTAg expression was assessed by quantitative RT-PCR of 18 CTAg and by immunohistochemistry of cell blocks where antibodies were available. Cell biology parameters of proliferation, apoptosis, and necrosis were assessed using MTS- and CFSE-based assays.

Results: Aza significantly upregulated expression of CTAg in the 5637, LM-TCC02 and HT1197 cell lines in a dose-dependent manner, but caused no change in CTAg expression in HT1376. Cell proliferation was inhibited by higher concentrations of Aza in all cell lines, while apoptosis and necrosis were increased. The 5637 cell line was most sensitive to Aza treatment both in terms of CTAg changes and in growth inhibition.

Conclusions: Aza treatment at doses with little cytotoxic effect led to effects on CTAg expression that varied between cell lines. Ongoing experiments will address the biological effects of changes in CTAg expression, with a view to incorporating Aza treatment into an immunotherapy strategy.

193

THE EXPRESSION OF CALCITONIN RECEPTOR IN THE BRAIN TUMOUR GLIOBLASTOMA MULTIFORME

S. Nguyen, C. A. McLean, P. Hwang, S. G.M. Furness, J. V. Rosenfeld, P. J. Wookey

Medicine, University of Melbourne, Heidelberg, Australia

Calcitonin receptors (CTR) are found widely expressed in foetal tissues, normal and diseased adult tissues. CTR has been reported to be expressed in primary tumour cells associated with leukaemia, breast and prostate cancer and in the transfected cells CTR has been shown to influence cell cycle progression.

The aim of this study was to examine the expression of CTR in the brain tumour glioblastomas multiforme (GBM).

The biopsies from 12 patients diagnosed with GBM were processed for immunohistochemical staining using anti-CTR antibodies to localise CTR expression in relation to malignant cells. To further focus on CTR expression and its possible function, the glioma cell line U87 MG was investigated. CTR expression in U87MG was determined by immunoblotting, immunofluorescent staining and confocal microscopy. RT-PCR was used to investigate the DNA sequence corresponding to CTR mRNA. Furthermore, a cell proliferation assay was used to assess the possible function of CTR on U87MG cells.

11/12 of the tumour samples were positive for CTR with expression localised on malignant cells and with minimal expression on adjacent structures such as blood vessels. Immunoblotting and immunofluorescent staining demonstrated CTR protein expression in U87MG. RT-PCR and DNA sequencing confirmed the presence of hCTR. In functional assays the potent ligand salmon CT appeared to promote cellular proliferation.

In conclusion, we have demonstrated the expression of CTR in primary GBM tumour cells and glioma cell line U87 MG and CTR may moderate the growth of U87 MG.

194

CANCER-ASSOCIATED FIBROBLAST EXPRESSION OF SERINE PROTEASE FIBROBLAST ACTIVATION PROTEIN INCREASES TUMOUR METASTATIC POTENTIAL AND ALTERS CELL DEATH MECHANISMS

S. Baird[1], C. Renner[2], F. E. Smyth[1], A. M. Scott[1]

[1]Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia
[2]Oncology Department, University Hospital Zürich, Zürich, Switzerland

Fibroblast activation protein (FAP), an integral membrane serine protease, is found on cancer-associated fibroblasts (CAFs) in over 90% of human epithelial cancers. In normal adult tissues, FAP is expressed only in wound healing, and is therefore an attractive target for antibody immunotherapy of cancer. We aim to clarify the role and mechanisms of action of FAP in tumour development and metastasis.

We have transfected HT1080, a fibrosarcoma line used as a model of CAFs, with FAP and explored the metastatic potential of the FAP-expressing cells. Cells were also treated with doxorubicin and cell death markers monitored over time.

Our results demonstrated that FAP-expressing cells had higher levels of adhesion to extracellular matrix proteins, as well as being more migratory and invasive, which could be blocked using an antibody. Arrays showed altered expression of integrin-related signaling proteins and lower inflammatory cytokine secretion. Cells expressing FAP were more susceptible to doxorubicin-induced cell death, which did not resemble classical apoptosis. FAP-positive cells exhibited altered mitochondrial metabolism and increased levels of oxidative stress.

In conclusion, as expected from the enzyme activity of FAP, expression of the protease increases invasive potential. In this system, FAP has additional effects on cell signaling, metabolism and cell death mechanisms, which may modulate inflammation in the surrounding area. These observations will be explored further *in vivo* with the aim of modulating FAP by antibody therapy.

EXPRESSION OF A NOVEL GROWTH FACTOR INHIBITOR, ATIP, IN HUMAN PROSTATE CANCERS

S. N. Louis, L. Chow, L. Rezmann, A. G. Frauman, W. J. Louis

Clinical Pharmacology Unit/Dept of Medicine, University of Melbourne AH/NH, Heidelberg, VIC, Australia

Angiotensin II (Ang II), the main effector of the renin angiotensin system, acts upon two distinct transmembrane receptors, the Ang II type 1 (AT_1-) and the type 2 (AT_2-) receptor, to induce a wide range of effects in man. The AT_2-receptor through an interaction with its putative signalling partner ATIP (AT_2-receptor interacting protein) inhibits the mitogenic effects of EGF in prostate cancer cell lines representing both early and late stage disease [1 & 2]. This pilot study examines for the first time the expression of ATIP in malignant human prostatic biopsies.

We examined ATIP protein expression in fixed LNCaP and PC3 prostate cancer cells and sections of human biopsies containing benign prostatic hyperplasia (BPH), high grade prostatic intraepithelial neoplasia (HGPIN) and well, moderately and poorly differentiated prostate cancer.

ATIP immunostaining was not detected in either the basal or columnar epithelial cell layers surrounding BPH glands, however, it was observed in the neoplastic epithelial cells of HGPIN, and in the cytoplasms of the malignant cells in all grades of prostate cancer, as well as the cytoplasms of LNCaP and PC3 prostate cancer cell lines. In addition, ATIP immunostaining was identified in the stromal cells of healthy prostate tissue but this expression was lost during the pre-malignant and malignant phases.

The consistent appearance of ATIP staining in the precancerous epithelial cells and its absence in normal epithelial cells strongly suggests that re-expression of ATIP occurs at an early phase in the malignant process and that ATIP re-expression may be an early indicator of malignant development. The continued expression of ATIP in all grades of prostate cancer suggests that the AT_2-receptor/ATIP signalling pathway is present and that it may continue to be a potential therapeutic target even following the development of androgen-independence.

(1) Chow L et al. Functional angiotensin II type 2 receptors inhibit growth factor signalling in LNCaP and PC3 prostate cancer cell lines. Prostate 2008;68:651-60

(2) Louis SN et al. Expression and function of ATIP/MTUS1 in human prostate cancer cell lines. Prostate 2010; Jun 1 (Epub)

COMPARISON OF SET-UP ERROR OF TWO STABILISATION DEVICES IN HEAD & NECK IMRT AND EVALUATION OF IMAGE GUIDANCE STRATEGY

M. Rolfo, N. Anderson, D. Lim Joon, M. Wada

Austin Radiation Oncology, Heidelberg Heights, Australia

Purpose/Objective(s): The clinical consequences of set up variations in IMRT of head and neck is increasingly apparent. Robust stabilization together with appropriate image guidance strategies are imperative to minimize set up errors. This study compared the daily set up error of the institutional stabilization devices leading to an assessment of the current institution image guidance (IG) protocol.

Materials/Methods: Twenty patients were stabilized using the conventional immobilization device (CID) consisting of a customized head and neck support with a head and neck thermoplastic mask. Twenty patients were stabilized using a new immobilization device (NID) consisting of a body cradle encompassing the upper arms and shoulders together with the mask. Daily Cone-Beam-CT were acquired and displacements in medio-lateral (M-L), cranio-caudal (CC) and antero-posterior (A-P) directions were analyzed. A volume-of-interest (VOI) placed around upper cervical vertebrae (C1-6) and displacement figures calculated using the automated bony match algorithm. Daily set up error statistics were then summarized.

Results: 1164 data sets were analyzed. Using the NID, systematic displacement of the group reduced from 1.44 to 0.89mm in M-L; 1.5 to 0.98 mm in CC; and 1.4 to 0.97mm in A-P directions compared to the CID cohort. The random displacement reduced from 1.92 using CID to 1.06 mm using NID in M-L; 2.00 to 1.12mm in C-C and 1.90 to 1.17mm in A-P directions. Displacements of 3mm or greater in M-L was 19.4% of fractions with CID compared to 3.8% for the NID, 21.2% (CID) and 7.4% (NID) in C-C and 20.3% (CID) 3.4% (NID) in A-P plane.

Conclusion: This study demonstrated the improvement in daily set up error with the NID device. An analysis of 3D data sets confirmed a reduction in both systematic and random error, thus minimizing the level of set-up uncertainty. Trend analysis of the positional and distortional stability of the initial five fractions enables customized patient specific IGRT strategies and tailored CTV-PTV margins.

IN VIVO COMPARISON OF THE DNA PROLIFERATION TRACER [18F]-FLETT WITH [18F]-FLT IN A MOUSE TUMOR MODEL

G. A. Cartwright[1,3], U. Ackerman[2,3], G. O'Keefe[2,3], S. Lee[1,2], S. Gong[2], H. J. Tochon-Danguy[2,3], A. Rigopoulos[1], D. Cao[1], J. I. Sachinidis[2], A. M. Scott[1,2,3]

[1]Ludwig Institute For Cancer Research, Heidelberg, VIC, Australia
[2]Centre For PET, Austin Health, Heidelberg, VIC, Australia
[3]The University of Melbourne, VIC, Australia

Imaging techniques such as PET are proving to be sensitive and specific tools for the diagnosis, monitoring and subsequent management of cancer. In particular, the tumour metabolism of glucose and thymidine through the use of [^{18}F]-fluoro-2-deoxy-D-glucose (FDG) and [^{18}F]-3'-fluoro-3'-deoxy-L-thymidine (FLT) have been used extensively to study tumour growth and DNA synthesis respectively. Such imaging however may be enhanced by the development of new imaging compounds with improved pharmacokinetic properties.

Objectives: This study compares the pharmacokinetics of [^{18}F]FLT and the novel [^{18}F]FLETT tracer uptake via imaging studies.

Methods: [^{18}F] 2-Fluoroethyl azide was synthesised from toluenesulfonic acid-2-azidoethyl ester and dried K[^{18}F] kryptofix 2.2.2 complex. The reaction mixture was distilled into a reaction vessel where click chemistry was performed at 80ºC for 20 minutes using copper (I) iodide with L-ascorbic acid. The reaction mixture was purified by reversed phase HPLC, giving [^{18}F]FLETT in 70% radiochemical yield.

Results: For *in vivo* evaluation, A431 (human epithelial carcinoma) tumours were established in BALB/c nude mice. Tumours were allowed to grow to a minimum size of 300mm^3. For comparison purposes, the FLT scans were performed on two mice four days after the FLETT scan.

For the FLETT images, the mice showed high bowel and cardiac uptake. The FLT, on the other hand, presented low bowel uptake but more generalised uptake whilst the bladder region showed significant uptake. There was lesion uptake in both FLETT and FLT images but unlike FLETT, FLT images indicated central necrosis.

Conclusions: FLETT seemed to be a promising tracer uptake. However, further studies need to be done to analyse potential metabolites which could be responsible for the high cardiac uptake. The usefulness of FLETT as a diagnostic tool to image tumour response to treatment will also be evaluated. Future studies will evaluate FLETT uptake with xenografts of similar size.

A STATISTICAL TREATMENT OF THE INTEGRAL DVH

C. S. Hamilton[1], V. Mak[2], T. Kron[3], S. Senthi[3]

[1]Radiation Oncology Centre, Austin Health, Heidelberg West, VIC, Australia
[2]School of Information Technology, Deakin University, Burwood, VIC, Australia
[3]Radiation Oncology Centre, Peter MacCallum Institute, East Melbourne, VIC, Australia

Background and Purpose: The delivery of high quality radiotherapy requires a homogeneous dose distribution which is conformed closely to the planning target volume. Several mathematical tools have been used over the years to assess the quality of the dose distributions (cold and hot spots). The Cumulative Dose Volume Histogram (cDVH) has several graphic and mathematical limitations in the assessment of Planning Target Volume (PTV) homogeneity. We present a statistical treatment of the typical PTV DVH using kurtosis, skewness, and an Mu-factor as an improved approach to existing indices.

Materials and Methods: Eight DVH displays from prostate IMRT patients are analysed using previously published measures of homogeneity and kurtosis, skewness and Mu-factor.

Results: Typical dose distributions from prostate and IMRT patients are shown to be non normally distributed and measures such as mean dose and standard deviation may not be appropriate. Kurtosis proves to be a better discriminant in this dataset than existing indices. The use of the Standard Deviation, Conformity Index, Homogeneity Index in this dataset are shown to have limitations.

DVH Descriptor	Coefficient of Variation	Coefficient of Correlation*	Slope
Kurtosis	40.8%	N/A	N/A
Sigma Index	17.8%	0.03	-0.08
Skewness	15.3%	0.96	0.28
Tail EUD	27.8%	0.27	-0.43
Peak Gradient	31.7%	0.09	0.39
Flexure Dose	0.4%	0.13	11.6
Mean Dose	0.8%	0.29	35.6
Homogeneity Index	1.7%	0.07	0.005
Conformity Index	23.7%	0.29	0.05
V95%	2.1%	0.37	96.2
V1%	1.1%	0.05	18.8
X Index	37%	0.25	24

Figure 1. The coefficient of variation for each of the PTV DVH descriptors and the linear regression values (RHO and slope) for each descriptor against kurtosis.

Conclusions: The integral DVH (iDVH) is shown to be the preferred plan comparison tool over the cDVH. The Tail EUD and Kurtosis both have discriminatory value for assessing the PTV homogeneity. The modal dose may represent a valuable dose reporting point in the IMRT era.

CANCER TESTIS (CT) ANTIGEN (AG) EXPRESSION IN NON-SMALL CELL LUNG CARCINOMA (NSCLC) PAIRED PRIMARY (1°) AND SECONDARY (2°) TUMOURS.

P. Mitchell[1], F. J. Chionh[1], T. John[1,2], A. Chakrabarti[3], S. Deb[4], S. Knight[5], J. S. Cebon[1,2]

[1]Medical Oncology, Austin Health, Heidelberg, VIC, Australia
[2]Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia
[3]Monash Medical Centre, VIC, Australia
[4]Pathology, Austin Health, Heidelberg, VIC, Australia
[5]Thoracic Surgery, Austin Health, Heidelberg, VIC, Australia

Background: The importance of CTAgs in NSCLC was recently highlighted with evidence that MAGE-A3 expression is not only a poor prognostic factor but also a promising target for adjuvant immunotherapy (Vansteenkiste, Proc ASCO 2007; abs 7554). With progressive stage, loss of or failure to acquire CTAg expression may indicate susceptibility of the tumor to immunoediting, where selective pressure from the host immune response results in Ag-negative variants that evade immune recognition. This may facilitate disease progression. This has implications for the effectiveness of cancer vaccination. We therefore compared expression of CTAgs in paired NSCLC 1° and metastatic 2° tumour tissue.

Methods: 43 patients (pts) with histologically confirmed 1° NSCLC resected at Austin Hospital from April 1, 1992 to April 1, 2009, with available paired 2° lung or brain metastases were studied. Immunohistochemical staining was performed on tissue microarrays of formalin-fixed paraffin-embedded tissues, with human testis as the positive control. We examined the proportions of tumours expressing 4 CTAgs (MAGE-A3, MAGE-A1, NY-ESO-1, MAGE-C1) in the paired samples.

Results: Among all 1° or 2° tumours MAGE-A3 was most frequently expressed in 65%, followed by MAGE-C1 in 56%, MAGE-A1 in 47% and NY-ESO-1 in 40%. Expression of any CTAg was seen in 22/27 (81.5%) 2° NSCLC brain metastases and in 10/16 (62.5%) 2° NSCLC lung metastases, p = 0.25. CTAg expression occurring in both 1° and 2° tumours was most common for MAGE-A3 (44%) and MAGE-C1 (26%). Acquisition of expression in 2° tumours occurred most commonly for MAGE-A1 (26%) and least commonly for NY-ESO-1 (9%), whereas loss of CTAg expression in 2° tumours was most common for NY-ESO-1 (19%) and MAGE-C1 (14%).

Conclusions: With progression to metastatic NSCLC, NY-ESO-1 expression was most frequently lost and least frequently acquired. In contrast, MAGE-A1 was the CTAg most frequently acquired in 2° tumours. Results of this small series support previous observations in malignant melanoma that the highly immunogenic CTAg, NY-ESO-1 may be particularly susceptible to immunoediting, whereas MAGE-A1 may be less so (Barrow et al 2006).

No significant financial relationships to disclose.

(1) Barrow. Clin Cancer Res. 2006;12:764-71

OPTIMISING POSACONAZOLE USAGE AS ANTIFUNGAL PROPHYLAXIS IN ACUTE LEUKAEMIA

L. Ho, J. Eyles

Pharmacy, Austin Health, Heidelberg, VIC, Australia

Background: The implementation of guidelines for antifungal prophylaxis in patients with acute myeloid leukaemia or acute lymphoblastic leukaemia pre-allogeneic stem cell transplant resulted in a greater than anticipated increase in posaconazole expenditure .

Aim: To evaluate the effectiveness of a multi-faceted intervention aimed at optimising cost-effective use of posaconazole.

Methods: Patients who received posaconazole for antifungal prophylaxis according to the guideline were identified from pharmacy dispensing records. A pharmacist extracted data retrospectively from medical and dispensing records using an explicit data collection tool. Pre-intervention data was collected between June 2009 and February 2010. Post-intervention data was collected between March and May 2010.

Pre-intervention data informed the content of the intervention. The intervention involved optimising use of the PBS scheme for eligible patients and educating pharmacy and nursing staff about the cost of posaconazole and methods to minimise wastage (e.g. storing individually dispensed in-patient stock in the pharmacy for use during the patient's next admission).

Results: 19 and 11 patients in the pre- and post-intervention periods, respectively, were commenced on posaconazole for antifungal prophylaxis according to the guidelines. Pre-intervention, 47% of posaconazole expenditure was PBS reimbursable. This increased to 70% after the introduction of the intervention ($p<0.001$). Monthly net expenditure has significantly declined from \$17,852 ± 3,776 pre-intervention to \$5,254 ± 2010 post intervention ($p=0.001$). Qualitative data suggests that staff awareness of posaconazole costs and wastage minimisation has greatly improved.

Conclusion: Targeted interventions have been effective in optimising posaconazole usage and minimising hospital expenditure.

DEVELOPMENT OF A HOSPITAL PHARMACIST PREPARED INTERIM RESIDENTIAL CARE MEDICATION ADMINISTRATION CHART TO FACILITATE CONTINUITY OF MEDICATION ADMINISTRATION AFTER DISCHARGE TO RESIDENTIAL CARE

R. Elliott[1,2], T. Tran[1], P. Harvey[3], S. Taylor[1], S. Cheung[1], L. Carter[3], M. Belfrage[4]

[1]Pharmacy Department, Austin Health, Heidelberg, VIC, Australia
[2]Centre for Medicine Use and Safety, Monash University, Parkville, VIC, Australia
[3]Aged Care, Northern Health, Bundoora, VIC, Australia
[4]North East Valley Division of General Practice, Heidelberg, VIC, Australia

Aim: To describe development of a hospital pharmacist prepared interim residential care medication administration chart (IRCMAC) to address gaps in continuity of medication management during transition from hospital to residential care.

Methods: Development of the IRCMAC was informed by analysis of data from a pre-intervention study of medication errors following discharge to residential care (RC), and extensive stakeholder consultation. Stakeholders included the Aged Care Accreditation and Standards Agency, Australian Nurses Federation, Victorian Drugs and Poisons Unit, RC staff, general practitioners, hospital doctors, and community and hospital pharmacists. To ensure sustainability and maximise patient safety, a semi-automated process for producing the IRMCAC was developed, designed to:

· have minimum possible impact on hospital staff workload

· avoid manual transcription of discharge medication lists

· ensure the IRCMAC was produced *after* pharmacist reconciliation and review of discharge prescriptions.

Results: The IRCMAC was designed to last for 7 days, enabling medication administration to proceed while waiting for the patient's GP to attend the RC facility and write a new medication chart. Sections on the IRCMAC for communicating medication changes were also included to address identified discharge communication gaps. Software modules were developed that could link or integrate with the hospitals' pharmacy dispensing software, to automatically populate the IRCMAC with the medications prescribed on discharge.

Conclusion: A semi-automated electronic process enabling hospital pharmacists to produce IRCMACs was successfully developed and implemented. Stakeholder consultation (and post-intervention evaluation - presented separately) indicate the IRCMAC is an efficient, highly effective method for improving continuity of medication management.

202

IMPACT OF A HOSPITAL PHARMACIST PREPARED INTERIM RESIDENTIAL CARE MEDICATION ADMINISTRATION CHART ON MEDICATION ADMINISTRATION ERRORS AFTER DISCHARGE FROM HOSPITAL TO RESIDENTIAL CARE (MEDGAP PROJECT)

R. Elliott[1,2], T. Tran[1], P. Harvey[3], S. Taylor[1], M. Belfrage[4,5], J. Marriott[2], R. Jennings[3], L. Carter[5]

[1]Pharmacy, Austin Health, Heidelberg, VIC, Australia
[2]Centre for Medicine Use and Safety, Monash University, Parkville, VIC, Australia
[3]Aged care, Northern Health, Bundoora, VIC, Australia
[4]North East Valley Division of General Practice, Heidelberg, VIC, Australia
[5]Pharmacy, Northern Health, Epping, VIC, Australia

Aim: To test the impact of a hospital pharmacy-provided interim residential care medication administration chart (IRCMAC) on medication errors and use of locum medical services.

Methods: Patients discharged from inpatient wards to a residential care facility (RCF) over a 3-month period were studied. Data were collected from hospital records and structured telephone interviews with RCF staff approximately 24 hours after discharge. This was followed by extensive stakeholder consultation and development of an electronically-generated IRCMAC and associated policies and procedures for RCFs and hospitals. Following implementation of the IRCMAC, data collection was repeated over another 3-month period. The primary endpoint was: proportion of patients experiencing a medication administration error, defined as a missed dose, significantly delayed dose (more than 50% of prescribed dose-interval), or wrong drug/dose. A secondary endpoint was: proportion of patients who required locum medical attendance at the RCF.

Results: The number of patients who experienced one or more medication administration errors fell from 41/202 (20%) in the pre-intervention period to 5/226 (2%) following introduction of the IRCMAC (difference in percentages 18%, 95%CI 12-24%, $p<0.001$). The majority of medication errors in both groups were missed doses (80%). The number of patients who received a locum medical attendance fell from 66/202 (33%) to 25/226 (11%) patients (difference in percentages 22%, 95% CI 13-30%, $p<0.001$).

Conclusion: Lack of an updated medication chart at RCFs was a barrier to continuity of care and source of unnecessary use of locum medical services. A hospital pharmacy-provided IRCMAC significantly reduced errors and locum attendances.

203

DETERMINATION OF MEDICATION STORAGE TEMPERATURES IN HOSPITAL-IN-THE-HOME (HITH)

L. A. Williams[1], T. To[1], S. Taylor[1], K. Bowler[2], N. Burnett[2]

[1]Pharmacy, Austin Health, Heidelberg, VIC, Australia
[2]HITH, Austin Health, Heidelberg, VIC, Australia

Aim: To determine the temperatures to which medications are exposed when used in HITH.

Methods: Patients requiring HITH services for antibiotic therapy between January-March 2010 (summer) were invited to participate. Participants placed temperature data-logger(s) with their medications. The number of loggers varied depending on the type and/or where medications were stored during HITH e.g., fridge, bedroom, with infusion pump. Loggers recorded the temperature every 30 minutes and remained with the medications for duration of HITH treatment or for a maximum of 6 weeks. Participants also completed a questionnaire regarding medication storage and air-conditioning in their home.

Results: Twenty-four participants completed the study. The logger recordings for medications stored at 'room temperature' or infusion pumps were mainly between 17-27ºC. However, for 13 participants the temperature exceeded 30ºC (at least 1 reading), with 4 having readings above 35ºC and 1 logger recording 41ºC. Refrigerated temperatures ranged from -10ºC to +15ºC.

Questionnaires were completed by 18 participants (75%) and showed that medications were stored in a variety of locations. Twelve of these 18 participants (67%) would 'always' enquire about medication storage and 17 (94%) had air-conditioning in their home.

Conclusion: Temperatures to which medications are exposed in HITH varied greatly. Of particular concern were the readings above 30ºC for medications or infusion pumps intended for storage at 'room temperature', and the -10ºC and +15ºC readings for refrigerated items. Although participants might enquire about medication storage and many have air-conditioning, the results indicate that medications can be exposed to extremes of temperature in HITH.

204

A COMPARISON OF ORAL AND INTRAVENOUS SODIUM BICARBONATE ON URINARY PH IN PATIENTS AT RISK OF CONTRAST-INDUCED NEPHROPATHY: A PILOT STUDY.

T. To[1], F. Chahadi[2], M. Freeman[2], M. Pan[3], O. Farouque[2], P. Mount[3]

[1]Pharmacy Department, Austin Health, Heidelberg, VIC, Australia
[2]Cardiology Department, Austin Health, Heidelberg, VIC, Australia
[3]Renal Department, Austin Health, Heidelberg, VIC, Australia

Aim: Intravenous sodium bicarbonate infusion has been shown to reduce the risk of contrast-induced nephropathy (CIN). We sought to determine whether oral sodium bicarbonate has a similar effect on urine pH to intravenous sodium bicarbonate in patients at risk of CIN.

Method: Cardiology and Renal unit patients scheduled for a non-emergency contrast-enhanced procedure and who were eligible for intravenous bicarbonate administration per Austin Health guidelines to prevent CIN were invited to participate. Participants received intravenous bicarbonate (150 mmols/L) at 3 mL/kg/hour one hour prior, then 1 mL/kg/hour during and for 6 hours after the contrast procedure. Participants returned to the hospital 2 to 3 weeks following their contrast procedure for oral bicarbonate administration. Participants took 16 x 840 mg bicarbonate capsules (160 mmol) over a 7-hour period with 1L of water. Urine pH was measured before and after both intravenous and oral bicarbonate using a handheld pH meter.

Results: Eight participants completed the study. Intravenous bicarbonate increased urine pH from 6.15 to 6.81 ($p = 0.002$), while oral bicarbonate increased urine pH from 5.68 to 6.55 ($p = 0.013$) ($n = 8$). The mean change in urine pH was 0.66 ± 0.38 with intravenous bicarbonate and 0.87 ± 0.74 for oral bicarbonate ($p = 0.43$).

Conclusion: These data suggest that oral sodium bicarbonate has a similar effect on urine pH to intravenous sodium bicarbonate when administered to patients at risk of CIN. A randomised controlled study is required to determine if oral sodium bicarbonate can reduce the risk of CIN.

205

RENIN SECRETION IS AUGMENTED IN ACETYL-COA CARBOXYLASE KNOCKIN MICE IN RESPONSE TO A SALT DEFICIENT DIET

S. Choy[1,2,3], S. A. Fraser[3], N. Cook[1], M. Katerelos[3], B. Van Denderen[4], K. Gleich[3], P. Mount[1], B. Kemp[4], D. A. Power[1,2,3]

[1]Nephrology, Austin Health, Heidelberg, VIC, Australia
[2]Medicine, The University of Melbourne, Heidelberg, VIC, Australia
[3]IBAS, Austin Health, Heidelberg, VIC, Australia
[4]Protein Chemistry and Metabolism Unit, St Vincent's Institute of Medical Research, Fitzroy, VIC, Australia

Aim: To determine whether the effect of AMP-activated protein kinase (AMPK) on renin secretion is mediated through phosphorylation of acetyl-CoA carboxylase (ACC), the major controller of fatty acid metabolism.

Background: As part of its function to increase energy supply during energy stress, AMPK increases fatty acid oxidation by phosphorylating ACC. We have previously demonstrated that AMPK binds the sodium co-transporter NKCC2, a major regulator of renin secretion. AMPK-β1 null mice have an exaggerated increase in plasma renin concentration in response to salt restriction. Since ACC is a major phosphorylation substrate for AMPK, we aimed to determine whether AMPK regulates renin secretion through its phosphorylation of ACC.

Methods and Results: Co-immunoprecipitation experiments demonstrated that ACC and AMPK both bind NKCC2. Pull-downs of ACC showed that it binds primarily to NKCC2 and not AMPK. In salt-restricted mice, there was increased AMPK activation and phosphorylation of ACC in WT mice, which was significantly reduced in AMPK-β1 null mice. Similarly, salt restriction in knockin mice with a mutation in the ACC phospho-site for AMPK showed a significant increase in renin compared to controls.

Conclusions: These studies indicate that both AMPK and ACC bind the salt co-transporter NKCC2. AMPK is activated by a salt deficient diet and phosphorylates ACC. ACC knockin mice have an augmented renin secretion in response to a salt deficient diet. These results suggest that AMPK suppresses renin secretion in response to a low salt diet by regulating fatty acid metabolism.

THE USE OF IOHEXOL CLEARANCE TO CALCULATE REFERENCE GFR IN AN EVALUATION OF THE UTILITY OF EGFR EQUATIONS IN THE INDIGENOUS AUSTRALIAN POPULATION.

A. Ellis[1], L. Maple-Brown[2,3], P. Lawton[3], J. Hughes[2], S. Sharma[2], G. Jones[4], R. MacIsaac[6], S. Panagiotopoulos[6], K. O'Dea[5], G. Jerums[6]

[1]Clinical Pharmacology and Therapeutics, Austin Health, Heidelberg, VIC, Australia
[2]Menzies School of Health Research, Darwin, NT, Australia
[3]Division of Medicine, Darwin Hospital, Darwin, NT, Australia
[4]Chemical Pathology, St Vincent's Hospital, Sydney, NSW, Australia
[5]Sansom Institute, Adelaide, SA, Australia
[6]Endocrinology, Austin Health, Heidelberg, VIC, Australia

Aim: to provide a practical reference measure of glomerular filtration rate (GFR) suited to application in remote areas of Australia.

Background: The 'eGFR Study'(1) is an ongoing large cross sectional study of the Indigenous Australian population comparing the accuracy of the following GFR estimates (MDRD 4-variable formula, CKD-Epi equation, Cockcroft-Gault formula and estimates derived from cystatin C levels) with a reference GFR measure. Reference GFR measured commonly by radio-isotopic markers is unsuitable for application in remote areas of Australia. Iohexol is an inert stable substance cleared from the body by glomerular filtration and measurement of its clearance from blood provides a practical, validated measure of GFR(2-4).

Methods: an RP-HPLC assay was developed and validated to measure plasma Iohexol. Analysis of samples collected 2-, 3- and 4-hours after Iohexol dose permitted calculation of reference GFR by the slope-intercept method.

Results: Validation using spiked plasma displayed within- and between-day precision of <=1.0% and inaccuracy <=4.5%, using matrix matched standards. Freeze/thaw stability was also confirmed. Samples from approximately 300 subjects have been successfully analysed.

Conclusions: A suitable procedure for plasma Iohexol measurement and reference GFR calculation has been developed and applied to approximately half of the planned 600 subjects for the 'eGFR Study'. Recruitment is expected to be complete by late 2010.

(1) 1. Maple-Brown L et al (2010) Study Protocol - Accurate assessment of kidney function in Indigenous Australians: aims and methods of the eGFR Study. Biomed Central - Public Health 10:80
(2) 2. Krutzen E et al (1984) Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. J Lab Clin Med 104:955–961.
(3) 3. Houlihan C et al. (1999) A comparison of the plasma disappearance of iohexol and 99mTc-DTPA for the measurement of glomerular filtration rate (GFR) in diabetes. Aust N Z J Med. 29:693-700
(4) 4. Brown SC and O'Reilly PH. (1991) Iohexol clearance for the determination of glomerular filtration rate in clinical practice: evidence for a new gold standard. J Urol 1991; 146:675–679.

207

ANGIOTENSIN 1-7 INCREASES CARDIAC FIBROSIS IN RENAL INJURY

E. Velkoska, R. G. Dean, L. J. Burchill, K. Griggs, L. M. Burrell

Medicine, The University of Melbourne, Heidelberg, VIC, Australia

Angiotensin converting enzyme (ACE) 2 is expressed in the heart and kidney, and metabolises angiotensin (Ang) II to Ang 1-7, a peptide with anti-fibrotic effects, that acts via the Ang 1-7 or mas receptor. This study assessed the effect of Ang 1-7 on blood pressure and cardiac remodelling in a rat model of renal mass ablation.

Male Sprague Dawley rats underwent subtotal nephrectomy (STNx) and were treated for 10 days with vehicle, the ACE inhibitor, ramipril (oral, 1 mg/kg/d) or Ang 1-7 (s.c 24 µg/kg/hr) (all, n=15 /gp). A control group (n=10) of sham-operated rats were also studied.

STNx rats were hypertensive ($P<0.01$) with renal impairment ($P<0.001$), cardiac hypertrophy ($P<0.001$) and fibrosis ($P<0.05$), and increased cardiac ACE ($P<0.001$) and ACE2 activity ($P<0.05$). Ramipril reduced blood pressure ($P<0.01$), improved cardiac hypertrophy ($P<0.001$), and inhibited cardiac ACE ($P<0.001$). By contrast, Ang 1-7 infusion in STNx caused further increases in blood pressure ($P<0.05$), cardiac hypertrophy ($P<0.05$) and fibrosis ($P<0.01$). Ang 1-7 also increased cardiac ACE ($P<0.001$), and reduced cardiac ACE2 activity ($P<0.05$) compared to STNx-vehicle rats.

Our results show that treatment with Ang 1-7 has adverse cardiovascular effects in rats with kidney failure due to renal mass reduction. Our results add to the increasing evidence that Ang 1-7 may have deleterious effects in kidney disease, and highlight the need for further in vivo studies of the ACE2/Ang-7/mas receptor axis in this increasingly common condition.

209

DEFECT IN ALBUMIN DEGRADATION IN THE PROXIMAL CONVOLUTED TUBULE OF LIMP2 KNOCKOUT MICE

D. H.K. Lee[1,2], M. J. Desmond[1,2], S. A. Fraser[1], M. Katerelos[1], K. Gleich[1], S. F. Berkovic[2,3,4], D. A. Power[1,2]

[1]Department of Nephrology, Austin Health, Heidelberg, VIC, Australia
[2]Departmentof Medicine, University of Melbourne, Parkville, VIC, Australia
[3]Epilepsy Research Centre, University of Melbourne, Parkville, VIC, Australia
[4]Departmentof Neurology, Austin Health, Heidelberg, VIC, Australia

Mutations of the lysosomal membrane protein SCARB2/Limp2 cause Action Myoclonus Renal Failure Syndrome (AMRFS) in humans, with collapsing focal segmental glomerulosclerosis (FSGS) manifesting as heavy proteinuria and kidney failure. Limp2 KO mice, however, have a milder renal phenotype and develop tubular proteinuria and albuminuria.

To identify the defect in tubular protein handling, Limp2 KO mice were injected with horseshoe peroxidase (HRP) and fluorescein-conjugated albumin.

Uptake of HRP by the proximal tubule (PCT) at 7 minutes showed no difference between KO and WT mice, indicating normal fluid phase endocytosis. Uptake of albumin at 7 minutes was also similar; however, at 30 minutes, there was persistence of albumin in the PCT of KO mice with a basolateral distribution compared to the original uptake, indicating a failure of albumin degradation. Normal tubular uptake in the KO mice was confirmed by similar expression of the proteins taken up by the receptors megalin and cubilin along the brush border of the PCT. Co-staining of retinol binding protein (RBP) taken up by PCT, and cathepsin B, a lysosomal enzyme, showed similar expression of cathepsin B in the PCT of KO mice, but greater amount of RBP not co-localising with lysosomes. Electron microscopy demonstrated an accumulation of large vacuoles in the PCT of KO mice, some apparently in contact with the luminal and/or basolateral surface of the cells. The appearance was similar to that seen in EBV-transformed lymphoblastoid B cells from 2 patients with AMRFS.

This data suggests that the absence of Limp2 in the PCT leads to a failure of fusion of endosomes with lysosomes, persistence of the proteins reabsorbed by the PCT, and their eventual release from the surface of the cell into the urine. This data does not support the proposed ability of the PCT to reabsorb significant quantities of albumin without lysosomal degradation.

SECULAR TRENDS IN CARDIOVASCULAR MORTALITY RATES OF PATIENTS RECEIVING DIALYSIS COMPARED TO THE GENERAL POPULATION

M. A. Roberts[1,2], K. R. Polkinghorne[3], S. P. McDonald[4], F. L. Ierino[1,2]

[1]Nephrology, Austin Health, Heidelberg, VIC, Australia
[2]Medicine, University of Melbourne, Heidelberg, VIC, Australia
[3]Nephrology, Monash Medical Centre, Clayton, Australia
[4]Nephrology, The Queen Elizabeth Hospital, Adelaide, SA, Australia

We analysed cardiovascular mortality rates (CVMR) of dialysis patients over time and compared these to the general population.

CVMR were calculated from ANZDATA Registry data for Australian patients receiving dialysis from 1992-2005, and from Australian Bureau of Statistics data for the comparable general population. Patients were included if dialysis was the first renal replacement therapy, and censored at transplantation or three years from commencing dialysis. Age-stratified CVMR were reported per hundred person-years and Relative Risks (RR) calculated relative to the Australian population rates. Secular changes in CVMR were analysed with negative binomial regression.

The analysis included 24,352 patients with 68,407 person-years of follow up and 5,134 cardiovascular deaths. CVMR declined over time in the general population (not shown). Age-stratified CVMR declined in patients on dialysis aged 55-74 years but the corresponding RR increased over this time (Table). The RR of dialysis patients increased markedly with younger age, a trend which did not alter over time. The risk associated with dialysis compared to the general population increased over time (p=0.044 for interaction). CVMR in Australia were comparable to those in the United States and Europe.

Although cardiovascular mortality rates are falling, the excess cardiovascular risk in dialysis patients compared to the general population is increasing over time.

Age-stratified CVMR and RR (95% CI) in 1992-1994 and 2004-2005

Era:	CVMR per 100 person-years		RR	
Age	1992-1994	2004-2005	1992-1994	2004-2005
35-44	2.5 (1.4-4.4)	3.3 (2.3-4.9)	122 (69.1-214)	192 (130-284)
45-54	3.5 (2.4-5.3)	3.2 (2.4-4.4)	46.9 (31.4-70.0)	69.0 (51.0-93.4)
55-64	7.3 (5.8-9.1)	6.0 (5.0-7.3)	26.3 (20.9-33.1)	49.2 (40.6-59.5)
65-74	9.4 (7.7-11.4)	6.6 (5.6-7.8)	10.5 (8.6-12.7)	16.6 (14.1-19.5)
75-84	14.2 (9.8-20.6)	10.2 (8.8-11.8)	4.8 (3.3-6.9)	6.4 (5.5-7.4)