Research Week
15 - 19 October 2012

Abstracts
Austin LifeSciences Research Week 2012 Program

Monday 15 October
Lunchtime forum
12 noon - 1.00pm
John Lindell lecture theatre

Professors professing  Speakers: Recent recipient of AMRF Distinguished Scientist Award, Prof David L Hare (2011 winner) and Prof Sue Walker (Mercy Hospital for Women), followed by lunch.

Mini oral sessions
2.00 - 4.00pm
Education Precinct L4 Austin Tower
Concurrent mini-oral sessions featuring the work of junior research staff.

Tuesday 16 October
Lunchtime Debate
12 noon - 1.00pm
John Lindell lecture theatre

“The problem with research is that 95% of it never cures anything” Moderated by Dr Brendan Murphy.
Followed by lunch

Poster session; Group I categories
2.30 - 3.30pm
Rooms 4.4 and 4.5, Education Precinct
L4 Austin Tower
AND
Collaboration Space L5 Olivia Newton John Cancer and Wellness Centre

Wednesday 17 October
Melbourne Brain Centre Symposium
Platform Technologies
10.30 - 11.30am
Lecture theatre L2
Melbourne Brain Centre
Morning tea from 10.00am

Austin LifeSciences Symposium
Lunchtime Lecture
12 noon - 1.30pm
John Lindell lecture theatre
Austin Medical Research Foundation Young Investigator Award Finalists present their work, followed by lunch.

Austin by Design Showcase Event
1.30 - 4.00pm
Education Precinct Lecture theatre/breakout area.
The Austin by Design Showcase event is a great opportunity to share improvements of patients and staff experiences with the broader Austin Health community
Thursday 18 October
Plenary session
and Awards Presentation
12 noon - 1.15pm
John Lindell Lecture theatre
Speaker: James F Bishop AO MD MMed MBBS FRACP FRCPA Executive Director, Victorian Comprehensive Cancer Centre; Professor of Cancer Medicine University of Melbourne, followed by lunch

Poster session; Group 2 categories
2.30 - 3.30pm
Rooms 4.4 and 4.5, Education Precinct
L4 Austin Tower
AND
Collaboration Space L5 Olivia Newton John Cancer and Wellness Centre

Friday 19 October
RJ Pierce Symposium
Cancer genomics revolution and its implications for therapy
12 noon - 1.00pm
John Lindell Lecture theatre
An overview of the cancer genome sequencing initiatives currently underway: Prof. David Bowtell
Use of genomics for improving cancer therapy: Prof Jonathan Cebon
BRAFi in melanoma. The complexities of using targeted therapies: Dr Miles Andrews.
Austin LifeSciences Research Week 2012

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<td>AMRF Distinguished Scientist Award</td>
<td>To honour Professor Scheffer’s contribution to the research community at Austin Health, in both clinical and basic research and her commitment to fostering young people into research and supervision of post-graduate degree students.</td>
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<td>Jeannette Milgrom</td>
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<td>A Neonatal Intervention to Improve Development in Preterm Infants: outcomes of a randomised controlled trial to 6 months of age</td>
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INTRODUCTION

Welcome to the 20th annual Austin LifeSciences Research Week. Once again we take time out to recognise and celebrate the quality and the breadth of the research being performed on this site through Austin health and its departments, its affiliated universities and research institutes, and also Mercy Hospital for Women. It is a time when people can share experiences and set up new collaborations, and to think towards the future in terms of cooperative grant applications, attracting future students and scientists, and cementing our place as one of the leading research centres in Australia. The research culture at Austin is vibrant and part of what we do every day. Already this is leading to changes in health care policy and practice and ultimately to better health outcomes for all of us.

Once again the Research Week Committee has considered your feedback very carefully and made changes to the format. Not all of the suggestions were physically possible or even legal, and certainly not without appropriate ethics approval! In 2011 we had a record number of abstracts submitted leading to unprecedented challenges in terms of how to accommodate everyone. We recognise that not every solution is perfect for everyone and we will gratefully consider suggestions for future years, once any packages have been cleared by the bomb squad.

In 2012 we have:
- continued the format of two separate poster sessions;
- provided a significant number of opportunities for researchers to present their work orally during the mini-oral sessions;
- continued the RJ Pierce Symposium, an event first held in 2009 to honour the contribution of the late Professor Rob Pierce;
- continued a Research Week debate that will be both entertaining and thought-provoking.

Other successful initiatives such as the Professors Professing seminar are also continuing. Several excellent abstracts have been chosen for oral presentation and will be competing for a substantial prize 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.

Our plenary session this year is highlighted by Professor James F Bishop AO MD MMed MBBS FRACP FRCPA Executive Director, Victorian Comprehensive Cancer Centre; Professor of Cancer Medicine University of Melbourne. As always, 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 2012 to be most interesting and enjoyable.

A/Prof Anthony Verberne
Chair
Austin LifeSciences Research Week Committee
Rifaximin for refractory hepatic encephalopathy: Impact on hospital admissions and length of stay at a liver transplant centre.
Zaid Ardalan, Sujievvan Chandran, Paul Gow, Adam Testro, Peter Angus

Risk stratification of upper GI bleeding with an esophageal capsule
Sujievvan Chandran, Adam Testro, Siddarth Sood, Rhys Vaughan, Paul Froomes

The utility of upfront double wire guided biliary cannulation technique following early unintentional pancreatic wire cannulation in patients undergoing endoscopic retrograde cholangiopancreatography.
Sujievvan Chandran, Mehrdad Nikfarjam

The safety and efficacy of tenofovir disoproxil fumarate (TDF) substitution for HBIG in liver transplantation patients receiving long-term low dose IM HBIG / lamivudine (LAM) prophylaxis.
Ilana Gory, Edward Gane, Paul Gow, Julie Pavlovic, Peter Angus

Different measures of HCV rapid fibrosis post liver transplant have different abilities to predict rapid graft cirrhosis
Jessica Howell, Michael Fink, Rohit Sawnhey, Peter Angus, Peter Crowley, Kumar Visvanathan, Robert Jones, BZ Wang, Paul Gow

NK cell marker expression is dysregulated post liver transplantation and correlates with HCV viral load
Jessica Howell, Rohit Sawnhey, Narelle Skinner, Dilip Ratnam, Peter Angus, Paul Gow, Kumar Visvanathan
Cyclosporine and tacrolimus have different inhibitory effects on toll-like receptor signalling post liver transplantation

Jessica Howell, Rohit Sawhney, Narelle Skinner, Dilip Ratnam, Peter Angus, Paul Gow, Kumar Visvanathan

Advanced glycosylation endproducts in nonalcoholic steatohepatitis: A second hit that drives hepatic stellate cell activation?

Christopher Leung, Chandana B Herath, Jia Zhiyuan, Michelle Goodwin, Kai Y Mak, Josephine M Forbes, Peter W Angus

Hepatoma can occur in fatty liver disease without cirrhosis or raised alpha fetoprotein.

Christopher Leung, Sern W Yeoh, Desmond Patrick, Shara Ket, Kaye Marion, Louise M Burrell, Paul Gow, Peter W Angus

To toe or not to toe? That is the question in cirrhotic patients with varices.

Christopher Leung, Sern W Yeoh, Lucy Y Lim, Ray Boyapati, Rhys B Vaughan, Adam G Testro, Kaye Marion, Louise M Burrell, Peter W Angus

Hepatitis C, cyclosporine, and mycophenolate mofetil enhance cell death in human liver cells, with partial reversal by inhibitors of apoptosis and necroptosis

Eu Jin Lim, Ruth Chin, Peter W Angus, Joseph Torresi

Hepatitis C, cyclosporine and Mycophenolate mofetil reduce cell viability and induce apoptosis in primary mouse hepatocytes

Eu Jin Lim, Ruth Chin, Peter W Angus, Joseph Torresi
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Kai Yan Mak, Chandana B Herath, Ruth Chin, Joseph Torresi, Peter W Angus

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Rohit Sawhney, Jessica Howell, Narelle Skinner, Peter Angus, Paul Gow, Kumar Visvanathan, Adam Testro

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Siddharth Sood, Sim Ong, Paul J Gow, Peter W Angus, Kumar Visvanathan, Adam G Testro

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Antibiotic specific sRNA responses exist in multi-resistant methicillin-resistant staphylococcus aureus

Benjamin P Howden, Marie Beaume, Paul Harrison, David Hernandez, Jacques Schrenzel, Torsten Seemann, Patrice Francois, Timothy Stinear

Comparison of new liofilchem® mic test strips with etest

Kit M Liu, Janet M Montgomery

Evaluation of a Newly Formulated Chromogenic Brilliance MRSA Agar for the Detection of Nasal and Cutaneous Groin Colonization by Methicillin-Resistant Staphylococcus aureus (MRSA)

Patricia B Szczurek, Elizabeth A Grabsch, Shirley Xie

Comparison of the BD GeneOhm MRSA Assay to BD GeneOhm MRSA ACP Assay Using Combined Swabs for the Detection of Nasal and Cutaneous Groin Colonization by Methicillin-Resistant Staphylococcus aureus (MRSA)

Patricia B Szczurek, E A Grabsch, S Xie, M L Grayson, B P Howden

Vancomycin dosing and monitoring – are we there yet?

Karen Urbancic, Lisa Hui, Alison Tyedin

Rapid Identification of Microorganisms using Matrix Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS)

Heather L Young, Elizabeth Grabsch, Lee Taylor, Darrow Wendoloski, Peter B Ward
A novel nitrofuran for the imaging of tumor hypoxia
Uwe Ackermann, L Chong, S Yeoh, A Rigopoulos, H Tochon-Danguy, J White, G O'Keefe, A Scott

MR Imaging Features of DNETs and Gangliogliomas. A retrospective review
Farah Al-Rawi, Yuliya Perchyonok, Greg Fitt

Detection of Activated Platelets in Carotid Artery Thrombosis in Mice with radiolabeled single chain antibodies for PET
Katie Ardipradja, Shinn Dee Yeoh, Uwe Ackermann, Graeme O'Keefe, Karlheinz Peter, David W Howells, Christoph Hagemeyer

Avoidable DVT Ultrasounds
Gregory Compton, Patrick Nowlan, Angel Wu

Longitudinal Analysis of Cortical Thickness in PiB+ and PiB- Healthy Elderly Controls
Vincent Dore, Victor L Villemagne, Jurgen Fripp, Pierrick Bourgeat, Luping Zhou, Parnesh Raniga, Ralph Martins, Lance Macaulay, Katherine A Ellis, Colin L Master, David Ames, Christopher C Rowe, Olivier Salvado

Audit of Pre-test probability (Well's score) and D-Dimer use prior to ordering CTPA in the emergency setting at the Austin Hospital.
Mark Emmerton

Primary Cerebral Lymphoma: Neuroradiology-Pathology Audit
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PET Image-derived Biodistribution and Dose Assessment of C-AG1478 in BALB/c Mice with A431 EGFR-positive Xenografts

Sylvia J. Gong, Uwe Ackermann, Graeme O'Keefe, Glenn Cartwright, Angela Rigopoulos, Diana Cao, Katie Ardipradja, FT Lee, Henri Tochon-Danguy, Andrew Scott

A Feasibility Study of Using InstaDose Personal Radiation Dosimeter In a PET Facility

Sylvia J Gong, Lisa Mong, Kenneth Young, Jessica Welch, Kevin Hickson, Kunthi Pathmaraj, John Sachinidis, Gordon Chan, Henri Tochon-Danguy, Paul U, Graeme J O'Keefe, Andrew M Scott

A Gibbs artifact in PET images reconstructed with the TrueX algorithm

Kevin Hickson, Graeme O'Keefe, Jason Bradley, Shakher Ramdave, John McKay, Andrew Scott

Automated Analysis of AV133 PET Images

Gareth Jones, Victor L Villemagne, Svetlana Pejoska, Graeme J O'Keefe, Christopher R Rowe

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Xiaoyun Liang, Alan Connelly, Fernando Calamante

Deblurring in 3D GRASE ASL by using variable flip angles and k-space demodulation

Xiaoyun Liang, Alan Connelly, Donald Tournier, Fernando Calamante

Highly Accelerated Single Breath-hold Non-contrast Thoracic MRA: Evaluation in a Clinical Population

Ruth P Lim, Priscilla A Winchester, Mary T Bruno, Jian Xu, Pippa Storey, KellyAnne McGory, Daniel K Sodickson, Monvadi B Srichai
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Kevin Ong, Victor L Villemagne, Alex Bahar-Fuchs, Fiona Lamb, Narelle Langdon, Cornelia B Reininger, Barbara Putz, Colin L Masters, Christopher C Rowe

Ongoing strategies to manage radiation exposure to PET staff during dose dispensing, dose administration and patient positioning.

Kunthi Pathmaraj, Jessica Welch, Sylvia Gong, Kevin Hickson, Graeme O'Keefe, Gordon Chan, Andrew Scott

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Kera Pethybridge, Bridget Chappell, Aurora Poon, Sze Ting Lee, Salvatore Berlangieri, Christopher Rowe

Implementation of a Solid Target facility at Austin Health for the production of Cu-64 and I-124.

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Davina A Cossigny, Effie Mouhtouris, Augusto Gonzalvo, Gerald M Quan

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Shik Nie Kong, Christopher Christophi, Patricia Luiza Nunes Costa

PAK1: a Therapeutic Target for Pancreatic Cancer
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Rose F Bogdal, Fook T Lee, Pece Kocovski, Nancy Guo, Malarmathy Ramachandran, Angela Rigopoulos, Diana Cao, Fiona E Scott, Andrew M Scott, Graeme O'Keefe, Glenn A Powers, Michael P Wheatcroft, Peter J Hudson

Can cone-beam computed-tomography measurements be used in place of in-room optical distance indicator source-to-skin measurements?

Kerryn Brown, Tom Kupfer, Drew Smith, Mary ann Marr

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Jenny Catimel, Tracy Cardwell, Mark R Frewin

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Hypoxia-targeted radiotherapy dose painting for head and neck cancer using F-FMISO PET improves predicted outcomes

Joe H Chang, Morikatsu Wada, Nigel Anderson, Daryl Lim Joon, Sze Ting Lee, Sylvia Gong, Vincent Khoo, Andrew M Scott

Oral versus intravenous fluoropyrimidines for colorectal cancer

Fiona Chionh, Yvonne Yeung, Timothy Price, Niall Tebbutt
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Rifaximin for refractory hepatic encephalopathy: Impact on hospital admissions and length of stay at a liver transplant centre.

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Aims: Rifaximin has recently become available at our centre for the management of patients with recurrent HE. We therefore sought to audit our experience, particularly with regards to impact on hospitalization and length of stay, in order to assess both efficacy and cost-effectiveness.

Methods: Rifaximin has been available at Austin Health since October 2011. Due to the cost of the drug (approximately A$160/patient/month) commencement of rifaximin currently requires approval by the Clinical Pharmacology Unit. The Clinical Pharmacology database was therefore used to identify all patients who were prescribed rifaximin for the indication of HE. The medical record, correspondence and pathology results were reviewed to directly compare the 6-month periods pre- and postcommencement of rifaximin with regards primarily to number of hospital admissions, cause of admission, and length of stay.

Results: 12 patients have commenced rifaximin at a dose of 1000mg daily in divided doses. Median age is 61.5 years with a male predominance (83.3%). Aetiology of liver disease included NASH (4 patients), HCV (4 patients), alcohol (3 patients) and combination of HCV/alcohol (1 patient). 2 patients died at 0.5 and 1-month post commencement of rifaximin, and were therefore excluded from further analysis due to inadequate follow-up. All other patients (10) were followed up for 6 months.

Results demonstrated in the table below represent the median (range). Non-parametric statistical analysis using the Mann-Whitney U test was performed.

| Comparison of 6-month period pre- and post-rifaximin administration |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Pre-Rifaximin               | Post-Rifaximin              | P~                          |
| Child-Pugh Score (<6-12)    | MELD Score (<6-32)          | Total daily lactulose dose mL | Admissions with encephalopathy per patient | Length of stay (days) per admission |
| 10                          | 17.5                        | 80                          | 2                          | (1-5)                       |
| (6-32)                      | (48-990)                    | (0-430)                     | (1-5)                      | (3-46)                      |
| 10                          | 15                          | 85                          | 1                          | (0-7)                       |
| (6-30)                      | (0-480)                     | (0-7)                       | (0-7)                      | (2-37)                      |
| 0.80                        | 0.40                        | 0.40                        | 0.07                       | 0.005                       |
| 0.30                        |                             |                             |                             |                             |

Conclusion: Within the limitations of this small audit, the introduction of rifaximin has led to a 50% reduction in both the number of admissions with HE and the length of stay, supporting the available literature and suggesting that when HE does occur, the severity of the encephalopathic episode is reduced. With a daily bed cost exceeding A$1000, rifaximin appears to be highly cost effective in this cohort of patients.

Risk stratification of upper GI bleeding with an esophageal capsule

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Background: Analysis of upper gastrointestinal bleeding (UGIB) presentations to our institutions suggests many patients admitted for endoscopic investigation could be safely managed as outpatients.

Objective: Whether an esophageal capsule (EC) could identify a low risk group of patients with UGIB that could safely wait for elective gastroscopy. Design: Diagnostic, non-randomized, single blind (investigator) study.
Setting: Three tertiary-care referral centres.
Patients: 83 consecutive adult patients referred for management of UGIB.
Intervention: EC was performed prior to gastroscopy for the investigation and management of UGIB.
Main Outcome Measurements: Detection rates of UGIB source and identification of a low risk group of patients who would have been suitable for outpatient endoscopy based on EC findings.
Results: In total, 62/83 (75%) patients had a cause for bleeding identified. Findings were concordant across both modalities in 34 (55%) patients. 21/55 (38%) of patients with a positive gastroscopy had a negative EC study, 7 of whom were due to lack of duodenal visualization alone. However 7/28 (25%) of patients with a normal gastroscopy had a positive EC study. Within the subgroup of patients with a complete EC, 23/25 (92%) were concordant with gastroscopy for low-risk lesions that would have been suitable for outpatient management.
Limitations: Low duodenal visualization rates with EC.
Conclusion: When complete, the EC study correlated well with gastroscopy findings and correctly identified 92% of patients who may have been managed as outpatients. Future studies are required as duodenal visualization rates are expected to improve with additional battery life in future generations of EC.

3

The utility of upfront double wire guided biliary cannulation technique following early unintentional pancreatic wire cannulation in patients undergoing endoscopic retrograde cholangiopancreatography.

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Background and study aims: Double guide wire technique (DGT) shows no significant advantage to standard cannulation technique (SCT) in difficult biliary cannulation on review of large series. The utility of upfront DGT following inadvertent early pancreatic duct cannulation is however unknown.

Methods: Patients with an inadvertent early pancreatic wire cannulation undergoing ERCP by the study endoscopist between 2010 and 2011 were included. DGT was utilized in the first 25 patients followed by SCT in the next 25 cases.

Results: 300 ERCP cases were performed with 17% fulfilling initial inclusion criteria. Two patients were excluded therefore 48 patients were analysed in total. The DGT group had a lower ASA classification than the SCT group, but were otherwise similar. A significantly lower mean rate of repeat pancreatic cannulation was noted in the DGT group (1 (0-5) versus 3 (0-6); p=0.013), however the pancreatitis rate was similar (2(9%) DGT versus 1(4%) SCT; p=0.601). Successful biliary cannulation was eventually achieved in all patients. The overall need for pancreatic stenting was also similar between DGT and SCT groups (24% versus 35%; p=0.849). In the SCT group 15 of 25 (60%) required a DGT to achieve biliary cannulation.

Conclusion: Early use of the double wire technique resulted in a significant reduction in unintentional pancreatic cannulation but did not translate into a reduction in pancreatitis in this small cohort. Whether the eventual use of a double wire technique in many patients in the SCT group contributed to similar complication rates is uncertain.
The safety and efficacy of tenofovir disoproxil fumarate (TDF) substitution for HBIG in liver transplantation patients receiving long-term low dose IM HBIG / lamivudine (LAM) prophylaxis.

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**Background:** Prevention of graft reinfection remains the most important issue in liver transplantation of hepatitis B (HBV) infected patients. When prophylaxis is not given, reinfection is almost inevitable. Standard protocol comprises use of lamivudine/HBIG, however HBIG is expensive and requires lifelong injections. The primary objective of this study is to assess the safety and efficacy of the substitution of TDF for HBIG in preventing the recurrence of HBV post liver transplantation.

**Methods:** We performed an open-label, multi-centre switch study. Patients were switched from combination therapy to TDF 300mg plus LAM 100mg. Exclusion criteria included evidence of HBV viral recurrence or baseline creatinine>150 μmol/L. The primary endpoint is at 96 weeks with continuation to 5 years.

**Results:** 18 patients received TDF plus LAM with a mean follow up of 20 months (range 12–24). There was no case of treatment failure (defined as reappearance of HBsAg or HBV DNA) and no side effects reported. There was no significant loss of kidney function (cr >50μmol/L or >25% above baseline) or evidence of phosphate loss from commencement of TDF. Mean creatinine at baseline was 109.1±24. The mean difference in renal function was -3.14 μmol/L (p=0.36, CI-10. 3 to 4.01). The mean PO4 at baseline was 1.12 ±24mg/L with a mean difference of 0.07 (p<0.21, CI-0.04-0.19). Four patients had dose reductions of TDF and one patient had the drug stopped due to minor declines in creatinine. However, all 5 patients had significant co morbidities known to contribute to nephrotoxicity. Subsequent to dose reduction 3 of these patients’ creatinine returned to baseline.

**Conclusions:** Replacement of HBIG with tenofovir appears to be an effective strategy in patients receiving HBIG/LAM prophylaxis post liver transplantation. Further follow-up is required to confirm the long term safety and efficacy of this approach.

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Different measures of HCV rapid fibrosis post liver transplant have different abilities to predict rapid graft cirrhosis

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**Background:** Hepatitis C (HCV) is the commonest indication for liver transplantation, recurrence post transplant is universal, with a subgroup of patients developing rapid fibrosis progression. Various definitions of rapid fibrosis have been used to identify risks for rapid progression, but their ability to predict poor outcomes has not been compared.

**Methods:** Prospective data analysis was conducted on 131 adult patients with HCV who underwent liver transplantation at a single centre. We measured fibrosis rate (calculated using liver biopsies graded by Metavir scoring F0-4; FR= fibrosis stage/ year post transplant), year one fibrosis progression (rapid fibrosis defined as Metavir F score ≥1 at 1yr liver biopsy) and time to Metavir F2 stage fibrosis (years). We determined correlation between measures of fibrosis progression and time to cirrhosis and the ability to predict cirrhosis development within five years of transplantation.

**Results:** 131 adult patients with HCV were included in the study; 100 had liver biopsies allowing determination of fibrosis rate (minimum post-transplant follow-up 6mths, mean 6.5 yrs). There was a significant correlation between fibrosis rate (p<0.0001, r=−0.76), time to F2
stage fibrosis ($p<0.0001$, $r=0.92$) and fibrosis stage on year one biopsy ($p=0.012$, $r=-0.67$) and time to cirrhosis. Fibrosis rate was the strongest predictor of rapid graft cirrhosis development ($p<0.0001$, AUC 0.979). Fibrosis rate calculated at year 3 post transplant and time to F2 fibrosis were also predictive of rapid graft cirrhosis ($p=0.004$, AUC 0.848, $p=0.012$, AUC 0.791 respectively). F stage on year one liver biopsy was not predictive of rapid graft cirrhosis ($p=0.06$).

Conclusion: Fibrosis rate measured on the most recent liver biopsy and at 3 years post transplant was the most accurate predictor of the rapid development of graft cirrhosis after transplantation for HCV.

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**6**

**NK cell marker expression is dysregulated post liver transplantation and correlates with HCV viral load**

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**Background:** Hepatitis C (HCV) recurrence post liver transplantation is universal, with a subgroup developing rapid hepatic fibrosis. Natural killer (NK) cells are critical to anti-viral immunity and the role of NK cell function in HCV rapid fibrosis post transplant is unknown.

**Methods:** PBMCs from 35 HCV patients post transplant, 10 non-HCV post transplant and 8 healthy controls were labelled for CD3 and CD56, activation markers CD69 and NKGD2, degranulation marker CD107a, inhibitory KIR 2DL2/2DL3 (CD158b) and baseline intracellular TNFα and IFNγ for flow cytometry. Rate of fibrosis progression was calculated using post transplant liver biopsies graded by Metavir scoring (F0-4; R= fibrosis stage/ year post transplant). Rapid fibrosis was defined as >0.4 units/yr.

**Results:** Both HCV and non-HCV patients post transplant had reduced NK cell CD158b (NK CD56bright cells $p=0.0081$) and NKGD2 expression (NK CD56dim cells $p=0.003$) compared with healthy controls. NK cell NKGD2 expression inversely correlated with HCV viral load ($p=0.012$, $r=-0.57$). HCV NK CD56dim cells had greater CD69 expression compared with post transplant controls ($p=0.043$) and CD69 expression correlated with both TNF and IFNγ production (TNF $p=0.043$, $r=0.43$; IFNγ $p=0.004$, $r=0.58$). HCV NK cells produced greater TNFα (NK CD56 dim $p=0.005$, NK CD56bright $p=0.044$) and IFNγ (NK CD56dim $p=0.008$, NK CD56bright $p=0.016$) at baseline compared with non-HCV post transplant patients. There was no relationship between NK cell marker expression and HCV fibrosis progression post transplant.

**Conclusion:** Impaired NK cell NKGD2 expression post transplant may contribute to poor immune control of HCV, whilst reduced NK cell CD158b expression contributes to greater NK-mediated cytokine production in HCV post transplant.

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**7**

**Cyclosporine and tacrolimus have different inhibitory effects on toll-like receptor signalling post liver transplantation**

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². Medicine, Monash Medical Centre, Clayton, Victoria, Australia

**Background:** Hepatitis C (HCV) recurrence post liver transplant follows a more aggressive course than pre-transplantation and the mechanisms remain poorly understood. Toll-like receptors (TLRs) are critical to innate immune antiviral responses. The interplay between TLR function and immunosuppressive drugs is unknown.

**Methods:** PBMCs from 136 post liver transplant patients and 10 healthy controls were stimulated with TLR specific ligands for 24 hrs. Production of IL-6, TNF and IFNα were
measured using ELISA. Flow cytometry was used to measure intracellular cytokine production by monocytes, NK, NKT and T cells.

Results: Cyclosporine levels correlated with reduced TLR3-induced IFN production by NK cells (NK cells p=0.011, CD56bright NK cells p=0.017). Cyclosporine levels also correlated with reduced TLR3-induced monocyte IL-6 (p=0.028) and TNF (p=0.070) production. Cyclosporine level correlated positively with T cell frequency (p=0.040).

In contrast, tacrolimus levels correlated inversely with TLR4-induced PBMC IL-6 production (p=0.030), TLR3-induced TNF production by PBMCs (p=0.027) and TLR7/8-induced NK cell TNF (NK cells p=0.009, CD56dim NK cells p=0.013). Tacrolimus levels correlated inversely with NKT cell frequency (p=0.032).

Conclusion: NK cells and monocytes have impaired TLR3-induced cytokine production that varied with increasing cyclosporine level. In contrast, tacrolimus levels correlated inversely with TLR4-induced IL-6 by PBMCs, TLR3-induced TNF from monocytes, and TLR7/8 induced TNF from NK cells. These data demonstrate important relationships between TLR function and calcineurin inhibitor levels that may contribute to disease recurrence post liver transplant, including aggressive HCV recurrence. Importantly, our data also suggest that tacrolimus and cyclosporine have different effects on innate immune signaling, which may influence choice of therapy post liver transplantation in different clinical contexts.

Advanced glycosylation endproducts in nonalcoholic steatohepatitis: A second hit that drives hepatic stellate cell activation?

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Introduction: Advanced-glycosylation-endproducts (AGEs) are compounds present in large amounts in our diet. Their production is also increased in diabetes where they increase oxidative stress via RAGE (receptor-for-AGEs). AGEs are implicated in non-alcoholic-fatty-liver-disease(NAFLD), the most common liver disease worldwide. However, it is unclear whether AGEs contribute to non-alcoholic-steatohepatitis(NASH) via direct effects on hepatic-stellate-stellate(HSCs), the primary effector cells for liver fibrosis.

Aims: To determine the role of the AGE/RAGE axis in HSCs from healthy livers and livers with NASH.

Methods: 10-week-old Sprague-Dawley rats were randomised into 2 groups. (1)NASH induced by the methionine choline deficient (MCD) diet and (2)methionine choline replete (MCR) diet as control. After 12 weeks, primary HSCs were isolated via portal vein perfusion, lysis and Nycodenz-separation. Preactivated HSCs (from MCD-animals) and healthy HSCs (from MCR-animals) were treated with BSA vehicle(100ug/mL) and AGE-BSA(100ug/mL). Two additional groups had AGE-BSA with NADPH oxidase inhibitor, diphenyleneiodonium(DPI) or RAGE antibody. Cell proliferation was measured via a bromodeoxyuridine chromogenic assay and reactive-oxygen-species(ROS) generation was determined using a fluorescence-based assay. Cells were also collected for determination of gene expression.

Results: HSCs from NASH livers had greater ROS generation, IL-6 and alpha-smooth-muscle-actin(aSMA) expression than HSCs from healthy livers(p<0.05). HSCs from healthy livers did not respond to AGEs. However, in HSCs from animals with NASH, AGEs markedly increased cell proliferation(p=0.01), ROS production(p=0.02) and gene expression of monocyte-chemoattractant-protein-1(MCP-1), IL-6 and aSMA(p<0.05). These effects were abrogated by both RAGE blockade and NADPH oxidase inhibition with DPI(p<0.05).

Conclusions: The finding that AGEs only affect HSCs from diseased livers helps explain why previous studies have produced conflicting results regarding the role of the AGE/RAGE axis in HSC activation. They also suggest that AGEs may provide a “second hit” that drives progression of simple steatosis to fibrosis in NAFLD.
Hepatoma can occur in fatty liver disease without cirrhosis or raised alpha fetoprotein.

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**Background:** Non-alcoholic fatty liver disease (NAFLD) now affects up to 1/3 of the population in Australia. This condition can lead to the development of hepatocellular cancer (HCC), however the rate at which this occurs, risks factors that predispose to HCC and whether HCC only occurs in the presence of cirrhosis are unclear.

**Aims:** We aim to describe the characteristics of patients with NAFLD who develop HCC.

**Methods:** An audit was conducted of patients with a diagnosis of HCC and NAFLD between 2000 and 2012 using ICD coding. Patients with cryptogenic cirrhosis were included since almost all patients with this diagnosis have NAFLD. Information collected included demographics; histology; size and number of HCC; alpha-fetoprotein (AFP); BMI, and the presence of diabetes, hypertension, dyslipidaemia and smoking.

**Results:** Of 310 patients with HCC, 54 had NAFLD or cryptogenic cirrhosis. Mean age was 64 with 87% male. 9%, 24% and 50% had a BMI of <25kg/m², 25-29kg/m² and ≥30kg/m² respectively. 74% were overweight or obese, 59% diabetic, 44% hypertensive and 26% hyperlipidaemic. 6%, 20%, 19%, 4% and 3% had 0, 1, 2, 3 and 4 of these risk factors respectively. Importantly, 15% of patients did not have cirrhosis (7% had no fibrosis (F0) and 8% had early fibrosis). 85% were cirrhotic. 54% had single HCCs (mean diameter 3.5cm). Median AFP was 12.2KU/L (normal < 10) and 33% of patients had normal levels.

**Conclusions:** This study provides concerning evidence that HCC develops in the absence of cirrhosis in NAFLD. AFP screening is unhelpful since it is normal or minimally raised in most patients with HCC. Furthermore, most patients had only one or two metabolic risk factors making it difficult to identify a profile that predisposes to HCC. Prospective studies are urgently needed to clarify the risk of HCC and role of screening in NAFLD.

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**To toe or not to toe? That is the question in cirrhotic patients with varices.**

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**Background:** The presence of oesophageal and/or gastric varices is considered a relative contraindication to performing a transoesophageal-echocardiogram (TOE) due to the perceived risk of post-procedural bleeding. The safety and clinical efficacy of TOEs in this setting is unclear.

**Aims:** To audit post-TOE bleeding rates in patients with varices or portal hypertension and to determine if TOEs were clinically useful in this setting.

**Methods:** We audited patients who had both a TOE and varices based on ICD-coding from 1995-2010. Information collected included demographics, indications, proximity of TOE to variceal diagnosis, cause of cirrhosis and Child Pugh score. Kruskal-Wallis analysis was performed to determine any correlations.

**Results:** 51 patients with varices had 60 TOEs performed. 70% were male with an average age of 56 years. Child-Pugh scores of A, B and C were 30%, 36% and 33% respectively, and
the median MELD was 17.1. Causes of portal hypertension included alcoholic cirrhosis (27%), hepatitis C (32%), hepatitis B (12%), non-alcoholic steatohepatitis (8%) and cryptogenic cirrhosis (8%). 78% had varices endoscopically and 22% had radiological evidence. On endoscopy, 30% also had gastric varices, 57% had varices >5mm and 26% had stigmata of acute bleeding. TOEs were performed at a median of 150 days from variceal diagnosis and 73% were for investigation for endocarditis. There was no variceal bleeding after any TOE. 47% were performed after variceal treatment (liver transplantation, endoscopic banding or transjugular intrahepatic porto-systemic shunting). TOEs changed clinical management in 50% of cases by guiding changes to medication regimens or surgical procedures. Multivariate analysis did not reveal any factors that predicted whether a TOE changed clinical management.

**Conclusions:** In our cohort, a TOE can be safely performed in patients with varices or portal hypertension, even if large varices and/or acute stigmata of bleeding are present.

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**Hepatitis C, cyclosporine, and mycophenolate mofetil enhance cell death in human liver cells, with partial reversal by inhibitors of apoptosis and necroptosis**

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Severe recurrent hepatitis C (HCV) disease post-liver transplantation results in rapidly progressive liver fibrosis that might in part be due to reduced hepatocyte viability and enhanced apoptosis driven by both HCV and immunosuppressants. We investigated the effect of MMF and cyclosporine on HCV-induced hepatocyte apoptosis, and determined the effect of apoptosis and necroptosis inhibitors on this system.

Huh7 cells were exposed to MMF and cyclosporine, and viral constructs made using the AdEasy system, expressing the structural (rAdHCV-CoreE1E2) or non-structural (rAdHCV-NS3-5B) HCV proteins. Q-VD-Oph (Q-VD) and necrostatin-1 (Nec-1) were used to inhibit apoptosis and necroptosis respectively. Cell viability was evaluated using crystal violet assays. Apoptosis was evaluated using Western immunoblots on cell lysates probed for cleaved PARP.

Infection with HCV constructs reduced cell viability by 1.6 fold, but addition of Q-VD and Nec-1 improved viability by 1.5 and 1.4 fold respectively. Cyclosporine 1 mcg/mL had no effect on cell viability. MMF 5 mcg/mL improved cell viability by 1.2 fold. However, combination of cyclosporine and MMF reduced cell viability by 1.9 fold. Addition of immunosuppressants to infection with rAdHCV-CoreE1E2, rAdHCV-NS3-5B and both viral constructs further reduced cell viability by 2.2-2.6 fold compared to respective infections alone. Infection with HCV constructs increased cleaved PARP by 3.3 fold. Addition of Q-VD significantly reduced cleaved PARP, but Nec-1 had no effect. Cyclosporine and/or MMF alone did not increase cleaved PARP compared to mock. However, addition of immunosuppressants to HCV infection increased cleaved PARP by 1.4-1.7 fold.

HCV infection reduces cell viability and increases apoptosis which is worsened by addition of immunosuppressants, perhaps contributing to accelerated liver disease in post-transplant HCV recurrence. Apoptosis and necroptosis inhibition partially improved cell viability, suggesting that cell death in infected cells may occur by both apoptosis and necroptosis.

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**Hepatitis C, cyclosporine and Mycophenolate mofetil reduce cell viability and induce apoptosis in primary mouse hepatocytes**

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Severe recurrent hepatitis C (HCV) disease post-liver transplantation results in rapidly progressive liver fibrosis, but the underlying mechanism remains uncertain. The effect of HCV, cyclosporine and mycophenolate mofetil (MMF) on cell viability and apoptosis was investigated in primary mouse hepatocytes (PMoH).

PMoH harvested from C57BL/6 mice were exposed to AdEasy system viral constructs expressing structural (rAdHCV-CoreE1E2) or non-structural (rAdHCV-NS3-5B) HCV proteins, ± CyA 1 mcg/mL and/or MMF 5 mcg/mL. Treated cells were compared to mock. PMoH viability was evaluated using crystal violet assays. Apoptosis was evaluated using Western immunoblots performed on cell lysates for cleaved caspase 3 (CC3) and cleaved PARP (CP).

Cyclosporine had minimal effect on cell viability and apoptosis. MMF improved cell viability and reduced CC3 and CP by 1.9 and 2.9 fold respectively. Combination of cyclosporine and MMF reduced cell viability by 2.1 fold and increased CP by 2.8 fold. Infection with HCV constructs reduced cell viability by 1.6 fold and increased CC3 by 2.4-3.2 fold, and CP by 2.6-2.7 fold. Addition of cyclosporine to HCV infections reduced cell viability by 2.1 fold and increased CC3 and CP by 2.6-2.8 and 6.3-6.7 fold respectively. Addition of MMF to respective HCV infections reduced cell viability by 1.8 fold and increased CC3 and CP by 1.9-2.2 and 3.5-4.5 fold respectively. Addition of both cyclosporine and MMF to HCV infections reduced cell viability by 3.9 fold and increased CC3 and CP by 7.6-9.1 and 8.3-10.4 fold respectively.

HCV infection reduced cell viability and increased apoptosis, as did the combination of cyclosporine and MMF. Addition of cyclosporine and/or MMF to HCV infection further reduced cell viability and increased apoptosis, perhaps contributing to accelerated liver disease in post-liver transplant HCV recurrence.

Tenofovir rescue therapy achieves long-term suppression of HBV replication in patients with multi-drug resistant HBV: 4 year follow-up of the TDF-109 cohort

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Multi-drug resistant (MDR) hepatitis B virus (HBV) presents a management challenge, particularly for advanced liver-disease patients. We present the long-term follow-up data from the TDF 109 study that investigated use of tenofovir (TDF)+/-lamivudine (LAM) for the treatment of well-characterized patients with chronic hepatitis B who had previously failed LAM and adefovir (ADV) therapy.

TDF-109 was an investigator-initiated, prospective, multi-centre, open-label study of 60 patients treated with TDF. The year 2 data have been previously reported (Patterson, GUT 2011). At study entry, 38/60 patients (63%) were switched from ADV monotherapy to TDF, 22/60 (37%) were switched from ADV/LAM to TDF/LAM combination. Patients treated with TDF monotherapy with persistent viraemia at or after 24 weeks were switched to TDF/LAM. The primary end point for the current analysis was number of patients with HBV DNA below the lower limit of quantification (LLOQ, DNA=<20IU/ml) after 4 years. We also analysed the rate of viral decline based on the HBV-resistance associated substitutions at baseline.

At year 2, 64% had serum HBV DNA levels below LLOQ. At 4 years, 63% had achieved complete viral suppression by intent-to-treat analysis. Four patients had discontinued TDF and four patients lost follow-up. 75% had achieved viral suppression per protocol. In patients with quantifiable serum HBV-DNA levels at 4 years, median viral load was
1.72 log IU/ml. 13/39 experienced HBeAg-seroconversion. Using population-based sequencing, substitutions associated with ADV resistance (rtA181T/V-/rtN236T) were present in 21 patients at baseline. Patients with rtA181T/V-containing HBV at baseline had a higher baseline viral load and took longer to achieve undetectable HBV-DNA. However the rate of viral decline was the same as patients without these ADV-R substitutions.

In patients with MDR HBV, 4 years of TDF therapy resulted in significant viral suppression, but not necessarily undetectability, in the majority of patients, including those infected with HBV containing ADV-associated resistance substitutions.

**Production and validation of liver specific adeno-associated virus expressing mouse angiotensin converting enzyme 2 (mACE2) for liver fibrosis treatment**

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**Background:**
The renin-angiotensin system plays a key role in the pathogenesis of liver fibrosis and cirrhosis. Recently identified major component of the alternate axis, angiotensin converting enzyme 2 (ACE2) has been shown to be crucial in the balance of the system and degrades the deleterious peptide, angiotensin II to angiotensin-1-7 which counteracts many of angiotensin II harmful effects. This has stimulated a major interest in the potential therapeutic role of ACE2 in liver disease. We therefore constructed a liver-specific recombinant adeno-associated viral vector system to overexpress ACE2 gene/protein and characterized the expression pattern in vivo in healthy mice.

**Methods:**
In this study, an ACE2 viral vector using mouse ACE2 gene sequence and AAV2 genome pseudotyped with type 8 capsid was constructed (rAAV2/8-mACE2). A single injection of rAAV2/8-mACE2 was administered to healthy C57BL/6 mice by both intraportal and peritoneal routes and ACE2 expression levels were studied 2, 4 and 8 weeks after injection. qPCR was performed to characterise the ACE2 gene expression in several major organs. Protein activity of ACE2 was also evaluated by using ACE2 activity assay.

**Results:**
Intraportal and interperitoneal injections of rAAV2/8-mACE2 in mice showed significant upregulation of mACE2 gene expression in the liver at 2, 4 and 8 weeks compared to saline injected controls. Importantly, ACE2 expression was liver-specific following both routes of administration, and found minimal expression levels in other major organs. In consistent with gene expression data, liver tissues harvested at week 2, 4 and 8 showed significantly increased ACE2 protein activity compared to that in saline injected control.

**Conclusion:**
We successfully characterized the construction of a recombinant viral vector system that can selectively overexpress ACE2 gene and protein in the mouse liver. High expression levels of ACE2 in the fibrotic liver of mice would be expected to improve the degree of fibrosis.
Pre-transplant monocyte toll-like receptor 4 expression predicts risk of acute allograft rejection in liver transplant recipients

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Background: Toll-like receptor (TLR) 4-mediated signaling has been associated with rejection in multiple solid-organ transplant studies. We have previously reported increased monocyte TLR4 expression prior to liver transplantation in patients who subsequently developed rejection. This study aimed to determine the ability of pre-transplant TLR4 expression to predict liver allograft rejection and validate these findings prospectively.

Methods: There were 26 patients in the original cohort and 31 in the subsequent validation cohort who underwent transplantation. Subjects were well matched for clinical variables between groups. Blood was collected prior to transplantation and flow cytometry used to determine TLR4 expression on monocytes. Results were reported as ratios to corresponding isotype controls. ROC curve analysis was performed on the original cohort to determine the optimum TLR4 threshold for predicting rejection. In the validation cohort, data was compared between groups using Mann-Whitney and Fisher’s exact test as appropriate.

Results: ROC curve analysis of the pilot cohort demonstrated good accuracy of pre-transplant TLR4 expression for predicting rejection (AUC=0.772, p=0.024). Based on this analysis, a cut-off of 1.03 was chosen to optimize sensitivity (88.9%) and specificity (72.2%). In the validation cohort, 9 patients (29%) experienced rejection. Pre-transplant TLR4 expression was significantly higher in patients who subsequently developed rejection (median 1.4 vs 0.94, p=0.016). The selected cut-off of 1.03 was significantly predictive of rejection in the validation cohort (p=0.016). The rate of rejection was 8/16 (50%) in patients with TLR4 >1.03 but only 1/15 (6.67%) for those with TLR4 <1.03.

Conclusions: We confirm that elevated pre-transplant monocyte TLR4 expression predicts acute rejection post liver transplant. The ability to stratify rejection risk pre-transplant may allow tailoring of immunosuppressive regimens and suggests targeting TLR4-mediated pathways to prevent rejection may be worthwhile.

STAG – A world-first use of a whole blood immune function assay in a transplant setting.

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Aim and background:
Long-term side-effects of immunosuppression result in a significant burden of morbidity and mortality post liver transplantation. Currently no objective measure of immune function is available and hence there is a tendency for clinicians to rely on drug levels as a surrogate for drug efficacy. In this world-first, proof of concept study, we aim to evaluate a new whole blood immune function assay (STAG – CST007_07, Cellestis Ltd, Australia) in a liver transplantation patient population.

Methods:
Patients were enlisted from the Victorian Liver Transplant Unit and defined as pre-transplant, early post-transplant (<3 months), and late post-transplant (>12 months). Healthy volunteers were enlisted separately. A single lyophilised ball containing ligands specific for both innate and adaptive immune systems was added to whole blood and incubated for 24 hours.
following which plasma was harvested and IFNγ (IU/ml) measured by ELISA. Higher IFNγ levels suggest a more robust immune response.

**Results:**
Healthy controls (n=212) were compared with pre-transplant patients (n=16), early post-transplant patients (n=18, 66 samples, median time post-transplant 31 days) and late post-transplant patients (n=12, median time post-transplant 2,290 days). Mean IFNγ from pre-transplant (64.26 IU/ml) and early post-transplant patients (3.76 IU/ml) both suggested significant immune dysfunction compared with healthy controls (555.2 IU/ml, p<0.001). As expected, late post-transplant patients (256.1 IU/ml) had higher values compared with early post-transplant patients (p<0.01). Furthermore, STAG suggests an incremental increase in immune function over the first 12 months post-transplant.

**Conclusion:**
STAG appears to be an objective marker of net immune response in a liver transplant setting. Based upon these results, a prospective study linking clinical events (rejection, infection, recurrent disease) with immune function is underway. Further, STAG may have utility in the broader transplant setting as well as other fields of medicine.

**Inflammatory changes in human small intestine following ischaemia-reperfusion: Potential mechanisms for dysmotility post intestinal transplantation**

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**Background:** Intestinal transplantation is offered to patients with intestinal failure and life-threatening complications of parenteral nutrition. Ischaemia-reperfusion (I/R) injury to the intestine is universal following transplantation, often resulting in intestinal dysmotility, the mechanism of which remains unclear.

**Aims:** To describe the histologic and molecular changes occurring in the human intestine following I/R injury to elucidate mechanisms explaining post transplant dysmotility.

**Methods:** I/R was applied to a segment of jejunum in patients undergoing pancreaticoduodenectomy. Adjacent segments of intestine were isolated. One segment was subjected to vascular occlusion for 30 minutes, followed by a subsequent reperfusion for either 30 minutes or 2 hours prior to removal. The adjacent (control) region had no vascular occlusion. Fresh tissue was processed for histology, immunohistochemistry and molecular analyses.

**Results:** 15 patients were included. 7 patients were exposed to 30 minutes reperfusion whilst 8 were exposed to 2 hours. With 30 minutes reperfusion histological examination revealed structural damage centred at the tips of the villi. There was villus neutrophil and macrophage infiltration (P<0.05) and inflammation present around the venules of the longitudinal muscle and serosa, but sparing submucosal vessels. With 2 hour reperfusion there was further infiltration of neutrophils and macrophages into the serosa and longitudinal muscle, with conspicuous neutrophil aggregates around myenteric ganglia. mRNA levels of inflammatory cytokines were significantly increased in external muscle layers including IL-1, IL-6, and IL-8 (P<0.001), but not in the mucosa.

**Conclusion:** This study clearly shows in human intestine that following I/R there is a rapid and targeted immune cell infiltration of the serosal and external muscle layers associated with significant local up-regulation of inflammatory cytokines. Within 2 hours ganglionitis is present. These findings highlight the exquisite sensitivity of the intestine to relatively brief periods of I/R and the need to further characterise the extremely complex association between I/R injury, inflammation and damage to intestinal ganglia.
Falls and sleep: subjective sleep difficulties in community-dwelling older fallers and relationship between sleep and falls
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Aim: To determine the prevalence of subjective sleep difficulties in community-dwelling older fallers, and to evaluate the relationship between sleep quality and falls.
Method: Data from a randomised controlled trial1 (Falls Aren't Us study) were analysed (n=698, mean age 75 years, 70% female). The original study evaluated a multi-factorial falls prevention program for community-dwelling older people presenting to emergency departments after a fall. The effects of baseline sleep (Assessment of Quality of Life sleep item) on falls over the subsequent 12 months was analysed using negative binomial regression.
Results: At baseline, sleep interruption was reported by 491 participants (70%). A high prevalence of nocturia (75%) was also found. Self-reported sleep quality and sleep-related factors remained stable over 12 months, apart from sedative use which significantly decreased (15% at baseline; 11% at follow-up). No independent effect of sleep quality on falls rate was found; however, there was a trend towards those who reported waking occasionally to have lower falls rates than those who reported not waking at all (incidence rate ratio: 0.61; p=0.06).
Conclusion: A high prevalence of sleep interruption and nocturia in this sample of community-dwelling older fallers was found. The study highlighted the complex nature of the relationship between falls and sleep. Further research investigating the relationship between frequency of night-time waking (subjectively, using a more detailed tool, and objectively assessed using polysomnography) and falls is required. Assessment of sleep should be considered in people presenting with falls.


Equity of access to and accuracy of neurodegenerative diagnoses in an Australian memory clinic – a new look at an old problem
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Background
Diagnosis in dementia has made recent progress with developments such as the integration of biomarkers including fluorodeoxyglucose positron emission tomography (FDG PET) into routine practice in memory clinics. Memory clinics are increasingly being established as centres of diagnostic excellence. Initial publications over 10 years ago recognized that non-English speaking background (NESB) patients may undergo different diagnostic journeys in these clinics.
Objectives
To compare presentations and diagnostic trends in cognitive disorders in those of NESB and English speaking background (ESB) and to contrast this with patterns 10 years ago.
Methods
Information on 54 patients attending an Australian memory clinic between 2009 and 2011 were retrospectively collected. This clinic extensively uses fluorodeoxyglucose positron emission tomography (FDG PET) in the diagnosis of cognitive concerns.

Results
Contrary to previous reports, rather than having increased rates of psychiatric disorders, NESB patients were less likely than ESB patients to present with AD (Alzheimer's disease) and more likely to present with non-AD dementias. The clinical work-up and neurodegenerative diagnoses on NESB patients relied more heavily on FDG PET than for those of ESB. Compared to ESB patients, NESB patients experienced symptoms more than a year longer before receiving a formal diagnosis, and there was a significant under representation of older elderly NESB females.

Conclusion
 Whilst presentation trends have not changed, altered patterns of NESB patients’ diagnoses may be accounted by improvement of diagnostic accuracy with the use FDG PET. Memory service provision to NSEB patients, especially older elderly NESB females continues to be under utilised.

Correlating the Frontal Presentation of Alzheimer's Disease with frontal metabolism as measured by FDG-PET

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Alzheimer's disease, the commonest cause of dementia, can present with memory, language or visuospatial features. We surmised that there is also a "frontal" presentation defined by high scores on the Frontal Behavioural Inventory (FBI) and presenting clinically as marked behavioural changes. We arrayed 53 patients with a diagnosis of AD, determined by an expert in this field, into "frontal" (n= 10) and "non frontal" (n=43) groups, on the basis of their FBI scores, and measured frontal metabolism with FDG-PET.

We used FDG-PET scans scaled using the cerebellar cortex as reference region and normalized into standard stereotactic space. A standard Regions Of Interest (ROI) template (AAL) was used to assess glucose metabolism in frontal and anterior temporal cortical regions. ROIs were aggregated to lateral frontal, superior lateral frontal, orbitofrontal and medial frontal regions. Z scores were used to evaluate difference between subject’s metabolism and that of a pooled group of non demented age-matched controls for the ROI.

We found that frontal metabolism in the orbito frontal ( Z= 2.64 versus 2.11, p=.03) and medial frontal (Z= 2.38 versus 1.82, p<.003) regions were significantly less in the frontal AD group compared to the non-frontal AD group. We surmised that this represented increased AD pathology in these regions in the frontal AD group, and that there is indeed a biological basis to the frontal AD presentation.
Rapid Detection of Toxigenic Clostridium difficile Infection

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Introduction: Clostridium difficile is a major cause of nosocomial diarrhoea and pseudomembranous colitis. Due increasing concern about hyper-virulent strains, rapid and accurate diagnosis toxigenic C. difficile infection is crucial. Laboratory diagnosis has precisely used culture and cytotoxin assays, techniques that are slow, insensitive and labour intensive requiring 24-48 hrs. Molecular amplification now offers rapid, sensitive and specific alternatives.

Method: Unformed Stool samples were used which C. difficile, all specimens were tested using 1) Illumigene, 2) Chromogenic agar culture, 3) cytotoxin B assay direct from stool using cell culture and neutralisation and 4) in-house PCR for C. difficile.

Cytotoxigenic culture (after alcohol shock to Chrom agar and Anaerobic-taurocholate agar without antibiotics) and GeneXpert were used to resolve discrepant result between methods. Any positive culture, illumigene negative specimens had a toxigenic culture performed. Any positive culture, illumigen and cytotoxic negative toxigenic culture performed.

Results: 256 total specimens were tested and 17 were positive for C. difficile by culture, Illumigene assay, in-house PCR and cytotoxic assay. 10 specimens were negative by cytotoxic assay but positive by illumigene. 7 of these samples were positive by culture, in-house PCR and toxigenic culture.

4 specimens were culture positive, negative by illumigene. 3 of these were negative by PCR and toxigenic culture. 1 was positive by PCR, geneXpert and toxigenic culture.

3 specimens were culture negative but positive by illumigene, 2 of these were also PCR positive, 1 specimen was GeneXpert pos, and another was positive on repeat culture (alcohol shock and media without antibiotics) and toxigenic culture positive.

Conclusion: The Illumigene assay was 96.3% sensitive and 99.6% specific, with PPV 96.3% NPV 99.6% when compared with toxigenic culture resolved with concordance of PCR. Illumigene is a rapid, sensitive and specific assay for detection of infection with toxigenic C. difficile, enabling same day results, improving patient management with C. difficile disease.

Subtle genetic changes in clinical staphylococcus aureus promote co-resistance to antimicrobials and innate immune responses

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Using whole genome sequencing we detected in vivo derived mutations including rpoB H481Y in a clinical S. aureus strain associated with acquired drug resistance and persistent infection. We hypothesized that this rifampicin resistance mutation also impacts vancomycin susceptibility and host pathogen interactions.

Aim: To determine the impact of S. aureus rpoB mutations on vancomycin resistance, virulence, and innate immunity.

Unmarked rpoB H481Y and capsule deletion mutants were generated using pKOR1. Mutants were assessed by vancomycin population analysis, microarray transcriptomics, virulence analysis (murine septicaemia model), whole blood killing, and human antimicrobial peptide resistance (hNP-1, hBD-2) using radial diffusion with propidium iodide staining and flow cytometry.
Results: A single rpoB H481Y mutation led to high-level rifampicin resistance and hVISA phenotype. Significant global transcriptional changes were identified; including highly up-regulated capsule biosynthesis, with changes suggesting this mutation may lead to innate immune resistance. The virulence of the rpoB H481Y mutant was attenuated in the murine model, however it was resistant to whole blood killing and demonstrated significantly reduced susceptibility to hNP-1 and hBD-2. Notably, capsule deletion of the clinically derived strain containing the rpoB H481Y mutation restored susceptibility to whole blood killing and partially restored hNP-1 susceptibility. The rpoB H481Y mutation prevented cell membrane permeabilisation with hNP-1 and hBD-2 treatment, and capsule deletion restored the permeability induced by both peptides. Reversion of the rpoB mutation restored all phenotypes to wild-type.

Conclusions: A dramatic link between antimicrobial resistance, virulence and resistance to host immune responses exists in S. aureus. Excess capsule expression partly drives these effects, and can be induced by minor genetic changes such as a single base substitution in rpoB. These findings further highlight the potential adverse effects of inappropriate antimicrobial therapy, leading not only to antimicrobial resistance, but effects on host-pathogen interactions.

Organism factors associated with elevated vancomycin minimum inhibitory concentration in *Staphylococcus aureus* bacteraemia.

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Background

Elevated vancomycin minimum inhibitory concentration (V-MIC) is associated with poor outcome in *Staphylococcus aureus* bacteraemia (SAB), irrespective of antibiotic treatment. We hypothesised that elevated V-MIC may be a marker of host or organism factors that impact on treatment outcome.

Methods

Organism characteristics were assessed in a subset of SAB isolates from our original cohort, including strain typing using pulsed-field gel electrophoresis, and detection of genes encoding *S. aureus* virulence factors by DNA microarray. We then examined whether genetic differences or patients’ disease severity scores were associated with elevated V-MIC.

Results

One hundred blood culture isolates from individual patients (18.8% of our total cohort) were included. Demographics included: age ≥ 70 years (40%), male (68%), methicillin-susceptible *S. aureus* (MSSA) (67%), hospital onset (47%). Elevated V-MIC, defined as Etest > 1.5 μg/mL, was present in 63%. The genetic diversity in clones was greater among MSSA than MRSA (18 versus 7, respectively). Only one isolate contained Panton-Valentine leukocidin. Enterotoxin A was associated with elevated V-MIC (p=0.009) however there were no differences in other virulence determinants. Disease severity scores and ICU admission were not different in patients with low versus elevated V-MIC isolates. Isolates belonging to accessory gene regulator (agr) group I were common overall (59%), however agr group II was associated with elevated V-MIC (p=0.007). Elevated V-MIC was associated with CC5
(including MSSA, \( p=0.045 \)) and CC8 (including MSSA, \( p=0.025 \)), and low V-MIC was associated with CC22 (exclusively MRSA, \( p<0.001 \)) and CC188 (exclusively MSSA, \( p=0.010 \)).

**Conclusions**

These results suggest that organism factors, in particular the genetic background, impact upon on V-MIC. These factors, rather than failure of vancomycin treatment, may contribute to increased mortality seen in patients with elevated V-MIC SAB. Prospective studies are required to fully understand the relative contribution of organism factors, patient factors and V-MIC to outcome in SAB.

### Antibiotic specific sRNA responses exist in multi-resistant methicillin-resistant staphylococcus aureus

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**Introduction:** Little is known about the role of S. aureus sRNAs in antimicrobial resistance and tolerance, however it is likely they form part of the global response to antibiotics.

**Aim:** To determine the global transcriptional profile, focussing on the sRNA response, of the ST239 MRSA strain JKD6009 and its VISA derivative JKD6008, which emerged in vivo following glycopeptide therapy, after exposure to last line antimicrobials.

**Methods:** RNAseq was employed to assess RNA expression under 20 conditions, including both isolates at 2 and 6 hours, without antibiotic exposure and after exposure to 0.5 x MIC for each of ceftobiprole, linezolid, tigecycline, and vancomycin, and using RNA isolation procedures to enrich for sRNA or mRNA. Differential RNA expression analysis was performed using EdgeR and limma. Non-negative matrix factorization (NMF) was used to explore this complex dataset to determine if condition-specific class responses were occurring.

**Results:** 410 sRNAs were identified in the ST239 MRSA strain. Fifty-two of these have been previously verified using Northern blot or RT-PCR. Global analysis revealed that antimicrobial exposure was the dominant factor driving differential sRNA expression (47 sRNAs differentially regulated), while for mRNA transcriptional analysis time and strain were the dominant factors. Analysis of differential expression after exposure to individual antibiotics, and the NMF both demonstrated a very specific antibiotic sRNA response in ST239 S. aureus. Between 11 - 24 sRNAs were differentially regulated after antibiotic exposure, including a large number of ribosomal antisense sRNAs, and antisense sRNAs to the translation elongation factor.

**Conclusions:** These data demonstrate a coordinated sRNA transcriptional response to antimicrobial exposure in MRSA, providing a potential new avenue for enhancement of antimicrobial efficacy through the specific inhibition of drug responsive sRNAs.

### Comparison of new liofilchem® mic test strips with etest

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**BACKGROUND**

Precise Minimum Inhibitory Concentration MIC results are required for some organism/antibiotic combinations, predicting treatment failure and guiding therapy. The quantitative assay with a predefined concentration gradient has been available for some time. It is simple, convenient to perform and good agreement with Clinical Laboratory Standards Institute (CLSI) broth micro-dilution demonstrated. The new Liofilchem® MIC strips uses paper rather than plastic. This study evaluated the new Liofilchem® MIC strips (LM) compared with Etest.

**METHODS AND MATERIALS**
Two-hundred forty nine isolates were retrieved from -80°C. Included were: 99 Staphylococcus aureus, of which, 28 were methicillin-susceptible, 56 resistant and 15 were heterogenous vancomycin resistant; 50 Streptococcus pneumoniae; 50 Enterococcus 35 vanB resistant and 15 vancomycin susceptible; 50 Enterobacteria were tested for presence of an ESBL. The method of testing followed manufacturer’s guidelines and were interpreted with CLSI recommendations. Appropriate ATCC organisms were included for each antibiotic.

RESULTS
Results are shown in the table below. Discrepancies were one S. pneumoniae penicillin Etest sensitive but LM intermediate; one ceftriaxone resistant Etest but LM susceptible; three VanB Enterococci were susceptible with LM and resistant by Etest and one susceptible Etest but resistant with LM. ESBL strips showed no discrepancies. There were no false positives but 16 were non-interpretable. All ATCC organisms were within acceptable ranges.

CONCLUSION
LM is an acceptable alternative to Etest.

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Evaluation of a Newly Formulated Chromogenic Brilliance MRSA Agar for the Detection of Nasal and Cutaneous Groin Colonization by Methicillin-Resistant Staphylococcus aureus (MRSA)

Patricia B Szczurek¹, Elizabeth A Grabsch, Shirley Xie

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Chromogenic agars have become the standard media for detecting methicillin-resistant Staphylococcus aureus (MRSA). A new formulation of Brilliance agar (bMRSA-2) claims to provide results in 18-24hr with improved sensitivity and specificity. This study assessed the performance of bMRSA-2 for detecting MRSA colonisation from patient nose and groin samples, compared to the original (bMRSA) and molecular testing. Nose and groin broth cultures with known molecular results (tested with IDI-MRSA/BD GeneOhm Assay) were retrieved from -80°C and 10µl of homogenised broth was inoculated onto both media. Cultures were incubated then examined at 18 and 24hr for the presence of blue colonies (suggesting MRSA) and other flora. Presumptive MRSA isolates were subcultured; their identification confirmed by DNAse, tube coagulase and cefoxitin disc susceptibility testing.

120 cultures from 60 patients (nose and groin from each) were assessed. 53/120 (44.2%) specimens were MRSA-positive by molecular testing. At both 18 and 24hr, MRSA was confirmed in 46/120 (38.3%) bMRSA and 49/120 (40.8%) bMRSA-2 cultures. However, false positive rates for the respective agars at 18hr were 6/120 (5%) and 8/120 (6.7%) but increased by 24hr to 9/120 (7.5%) and 15/120 (12.5%). After confirmatory testing, at 24hrs bMRSA had 71.7% sensitivity, 88.1% specificity, 79.7% NPV and 82.6% PPV whilst bMRSA-2 had 79.2% sensitivity, 89.5% specificity, 84.5% NPV and 85.7% PPV when compared to molecular testing. Less other flora (non-blue colonies) was isolated on bMRSA-2 (68/120 [56.7%]) compared to bMRSA (81/120 [67.5%]). In summary, bMRSA-2’s performance is comparable to its predecessor with good sensitivity and specificity even at 18hr. Although less other flora is isolated on bMRSA-2, the false positive rates obtained from both media suggest that confirmatory testing is required.
Comparison of the BD GeneOhm MRSA Assay to BD GeneOhm MRSA ACP Assay Using Combined Swabs for the Detection of Nasal and Cutaneous Groin Colonization by Methicillin-Resistant Staphylococcus aureus (MRSA)

Patricia B Szczurek¹, E A Grabsch¹, S Xie¹, M L Grayson¹, B P Howden¹

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Detection and prevention of hospital acquired MRSA (methicillin-resistant Staphylococcus aureus) is an important infection control strategy. The BD GeneOhm™ MRSA Assay (BD-MRSA) is used widely for rapid testing of nasal swabs. We have previously shown combined nose and groin swabs (CNG) can also be processed as one test. A modified, easier extraction method using achromopeptidase (ACP) has been incorporated into the BD GeneOhm MRSA ACP Assay (BD-MRSA-ACP). In this study we assessed BD-MRSA-ACP for detection of MRSA-colonization against BD-MRSA, using CNG swabs.

Nose and groin swabs were collected from ICU patients using dual swabs for each site. One each of the nose and groin swabs were broken into individual sample buffer tubes. 250µl of nose and groin buffers were combined (total 500µl) in the lysis tube for BD-MRSA. 0.45µl aliquots of each of the same nose and groin buffers were combined for BD-MRSA-ACP (total 90µl). Lysis was performed according to protocol and samples run using the Smart Cycler II (Cepheid). PCR-positive samples were tested by culture; 1ml of tryptone soya broth+6.5% sodium chloride (TSB NaCl) was added to each nose and groin buffer tubes. After 48h incubation, the TSB NaCl was subcultured onto chromogenic MRSA-agar (cMRSA). The spare nose and groin swabs were also plated directly to cMRSA. These swabs were also combined (after culture) for testing using the XpertMRSA-Assay, if the two PCR-assay results differed.

233 CNG samples (153 patients) were assessed for MRSA-colonization using the two assays; 6/233 (2.6%) and 7/233 (3.0%) were MRSA-positive by BD-MRSA and BD-MRSA-ACP respectively. Using CNG specimens, BD-MRSA-ACP had 100% sensitivity, 99.6% specificity, 100% NPV and 85.7% PPV compared to BD-MRSA.

Testing CNG specimens using BD-MRSA-ACP has excellent sensitivity and specificity. Like its predecessor, it allows for a more cost-effective approach to rapid molecular testing without the additional cost of processing each specimen separately.

Vancomycin dosing and monitoring – are we there yet?

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Background:
Vancomycin dosing recommendations have been revised to include higher target trough serum concentrations and administration of loading doses for rapid attainment of therapeutic levels¹. In September 2010, vancomycin guidelines at a tertiary teaching hospital were reviewed.

Aims:
Describe concordance with dosing and monitoring recommendations, achievement of therapeutic targets, and identify safety concerns with current vancomycin therapy.

Methods:
A prospective audit of non-ICU patients, prescribed intravenous vancomycin was conducted over 6-weeks. Data included demographics, indication, dosing, renal function, timing and results of vancomycin serum concentrations. Reasons for withheld/delayed doses and changes were identified by reviewing clinical charts and staff discussion.

Results:
For the 50 patients included, two-thirds of initial loading doses and maintenance doses were concordant with the guidelines and 84% of initial vancomycin serum concentrations were
drawn appropriately. However, there was variability in timing and follow-up of subsequent concentrations. Three-quarters of the patients recorded a therapeutic vancomycin concentration (15-20mg/L), achieving this in a mean of 4 days. Supra-therapeutic vancomycin levels were recorded during prolonged courses and 6 (12%) patients experienced significant deterioration in renal function.

Conclusions:
Whilst uptake of local vancomycin dosing and monitoring recommendations has occurred, this audit has identified clinical staff knowledge gaps and educational opportunities.


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Rapid Identification of Microorganisms using Matrix Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS)

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Introduction: Traditional methods for identifying infecting organisms from cultures often require 18 to 48 hours or longer, these techniques are slow, insensitive and labour intensive. Recent improvements in genomic computing and technology for proteomics have made rapid identification or organisms possible using Matrix Assisted Laser Desorption/Ionisation Time of Flight Mass Spectroscopy (MALDI-TOF-MS). MALDI uses the spectra of laser vapourised/ionised proteins to compare with extensive libraries of know spectra to obtain a reliable identification of bacteria yeasts, and fungi.

Method: We compared identifications obtained on over 400 organisms isolated from clinical specimens. Compared systems included routine biochemical identification by replicating system, Vitek2 biochemical identificaion, laboratory spot tests, antimicrobial susceptibility and latex agglutination systems.

The data was compared and discrepancies resolved with additional systems, including the above and the comparison of MALDI-TOF-MS systems from 2 companies (Bruker and BioMerieux).

Results:
Over 400 total cultures were tested, there was concordance at species level at >95% and at genus level at >98% where an identification was obtained from available data.

Results were available rapidly (usually within 1 hour preparation), the instrument is user friendly and will soon provide identification within hours of reading cultures.

Conclusion: MALDI-TOF-MS provides identification of micro-organisms rapidly and with a high level of concordance to traditional methods but significantly lower costs.
A novel nitrofuran for the imaging of tumor hypoxia

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3. Chemistry, University of Melbourne, Melbourne, Vic, Australia

Background: Positron Emission Tomography (PET) imaging of hypoxia is of great significance for the management of cancer patients. To date, the most commonly used radiotracers for PET imaging of tissue hypoxia are the nitroimidazole based compounds [18F]FMISO and [18F]FAZA. However, slow accumulation in hypoxic tissue and slow clearance from normoxic tissue results in a low target to background ratio and a 2 h delay between tracer administration and the actual scanning of a patient needs to be observed. In our search for novel imaging agents for hypoxia we have labelled a PEGylated nitrofurane with fluorine-18 and investigated the uptake of this putative tracer in transplanted SK-RC-52 tumor bearing mice.

Method: The radiolabelling of the precursor molecule was achieved via a click chemistry approach using F-18 fluoroethyl azide. A rat S9 liver fraction assay was employed to investigate the metabolic stability of the putative tracer in vitro.

In vivo, transplanted SK-RC-52 tumor bearing BALB/c nude mice were used to determine tracer kinetics and uptake in hypoxic tumors. Dynamic imaging using a Mosaic small animal PET scanner was performed over a period of 2 h by acquiring 12×10 min frames from the start of tracer injection. Animals were injected with 9.25 MBq of radiotracer in 100 µL of the final formulation and anaesthetized using isoflurane delivered by the Minerva Biovet animal imaging system before scanning.

Results: F-18 labelled LC1 was synthesised in 78±3% radiochemical yield and was found to be metabolically stable when subjected to our S9 liver fraction assay. In vitro uptake studies in hypoxic SK-RC-52 tumor bearing BALB/c mice showed no retention of LC1 in tumors. However, fast clearance from muscle was observed.

Conclusion: Changes to the chemical structure of LC1 need to be made in order to improve trapping of this nitrofurane derivative in hypoxic tumors in vivo.

MR Imaging Features of DNETs and Gangliogliomas. A retrospective review

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¹. Austin Health, Heidelberg, Vic, Australia

Objective / Introduction: Dysembryoplastic neuroepithelial tumours (DNETs) and gangliogliomas are epileptogenic lesions which share similar imaging characteristics making them difficult to distinguish radiologically. The aim of the study is to review the MR imaging features that can help differentiate these two entities.

Methods: The MR images of all patients with histopathologically proven DNET and gangliogliomas imaged at Austin Health between July 2003 and December 2011 were reviewed. T1 and T2 signal characteristics, presence of cystic components, focal susceptibility, cortical dysplasia and parenchymal mass effect as well as contrast enhancement and lesion location were recorded for each case.

Results: DNETs demonstrated low T1 and high/heterogenous T2 signal with a parenchymal mass effect and a cystic component present in 67% of the cases. Gangliogliomas demonstrated mainly low T1 and high T2 signal with a parenchymal mass effect and cystic components present in 80% of the cases. The presence or absence of a susceptibility effect adjacent dysplasia and skull deformity was not specific to either. Contrast enhancement and diffusion restriction were inadequately assessed due to technical factors.
Conclusion: DNETs and gangliogliomas share similar MRI features. MRI cannot reliably distinguish between these two entities. When either DNET or ganglioglioma are included in the differential diagnosis, both should be mentioned.


Detection of Activated Platelets in Carotid Artery Thrombosis in Mice with radiolabeled single chain antibodies for PET

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The chronic inflammatory disease, Atherosclerosis is a major underlying cause of ischaemic heart disease. Rupture of unstable atherosclerotic lesions, causing heart attack and stroke, is the most common cause of sudden death in the developed world. Activated platelets play a major role in development of lesions, in both early stages and formation of thrombi after plaque rupture. We previously generated a single-chain antibody (scFv) that specifically binds platelet glycoprotein integrin receptor IIb/IIIa in its activated, ligand-bound form (LIBS) 2. The purpose of this study is labelling LIBS-scFv with F18 to investigate its potential as an imaging agent to detect activated platelets in thrombi.

Radiolabeled LIBS-scFv was prepared by reacting with N-succinimidyl-4-[18F]fluorobenzoate (S[18F]FB). Radiolabeled scFv was incubated with in vitro formed platelet clots. Excess unbound [18F]scFv was washed off. Clots were imaged in PET scanner and bound radioactivity was measured using an ionisation chamber and image analysis. Both measurements show increased radioactivity in clots exposed to [18F]SFB-LIBS compared to non-binding control scFv (p = 0.04, n=9). Clots incubated with [18F]SFB-LIBS retained on average of 3.5% of radioactivity compared to clots incubated with the control scFv which retained 1.3% of radioactivity.

Assessment of vessel injury and biodistribution of the radiolabeled scFv was studied in a mouse model of thrombosis, where a platelet-rich clot is induced in the carotid artery. High uptake of [18F]SFB-LIBS in the injured vessel compared to non-injured vessel is observed. Whilst no difference between the vessels is observed in mice injected with radiolabeled control scFv. Presence of platelets in the injured vessel was determined by histology subsequently.

The novel radiotracer [18F]SFB-LIBS might be useful for detecting activated-platelets associated with rupture-prone plaques. The potential to determine if patients have vulnerable plaques would allow early initiation of medical treatment to stabilize plaques 2.

Avoidable DVT Ultrasounds

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Purpose:
The majority of Australian Emergency Departments do not routinely use a diagnostic algorithm (eg Well's Score [2]) to aid in the diagnosis of DVT (1). We seek to confirm this and to show that this leads to high rates of potentially avoidable ultrasound scans.

Methods and Materials: Retrospective audit of all Emergency referrals and results for ultrasound examinations for DVT between 1/1/2010–31/12/2011.

Inclusion criteria was: all patients over 18 years of age; all referrals from an ED doctor; referral for one or both legs to be scanned; scanned at one of our two campuses; and, scanned by a Radiology Registrar or Sonographer. The exclusion criteria included: non-acute presentation (eg follow-up scan); and, non-diagnostic or incomplete scans. All referrals were assessed for presence or absence of a Well’s criteria or score.

Results: The total number of included US scans performed in the specified time period was 787, 87% of which returned a negative result. Of the total number of scans performed 83% of the referrals did not include a Well’s Criteria or Score. When one or more Well’s Criteria was included in the referral from ED, this more than doubled the positive predictive value of the US.

Conclusion: Lack of use of a diagnostic algorithm for the diagnosis of DVT in our hospital leads to unnecessary test ordering. Use of at least one Well’s Criteria improves the positive predictive value of ultrasound, thus eliminating unnecessary scans.


Longitudinal Analysis of Cortical Thickness in PiB+ and PiB- Healthy Elderly Controls

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Previous studies have shown that Aß plaques are likely to exhibit local effects on cortical grey matter only at early stages of the disease, sometimes even before cognitive symptoms arise. Understanding when and where cortical neurodegeneration starts may provide insights into the pathogenesis of AD. In this study, we examined cognitively unimpaired elderly subjects, termed healthy controls (HC). While the majority of the HC present with low neocortical PiB retention, ~30% present with high PiB retention. We evaluated the progressive loss of cortical thickness (CTL) in HC with large and low PiB retention independently.

As part of the Australian Imaging, Biomarker and Lifestyle study, 53 HC underwent MRI and 11C-PiB PET scans every 18 months over 3 years. PiB scans were normalised using the standardized uptake value ratio (SUVR) method. A SUVR threshold of 1.5 was use to
differentiate subjects with high PiB retention (PiB+) from those with low retention (PiB-).
Based on this threshold, 15 HC (28%) were classified as PiB+, while 38 HC (72%) were
deemed PiB-. The cortical thickness values from the baseline and follow-ups MRI were
mapped onto their associated baseline WM/GM interface surface and corrected for age. All
baseline surfaces were registered to a common surface to generate population statistics.
While the PiB- HC group exhibited some CTL, CTL was faster in PiB+ HC than in PiB- HC
especially in the temporal (p=0.05), posterior cingulate, and hippocampal regions (Fig 1).
When compared to the 18 month scans, CTL was more extensive at 36 months in PiB+ HC
but no significant progression was observed in PiB- HC.
High Aβ burden is associated with faster CTL, suggesting an early neurodegenerative effect
of Aβ in asymptomatic individuals. The pattern of CTL also suggests that certain areas of the
brain, e.g. the temporal lobe, might be more susceptible/vulnerable to Aβ than others.

**Audit of Pre-test probability (Well's score) and D-Dimer use prior to ordering CTPA in the emergency setting at the Austin Hospital.**

**Mark Emmerton**

1. Austin Health, Heidelberg, VIC, Australia

We audit the use of pre test probability calculation (Well's score) and D-Dimer prior to the
ordering of CTPA for investigation of pulmonary emboli in the emergency setting at the Austin
hospital. Patterns of CTPA ordering, outcomes and involved patient characteristics are
explored. By using Well's Score and D-Dimer results, the number of CTPAs ordered in low
risk patients can be reduced. Reducing the number of CTPAs ordered reduces unnecessary
radiation exposure to patients and provides cost benefit; improving overall quality control.

**Primary Cerebral Lymphoma: Neuroradiology-Pathology Audit**

**Tom Entwisle**

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**AIM**

To assess the accuracy of MRI diagnosis of primary cerebral lymphoma (PCL) at Austin
Health.

To identify and highlight the typical MR imaging features of PCL.

To analyse the false negative and false positive cases to identify confounding imaging
features of PCL.

To present a case series of PCL highlighting confounding or atypical imaging features.

**METHOD**

All cases of PCL in immunocompetent patients in the Austin neuroradiology-pathology audit
from 2003 to mid-2012 were retrospectively reviewed. The pre-operative diagnostic MRI
report and subsequent histopathological diagnosis were compared with each case
categorised as true positive, true negative, false positive or false negative.
RESULTS
The Austin neuropathology audit includes 1174 cases from 2003. There were 25 cases of primary cerebral lymphoma, of which there were 16 correct and 9 incorrect initial MRI diagnoses. There were 23 cases where PCL was raised in the differential but was subsequently shown to be incorrect at histopathology.

<table>
<thead>
<tr>
<th>MRI Status</th>
<th>Primary Cerebral Lymphoma</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>16</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>True Positive</td>
<td></td>
<td>False Positive</td>
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<tr>
<td>Negative</td>
<td>9</td>
<td>1126</td>
<td>1135</td>
</tr>
<tr>
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<td>True Negative</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>1149</td>
<td>1174</td>
</tr>
</tbody>
</table>

Sensitivity 64%
Specificity 98%
Positive Predictive Value 41%
Negative Predictive Value 99%
PCL represents 2.1% of cases in the Austin neuroradiology-pathology audit (Up-to-Date states 4% of newly diagnosed primary CNS tumours).
Important discriminating MRI features include contact to a CSF surface, avid/homogeneous contrast enhancement, isointense with cortex on T2 weighted images and restricted diffusion. Mild mass effect and vasogenic oedema for the extent of disease are also suggestive.

CONCLUSION
Primary cerebral lymphoma is uncommon. Familiarity with the typical distribution and MRI signal characteristics, and knowledge of particular discriminating features, is essential for the accurate diagnosis of PCL. As PCL is optimally treated with biopsy and chemoradiotherapy rather than resection, it is important to include it in the differential diagnosis to avoid the higher morbidity associated with neurosurgical resection.

VOI markup and analysis were performed using PMOD. Preclinical pharmacokinetic data were extrapolated to its human-equivalent and dose assessment was implemented using OLINDA 1.0|EXM.

RESULTS: The $^{11}$C-AG1478 uptake was initially high in the liver and stomach, followed by gradual clearance (11.6%ID/g and 13.95%ID/g at 60-min post-injection). A steady and substantial accumulation of radiotracer was observed in the gallbladder and urinary bladder. Uptake in heart dropped rapidly to a plateau (2.3%ID/g) by 20-min post-injection. Tissue distribution revealed similar uptake patterns in major organs. The highest absorbed dose in human was determined to be to the gallbladder wall (1.12E-03±9.89E-05 mGy/MBq), followed by the urinary bladder wall (8.53E-04±1.67E-04 mGy/MBq) and liver (8.06E-04±1.57E-04 mGy/MBq). The human-translated EDE was 5.84E-03 mSv/MBq for a male and 7.19E-03 mSv/MBq for a female.

CONCLUSION: Tracer kinetics and biodistribution estimated via small animal PET imaging correlated well with a cut-and-count method. Both dynamic imaging and dose assessment confirmed that $^{11}$C-AG1478 was principally metabolized and excreted through the hepatobiliary and renal pathways.

A Feasibility Study of Using InstaDose™ Personal Radiation Dosimeter In a PET Facility

Sylvia J Gong, Lisa Mong, Kenneth Young, Jessica Welch, Kevin Hickson, Kunthi Pathmaraj, John Sachinidis, Gordon Chan, Henri Tochon-Danguy, Paul U, Graeme J O'Keefe, Andrew M Scott

INTRODUCTION: Legal records of occupational dose are commonly provided by approved TLD services. InstaDose™ is a direct ion storage dosimeter with reportable dose range from 0.03mSv to 5Sv. Its USB connectivity enables instant reading of accumulative dose on any computer connected to InstaDose server via internet. This project studied the feasibility of using InstaDose in our PET facility.

METHOD: The measurement characteristics of InstaDose was assessed in the low dose range. Four zeroed InstaDoses along with two personal TLDs were irradiated to individual radiation point sources ($^{99}$mTc, $^{111}$In, $^{131}$I, $^{18}$F and $^{137}$Cs). Consistency tests were conducted by exposing individual InstaDose to a 740MBq $^{137}$Cs point source along with either a pair of AEGIS ED2 probes or a Vertec Bleeper. InstaDoses were worn side-by-side with trunk TLDs by 9 department staff for 3 months, and worn on wrist by 2 radiopharmacy staff for 2 months. The integrals of InstaDose periodical read-outs were compared with trunk TLDs’ quarterly readings and extremity TLDs’ monthly readings.

RESULTS: InstaDose reported -7.9% to 28.7% dose differences from the TLDs over 140-662 keV radiation energies at the low dose range (70-300µSv). Bland-Altman analysis showed good agreement between the energy-corrected InstaDose and the TLD dose results. The InstaDose responses varied by 3-5% in repeatability and reproducibility tests. Operational-based exposures of InstaDose show average weekly dose of 71±8.3µSv in technologists and 53±32µSv in radiopharmacists.

CONCLUSION: InstaDose with its on-line account service provides a complementary approach to monitor and manage occupational dose digitally with flexibility, minimum waiting time and low cost. Applications in dose assessment and operation optimization which were not previously feasible with TLDs can be achieved with InstaDose. However its readout-and-record sampling scheme is a limitation in situations where an electronic personal dosimeter with secure data storage is more suitable.
A Gibbs artifact in PET images reconstructed with the TrueX algorithm

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BACKGROUND: Iterative reconstruction methods that incorporate accurate system modeling are beginning to see increased use in clinical PET imaging. For example, depth-of-interaction (DOI) effects are corrected in the Siemens Biograph PET scanner by incorporating a scanner specific point spread function (PSF) into the system matrix. This paper aims to show the introduction of a ‘Gibbs-like overshoot’ artifact in images reconstructed with the Siemens TrueX algorithm and the possible implications to clinical imaging.

METHOD: A series of Jaszczak phantom measurements have been performed with varying foreground / background activity concentrations. Phantom images are reconstructed using both OSEM-3D and TrueX algorithms. Visual comparisons of the reconstructed images have been conducted to view the presents of the Gibbs artifact. Image quality was quantified by calculating the percentage contrast of each of the hot and cold spheres as well as the percentage background variability using the equations defined in the NEMA NU2 protocol. The recovery coefficients for the different sphere volumes have also been calculated.

RESULTS: Noticeable artifacts are seen in the TrueX reconstructed images. It has been found that the TrueX reconstruction overestimates the activity concentration (and therefore SUV) compared to the true activity and that in general there are some improvements in image contrast and background variability compared to OSEM-3D.

CONCLUSION: It is recommended that particular care should be taken when using the TrueX algorithm for quantitative SUV measurements. Clinicians should also be aware of the possible presence of this artifact in algorithms that incorporate direct modeling of the system matrix.

Automated Analysis of AV133 PET Images

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2. The University of Melbourne, Parkville, Australia

Objectives: To validate the an automated method of analysing PET images produced using the ¹⁸F-Fluoropropyldihydrotetrabenazine ([¹⁸F]AV-133) vesicular monoamine transporter type 2 (VMAT2) radioligand, commonly used to distinguish dementia with Lewy bodies (DLB) from Alzheimer’s disease (AD).

Method: AV-133-PET images were obtained from 50 volunteer subjects. An SPM8 script was developed which reoriented and spatially normalized the image to a template developed in-house from nine healthy-control AV-133-PET images. Volumes of interest (VOI), drawn on the standard space of the template, then sampled the Caudate, Anterior and Posterior Putamen, and Midbrain. The sampled regions were then thresholded by excluding the 10% most active and 50% least active voxels. These thresholded regions were then normalized to the AAL Primary Visual Cortex and presented with the respective Z-score. These calculated ratios were then compared to ratios produced manually.

Results
The correlation found between the automated striatal ratios and the manually derived results was found to be over 0.95.

Conclusions
This method of automated analysis closely correlates to the manual analysis.
Improved partial volume correction for single inversion time ASL data

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Aim: Partial volume effect (PVE) causes significant inaccurate CBF quantification in arterial spin labeling (ASL) due to its relatively low spatial resolution. To correct for PVE in ASL, a linear regression (LR) method has been proposed by Asllani et al., but with large spatial blurring. In this study, we propose a modified LTS method for PVE correction and reduced blurring.

Methods: (1) For a 5×5×1 kernel, given that data have properties of structural similarity in the local area, the absolute differences of ΔM between any other voxel and the central voxel were sorted in ascending order; (2) a subset was built by adding the ΔM value to the p-subset until its rank reaches 2; (3) the initial estimation of the two parameters was obtained and it was subsequently used to calculate the residuals rsd(i), for voxels i=1,2,...,25; (4) sort the residues in ascending order and choose h (h=25*alpha) voxels of smallest residuals to form the new h-subset (alpha-proportion of trimming); (5) estimate the parameters based on the current h-subset; (6) repeat step (4) and (5) until the prescribed number of iterations is reached (iterations=10). Two simulated datasets were created; 2 in vivo datasets were acquired with 3D GRASE ASL sequence. For comparisons, both the proposed method and LR method were applied to simulated and in vivo data. To investigate the optimal alpha, 3 different alpha=0.3, 0.4 and 0.5, were each applied to the simulated datasets.

Results and conclusions: Both simulated and in vivo results demonstrate the proposed method can correct the partial volume effects and produces less blurring than the LR method. The proposed method should therefore play an important role in ASL studies.

Deblurring in 3D GRASE ASL by using variable flip angles and k-space demodulation

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Aim: Arterial spin labeling (ASL) can measure cerebral blood flow (CBF) directly and noninvasively. However, ASL suffers from low signal-to-noise ratio (SNR). Three-dimensional imaging techniques, such as 3D gradient- and spin-echo (GRASE), can increase the SNR and measure the perfusion signal in the whole brain at a single inflow time, which greatly benefits CBF quantification. However, a major limitation of this technique is that the images in inferior-superior direction are significantly blurred due to the T2 decay during the relatively long readout time required for whole-brain coverage. In this study, a method based on variable flip-angle and k-space demodulation was proposed for 3D GRASE to manipulate the echo signal and to collect images with less blurring.

Methods: The proposed method was based on our 3D GRASE sequence. To maintain image contrast, high flip-angles were employed for central k-space while low flip-angles for high frequency k-space. A Kaiser-window approach was used to modulate flip-angles. A method proposed by Busse et al. was employed to correct the echo signal. To further enhance the later echo signal, a method based on k-space demodulation was proposed as well. In vivo whole-brain datasets were acquired on a 3T scanner with the proposed sequence.

Results and conclusions: Numerical simulations show that later echoes maintain higher signal than using the proposed method than for methods using either CFA or VFA without k-space demodulation, which leads to higher peak and narrower full-width-half-maximum (FWHM) of the point-spread function (PSF) for the proposed method, and thus less blurring. In addition, the simulations demonstrate that our method is relatively robust to varying T1 and T2, provided T2 is less than 125ms, which is usually true for GM and WM in normal subjects. In
vivo results show that the proposed method has much less blurring than the other two methods.

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Highly Accelerated Single Breath-hold Non-contrast Thoracic MRA: Evaluation in a Clinical Population

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Aim: Contrast-enhanced MRA (Gd-MRA) of the thoracic aorta is useful clinically¹, but is relatively contra-indicated in renal impairment². We evaluate breath-hold 3D electrocardiographically (ECG) gated non-contrast enhanced steady state free precession MR angiography (NC-MRA)³ in a clinical population, compared with ECG-gated Gd-MRA.

Methods: 30 patients were prospectively imaged at 1.5T with NC-MRA and Gd-MRA. Images were reviewed by 2 readers for aortic pathology, image quality, artefacts, diagnostic confidence and aortic dimension. Diagnostic confidence and image quality scores were scored on a 5-point Likert scale (1=worst, 5=best) and evaluated with the Wilcoxon signed-rank test. Paired Student t-test and Bland-Altman analysis were used to compare aortic dimensions.

Results: NC-MRA vascular pathology findings, including aortic aneurysm (n=21) and residual coarctation (n=1), were concordant with Gd-MRA in 29/30 (96.7%) and 28/30 (93.3%) of patients for Readers 1 and 2 respectively. There was high diagnostic confidence (mean 4.35±0.77), not significantly different from Gd-MRA (4.38±0.64), p=0.74. Image quality scores were comparable to Gd-MRA on a segmental basis, with differences observed at the ascending aorta (NC-MRA 3.80±0.88 vs. Gd-MRA 4.13±0.73), left main coronary artery (3.38±1.47 vs Gd-MRA 2.78±1.21) and right coronary artery (3.55±1.40 vs Gd-MRA 2.32±1.16), p<0.05 for all three comparisons. Aortic dimensions were comparable, with the only significant difference observed at the ascending aorta, with mean NC-MRA dimension (4.05±0.76cm) slightly smaller than Gd-MRA (4.12±0.70), p=0.04.

Conclusion: Breath-hold non-contrast enhanced thoracic MRA yields good image quality comparable to gadolinium-enhanced MRA with high accuracy for aortic pathology. It can be considered an alternative to contrast-enhanced MRA in patients with contra-indications to gadolinium or problematic venous access.

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Comparison of blood pool and extracellular gadolinium contrast for functional MR evaluation of vascular thoracic outlet syndrome

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Purpose: Functional MR angiography (MRA) of thoracic outlet syndrome is useful for determining degree/ reversibility of vascular compression in arm abduction and adduction for treatment planning¹. Extracellular gadopentate dimeglumine (ECA) is used in clinical practice, requiring injections in both arm positions, high dose contrast (0.15-0.2mmol/kg), and prolonged scan time. We compare functional MRA using a single injection low-dose blood pool agent (gadofosveset trisodium, BPA) to ECA.

Methods: 11 patients with suspected TOS underwent BPA (n=7) or ECA (n=4) MRA at 1.5T. 0.03mmol/kg BPA was injected once only during abduction, with steady state imaging only at adduction. For ECA, 0.075 mmol/kg was injected in each position (total 0.15mmol/kg). Imaging time was recorded. Two radiologists evaluated abduction-early (arterial), abduction-late (venous), abduction-early, adduction-late images. Image quality (1=worst, 5=best) and
vessel contrast (1=worst, 4=best) were graded and pathology recorded. A separate unblinded radiologist performed pathology reference assessment.

Results: Mean imaging time was 34.6±11.8min for ECA and 26±4.6min for BPA, p=0.1. Reference assessment revealed 1 functional arterial >50% stenosis, underestimated by 1 reader, with remaining findings (6 significant venous stenoses and 1 thrombus) concordant with the reference. Image quality scores were diagnostic for all studies, with significantly higher ECA score at adduction-early only (4.7±0.4 vs. 3.7±0.8, p<0.05). Arterial contrast scores were high (>3) for both agents at early imaging, with superior ECA contrast at adduction-early not statistically significant (3.9±0.4 vs. 3.4±0.6, p=0.11). Both agents scored well for venous contrast at late imaging (all scores>3), p>0.05.

Conclusion: Low-dose BPA MRA for vascular TOS can be efficiently performed with good image quality and diagnostic vessel contrast similar to dual injection ECA.

Assessment of changes in cognition and Aβ deposition in mild cognitive impairment subjects with serial 18F-Florbetaben PET over 2 years

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Background: Converging evidence from 11C-PiB PET studies show that Aβ accumulation precede and predict cognitive decline along the continuum of Alzheimer’s Disease (AD). Due to the very short t1/2 of 11C, novel Aβ tracers labeled with 18F have been developed for routine clinical use.

Objectives: To determine how well 18F-florbetaben (FBB) predicts which mild cognitive impairment (MCI) participant will progress to AD within 2 years, especially if there is significant logical memory (LM) impairment, and to explore the relationship of Aβ burden with cognitive decline and disease progression.

Methods: Clinical and neuropsychological examination, FBB PET and 3D MPRAGE MRI were performed at study entry and 24 months in 45 subjects with MCI. Aβ burden was quantified using neocortical standardized uptake value ratio (SUVR) normalized to the cerebellar cortex. Cut-off for high SUVR was ≥ 1.4. Hippocampal volume (HV) was assessed with Neuroquant®. HV below age adjusted 25th percentile defined hippocampal atrophy (HA). Cox proportional hazards models were applied. Analyses were adjusted for age, gender and years of education.

Results: At baseline, composite memory scores correlated with neocortical SUVR (r=-0.56, p=0.0002). At 24-month follow-up, increase in neocortical SUVR was observed in MCI with high SUVR (+4.1%, p=0.003), and in those who declined in composite memory scores (p=0.04). The hazard ratio for progression to AD was 11 in MCI with high SUVR (p<0.0001) and 4 in MCI with HA (p=0.03). The predictive accuracy for MCI progressing to AD with significant LM impairment who also had both high SUVR and HA was 94%.

Conclusions: FBB PET predicts progression from MCI to AD within 2 years more robustly than hippocampal atrophy, and can show progression in Aβ accumulation over 2 years. In MCI, the combination of significant LM impairment, high Aβ burden and HA likely represents prodromal AD.
Ongoing strategies to manage radiation exposure to PET staff during dose dispensing, dose administration and patient positioning.

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Background
The Austin PET centre has a busy clinical program as well as an extensive research program which poses challenges in managing and minimising radiation exposure to PET personnel. The three main sources of radiation dose in a PET facility are: dose dispensing (finger and whole body dose), dose administration and patient positioning. It is usually the technologists and radiopharmacists who are involved with these procedures; hence we have strict protocols and ongoing strategies to manage radiation exposure to these personnel.

Aims:
To develop innovative strategies to minimise radiation dose to PET Centre staff engaged in routine clinical practice.

Results
Radiation exposure to technologists is primarily due to patient positioning and injection. Through the initial introduction of syriporters, and then further modifying the syriporters with a body shield, we have been able to reduce dose from 2.3 ± 0.9 µSv per procedure to 0.7 ± 0.4 µSv per procedure during dose administration. By using a modified body shield, we have been able to reduce the dose during patient positioning from 1.3 ± 0.7 µSv per procedure to 1.04 ± 0.8 µSv per procedure using a modified body shield. Finger doses to radiopharmacists has been addressed through the installation of an automated dispenser for FDG doses, which has reduced finger dose from 420 µSv/GBq dispensed to 25 µSv/GBq dispensed.

Conclusion
Minimising radiation exposure to PET personnel continues to be an issue, particularly in the era of increasing PET utilisation. We have successfully implemented strategies to address radiation dose to staff, and this will be enhanced through the use of lower FDG doses with new generation PET/CT scanners.

The effect of dose reduction on bone scan image quality

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BACKGROUND: Nuclear Medicine needs to balance the requirement for high quality diagnostic images whilst adhering to ALARA principles. With advancements in imaging technology delivering better sensitivity our institution implemented a 10-20% reduction in administered doses for diagnostic imaging procedures to facilitate a reduction in effective dose to patients. This lead to bone scan doses being reduced from 800MBq to 700MBq.

AIM: To determine if a 100MBq reduction in administered dose of ⁹⁹mTc-MDP impacts on image quality and diagnostic interpretation.

METHOD: A retrospective review of patients having undergone whole body bone scans (WBBS) on the GE Infinia Gamma Camera at Austin Health in 2010-2011. Study Inclusion criteria were as follows: Patient having undergone two WBBS with comparable uptake times; administered doses of 800MBq(+/-10%) and 700MBq(+/-10%) with calculated difference of 70-140MBq and identical imaging parameters. Scans were excluded from analysis if there was evidence of patient movement, superimposition of bones, contamination and subcutaneous injection sites.
Patient studies were anonymised and paired WBBS randomized. Blind review of studies was performed by 3 experienced Nuclear Medicine Physicians. The readers qualitatively reviewed scans for overall image quality, ability to detect lesions and target:background ratio, using a 5-point likert scale (1=poor→5=excellent) to record results. Comparison and analysis of results was then performed.

RESULTS: A total of 30 patients were analysed with the comparison of 2 dose groups. Statistical modes derived from Likert analysis demonstrated no difference between image quality of 2 dose levels, with identical modes for each criteria. In comparison of paired image data only 1 high dose WBBS was determined to be superior in all categories by all reviewers.

CONCLUSION: A 100MBq reduction in the administered dose of 99mTc-MDP leads to a 12.5% reduction in the effective whole body dose from 4.56mSv(800MBq) to 3.99mSv(700MBq) without impacting on image quality or diagnostic interpretation.

Implementation of a Solid Target facility at Austin Health for the production of Cu-64 and I-124.

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BACKGROUND: Copper-64 (Cu-64; t½ =12.7h) and iodine-124 (I-124; t½ =4.2d) are long-lived PET radionuclides attracting increasing interest for clinical and animal studies. Although Cu-64 is available at reactor sites, it has now been produced using low energy proton beams. As well, I-124 commonly available from deuteron bombardment, is now successfully produced from proton bombardment. Therefore, development of cost-effective technologies for I-124 and Cu-64 production using a medium-energy cyclotron is warranted in Australia.

METHODS: A fully automated solid target system was installed, capable of loading, irradiating and transporting the target disk into hotcells for processing. A Nirta Solid Target (Ion Beam Applications, Belgium) is mounted at the end of our existing external 1.5m beam line on an IBA Cyclone 18/9. Separation and purification modules for both radionuclides were also sourced from IBA. Three hotcells, and a custom-built pneumatic transport system have been sourced from TEMA, Faenza, Italy (distributed by GMS, Australia). All equipment has passed acceptance tests. Radionuclide identification for Cu-64 and I-124 was carried out using a multi-channel-analysers coupled to a Canberra HPGe detector.

RESULTS: Initial production tests of Cu-64 were performed at 25uA for 6.3h and showed a good correlation between experimental measurements (170mCi at EO) and theoretical calculations (193mCi). Production tests of I-124 were performed at 12uA for 3.5h and showed good extraction yields (72%, 12.3mCi). The radioisotopic purity of I-124, two days post irradiation, was found better than 95%.

CONCLUSION: Installation of a solid target facility has been completed, with test production of Cu-64 and I-124 successfully demonstrated. The current system is also capable of producing Y-86 and Zr-89 which will be implemented in the near future.

A joint project of Austin Health, ANSTO and Ludwig Institute for Cancer Research.

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Introduction:
The routine daily chest radiograph (CXR) is a common protocol in many intensive care units (ICU). Daily CXRs are often performed unnecessarily, without clinical indication, resulting in inappropriate patient irradiation and additional cost. An alternative ‘on-demand’ system has received much research attention in recent years, with no demonstrable adverse outcomes seen, however there has been little investigation of the referral patterns, cost and radiation savings differences between the two systems.

Objectives:
This study aims to analyze the daily chest radiograph system in a mixed medical & surgical ICU – to determine (1) the average number of CXR per patient per ICU stay; (2) referral characteristics (3) the financial costs of this practice; and, (4) effective radiation dose for ICU patients as a result of this practice.

Method:
Data was collected via in-hospital RIS and ICU databases for all ICU patients over a consecutive 7 month period. Calculation of CXR per ICU stay, financial costs, and effective dose per CXR were performed using data from the ICU research database, RIS, hospital coding data, staff requirements and Medicare rebates.

Results:
4775 CXRs were performed on 877 patients in the 7 month period. 51% were performed as ―routine‖ CXRs. The average ICU patient received 1.6 CXR per day, 0.046mSv effective dose per day, for a total cost of $320000.

Discussion & Conclusion:
The daily routine chest radiograph burdens ICUs with unnecessary costs with no clinical gain. The performance of daily CXR on ICU patients also does not ascribe to the ALARA principle of radiation safety. A follow-up study will analyse an ‘on-demand’ system in the ICU and we hypothesise that our study will add further weight to the argument that the current system of daily ICU chest radiographs should be abandoned.

When is a high blood glucose level too high for FDG-PET brain imaging for dementia?

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2. Ludwig Institute for Cancer Research, Melbourne, VIC, Australia

Abstract
The demand for FDG-PET to investigate the presence and characterise the type of dementia has greatly increased over the past few years. Concurrent diabetes can result in a high blood glucose level (BGL) that can result in poor quality images and potentially interfere with accurate reporting of the study.

Aim: To determine the relationship between BGL and image quality in FDG PET brain studies and to establish a threshold BGL which will ensure diagnostic image quality.

Method: FDG-PET brain images from 266 patients (153 diabetic and 113 non-diabetic) who had presented for the investigation of dementia were retrospectively analysed. The images were analysed blinded to the patient’s BGL status. All scans were assessed qualitatively and rated as good, fair or poor quality.
Results: FDG-PET imaging when performed with a BGL <7 mmol/L, produced diagnostic quality images consistently. As the BGL increased above 7, the incidence of studies judged as fair or poor quality also increased. It was found that when the BGL was between 10.1 and 11 mmol/L, 14% of scans were of poor quality, whilst a BGL above 11.1 mmol/L increased the incidence of poor quality studies to about 44%.

Conclusion: Optimal quality brain FDG PET scans can be routinely obtained with BGL <7mmol/L, but a diagnostic quality FDG PET scan is also possible with a BGL between 7.1 and 11.0mmol/L. Patients with a BGL >11.1mmol/L are rescheduled or administered insulin prior to proceeding with the scan.

Lipid changes in pregnancy

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Introduction

Pregnancy is often listed as a secondary cause of hyperlipidemia although the magnitude and timing of changes is not well understood nor appreciated. Many studies report lipid concentrations at a single time-point in pregnancy and compare these to non-pregnancy levels; not recognising that changes in lipid concentrations are continuous throughout gestation. We aimed to characterise lipid concentrations for each trimester in pregnancy using two independent sources of data.

Methods

Total-Cholesterol and triglyceride results (Roche Modular) from 40,000 pregnant women were extracted from a private pathology database in Australia. Lipids (including HDL-Cholesterol and calculated LDL-cholesterol) were also measured (Beckman DxC) on stored serum from 154 pregnancies originally enrolled in a study for trimester-specific thyroid function reference intervals at the Mercy Hospital for Women, Melbourne.

Results

Results from both study populations showed significant increases in total-cholesterol and triglycerides, peaking in the 3rd trimester. Despite using different assays, the observed reference intervals, Median (2.5th -97.5th percentile), for both populations were similar.

<table>
<thead>
<tr>
<th></th>
<th>Roche Modular</th>
<th>Trimester 1 (9-13 weeks)</th>
<th>Trimester 2 (22-26 weeks)</th>
<th>Trimester 3 (35-39 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Chol, mmol/L</td>
<td>4.5 (3.1-5.9)</td>
<td>6.0 (4.1-7.9)</td>
<td>8.8 (4.5-9.1)</td>
<td></td>
</tr>
<tr>
<td>Trigs, mmol/L</td>
<td>1.0 (0.5-2.0)</td>
<td>1.8 (0.8-3.3)</td>
<td>2.8 (1.5-5.3)</td>
<td></td>
</tr>
<tr>
<td>Beckman DxC</td>
<td>Total Chol, mmol/L</td>
<td>4.7 (3.5-6.9)</td>
<td>6.3 (3.7-8.3)</td>
<td>6.9 (5.0-10.2)</td>
</tr>
<tr>
<td>Trigs, mmol/L</td>
<td>1.2 (0.6-2.9)</td>
<td>2.0 (0.9-5.0)</td>
<td>3.0 (1.5-5.8)</td>
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Fractionated cholesterol results from the Mercy population indicated that VLDL and LDL were the main contributors to the total cholesterol peak in the 3rd trimester. In contrast, HDL declined from the 2nd trimester onwards.

Conclusion

Lipid profiles change in pregnancy, not only from the non-pregnancy state but also between trimesters. Awareness of the timing and extent of these changes will allow clinicians to recognise when hyperlipidemia in pregnancy requires further assessment.
Development of a silver in-situ hybridization-based assay for the determination of ploidy status in molar pregnancy diagnosis

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Background: Hydatidiform moles (HM) are non neoplastic proliferations of the villous trophoblast, including complete hydatidiform moles (CHM) and partial hydatidiform moles (PHM). They have subsequent risk of developing persistent gestational trophoblastic disease and their prognosis and follow-up is significantly different from non-molar mimics. Genetically, CHMs are diploid without maternal contribution, whereas PHM are triploid with a maternal chromosome complement. The difference in their genetic composition is used in diagnostic techniques to differentiate between HM and their mimics, including hydropic products of conception (HP).

Aims: To establish a scoring method for ploidy analysis by silver in-situ hybridization-based assay (SISH) using the chromosome 17 probe

Methods: SISH using the chromosome 17 probe was performed on paraffin sections of archival cases of CHM, PHM and HP at Austin Pathology with previously confirmed ploidy status (determined by either flow cytometry or karyotyping at the Royal Women's Hospital, Victoria). A scoring method was developed based on the average number of signals per nucleus (SN) in fifty villous cytotrophoblasts and/or stromal cells per case.

Results: There was significant difference in the NS for confirmed diploid and triploid gestations, 1.93±0.05 and 2.76±0.07 respectively (Student t-test, P<0.0001). There was 100% interobserver concordance and 100% concordance of SISH scoring with known ploidy status.

Conclusions: This single probe SISH-based assay can reliably distinguish between diploid and triploid gestations.

Maternal morbidity in antenatal transfer and retrieval: The unknown cost of regionalised perinatal care.

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BACKGROUND: Safe and effective transfer systems are essential for ensuring equitable access to specialised care for all women with high risk pregnancies. The Victorian Perinatal Emergency Referral Service (PERS) co-ordinates in-utero transfer to higher level care for ~1200 women annually. These women may constitute a high risk group for maternal morbidity and mortality, however obstetric transport and transfer remains under-researched.

AIM: We reviewed the current local and international literature to establish the incidence, indications and outcomes of antenatal interhospital transfer in well resourced countries globally.

METHODS: A thematic search of computerised databases, textbooks and regional journals in obstetrics, emergency medicine and paramedic sciences was conducted. English language articles relating to in-utero transfer from 1970 to 2012 were identified and included.

RESULTS: 31 relevant articles were identified, 8 of which were were highly relevant. Only one (Australian) article listed maternal indications as well as neonatal outcomes. No articles
specifically examined maternal safety, physiological disturbance or well-being before, during and after transfer.

Key findings were that:
Interhospital transfer distances are longer for pregnant women than non-pregnant women.
The commonest indications for transfer are preterm prelabour rupture of membranes, preterm labour, pre-eclampsia and related conditions, and antepartum haemorrhage.
Air travel appears safe for transporting pregnant women, and in-flight delivery is uncommon.
A significant number of transfers do not result in delivery at the receiving hospital.
In-utero transfer is associated with social disruption, prolonged hospital stay, personal stress and financial cost.

CONCLUSION: Perinatal transfers, may constitute a group of pregnant women at high risk for morbidity and mortality, however more research is required. "The ITriP Study: Evaluating Interhospital Transfers in Pregnancy" is a new statewide data linkage project evaluating two years of maternal transfers to better characterise the maternal as well as perinatal risks and benefits of regionalised perinatal care with a centrally co-ordinated maternal transfer service.


MMP-17: a negative regulator of soluble endoglin production?

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Pre-eclampsia (PE) is a severe disease of pregnancy accounting for approximately 20,000 maternal deaths/year globally and many more neonatal deaths. Soluble endoglin (sEng) is an anti-angiongenic factor centrally responsible for severe early onset PE. It is significantly up-regulated in the serum of PE women and correlates with disease severity. Given sEng’s detrimental effects on maternal endothelium in preeclampsia, it is essential to fully elucidate the mechanisms governing its release.

We have recently identified MMP-14 as the protease that produces soluble endoglin from placenta, however noted that specific inhibition only partially repressed sEng production1. This implies that other undiscovered protease(s) might have a role in sEng production.

The objective of this study was to assess the expression and tissue localisation of other membrane-type (MT-)MMPs (MMP-15, -17, 25) in preeclamptic placentas and to determine their contribution to sEng production in vitro.

Real time RT-PCR and Western Blot on a cohort of severe early onset pre-eclamptic (n=20) and gestationally matched pre-term (n=10) placentas confirmed mRNA expression and revealed a significant (p<0.05) increase in MMP-15 and -17 protein expression in pre-eclamptic placentas. In vitro siRNA administration of MMP-15, -17 and -25 siRNA to human umbilical endothelial vein cells (HUVECs) demonstrated no effect of silencing MMP-15 and MMP-25 on soluble endoglin levels whilst inhibition of MMP-17 resulted in a significant increase in sEng levels (p<0.05). Importantly over-expression of MMP17, MMP14 and endoglin in HEK293Ts completely inhibited sEng production (compared to MMP14 and endoglin alone).

Together these results suggest that MMP-17 acts as a negative regulator of sEng production.

Targeted nanoparticle delivery of doxorubicin to placental tissue – a potential therapeutic for ectopic pregnancy

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Abnormal trophoblast growth can cause life-threatening diseases such as ectopic pregnancy, choriocarcinoma and placenta accreta. Engenic Delivery Vehicles (EDVs) are nanoparticles that promote tissue-specific delivery of drugs, and may be useful to medically treat such disorders. The objective of this study was to assess whether EDVs loaded with the chemotherapeutic doxorubicin and targeting the Epidermal Growth Factor Receptor (EGFR, very highly expressed on the placental surface) can regress placental cells in vitro, ex vivo and in vivo. In a murine xenograft model, intravenous injection of EDVs packaged with doxorubicin targeting EGFR induced a significant reduction (p<0.05) of JEG-3 placental tumour xenografts, compared to EDVs packaged with doxorubicin but targeting an irrelevant antigen (non-targeted EDVs) or naked doxorubicin alone (at 100x the dose contained within the EDVs). EGFR targeted EDVs were more readily taken up by human placental explants ex vivo and induced increased cell death (M30 antibody) compared to non-targeted EDVs. In vitro, EGFR targeted EDVs administered to JEG-3 cells resulted in a significant (p<0.05) dose dependent inhibition of cell viability (MTS assay), proliferation (CFSE by FACs) and increased apoptosis (Annexin V positive cells by FACs) following EDV treatment. Continuous monitoring using the xCELLigence system confirmed end-point assay findings. In conclusion, EGFR targeted EDVs loaded with doxorubicin significantly inhibited xenograft growth in vivo and increased apoptosis ex vivo and in vitro. Furthermore, they decreased cell viability and proliferation in vitro. Therefore, EDV’s may be a novel nanoparticle treatment for ectopic pregnancy and disorders of trophoblast overgrowth.

Corin, a protein involved in spiral arteriole remodelling in mice, is expressed in human endometrium and the villous cytotrophoblast

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Corin has recently been proposed as a new protein involved in trophoblast invasion and hypothesised to be a major contributor to the early pathogenesis of preeclampsia¹, a severe disease of pregnancy. Corin is best known for its role in activating a hormone important for regulation of blood pressure, atrial natriuretic peptide (ANP). Interestingly, pregnant mice deficient in corin or ANP developed characteristics of pre-eclampsia including high blood pressure and proteinuria and were shown to have deficient trophoblast invasion and uterine spiral artery remodelling. However, Corin expression in human gestational tissue remains very ill defined. The objective of this study was to further define Corin expression in human tissues. Corin immunohistochemistry on endometrial tissue from across the menstrual cycle indicated patchy glandular epithelial staining, whilst human endometrial stromal cells induced to decidualise ex vivo displayed increased corin mRNA expression compared to controls.
Similarly, in 1st trimester placental bed biopsies, strong corin expression was apparent in decidual cells surrounding spiral arteries. Corin mRNA levels did not alter between a cohort of severe preeclamptic (n=20) and gestationally matched pre-term placentas (n=10). Within the placenta Corin was localized to the villous cytotrophoblast, the same site as has been previously reported for ANP. Although Corin was readily detectable in early pregnancy sera, there was no change across 7-11 weeks gestation (n=10-15 per gestation), suggesting an extra-placental source during early pregnancy.

In conclusion, Corin expression in 1st trimester human decidua is consistent with Cui et al’s suggestion of a role in activating ANP in these cells, which may contribute to trophoblast invasion and early spiral artery remodelling.


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FOXO1 regulates contraction-associated proteins in human myometrial cells: implications for preterm birth

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Aim: One of the most important complications contributing to neonatal mortality and morbidity is spontaneous preterm birth. Infection and/or inflammation is commonly associated with preterm birth and thought to have a driving role in initiating uterine contractions; thus, anti-inflammatory regulators may be a therapeutic approach to block contractions. FOXO1 is a pro-inflammatory transcription factor which controls numerous physiological and pathological processes. The aim of this study was to determine the effect of FOXO1 inhibition on pro-labour mediators in primary myometrium cells.

Method: Smooth muscle cells were isolated from pregnant human myometrium (n=6 patients). Cells were transfected with 100 nM FOXO1 or non-specific (NS) siRNA for 72 h. After treatment with 1 ng/ml IL-1β for 24 h, pro-labour mediators were assayed (cytokines, cyclooxygenase (COX)-2, extracellular matrix remodelling enzymes).

Results: Incubation of myometrial cells with 100 nM FOXO1 siRNA resulted in 60% decrease in FOXO1 mRNA expression, without affecting the expression of other FOXO proteins (FOXO3 and FOXO4). FOXO1 inhibition by siRNA decreased IL-1β-induced pro-inflammatory cytokine expression (TNF-α mRNA expression, and IL-6 and IL-8 mRNA expression and release), COX-2 mRNA expression and subsequent prostaglandin release (PGE₂ and PGF₂α), and MMP-9 mRNA and pro MMP-9 enzyme activity.

Conclusions: Our studies have shown an important role for FOXO1 in the regulation of cytokines, prostaglandins and MMPs associated with preterm birth. This pro-inflammatory effect of FOXO1 in human myometrium provides a possible point of intervention for the management of preterm labour; namely that inhibition of FOXO1 could aid in the cessation of contractions.

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SIRT6 regulates key terminal effector pathways of human labour: possible therapeutic target for the management of infection-induced preterm birth

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Aim: Preterm birth (PTB), with its attendant morbidity and mortality, is one of the most significant health care issues in the world. Pre-labour rupture of fetal membranes (PROM) accounts for one-third of cases of PTB. Ascending infection stimulates inflammatory responses in the fetal membranes leading to the degradation and eventual rupture of the membranes. The mechanisms that regulate this are unknown; however, the pro-inflammatory
transcription factor nuclear factor-κB (NF-κB) and its target genes play an important role. In non-gestational tissues, sirtuin (SIRT) 6 exhibits anti-inflammatory actions by inhibiting NF-κB. The aims of this study were to determine the effect of (1) human preterm labour on SIRT6 expression; and (2) SIRT6 inhibition and overexpression on pro-inflammatory and pro-labour mediators in human fetal membranes.

**Method:** SIRT6 mRNA and protein expression was determined in fetal membranes from preterm (1) Caesarean section with no labour and (2) after spontaneous labour and normal vaginal delivery. In amnion cells extracted from fetal membranes, SIRT6 knockdown was achieved using siRNA and SIRT6 overexpression using a cDNA clone. After treatment with IL-1β, pro-labour mediators were assayed.

**Results:** SIRT6 mRNA and nuclear protein expression was significantly lower in fetal membranes from women after preterm labour compared to preterm not in labour. In primary amnion cells, SIRT6 inhibition increased IL-1β-induced cytokine expression (IL-6, IL-8, TNF-α), COX-2 mRNA and subsequent prostaglandin release, MMP-9 mRNA expression and release and NF-κB p65 mRNA expression. Conversely, SIRT6 overexpression decreased IL-1β-induced cytokine, prostaglandin, MMP-9 and NF-κB p65 mRNA expression.

**Conclusion:** Spontaneous PTB is associated with decreased SIRT6 expression. Functional studies demonstrate an important role for SIRT6 in the regulation of cytokines, prostaglandins and MMPs associated with PTB. These actions of SIRT6 appear to be mediated via its effects on NF-κB. Thus, SIRT6 could provide a candidate therapeutic target for the management of infection-induced PTB.

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**Biomarkers of impending preterm pre-labour rupture of fetal membranes**

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**Background:** Preterm pre-labor rupture of membranes (PPROM) is the rupture of the fetal membranes prior to the onset of labour at less than 37 weeks’ gestation. PPROM accounts for approximately 30% of preterm births and is associated with chorioamnionitis, neonatal sepsis and placental abruption, leading to high risks of perinatal morbidity and mortality. The objective of this study was to identify differentially expressed proteins in the cervicovaginal fluid (CVF) of asymptomatic women before the clinical manifestation of PPROM.

**Subjects:** Asymptomatic pregnant women were prospectively recruited for CVF sample collection. The PPROM group consisted of women with samples collected within 6-23 days before rupture of membranes and who subsequently delivered preterm (n=5). Women with normal spontaneous term delivery outcomes served as gestation-matched controls (n=10).

**Methods:** Two-dimensional difference in gel electrophoresis (2D-DIGE) was used to distinguish differential expression between the pooled cohorts. To examine confirm fold changes, each individual sample was subjected to 2D-polyacrylamide gel electrophoresis (2D-PAGE) analysis. Spots of interest were identified by mass spectrometry and validated by Western blot.

**Results:** Proteomic analysis of the CVF revealed differing expression profiles between the PPROM and term control groups. Proteins that were significantly decreased in the PPROM cohort included: thioredoxin, interleukin-1 antagonist receptor, fatty acid-binding protein 5, cystatin A, serpin B1 and serpin B3 (p<0.05). In contrast, annexin A3 and vitamin D-binding protein were significantly increased before PPROM (p>0.05). These proteins have known biological functions in oxidative balance, inflammation or protease inhibition that may impact on cervical dilatation and weakening of the fetal membranes.

**Conclusion:** We have identified several proteins that are differentially expressed before PPROM. The development of a test to assess the risk of an impending PPROM event in clinically asymptomatic women may in the future provide better management and improved maternal and fetal outcomes.
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PAPP-A2 in the Pathogenesis of Pre-eclampsia

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Pre-eclampsia (PE) is a severe disease affecting 4-8% of pregnancies and accounting for 20% of maternal deaths worldwide. PE is believed to arise as a result of persistent placental hypoxia due to insufficient placentation. Pregnancy-associated plasma protein-A2 (PAPP-A2, Pappalysin-2) is a novel homolog of PAPP-A of the metzincin superfamily. In silico analysis shows 700-fold placental PAPP-A2 expression compared to other human tissues, whilst our previous data indicates a 20-fold up-regulation in PE compared to term placental mRNA expression.

The objective of this study was to characterise PAPP-A2 protein expression and determine its functional role in the PE placenta. Real time RT-PCR and Western Blot confirmed PAPP-A2 mRNA and protein expression in a cohort of severe early onset PE placentas (n=17) and gestationally matched pre-term controls (n=12). Immunohistochemistry revealed PAPP-A2 protein was localised to the syncytiotrophoblast in PE and preterm placenta, however no expression was detected in term placental tissue. Using the choriocarcinoma BeWo cell line, we demonstrated a significant (p<0.05) decrease in PAPP-A2 mRNA and protein expression with syncytialisation (confirmed by hCG ELISA). Given PAPP-A2 has a putative hypoxic recognition site in its promoter region, we next assessed PAPP-A2 mRNA and protein expression in BeWo cells and primary placental explants following exposure to hypoxia (1% oxygen).

In both BeWo cells and placental explants, hypoxia induced a significant (p<0.001) increase in PAPP-A2 mRNA and protein expression. Together this data suggests that PAPP-A2 may become dysregulated during the early pathogenesis of PE when there is persistent hypoxia resulting in over-expression that contributes to the ongoing placental dysfunction observed in PE.

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Phytophenols as Therapeutic Agents in the Management of Infection-induced Preterm Birth

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Objectives: Preterm birth (PTB) is associated with a high incidence of perinatal morbidity and mortality. Inflammation is commonly associated with PTB and has a driving role in initiating uterine contractions and rupture of fetal membranes. Pro-inflammatory cytokines induce the production of (i) matrix metalloproteinases (MMPs) that degrade the extracellular matrix (ECM) within the cervix and fetal membranes, and (ii) prostaglandins which initiate uterine contractions that can lead to PTB. Studies from our lab group have shown a key role for nuclear factor-κB (NF-κB) in promoting the formation of these pro-labour mediators. In non-gestational tissues, naturally occurring plant compounds (phytophenols) such as luteolin (from celery) and kaempferol (from grapefruit) inhibit NF-κB and its downstream targets. The aim of this study was to determine if luteolin and kaempferol will reduce infection-induced pro-labour mediators in human gestational tissues.

Methods: Fetal membranes and myometrium were obtained at term Caesarean section. Fetal membranes and primary myometrial smooth muscle cells were incubated for 24 h with 10 μg/ml LPS or 1 ng/ml IL-1β in the absence or presence of 20 μM luteolin or 100 μM kaempferol (n=6-8). The endpoints examined were, gene expression by qRT-PCR; release of pro-inflammatory cytokines (IL-6 and IL-8) and prostaglandins (PGE₂ and PGF₂α) by ELISA; and MMP-9 activity by gel zymography.
Results: Luteolin and kaempferol significantly reduced LPS/IL-1β-induced expression and secretion of pro-inflammatory cytokines, cyclooxygenase gene expression and resultant prostaglandin production, and MMP-9 expression and activity.

Conclusion: Luteolin and kaempferol inhibit pro-inflammatory mediators in human gestational tissues. Given the central role of inflammation in provoking preterm labour, phytophenols may be a therapeutic approach to reduce the incidence of PTB. Related studies are now underway to determine their effect in an in vivo mouse model of infection-induced PTB.

A Neonatal Intervention to Improve Development in Preterm Infants: outcomes of a randomised controlled trial to 6 months of age

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BACKGROUND: Despite ongoing improvements in clinical care, preterm infants experience a variety of sources of stress in the first weeks of life, including necessary medical procedures, which may impact on development. A small number of stress-reduction programs based in the neonatal intensive care unit (NICU) have reported a positive impact on development of preterm infants. In particular, trials of the Mother-Infant Transaction Program (MITP) have shown promise, and are based on parent sensitivity training beginning while infants are in NICU. In a randomised controlled trial of a MITP-type program (PremieStart) involving 109 women with 123 very preterm infants born at <30 weeks gestation, we assessed the efficacy of the intervention, initially at term equivalent age and at 6 months corrected age.

RESULTS: Some differences between intervention and control conditions were demonstrated by term equivalent age, and included later preterm infants (≥28 weeks gestation) in the intervention group being significantly heavier than later preterm control group infants after controlling for birth weight (p<.05). Intervention mothers were more sensitive in providing care to infants, stressed their preterm infants less, and responded more appropriately to both positive and negative infant cues (p<.05 in each case).

At 6 months corrected age, intervention infants showed higher mean scores on the Communication and Symbolic Behavior Scale (CSBS). The strongest effects appeared in Symbolic behaviour (p<.05) and this was reflected in the Total score (p<.05).

CONCLUSIONS: Previous research has suggested that the benefits of some NICU-based interventions tend to emerge at later ages. Given the later cognitive and language deficits reported in longitudinal studies of preterm infants, an intervention that improves an early marker of infant communication abilities (CSBS) is promising. A follow-up study of this cohort is underway to assess child cognitive, emotional and behavioural development until school age.
The Effectiveness of a Restart Break Containing Two Biological Nights for Maintaining Simulated Driving Performance across Consecutive Weeks of Night Work

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Current hours of service regulations stipulate that U.S. commercial motor vehicle drivers must have a 34h “restart” period for recovery after accumulating 70h on duty before commencing another work period. We recently showed that in night shift workers, where the 34h restart period contains only one biological night, recovery is incomplete. The current study aimed to determine whether extending the restart period to 58h, so as to include two biological nights, would help sustain performance in night shift workers.

Sixteen healthy men (ages 27.5±5.6y) participated in a 16-day in-laboratory experiment. After two baseline days with nocturnal sleep, subjects were given a 5h nap, and underwent 5 days of night work and daytime sleep (TIB 10:00-2:00). This was followed by a 58h restart period, which included a 5h nap, two days with 10h nocturnal sleep, and another 5h nap. Subjects then underwent another 5 days of night work and daytime sleep. On night work days, subjects performed four 30min drives on a high-fidelity driving simulator. Mixed-effects ANOVAs were performed to examine the effect of the restart period on performance.

There was a significant effect of work week for speed variability (F[1,618]=18.1; P<0.001) and lane deviation (F[1,618]=21.3; P<0.001), with improved performance following the restart period. When comparing to results from our earlier study with a 34h restart period, there was a significant interaction for speed variability, with better performance following the 58h restart period (F[1,1123]=8.1; P=0.045).

An extension of the current 34h restart provision for U.S. commercial motor vehicle drivers in night work operations, to include an extra biological night, appears to be an efficacious, albeit perhaps not cost-effective, approach for sustaining some aspects of simulated driving performance.

Traumatised police and effective psychological treatment

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Post Trauma Victoria (PTV) is a specialised assessment and treatment service for individuals suffering unwanted psychological effects of traumatic events. PTV operates on the basis of a philosophy of providing holistic, clinically-targeted and empirically-driven treatment and has thus far has delivered strong treatment outcomes for a range of workplace and Road Traffic Accident survivors. A special feature of its work, the Service has conducted a series of program-based interventions for current and ex-serving police officers with post-traumatic symptomatology. Typically conducted over 12 weeks, each group treatment cohort of 5-6 participants is provided with a manualised treatment program of interlocking treatment modules, targeted to the principal elements of PTSD and its comorbidities. This presentation reports on a preliminary analysis of treatment outcomes for police officers who have completed the treatment program. Participants typically show high levels of depression, anxiety, and alcohol use, in addition to the symptoms of PTSD pre-treatment. Preliminary analysis of treatment gains over the course of the program reveals significant reductions in measures of PTSD (p < .001), depression (p = .005), anxiety (p = .004), and anger (p = .003).

This is supported by participant feedback in which participants reported a degree of symptom...
improvement that compares favourably with other similar-PTSD affected populations, such as combat-veterans.

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The Role of Visual Imagery In Anger In PTSD - A New Model For Understanding Anger in PTSD

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Anger is a critical facet of PTSD that affects the disorders intensity and longevity. Recently, a ground-breaking theoretical model has been proposed for better understanding the crucial role it plays in PTSD. This model affords due recognition to the role visual imagery plays in the genesis and maintenance of PTSD.

This presentation will detail this model, describe well-established lines of evidence that support it and present early research data from clinical and non-clinical population studies that illustrate its existence. It will also discuss the implications of the model for treatment of anger in PTSD and problematic anger generally. Finally it will present two sources of preliminary data illustrating this relationship in a general and clinical population.

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The Delivery of Effective Public Mental Health Services Post Disaster: an Example from the 2009 Victorian Bushfire Crisis

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The Victorian Bushfires of February 2009 (the Fires) were a natural disaster of immense proportions causing loss of life, loss of housing and infrastructure, and widespread damage to forested environments. In responding to the Fires, the Victorian Government, through its Department of Human Services (DHS), and specifically the DHS Mental Health Branch, called upon Austin Health, through the specific expertise of its Psychological Trauma Recovery Service (PTRS), to play a lead role in the recovery process. Over the three years after the Fires, the PTRS implemented a comprehensive program of Trauma related Mental Health Service Delivery designed to meet the psychological needs of bushfire affected individuals, families and communities.

Services provided included:
1. Direct psychological and psychiatric outpatient and inpatient care &
2. Primary, secondary and tertiary consultation and education and training services for a range of organisations and individuals, including local government, education and disaster recovery agencies and an array of community groups, treating professionals and members of the public.

This presentation outlines the types of activity provided by PTRS and presents data that: describes the various activity outputs delivered; profiles the clinical presentation of clients treated; and illustrates treatment outcomes. Qualitative feedback illustrating key themes nominated by the range of clients assisted by PTRS will also be presented. Through this presentation, it is hoped that knowledge of PTRS activities will aid disaster-recovery services and agencies in better anticipating the post disaster needs of individuals and communities, in the future.
AMP-Activated Protein Kinase (AMPK) β1 null mice demonstrate marked vacuolisation of proximal convoluted tubule epithelial cells

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Aim: To determine the cause of isometric vacuolisation of the proximal tubules of AMPK β1−/− mice.

Background: AMP-activated protein kinase (AMPK) is a master metabolic controller whose best-known role during energy stress is increasing fatty acid oxidation by phosphorylating acetyl-CoA carboxylase (ACC). Recent studies suggest a role in cellular processes, not explained by control of metabolic activities, such as autophagy, cell polarity and controlling cytoskeletal dynamics.

Methods: Kidneys from AMPK β1−/− mice and mice bearing a knock-in mutation of the AMPK phosphosite in ACC1 (ACC1S79A) were assessed by immunohistochemistry.

Results: Isometric vacuolisation was observed in kidneys from 19/22 AMPK β1−/− mice but none of the wild type (WT) controls. The vacuoles were co-located with the proximal tubule marker megalin. There were no vacuoles in the thick ascending limb, when stained with anti-NKCC2 (Na/K/Cl co-transporter 2) or in the distal convoluted tubule stained with anti-TSC (thiazide-sensitive co-transporter), nor the collecting duct marker PNA. Vacuoles were not observed in ACC1S79A mice, suggesting they were not related to the metabolic activities of AMPK. This was confirmed by negative staining for Oil Red O, a lipid marker. The vacuoles co-stained with anti-GRP78, a histochemical marker of the endoplasmic reticulum (ER). This vacuolisation was similar to that seen in cyclosporin/tacrolimus nephrotoxicity, which is due to an enlargement of the endoplasmic reticulum and an increase in lysosomes. However, the lysosomal membrane protein, LAMP-1 and the autophagosomal marker (LC3) did not stain the vacuoles, suggesting they were not autophagic vacuoles.

Conclusions: Kidneys from AMPK β1−/− mice demonstrate isometric vacuoles in proximal tubule cells due to dilatation of the ER, similar to the location and origin of vacuoles seen in cyclosporin/tacrolimus toxicity. This data suggests AMPK has a previously unsuspected role in the function of the ER.

REGULATION OF NKCC1 by AMP-activated Protein Kinase (AMPK)

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The Sodium-Potassium-Chloride cotransporter NKCC1 has important roles in epithelial cell secretory function, regulation of cell volume and modulation of vascular tone. Phosphorylation at regulatory sites (including Thr212 and Thr 217) increases NKCC1 activity. The energy-sensitive kinase AMPK plays a key role in cellular metabolism. Its activity is altered in metabolic disorders such as obesity. We have previously shown that AMPK regulates the function of NKCC2 in the kidney. We sought to determine if AMPK also regulates the function of NKCC1.

We studied the effects of stimulating and inhibiting AMPK on the function and phosphorylation of NKCC1 in MDCK cells using bumetanide-sensitive Rb86+-flux and Western Blotting with R5 antibody, which recognises phosphorylated residues Thr212 and Thr217. The effect of AMPK on expression of NKCC1 was compared using transfection of Cos cells. Membrane localisation of NKCC1 was determined by immunofluorescence.
Stimulation of AMPK in MDCK cells with A-769662 resulted in a dramatic reduction of bumetanide-sensitive Rb86+-flux and reduced R5-phosphorylation of NKCC1. Co-treatment with Compound C abrogated these changes. Inhibition of AMPK with Compound C alone induced an increase in R5-phosphorylation of NKCC1. Surprisingly, co-transfection of AMPK with NKCC1 resulted in enhanced expression of NKCC1. However, active but not kinase-dead AMPK led to a reduction in membrane localisation of NKCC1 despite increased expression.

This data suggests that activation of AMPK leads to dephosphorylation of NKCC1 at key regulatory sites, possibly by recruitment of a phosphatase, so reducing its membrane localisation and co-transporter activity. Binding of AMPK appears to provide structural stability to NKCC1, indicating the importance of AMPK in its function. The data provides a new insight into the regulation of the important ion co-transporter NKCC1 and may help explain changes seen in metabolic disorders, such as increased vascular tone.

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**Advance Care Planning (ACP), and its impact in the renal unit.**

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**Aim:** To evaluate the impact of an ACP implementation in the Austin Health nephrology department during 2010

**Method:** Patient and ACP activity data was collected prospectively to audit the ACP process and assess its impact on patient outcome. Data was collected from 1/2/2010 to 1/8/2011, including review of deceased patients’ files.

**Results:** 197 nephrology patients were seen (median age 69 (21-91), 68% male). 81% were receiving dialysis, 8% pre-dialysis, 7% post-transplant and 4% were non-dialysis patients. 63% completed documentation: 16% appointed a substitute decision maker, 3% an advance care plan only, or 81% did both. Of those who completed an advance care plan 69% stated that they did not want CPR, 17% did not want life-prolonging treatment at all, and 78% only wanted life-prolonging treatment other than dialysis if their specified minimal outcome was anticipated. 31/197 (16%) patients (median age 80) died: 29/31 were dialysis patients (median dialysis duration prior to death 4.2 years). 72% of deceased dialysis patients had dialysis electively ceased prior to death (median time withdrawal to death 2 days), 7% patients died suddenly outside of hospital, 7% patients specifically requested dialysis continue, but died, and 14% patients had a cardiac arrest during dialysis and died. Deceased patients wishes were known and respected in 68%, unknown in 19%, or known and not respected in 13%.

**Conclusion:** Whilst ACP facilitates patient-initiated dialysis withdrawal it usually occurs late and too many patients are receiving unwanted treatment.
Abnormal processing of autophagosomes in transformed B-lymphocytes from SCARB2 deficient subjects

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Mutations of the intrinsic lysosomal membrane protein SCARB2 cause Action myoclonus-renal failure syndrome (AMRF syndrome), a rare disease characterised by renal and neurological manifestations. In this study, examination of Cos7 cells transfected with SCARB2 cDNA derived from 2 patients with AMRF syndrome showed that the protein was not incorporated into vesicular structures, as occurred with full-length SCARB2 cDNA. Mutant SCARB2 protein failed to co-localize with lysosomes and was found in the endoplasmic reticulum or the cytosol indicating a loss of function. Cultured skin fibroblast and EBV-transformed lymphoblastoid B cell lines (LCLs) were created from these two patients. Despite the loss of SCARB2 function, studies with LAMP1 and LAMP2 demonstrated normal lysosomal numbers in fibroblasts and LCLs. Immunofluorescence microscopy using anti-LAMP1 and LAMP2 Ab also showed normal lysosomal structures in fibroblasts. There was no change in the morphology of fibroblasts examined by electron microscopy when compared with cells from unaffected individuals. By contrast, LCLs from individuals bearing SCARB2 mutations had large intracellular vesicles with the appearance of autophagosomes, containing heterogeneous cellular debris. Some of the autophagosomes were seen to be extruding cellular contents into the media. Furthermore, LCLs had elevated levels of LC3-II, consistent with increased autophagy. These data demonstrate that SCARB2 mutations are associated with an inability to process autophagosomes in B-lymphocytes, suggesting a novel function for SCARB2 in immune function.

Deficiency of LIMP-2, an integral lysosomal protein, attenuates renal injury in experimental crescentic glomerulonephritis

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Deficiency of intrinsic lysosomal protein SCARB2 (Limp-2 as murine homologue) causes collapsing FSGS in humans, and tubular proteinuria in humans and mice. Limp-2 deficiency in mice, however, leads to failure of fusion of phagosomes with lysosomes, defective macrophage activation and innate immunity in response to Listeria infection.

To define the role of Limp-2 in experimental crescentic glomerulonephritis (GN), wild-type (WT) and Limp-2/- littermates received intraperitoneal injections of 2 mg of nephrotoxic sheep serum per gram of body weight. Renal injury and immune response were assessed at day 14.

Compared with WT, Limp-2/- mice had significantly reduced crescent formation (15.6±10.2% vs 40.2±22.5%, P<0.005), interstitial inflammation (P<0.05) and a trend for reduced tubulointerstitial injury. On day 1 after disease induction, urinary albumin/creatinine ratio was significantly increased in WT mice (4921±5180 vs 18±11 mg/mmol, P<0.05) but not Limp-2/-
mice (1655±1842 vs 315±173 mg/mmol) compared to baseline. At day 14, albuminuria and renal function were similar. There was, however, a significant reduction in the influx of glomerular macrophages (0.6±0.2 vs 1.2±0.4 cells per glomerular cross section (c/gcs), P<0.05) and CD4+ T cells (0.2±0.1 vs 0.6±0.2 c/gcs, P<0.05) in Limp-2/- versus WT mice. Renal MCP-1 mRNA expression was also reduced (P<0.05). The systemic humoral immune response, determined by glomerular mouse IgG deposition and mouse anti-sheep IgG subclass production, was similar in both groups.

The data suggest that Limp-2 is essential in mediating the local immune response in experimental crescentic GN. The likely reason is a failure of macrophage activation and cytokine release. This study identifies a novel role for a lysosomal protein in autoimmunity.

LIMP-2, a lysosomal membrane protein, is expressed in renin secretory granules, and inhibits proteolytic conversion of prorenin to renin

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Limp-2 (SCARB2 in humans) is a lysosomal membrane protein responsible for the intracellular trafficking of β-glucocerebrosidase. Renin processing and storage occurs in structures expressing lysosomal hydrolases, but the expression and role of lysosomal membrane proteins in renin granules are unclear.

To determine whether renin secretory granules express lysosomal membrane proteins, and the role of Limp-2 in renin processing and secretion, co-localisation studies of prorenin/renin with lysosomal membrane proteins SCARB2/Limp-2, LAMP-1 and LAMP-2, and lysosomal hydrolase cathepsin L, were performed in mouse and human kidney sections. Expression and secretion of prorenin/renin in WT and Limp-2/- mice were compared with and without stimulation.

Prorenin/renin co-localised with SCARB2/Limp-2, LAMP-1, LAMP-2 and cathepsin L in mouse and human kidney. Plasma renin concentration (PRC), but not total prorenin/renin concentration (TPRC), in Limp-2/- mice was increased compared with WT littermates (P=0.0057). No change in prorenin/renin expression, however, was noted in Limp-2/- mouse kidney cortex by immunofluorescence microscopy, Western blotting, qRT-PCR, or the ultrastructural appearance of renin secretory granules. Acute release of renin after isoprenaline or hydralazine stimulation was similar in WT and Limp-2/- mice. Following chronic stimulation with a salt deficient diet, there was significantly less prorenin but not renin by Western blotting in kidney cortex from Limp-2/- compared with WT mice (P=0.0056), and less prorenin/renin on immunofluorescence microscopy. However, both PRC and TPRC were similar in salt restricted WT and Limp-2/- mice.

Co-localisation studies demonstrated that prorenin/renin is stored in vesicles expressing lysosomal membrane proteins in mice and humans, confirming their long suspected identity as modified lysosomes. Limp-2 appears to have a role in controlling renin release, and the processing of prorenin to renin within the kidney upon chronic stimulation to compensate for the increased intrarenal loss of renin.
Tubular proteinuria induced by albumin overload is not due to reduced uptake but reflects the limited capacity of lysosomal degradation in the proximal tubule

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Increased glomerular leakage of proteins leads to proteinuria due to the reabsorptive capacity of the proximal convoluted tubules (PCT) being exceeded. It has been postulated to be due to reduced tubular uptake due to the competition from increased delivery of filtered proteins. We postulated that this is as a result of inadequate lysosomal degradation, which is the mechanism of tubular proteinuria in mice and humans lacking the intrinsic lysosomal protein Limp-2.

To determine whether tubular proteinuria in mice induced by protein overload is due to reduced protein uptake or inadequate proteolysis, wild-type (WT) mice received daily bovine serum albumin (BSA) injections intraperitoneally for 10 days, and untreated Limp-2\(^-\)/- mice were used as a positive control for inadequate tubular proteolysis.

Compared with baseline, BSA-treated WT mice at Day 10 developed a significant increase in mouse albuminuria (uACR: WT Day 0 17±17; WT Day 10 271±278 mg/mmol; P<0.01) and tubular proteinuria, similar to untreated Limp-2\(^-\)/- mice. Expression of the receptor, megalin, for tubular protein uptake, was unchanged. Tubular uptake of endogenous retinol-binding protein (RBP) and intravenously injected Alexa-conjugated BSA at 7 minutes was intact in treated WT mice. Increased basal distribution of endocytosed RBP (P<0.05) and persistence of Alexa-BSA at 30 minutes were evident in the PCT compared with untreated WT mice, suggestive of inadequate tubular proteolysis, with features resembling those in untreated Limp-2\(^-\)/- mice. Enlarged basolateral cathepsin B vesicles in the proximal tubules and up-regulation of cathepsin L in cortical kidneys suggested the activation of the degradation system in response to increased loads of endocytosed proteins for lysosomal processing.

The data suggest that tubular proteinuria in normal mice induced by albumin overload is not due to failure of uptake but reflects the limited capacity of the PCT to degrade increased loads of endocytosed proteins by lysosomes.

Angiotensin Converting Enzyme 2 plasma activity is lower in female hemodialysis patients and associations differ between males and females

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Background: Angiotensin converting enzyme 2 (ACE2) is a novel regulator of the renin-angiotensin system that counteracts the adverse effects of angiotensin II. The ACE2 gene is located on the X-chromosome. Plasma ACE2 predicts adverse events, and elevated levels are associated with more severe myocardial dysfunction, in heart failure patients. No studies have examined plasma ACE2 activity in chronic kidney disease (CKD) patients.

Methods: Patients groups included CKD Stage III/IV, hemodialysis patients (HD), and kidney transplant recipients (KTR). Plasma ACE2 activity was measured using a fluorescent substrate assay. Linear regression was performed in males and females separately to determine covariates associated with log-transformed ACE2.

Results: The median (inter-quartile range) plasma ACE2 activity was 15.9 pmol/minute/mL (8.4-24.2) in CKD (n=57), 9.2 (3.9-18.2) in HD (n=100) and 13.1 (5.7-21.9) in KTR (n=80;
p<0.01). In HD, males had levels of 12.1 (6.8-19.6) compared to 4.4 (2.5-10.3) in females (p<0.01). Log-transformed ACE2 plasma activity was associated with post-HD systolic blood pressure (SBP) in females (β-coefficient 0.04, 95% confidence interval 0.01-0.06, p=0.006). In males, log-transformed ACE2 plasma activity was associated with BNP (0.39, 0.19-0.60, p<0.001), diabetes (-1.01, -1.73 to -0.28, p=0.007) and log-transformed time on dialysis (-0.29, -0.53 to -0.05, p=0.018).

Conclusions: Plasma ACE2 activity is reduced in HD patients compared to CKD patients, and in female HD patients compared to male. In HD patients, the association of plasma ACE2 activity with BNP in males and SBP in females indicates that the role of ACE2 in cardiovascular disease may differ by gender.

Comparison of LIAISON Direct Renin Assay to DIASORIN RIA Renin Activity Assay and Evaluation For Routine Use in a Hospital Laboratory

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Introduction
Plasma renin, measured either as renin activity (PRA) or direct renin concentration (DRC), is used to monitor adrenal function and in screening for Primary Hyperaldosteronism (PHA). While suppressed renin activity is suspicious for PHA, calculation of the aldosterone-to-renin ratio (ARR) is the preferred screening tool, and has been validated using PRA or DRC. We evaluated the automated direct renin immunoassay on the Diasorin Liaison against our current Diasorin Gamma Coat plasma renin activity radioimmunoassay (RIA) for routine use in our hospital-based laboratory.

Methods
Plasma from >90 hospital patients in whom renin was requested and 20 volunteers were analysed for this study. In addition to a direct method correlation, we conducted serum-plasma correlations and investigated the effect of posture. PRA was measured using the Diasorin Gamma Coat RIA and DRC using the Diasorin chemiluminescent immunoassay on the Liaison. Aldosterone was assayed by the Siemens Coat-A-Count RIA.

Results
Although numerically different, overall agreement between PRA and DRC was reasonable with a correlation coefficient on Passing-Bablok regression analysis of 0.891 (slope=13.077, intercept=0.78). Of the 70 renin results with a corresponding aldosterone, 7% (5/70) had an abnormal ARR using PRA (cut-off >750), and 19% (13/70) by DRC (cut-off >35). These were reduced to 4% (3/70) and 9% (6/70) respectively if the additional criteria of Aldosterone >300 pmol/L was used.

Serum DRC was significantly lower than plasma DRC (average difference -48%) while DRCs obtained sitting were on average 36% higher than the paired sample collected supine, suggesting that like PRA, DRC is not robust enough to allow for variations in collection requirements.

Conclusion
The automated DRC assay on the Diasorin Liaison simplifies renin measurement when compared to PRA measurement by RIA. However collection and handling still require strict instructions especially if results are to be used in the ARR.
Social Indicators for Survivorship: A Review of Austin Cancer Patients.

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The opening of the Olivia Newton-John Cancer and Wellness Centre (ONJWC) in July 2012, presents a unique opportunity for the Austin Health Cancer Social Work team to examine current practice models. We are examining the implication for the significance of the social context on its contribution to, and management of, the diagnosis and treatment trajectory. We will describe the impact of Social Work intervention on survivorship after a diagnosis of cancer.

This presentation will describe the results of an exploratory retrospective data mining study, which gathered qualitative information on patient demographics and issues affecting social functioning. Data was collected on one hundred consecutive patients referred to Cancer Social Work during May – June 2012. A thematic analysis of the data was conducted to establish who the team are servicing, the presenting issues, and how Social Work contributes to patient and carer survivorship. Results revealed an association between depression and three domains of social capital; financial, carers and family & interpersonal. The primary stressor was found to be the financial domain, while family and interpersonal relationships was found to be the most supportive.

Analysis of the results highlighted the range of social issues experienced by Austin Health cancer patients and carers, which can significantly impact upon the quality of their survivorship. The expertise of Social Work in the psychosocial domain enables the team to positively impact on the lived experience of this patient group, by tailoring support to best meet their needs.

Analysis of medical emergency team calls on General Surgical patients in a tertiary hospital.

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Introduction: Medical emergency teams (MET) are now present in tertiary centres to intervene when a patient is acutely deteriorating. We analysed the patients who required a MET call during the calendar year of 2011 in our surgical unit.

Study aim: To identify which surgical patients are deteriorating on the surgical ward.

Study design: Retrospective review of a prospectively collected database between 1/1/2011 to 31/12/2011. We looked specifically at our upper gastro intestinal surgical unit at Austin Health Melbourne.

Results: We had 1215 surgical admissions (329 elective, 886 emergency) with 11 deaths. There were 49 MET calls. We assessed the triggers for the MET calls, the type of admission, the admission condition, if there was a surgical or medical complication, if the cause of the MET call was from delay in theatre/ delayed review on ward/ inappropriate admission under surgical unit and admission outcome (delayed discharge/rehab/nursing home/death).

Conclusion: The acutely deteriorating patient has multiple factors that contribute to their demise. Reviewing these cases in our unit helped us identify where we can improve.
Investigating the lymphatic patterns in liver metastases from colorectal cancer and in normal human liver

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BACKGROUND AND AIMS: Hepatic resection, in conjunction with chemotherapy remains the treatment of choice for patients with colorectal cancer liver metastases (CRCLM). Historically the focus on tumor spread to the liver has been based upon a haematogenous pathway. Emerging evidence suggests that lymphatics may play a significant role in the development and subsequent dissemination of CRCLM within the liver. This has both prognostic and therapeutic implications. This study investigates the localization of lymphatics within and adjacent to CRCLM, correlating the development of lymphatics with tumour spread and their potential prognostic influence.

METHODS: Established immunohistochemical protocols were used on human tissue retrieved from CRCLM patients following liver resection. Lymphatic markers were used including, D2-40, LYVE-1 and PROX-1, and CD34 for tumor microvessels. The primary endpoints from this were lymphatic vessel density (LVD) and microvessel density (MVD) at intratumoral, peritumoral, liver immediately adjacent to tumours and normal liver tissue. LVD and MVD at these sites will then be correlated to disease free survival and rates of recurrence. Ethical approval was obtained through Austin Health, approval number H2012/04618.

RESULTS: Changes in lymphatic vessel density were observed between the peritumoral, intratumoral and liver immediately adjacent to the tumour. Correlation studies are currently being conducted to relate LVD and MVD to patient prognosis.

CONCLUSION: The spatial differences in lymphatic and microvascular densities between the peritumoral and intratumoral sites may play a critical role in the dissemination of tumor cells; elucidating the role lymphatics play in tumour progression would have significant clinical implications.

Effect of pump prime on acidosis, strong-ion-difference and unmeasured ions during cardiopulmonary bypass: a randomized blinded clinical trial. (ANZCTR: 12612000022864) – Interim Results

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Introduction:
There are no studies comparing the mechanism of metabolic acidosis during cardiopulmonary bypass (CPB) using Hartmann’s solution and Plasmalyte as pump primes. We tested the hypothesis that the effects of these crystalloids on acidosis is a function of their individual strong ion differences (SID) and unmeasured anions.

Methods: With ethics approval we performed a blinded randomized trial of 38 adult patients undergoing elective coronary or valve surgery. Both groups received a prime solution of 2000mL; one group with anions lactate and chloride (Hartmann’s), the other with anions acetate, gluconate and chloride (Plasmalyte). Endpoints: standard base excess (BE), SID, total weak acids, strong ion gap (SIG). Serum electrolytes and arterial blood gases were collected at 6 time points: baseline (BL); then 2min (T2); 5min (T5); 10min (T10); 30min (T30); and 60min (T60) during CPB.
Results: CPB was associated with metabolic acidosis with Plasmalyte and Hartmanns solutions. Lactate remained unchanged in the Plasmalyte group, however peaked with Hartmanns at 2min: 0.15mmol/L (BL) to 4.5mmol/L (T2), P<0.001, returning to baseline by T60. There was hyperchloraemia with Hartmanns compared to Plasmalyte. The SID with Plasmalyte increased from 37.6mEq/L (BL) to 40.3mEq/L (T2), P<0.001, returning to baseline levels at T60 (36.7mEq/L). Conversely with Hartmanns, the SID decreased from 36.3mEq/L (BL) to 31.2mEq/L (T2) and remained decreased at T60 (33.3mEq/L). The SIG increased significantly from BL to T2 with Plasmalyte (1.1mEq/L to 11.9mEq/L, P<0.001), but marginally with Hartmanns (-0.6mEq/L to 2.0mEq/L).

Conclusion: The mechanism of acidosis during CPB with Hartmann’s solution was a combination of iatrogenic hyperlactaemia and hyperchloraemia. In contrast the mechanism with Plasmalyte was a production of unmeasured anions, most likely acetate and gluconate.

Establishment of a novel in vivo mouse model of spinal metastasis

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Aim: To establish a clinically relevant mouse model of human spinal metastasis.

Background: Patients with advanced cancer will eventually develop metastases to the spine, which if untreated causes intractable pain and paralysis. Breast cancer in women and prostate cancer in men are the most common primary cancer sources. Animal models are desperately needed to improve understanding of this devastating condition and test adjuvant therapies.

Methods: An orthotopic injection of human breast (MDA-MB-231) or prostate (PC-3) cancer cells was administered into the upper lumbar spine of female and male nude mice respectively (n=6). Animals were monitored daily with a numerical score based on neurological function, gait and general welfare. Plain radiographs, and micro-CT imaging of each mouse were taken at time of sacrifice, together with histological analysis of spine and tumour sections.

Results: Five mice developed evolving paralysis in their hind limbs between 3-5 weeks post-inoculation. All followed the same pattern of decline following onset of paralysis. Plain radiographs and micro-CT scanning confirmed that all six mice had tumour growth in their spine where the orthotopic injection was administered. Histological analysis confirmed cancer growth and spinal cord compression.

Conclusion: A novel in vivo mouse model of human spinal metastasis has been successfully established forming cancers that grow within the spine and cause spinal cord compression, resulting in reproducible and evolving neurological deficit and paralysis that precisely mimics the human condition. This enables us to investigate cancer growth in the spine and gives us a suitable platform to trial novel therapeutics.

The role of kinin receptors in the growth of colorectal cancer

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Introduction and aims: Colorectal cancer is the third most common cancer in males and the second in females, with over 1.2 million new cases diagnosed annually worldwide, resulting in 608,700 deaths. Evidence suggests that paracrine systems such as the kallikrein-kinin system (KKS) play an important role in tumour growth and metastasis. The kinins modulate events such as proliferation, migration, angiogenesis and inflammation, which are relevant for tumour progression. The active peptides of the KKS: bradykinin and des-Arg9-bradykinin (DABK) exert their action binding to the receptors: B2R and B1R, respectively. This study aimed to evaluate the effect of kinins on colorectal cancer cell proliferation.
Method: The expression of kinin receptors were determined in the colorectal cancer cell lines SW-480 and MoCR using immunocytochemistry. In vitro cell proliferation was determined after stimulus with bradykinin and DABK, either in the presence or absence of the antagonists SSR240612 and Hoe-140 (B1R and B2R antagonists respectively), and were analyzed by MTT and H3-thymidine assay.

Results: The SW480 and MoCR cells express both B1R and B2R in vitro. Our preliminary results show that bradykinin induce proliferation of these cancer cells, which were inhibited by the antagonist Hoe-140. DABK does not seem to have an effect on proliferation of these cells.

Conclusion: Bradykinin work as a mitogenic agent on colorectal cancer cells in vitro, through the action of the B2R. This receptor is upregulated in many different cancers. Our results suggest a potential role for bradykinin in progression of colorectal cancer.

In vivo studies are required to evaluate the use of B2R antagonists either alone or in combination therapy for the treatment of colorectal cancer.

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**Generation of a human gastrin knockout colorectal cell line**

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Progastrin and its derived peptides are growth factors implicated in the development of colorectal cancer. The gastrin gene codes for progastrin and has been knocked out in mice, providing a valuable tool for in vivo analyses. In contrast, there are no current models for gastrin gene knockout in human cell lines, as targeting many human genes has until recently been very technically challenging. However, a new technique has emerged that will allow the generation of a human gastrin knockout cell line.

**Aim:** To generate a human gastrin knockout colorectal cell line using Transcription Activator-Like Effector Nuclease (TALEN) technology.

**Method:** Two TALEN plasmids and targeting donor plasmids were constructed through molecular cloning using a new restriction enzyme digest method and PCR. The TALEN plasmids were designed to express TALEN proteins, which catalyse site-specific double stranded breaks within the gastrin gene. Targeting donor plasmids were designed with regions homologous to the gastrin gene flanking a selectable marker (puromycin or neomycin resistance). Plasmids were transfected into a human colorectal adenocarcinoma cell line (SW480) in a two-step process to target each allele. Clones for gastrin knockout cells were selected with antibiotics and screened for a successful knockout event using both PCR and Southern blot.

**Results:** TALEN and targeting donor plasmids were successfully cloned and used to establish a human gastrin knockout cell line.

**Conclusion:** TALEN technology can be used as an effective means to generate a human gastrin knockout cell line. Consequently, the technology can be applied to other human genes and/or other human cell lines, previously not amenable to knockout targeting.

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**Mixed epithelial and stromal tumour of kidney**

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Benign mixed epithelial and stromal tumour of the kidney (MESTK) is a recently described rare neoplasm. It shows a predilection for perimenopausal women. About fifty cases have been described in the English literature. The pathogenesis of MESTK is uncertain with deranged hormonal environment, including unopposed oestrogen implicated in the induction of periductal foetal mesenchymal cell proliferation. A translocation involving t(1;19)(p22;p13.1) was identified in one case. Recent evidence supports the theory of a single cell of origin with the capacity for epithelial and stromal differentiation. Though most MESTK are benign, a few cases with aggressive behaviour and malignant transformation have been reported. We
describe another two cases of MESTK occurring in two female patients. The mode of presentation, operative procedures and pathology is described.

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The role of kinin receptors in liver regeneration
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Introduction and aims: Liver regeneration involves the proliferation of remnant mature functioning cells of the organ after suffering lost of hepatic mass. Hepatocytes, the main parenchymal cells of the liver, have the regenerative ability to restore liver to its previous size. The kinin peptides from kallikrein-kinin system (KKS) are generated in physiological environment and up-regulated after tissue injury. They modulate events such as proliferation, migration, angiogenesis and inflammation, which are relevant for the process of liver regeneration. The active peptides of the KKS: bradykinin (BK) and des-Arg9-bradykinin (DABK) exert their action binding to the receptors: B2R and B1R, respectively. This study aimed to evaluate the effect of kinins on hepatocyte proliferation.

Method: The expression of kinin receptors was determined in murine and human hepatocyte cell lines (BNL CL.2 and NeHepLxHT respectively) using immunocytochemistry. In vitro hepatocyte proliferation was determined after stimulation with BK and DABK, either in the presence or absence of the antagonists, SSR240612 and Hoe-140 (B1R and B2R antagonists respectively), and were analyzed after 24, 48 and 72 hours by MTT and ³H-thymidine assay.

Results: Protein expression of kinin receptors are detected in both murine and human hepatocyte cell lines. Our preliminary results show that the proliferation pattern of these cell lines is dependent on the presence of fetal bovine serum (FBS). In the absence of FBS, the agonists seem to induce the proliferation of hepatocytes which can be inhibited by the specific antagonists. However, the agonists did not significantly alter the hepatocyte proliferation rate in the presence of 1% FBS.

Conclusion: The kinins seem to have an in vitro proliferative effect on hepatocytes in the absence of FBS, possibly through the B2R. This enables us to better understand the process of liver regeneration after hepatic resection such as colorectal cancer liver metastasis.

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PAK1: a Therapeutic Target for Pancreatic Cancer
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Introduction and Aim
Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most deadly forms of cancer. Treatment and management for PDAC are limited and have not significantly improved over the last few decades. KRAS mutation is universally observed (>95%) and activates the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K) pathways1. Inhibition of the p21-activated kinase 1 (PAK1) pathway in pancreatic cancer has not been previously investigated. This project aims to investigate the role of PAK1 in the development and progression of pancreatic cancer using a novel inhibitor of PAK1.

Methods
The effect of glaucarubinone (a novel PAK1 inhibitor) on the human pancreatic cell lines PANC-1 and MiaPaCa-2, and on a murine pancreatic cell line PAN-02 was examined in vitro. Tumour cell proliferation was determined by thymidine-incorporation, migration/invasion using a Boyden Chamber assay, and toxicity and survival using thymidine-withdrawal. Furthermore, the synergistic effect between glaucarubinone and a chemotherapeutic agent, gemcitabine, was assessed.

Results
Proliferation and migration/invasion were inhibited by glaucarubinone by at least 40% and 60% respectively at 500nM. Survival was significantly reduced by 20% (p < 0.05), but
glaucarubinone was not toxic at the same concentration. Together, glaucarubinone and gemcitabine (500nM and 1µM respectively) inhibited proliferation to 10% of control.

Conclusion
Glaucarubinone inhibited the growth and migration of pancreatic tumour cells in vitro and further inhibition of proliferation was seen when glaucarubinone treatment was combined with gemcitabine. In vivo confirmation of these findings is required. This study suggests that PAK1 may be a novel therapeutic target for the treatment of pancreatic cancer.


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Hypoxia-inducible factor 1α: A poor prognostic marker in prostate cancer?

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Objectives: We aimed to investigate the correlation between HIF1α and adverse outcomes in patients with prostate cancer (PC) and to predict which tumours will progress to castrate resistance (CRPC).

Methods: 100 human PC tumors were divided into Gleason grade ≤7 or >7 and stained for HIF1α using immunohistochemistry. The outcomes of CRPC, chemo-resistance, metastases and PC-specific death were retrospectively correlated with HIF1α expression using Kaplan-Meier estimates and using multivariate Cox regression analyses.

Results: HIF1α expression was independent of Gleason grade and tumor stage. All 8 patients who had chemo-resistance were positive for HIF1α. In patients whose tumors expressed HIF1α, CRPC-free survival and metastasis-free survival were significantly decreased on Kaplan-Meier analysis. PC-specific death was also reduced on Kaplan-Meier analysis (p=0.07). On Cox Regression multivariate analysis, HIF1α was an independent risk factor for development of CRPC (Hazard ratio (HR) 10.0 p=0.017), progression to metastatic PC (HR 9.8 p=0.017) when adjusted for Gleason score, age and PSA. The presence of HIF1α was highly sensitive (all 100%) with excellent negative predictive values (100% for all 3 outcomes), but had poor specificity.

Conclusions: HIF1α is likely to contribute to the high numbers of metastasis, chemoresistance and PC-specific death in CRPC patients. Additionally, HIF1α may be a useful predictor for development of CRPC and may play a vital role as a targeted therapy for CRPC.

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Regulation of expression of hypoxia inducible factor 1 alpha (HIF1α) in prostate cancer cells

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Introduction: We have previously shown the role of HIF1α in chemoresistance, metastatic potential and castrate resistance in prostate cancer (PC). The conventional belief is that HIF1α is upregulated in hypoxia by protein stabilisation. However, the mechanisms of upregulation of normoxic HIF1α expression in PC are unknown. Therefore we aimed to identify the mechanisms behind normoxic HIF1α expression in PCs.

Methods: The HIF1α protein and mRNA levels were measured in the PC cell lines PC3 and LNCaP in normoxia. A reporter plasmid was constructed in which the entire 5'-UTR and 238 bp promoter sequence of the HIF1α was cloned upstream of firefly luciferase coding sequences in the pGL4.10 reporter plasmid and was co-transfected with control renilla luciferase vector in the LNCaP and PC3 cells. Firefly luciferase activity driven by the HIF1α-
UTR reporter vector and Renilla-luciferase activity was determined following 24 hours of incubation. Luciferase mRNA was then measured in the transfected cells. Bisulfite sequencing was conducted to examine differences in methylation status of HIF1α promoter in the two PC cell lines. Translation efficiency was calculated by the ratio of Luciferase activity over Luciferase mRNA.

**Results:** PC3 cells had 12 ± 6 fold higher HIF1α protein expression but lower mRNA levels compared to LNCaP cells. The ratio of firefly luciferase activity over renilla luciferase activity was 6 ± 3 fold higher in PC3 cells compared to LNCaP suggesting that the upregulation occurred before protein stabilisation. There was no difference in promoter methylation indicating promoter driven HIF1α expression. However PC3 cells demonstrated a 2.9 ± 0.4 fold higher mRNA translation efficiency compared to the LNCaPs.

**Conclusions:** HIF1α in PC3 cells is upregulation in normoxia is likely to be due to increased mRNA translation efficiency regulated by a ‘GC rich’ region in the 5’UTR (previously shown1).


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**Primary Gastric Adenosarcoma**

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Introduction: Adenosarcoma is a rare biphasic polypoid neoplasm composed of a benign epithelial component and a sarcomatous mesenchymal component. Adenosarcomas most commonly arise in the uterus, but have been described in a few extragenital sites. We describe a case of a primary multifocal gastric adenosarcoma. We believe that this is the first bona fide reported case of this tumour in the stomach, and it expands the differential diagnosis of spindle cell tumours at this site. Only one other case of primary gastric adenosarcoma has been previously reported in literature, in 1993, but it differs from our case in several respects.

Case report: A 79 year old man underwent gastroscopy for investigation of iron deficiency anaemia and weight loss, which revealed ulcerated polyps on the greater curve. Biopsies were suspicious for a sarcomatoid neoplasm. Subsequent sleeve gastrectomy contained four polypoid masses up to 4.5cm in size. Microscopically, at low magnification these tumors bore a striking resemblance to phyllodes tumour of the breast, with leaf-like stromal fronds surrounding cleft-like glandular spaces. The epithelial component comprised benign gastric foveolar and pit epithelium. The stromal component consisted of high-grade pleomorphic sarcoma, which lacked morphological or immunohistochemical differentiation. All the tumours were mucosal, with only focal submucosal invasion in the largest tumour. In comparison, the single previously published case was composed of purely leiomyosarcomatous stroma surrounding columnar epithelium-lined glands, and involved the deep gastric wall.

Conclusion: There was no evidence of tumour recurrence at four months post operatively. Given the uniqueness of this tumour and a short follow up period, no conclusion about the prognosis could be made at this time.
The role of Angiotensin II on the growth of Pancreatic Ductal Adenocarcinoma

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Background:
Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most aggressive and lethal of all solid tumours and is poorly responsive to current chemotherapy options. The Renin Angiotensin System (RAS) has been shown to be involved in the growth and development of several tumour types and serve as a potential treatment target. This study investigates the expression and effect of a key mediator of RAS, Angiotensin II (ANG II), on the growth and proliferation of PDAC which has not been fully determined.

Methods:
Three pancreatic cancer cell lines [two human (PANC-1 and MiaPaca-2) and one murine (PAN-02)] were examined. Determination of Angiotensin Type 1 and 2 receptor (AT1R and AT2R) was performed by Immunocytochemistry. The effect of ANG II, AT1R antagonist (Irbesartan) and AT2R antagonist (PD 123319) on the proliferation was determined by MTT assay. Tumour cell migration was assessed by wound healing assay.

Results:
AT1R and AT2R were noted on all three cell lines. ANG II significantly increased the proliferation of PAN-02 at 24, 48 and 72 hours time points. Irbesartan showed no growth inhibitory effect in vitro however PD 123319 inhibits the proliferation of all cell lines at 1 micromolar (µM). Significant wound migration effects have not been noted to date in any of the cell lines following ANG II stimulation.

Conclusion:
ANG II has a direct proliferative effect in vitro on human and murine pancreatic cancer cells. AT2R antagonist, PD 123319, inhibits the proliferation. More studies are required in animal models to further understand the effect of ANG II and its receptor antagonist.

The Antagonistic Activities of Angiotensin II and Angiotensin-(1-7) in Macrophages: a Potential Target for Colorectal Cancer Liver Metastasis

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Background and Aim: The local renin angiotensin system (RAS) plays an important role in colorectal cancer liver metastasis (CRCLM). The key peptides of RAS, Angiotensin II (Ang II) and Angiotensin-(1-7) (Ang-(1-7)) are reported to exhibit antagonistic activities in regulating tumor growth and metastasis. However the role of these two RAS peptides in modulating tumour associated macrophages remains unclear. This study aims to investigate the role of Ang II and Ang-(1-7) in macrophage regulation and its impact on tumour.

Method: In-vitro, the murine macrophage cell line P338D1 cells were cultured with increasing concentrations of Ang II (0.01µM-1µM), or Ang-(1-7) (0.01µM- 2µM,), or control (0.1% FBS/RPMI [negative] or 5% FBS/RPMI [positive]) for 24 hours. The conditioned media was used to treat murine colorectal cell line MoCR cells for 24, 48 or 72 hours. Changes in MoCR cell viability, proliferation and migration/invasion were examined using MTT assay, [H³]-thymidine assay and Boyden Chamber Assay respectively. The levels of MMP-9, VEGF and iNOS in the conditioned media were investigated by western blot or ELISA.

Results: Ang II-treated P338D1 cells increased cell viability (p<0.01), cell proliferation (p<0.05) but decreased migration/invasion in MoCR cells. In contrast, Ang-(1-7)-treated P338D1 cells decreased cell viability (p<0.005), cell proliferation (p<0.001) but increased cells
migration/invasion. Ang II and Ang-(1-7) also altered the expression of MMP-9, VEGF and iNOS in P338D1 cells.

Conclusion: The results indicate that Ang II and Ang-(1-7) may differentially regulate tumour progression through their actions on macrophages. The underlying mechanisms of RAS in regulating liver macrophages could potentially become a target in the treatment of CRCLM.

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Adaptive resistance to hypoxia-inducible apoptosis in colorectal cancer cells is mediated by HIF-1α-dependent gastrin gene expression

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Introduction: Understanding the molecular processes mediating colorectal cancer (CRC) tumorigenesis will enable the development of targeted therapies that selectively disrupt the pathways responsible for tumour growth. The gastrin family of growth factors promote CRC growth, invasion and angiogenesis. Hypoxic microenvironments, caused by tumours outgrowing their local blood supply, stimulate aggressive tumour behaviour. However, the effect of hypoxia on gastrin expression in CRC is unknown.

Methods: Expression of the gastrin gene in human CRC cells was examined under conditions of normoxia and hypoxia. The effect of inhibiting expression of HIF-1α (the transcriptional master regulator of cellular responses to hypoxia) and of deleting HIF-binding sites in the gastrin promoter was investigated. The effect of inhibiting gastrin expression on CRC cell behaviour in vitro and on tumorigenesis in mouse xenografts was analysed.

Results: Gastrin gene expression in CRC cells is stimulated by hypoxia by binding of HIF-1α to the gastrin promoter. The viability of gastrin knockdown CRC cells in vitro is diminished under hypoxic (1% O₂) conditions due to loss of resistance against hypoxia-inducible apoptosis. The growth of tumour xenografts in mice exposed to hypoxia (10% O₂) for 21 days is significantly reduced by knocking down gastrin expression.

Conclusion: This work provides evidence that gastrin expression is involved in the adaptation of CRCs to hypoxic microenvironments through resistance to apoptosis. Shrinkage of CRC liver metastases by the angiogenesis inhibitor bevacizumab is dependent on hypoxia-induced apoptosis. Therapies that target gastrin may enhance the therapeutic efficacy of bevacizumab and increase secondary resectability rates in patients with CRC liver metastases.

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Anastomosing Haemangioma of the Kidney: A Rare Case Report and Literature Review

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Aim To identify the radiological and characteristic morphological features associated with the rarely encountered anastomosing haemangioma of the kidney.

Method A case report and comprehensive review of the literature.

Results We describe a case of a rare and recently recognised benign vascular tumour in a 74-year-old male with an irregular, enhancing, complex cystic lesion in the right kidney on triple-phase contrast-enhanced computed tomography (CT). A laparoscopic right nephrectomy was performed. Histology revealed a 5cm renal anastomosing haemangioma. Vascular tumours of the kidney are rare and most are benign. Fewer than 20 cases of renal anastomosing haemangioma have been reported in the literature. Most lesions are detected incidentally on imaging. Contrast-enhanced CT appearance of these lesions includes well-demarcated, heterogeneous, solid, enhancing lesions similar to renal cell carcinoma (RCC). Distinguishing these lesions from RCC pre-operatively is difficult; therefore conservative management is rarely an option. Angiography is of little use diagnostically.
Characteristic histological features include vaguely lobulated architecture, anastomosing sinusoidal capillary-sized vessels, with scattered hobnail endothelial cells. Vascular thrombi or infiltration of large veins is not uncommon. However, they lack features of malignancy, such as infiltrative growth and cytological atypia.

**Conclusion** Anastomosing haemangioma is a rare, recently recognised benign vascular tumour with distinctive morphology. Primary treatment in most cases is surgery due to suspicious malignant features on imaging.

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**KI67 expression in oestrogen receptor positive breast ductal carcinoma**

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Gene expression profiling has identified two biologically distinct oestrogen positive subtypes[1]. Luminal A tumours have a better prognosis than luminal B tumours, are tamoxifen sensitive and derive less benefit from adjuvant chemotherapy. A high Ki67 proliferation index (>14%) has been proposed as a cost-effective means to distinguish luminal B from luminal A tumours[2]. We aimed to assess the reproducibility of classifying ductal carcinomas into luminal subtypes by Ki67 expression using manual scoring and image analysis.

Immunohistochemistry for Ki67 was performed on ninety ER positive invasive ductal breast carcinomas diagnosed at Austin Pathology. The Ki67 index was assessed semi-quantitatively by pathologists and quantitatively by image analysis in the Aperio digital scanning system. Correlation between scoring methodologies and BRE grade was assessed. There was a statistically significant correlation between Ki67 scoring and BRE grade (ANOVA, p<0.0001). Ki67 scores obtained by manual and digital image analysis methods were highly correlated (Pearson product moment correlation coefficient, r > 0.8).

Addition of the proliferation marker Ki67 to the standard breast cancer immunopanel of ER, PR, and HER2 can help distinguish Luminal A from Luminal B invasive ductal carcinomas. Aperio digital scanning system is likely to be most useful in assessing Ki67 scoring in cases where manual scoring is equivocal.


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**Metastatic melanoma contains subclonal chromosomal abnormalities and somatic mutations affecting clinically relevant cancer genes**

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**Aim**

Solid tumours rapidly develop resistance to targeted therapies, suggesting either that some cells can adapt to the treatment or that minor resistant clones are present prior to therapy. We sought to characterize genetic and phenotypic heterogeneity in metastatic melanoma as both could contribute to therapeutic escape.

**Methods**

Genetic heterogeneity was assessed by extracting from different regions of melanoma tissue samples, and performing genome wide analysis of chromosome amplifications and deletion using SNP microarrays, and next-generation sequencing of amplicons covering a panel of clinically relevant cancer genes. Single cell clones from early passage melanoma cell lines
were also assessed for chromosomal changes and somatic mutations, as well as gene expression profile and functional differences.

Results
Melanoma featured regional differences in chromosomal abnormalities encompassing known melanoma oncogenes, and in somatic mutations in genes relevant to resistance of approved targeted therapies. Early passage cell line clones contained representative differences in DNA copy number, and displayed evidence of differential signaling pathway activation. Interestingly some minor clones were less sensitive to drug treatments than the parental cell line, and expressed higher levels of genes associated pathways known to mediate therapy resistance in melanoma, such as PI3K, NOTCH, and MET.

Conclusion
Metastatic melanomas are phenotypically and genetically heterogeneous, with minor cell populations containing mutations in known mediators of cell survival and drug resistance. These results have important implications concerning the application of targeted therapies based on biopsy-derived molecular diagnostics, as key changes influencing drug response could be missed due to sampling error.

Correlation of Oral Cavity Dose, Acute Mucositis and PEG Dependency for Head and Neck Intensity Modulated Radiation Therapy

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Purpose/Objective(s): Radiation induced toxicities have a strong correlation to delivered dose. The Quantitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) report generated an increased knowledge of dose volume/normal tissue outcomes. Oral mucosa dose and its contribution to both acute mucositis and late swallowing impediment, delivers a dose/volume/outcome relationship. A correlative study was undertaken to determine the relationship of oral cavity dose and acute mucositis as a predictor of enteral feeding dependence two-months post treatment.

Materials/Methods: Sixty-eight patients with locally advanced SCC of the head and neck were prospectively analysed in this study. Multiple oral cavity dose parameters were recorded. Patients were reviewed weekly for acute mucositis (CTCAEv3). Grade 3 toxicity was deemed clinically significant. At 2-month follow up, patients were divided via percutaneous endogastric tube (PEG) utilization (PY) and no-PEG utilization (PN). Oral cavity dose and acute mucositis incidence were analysed and compared.

Results: On comparison of the PY (n=35) v PN (n=33), a significant dose difference was observed on multiple oral cavity dose parameters. PY delivered a significant increase in dose to Dmean (PY=46.4±8.1Gy v PN=38.9±12.9Gy, p=0.007), V50Gy(p=0.008), V45Gy(p=0.004), V35Gy(p=0.001), and V25Gy(p=0.002). The PY cohort presented higher incidence of grade 3 acute mucositis at multiple treatment weeks (Week 6,PY=40.6% v PN=25.8%,p=0.212; Week 7,PY=56.5% v PN=45.0%,p=0.451).

Conclusion: The QUANTEC report presented a series of recommendations for future dose/volume/outcome studies. This study has demonstrated a relationship between oral mucosal dose (across multiple parameters) and PEG dependence post radiotherapy. The relationship between acute mucositis and PEG dependence warrants further investigation. Continued follow-up and an increased patient population may allow for contribution to future dose/volume/outcome recommendations.
Thrombopoietin Receptor Agonists are Effective in Acute Immune Thrombocytopenic Purpura

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Background: Two thrombopoietin (TPO) receptor agonists, eltrombopag and romiplostim, have recently become available under the PBS for the therapy of ITP in patients refractory to steroids, intravenous immunoglobulins (IVIg) and splenectomy (or in whom splenectomy is contraindicated). Patients must demonstrate that they are refractory over a 4-6 week period, regardless of treatment response (or lack of), the severity of thrombocytopenia or bleeding during that time. The major studies relating to these agents describe their use in patients with chronic (> 6 months) ITP. To our knowledge, the use of TPO mimetics has not been reported in acute ITP (duration ≤ 3 months). We present the results of compassionate use of these agents in this context.

Methods: Eltrombopag and romiplostim were used in 5 patients with acute ITP treated in 3 hospitals. Data collected included patient demographics and comorbidities, the nature of the ITP diagnosis (primary or secondary), bleeding symptoms, other therapies used and responses, as well as response and adverse effects to TPO mimetics.

Results: The patients were aged 40-75 years. Four patients had primary ITP with underlying hepatitis C in one. All had significant bleeding (including intracerebral haemorrhage in one case) and were refractory to several modes of therapy, including pharmacotherapy (e.g. steroids, azathioprine, vincristine, cyclophosphamide, rituximab), blood products (e.g. IVIg and platelets), and in some cases surgery (splenectomy). All patients responded rapidly with resolution of bleeding and an increase in platelet count within one and two weeks with durable responses with ongoing therapy. No significant adverse effects were reported.

Conclusion: TPO mimetics appear to be effective in acute ITP refractory to conventional therapy.

Slow-cycling and mtBRAF-inhibitor resistant melanoma cells share a common phenotype and gene-expression pattern linked to EMT

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Melanoma is intrinsically resistant to chemotherapy and can metastasize unpredictably to a variety of anatomical sites. While BRAF inhibitors show promise in the clinic, the majority of patients relapse within 6-9 months due to the acquisition of drug resistance. The clinical patterns of disease vary considerably, reflecting functional and phenotypic heterogeneity as well as adaptation potential among melanomas. Using the membrane-labelling dye, CM-Dil, we identified a slow-cycling subpopulation of cells within melanoma cell lines and xenografts. Acquisition of resistance to the BRAF inhibitors PLX4720 and dabrafenib was associated with an elevated expression of the LRC gene signature together with the acquisition of increased invasiveness. Furthermore, we demonstrate that LRC from multiple melanoma cell lines are invasive and survive exposure to cytotoxic drugs. Gene expression profiling of label-retaining cells (LRC) identified a network of overexpressed genes associated with the extracellular matrix (ECM) and related to epithelial-to-mesenchymal transition (EMT). This phenotype was characterised by the expression of thrombospondin-1 (TSP-1), transforming-growth factor beta induced (TGFβI) and a variety of other molecules. Since drug resistance is a
characteristic of LRC, we postulate that this subpopulation is a source of resistant cells, thus heterogeneity is a major hurdle to effective targeting. This study links LRC, treatment failure and increased tumor invasiveness and yields new potential targets for overcoming treatment failure.

Management impact of FDG PET/CT in patients with differentiated thyroid carcinoma: a comparative study with I-131 whole body scan and serum thyroglobulin

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\textbf{Aim:} To assess the clinical impact of FDG-PET/CT on the management of the patients with differentiated thyroid carcinoma who had elevated thyroglobulin (Tg) levels and negative I-131 WBS.

\textbf{Method:} 96 patients with differentiated thyroid carcinoma were included. Each patient had one of the following: elevated Tg level (≥1 pmol/L) (n=71), suspected tumour disease in other imaging modalities (n=17), or clinical suspicion of recurrent disease (n=8). FDG-PET/CT scan findings was compared with histopathology, follow up imaging, or clinical follow-up (including thyroglobulin level) results as a gold standard. The impact of FDG-PET/CT on management was also evaluated.

\textbf{Results:} 44 patients had positive findings on FDG-PET/CT; 41 were true-positives and 3 were false-positives. The overall sensitivity, specificity, accuracy, PPV and NPV of FDG-PET/CT was 75.9%, 92.8%, 83.3%, 93.2%, and 75%. The sensitivity of FDG-PET/CT at serum thyroglobulin levels of between 5–10, and more than 10 pmol/L was 9.7%, and 83.4%. FDG-PET/CT had a management impact in 78 (81.2%) patients. FDG-PET/CT identified 24 (75%) patients with cervical metastases/neck recurrence, 5 (71.4%) with mediastinal metastases, 9 (69.2%) with lung metastases, 6 (75%) with bone and 2 (67%) with liver metastases.

\textbf{Conclusion:} FDG-PET/CT has a high diagnostic accuracy and management impact in patients with suspected residual or recurrent differentiated thyroid carcinoma.

Preliminary Characterisation of $^{124}$I Radiolabelled anti-TAG72 Pegylated Diabody AVP0458

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The high affinity and specificity of antibodies for tumour specific antigens makes them an ideal platform for delivery of cancer killing cytotoxic drugs in the form of antibody-drug conjugates. AVP0458 is a diabody derived from the scFv domains of mouse monoclonal antibody CC49 that targets TAG 72 antigen, an oncofetal antigen found on the surface of many epithelial cancer cells including prostate, colon, ovarian and breast cancer. Diabody PEGylation achieves increased tumour uptake by reducing renal clearance, improving the serum half life and achieving high tumour: blood ratios\textsuperscript{11}.

A preclinical biodistribution and imaging study was conducted with iodine-124 trace radiolabelled PEG-AVP0458. In vitro analyses confirmed retention of antigen binding activity and serum stability. Female BALB/c nude mice bearing TAG-72 positive LS174T xenografts were administered $^{124}$I-PEG-AVP0458 via a single tail vein injection. Group 1 received low specific activity (1.01 mCi/mg) for biodistribution study; group 2, high specific activity (2.98 mCi/mg) for biodistribution and imaging analyses. High in vivo tumour uptake > 52% was
observed 24-48 hrs p.i with prolonged retention for both groups. Normal tissues showed minimal $^{124}$I-PEG-AVP0458 uptake, consistent with blood pool activity only (<3 %ID/g at 24 hrs p.i). Pharmacokinetic analysis calculated a serum half-life of $T1/2 \alpha = 6.7$ hrs, $T1/2 \beta = 29.72$ hrs. This compares with $T1/2 \beta = 72.2$ hr previously observed with the parent antibody, CC49, in the same LS174T human colon carcinoma xenografts model[2]. Specific tumour localisation of $^{124}$I-PEG-AVP-0458 was observed over 3 days by Positron Emission Tomography (PET) imaging.

In conclusion, the two $^{124}$I- PEG-AVP0458 radioconjugates of low and high specific activities demonstrated similar properties. $^{124}$I-PEG-AVP0458 shows good blood clearance with high specific and prolonged tumour uptake in vivo and is an ideal radioimaging product for planned Phase I PET bioimaging investigations.


Can cone-beam computed-tomography measurements be used in place of in-room optical distance indicator source-to-skin measurements?

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Introduction: Routine source-to-skin (SSD) measurements can be time and workload consuming as radiation therapists (RTs) are required to manually measure these in the treatment room at multiple gantry angles using the optical distance indicator (ODI). The aim of this study was to validate the process of streamlining routine SSD checks using the on-board cone-beam CT (CBCT) dataset which is routinely acquired to validate patient position prior to treatment.

Methods/Materials: We directly compared ODI and CBCT measurements for 11 prostate patients over multiple (1-9) fractions and gantry angles. This data was acquired on two matched Elekta Synergy linacs with XVI 4.5, using the bowtie filter (F1) and medium field-of-view (M10). These measurements were further validated with a phantom, for which a known isocentre depth could be directly measured with a ruler.

Results: Of the 230 matched patient data points, there was a statistically significant difference between the ODI and CBCT measurements ($p < 0.005$), with an average difference of -0.1 cm (SD = 0.4 cm) and a maximum difference of 1.9 cm. Measurements of the phantom showed a greater correlation between actual (ruler) and CBCT measurements (average absolute difference = 0.15 cm, SD = 0.12 cm, maximum difference 0.3 cm) than between ruler and ODI (average absolute difference = 0.25 cm, SD = 0.15 cm, maximum difference 0.5 cm).

Conclusion: CBCT appears to more accurately reflect SSDs than ODI measurements. Obtaining SSDs with this technique that includes the bowtie filter, has the potential to save between 3-7 minutes machine-time per routine SSD check, which is of benefit for patients and the clinic. This study validates the use of CBCT for routine SSD checks in the pelvis and has been adopted as standard practice in our department.
Molecular Imaging of Death Receptor 5 Receptor Occupancy and Saturation Kinetics In vivo by Humanized Monoclonal Antibody CS-1008

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Purpose: CS-1008 (tigatuzumab, phase I/II), an anti-human death receptor 5 (DR5) agonist induces apoptosis and has cytotoxic activity against human cancer cell lines. Tumour concentration of CS-1008 in vivo and the impact of dose on tumour saturation of DR5 receptors remain key questions in the determination of the optimal dosing schedule for therapeutic benefit. This study reports on the validation of radiolabelled anti-DR5 humanised antibody CS-1008 as a diagnostic tool to study the DR5 receptor occupancy in a colorectal cancer xenograft model, and establish dose ranges for receptor saturation kinetics in vivo.

Experimental design: CS-1008 was radiolabelled with 111In via the bifunctional metal-ion-chelating agent CHX-A^-DTPA and was characterized for DR5 binding and labelling efficiency on DR5-positive human colorectal cancer cells (COLO 205). Pharmacokinetic and biodistribution studies were performed in BALB/c nu/nu mice bearing COLO 205 or DR5-negative mouse colon tumour CT26 using antibody dose levels of 0.2, 1, or 10 mg/kg. Planar gamma camera imaging and computerized tomography (CT) images were obtained at 2 days post injection to study the receptor occupancy in vivo.

Results: Scatchard analysis showed high and specific binding affinity (Kd, 1.05 ± 0.12 nmol/L) of 111In-labelled CS-1008 and a low number of DR5 receptors expressed per COLO 205 cell (Bmax, 6578 ± 284 receptors/cell). 111In-labelled CS-1008 is specifically taken up in mice bearing COLO 205 tumour xenografts with prolonged tumour retention (21.05 ± 2.00 %ID/g; n = 5, SD). DR5 receptor saturation can be demonstrated in vivo via both biodistribution and planar gamma camera imaging of 111In-labelled CS-1008. Saturation of DR5 receptor corresponds to maximal preclinical efficacy, with saturation occurring between the 1 mg/kg and 3 mg/kg dose levels. 111In-CHX-A^-DTPA-CS-1008 is a potential novel SPECT imaging tracer that can be used to determine receptor occupancy and effective dose levels of CS-1008 therapy in the clinic.


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LICR has implemented a comprehensive Cell Bank management system. This management system has the following objectives:
• Establish an integrated searchable database for storage of vials in liquid nitrogen.
• Achieve reliable cell line storage.
• Achieve functional cell line accessibility.
• Maintain cell line integrity and stocks

A Cell Bank management system provides a secure storage site for biological material. This material is kept primarily for laboratory research use, although some may be used to support clinical trials. With the current LICR liquid nitrogen storage reaching near 100,000 vials, an integrated searchable database was required. In addition to storage, a Cell Bank
A management system would have an increasingly important role in the validation of existing cell lines and the elimination of cell culture contaminants. A review of existing peer review and GLP obligations was undertaken and commercial inventory databases were evaluated and a software package purchased. By dividing cell stocks into 3 groupings (Archival, Master and Working stocks), setting vial number alerts and restricting levels of access, valuable cell stocks are maintained. Additional cell line information is also recorded through key User Defined Fields. Reference material and photos are linked by establishing additional Source screens for each cell line. Furthermore, with the need to validate existing cell lines in storage and the continued testing for Mycoplasma, an ongoing program of Quality Control testing has been implemented.

11C-choline PET is superior to T2-weighted MRI for localising malignant intraprostatic lesions for guiding prostate cancer focal therapies

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Aim: Previous studies have proposed using MRI for guiding focal therapies in prostate cancer, which is limited in its ability to reliably localise malignant intraprostatic lesions (IPLs). We sought to determine whether or not 11C-choline (CHOL) PET is superior to T2-weighted (T2W) MRI for identifying IPLs.

Methods: Thirty patients with intermediate to high risk, localised prostate cancer were recruited into this prospective, single institution study. All patients underwent CHOL PET and MRI prior to radical prostatectomy. IPLs were identified by two expert observers for each of the CHOL PET and MRI scans, and also by automatic contouring algorithms for the CHOL PET scans. These contours were compared to the pathologist-defined contours of involved tumour regions on the radical prostatectomy specimens based on Dice similarity coefficient (DSC), sensitivity and specificity. Interobserver variability for each contouring method was calculated using Fleiss' kappa.

Results: The DSCs of the CHOL PET and MRI manual contours, and the CHOL PET automatic contours were 0.517, 0.310 and 0.600, respectively. CHOL PET automatic contouring had a significantly higher DSC than both of the manual contouring methods (p = 0.027, < 0.001). CHOL PET manual contouring also had higher DSC than MRI manual contouring (p < 0.001). The Fleiss' kappa values for CHOL PET and MRI manual contouring, and CHOL PET automatic contouring were 0.75, 0.31 and 1.0, respectively (p < 0.001). The sensitivity/specificity values were 52%/86%, 24%/95%, and 75%/70%, respectively.

Conclusion: CHOL PET using either manual or automatic contouring is superior to MRI for localising IPLs, and also has significantly lower interobserver variability. The accuracy of CHOL PET is sufficient for guiding focal boost therapy2, and its use should be further investigated in prospective clinical therapeutic trials.

Hypoxia-targeted radiotherapy dose painting for head and neck cancer using $^{18}$F-FMISO PET improves predicted outcomes

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Aim: Hypoxia has been identified as a major factor limiting locoregional control in radiotherapy, rendering cells up to three times more radioresistant than aerobic cells. Hypoxia in head and neck cancer is associated poorer locoregional control, disease-free survival, and overall survival. Hypoxic modification can however, improve all of these outcomes. This study investigates the use of $^{18}$F-fluoromisonidazole (FMISO) PET-guided radiotherapy dose painting for potentially overcoming the radioresistant effects of hypoxia in head and neck cancers.

Methods: The study cohort consisted of eight patients with HNSCC who were planned for definitive radiotherapy. Hypoxic subvolumes were automatically generated on pre-radiotherapy FMISO PET scans. Two radiotherapy plans were generated for each patient: a standard (STD) radiotherapy plan to a dose of 70 Gy and a hypoxia dose-painted (HDP) plan with dose escalation to the hypoxic subvolume to 84 Gy. Plans were compared based on tumour control probability (TCP), normal tissue complication probability (NTCP), and uncomplicated tumour control probability (UTCP).

Results: The mean TCP increased from 73.4% with STD plans to 93.0% with HDP plans ($p < 0.001$). There were no statistically significant differences between any of the NTCPs between the STD plans and HDP plans. The mean UTCP increased from 47.8% with STD plans to 65.7% with HDP plans ($p = 0.016$).

Conclusion: Hypoxia-targeted radiotherapy dose painting for head and neck cancer using FMISO PET is technically feasible, increases the predicted locoregional control without increasing predicted side effects, and therefore improves the therapeutic ratio. This strategy should now be tested in prospective clinical trials.


Oral versus intravenous fluoropyrimidines for colorectal cancer

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2. Medical Oncology, The Queen Elizabeth Hospital, Woodville, South Australia, Australia

Aims:
Perform a Cochrane Collaboration systematic review and meta-analyse, where appropriate, safety and efficacy outcomes of randomised controlled trials (RCTs) comparing oral fluoropyrimidines to IV 5-FU for the treatment of colorectal cancer (CRC).

Primary objectives- Compare (i) disease-free survival (DFS) (neoadjuvant/adjuvant) and (ii) progression-free survival or time to progression (advanced/metastatic CRC).

Secondary objectives- Compare overall survival and grade 3/4 adverse events (AEs) (early and advanced/metastatic CRC), and objective response rates (ORRs) (advanced/metastatic CRC).

Methods:
Main inclusion criteria- RCTs comparing an oral fluoropyrimidine to IV 5-FU. Fluoropyrimidines are given alone, or with other oral/IV cytotoxic agents and/or biologic
agents and/or radiotherapy which must be common to all treatment arms. Trials for which DFS is the only assessable outcome of interest have a median follow-up of \( \geq 3 \) years.

Search Methods and Data Collection- The search included the MEDLINE, EMBASE, Cochrane Library, and Web of Science databases; clinicaltrials.gov, Current Controlled Trials, ANZCTR and EORTC Clinical Trials registries; ASCO, ESMO and ECCO conference proceedings; contacting pharmaceutical companies; and hand-searching bibliographies of identified trials. Study selection, data extraction, and risk of bias assessments were performed by two independent reviewers, with disagreements resolved by a third reviewer. Analysis- Statistical analysis using aggregate data was performed using RevMan 5.1. Hazard Ratios (survival outcomes) and Odd Ratios (grade 3/4 AEs and ORRs) were estimated directly or indirectly from published data. Random-effects models were used to incorporate heterogeneity among studies.

Results: 50 studies were included (Table 1). Comprehensive results will be presented at the meeting.

Table 1: Characteristics of included studies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of oral fluoropyrimidine</td>
<td></td>
</tr>
<tr>
<td>Capecitabine</td>
<td>26</td>
</tr>
<tr>
<td>Tegafur/Fluraflur +/− uracil</td>
<td>11</td>
</tr>
<tr>
<td>Oral 5-FU +/− Eniluracil</td>
<td>6</td>
</tr>
<tr>
<td>Doxifuridine</td>
<td>5</td>
</tr>
<tr>
<td>S-1</td>
<td>2</td>
</tr>
<tr>
<td>Fluoropyrimidines given alone or in combination</td>
<td></td>
</tr>
<tr>
<td>therapy</td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>22</td>
</tr>
<tr>
<td>Combination</td>
<td>29</td>
</tr>
<tr>
<td>Treatment setting</td>
<td></td>
</tr>
<tr>
<td>Advanced/metastatic CRC</td>
<td>40</td>
</tr>
<tr>
<td>Neoadjuvant and/or adjuvant CRC</td>
<td>10</td>
</tr>
</tbody>
</table>

Apoptotic sensitivity of tumour cells to HDACi is determined by sustained induction of the immediate-early genes Jun and Atf3.

Anderly C Chueh\(^1\), Lars Tögel\(^1\), Ian Y Luk\(^1\), Matthew R Thompson\(^2\), Bryan R Williams\(^2\), John M Mariadason\(^1\)

1. Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia
2. Monash Institute of Medical Research, Clayton, VICTORIA, Australia

Background/Aim: Histone deacetylase inhibitors (HDACi) are a novel class of cancer therapeutics currently approved for treatment of cutaneous T-cell lymphoma (CTCL). We have previously established that drug sensitivity of colon cancer cells to HDACi is linked to induction of immediate-early (IE) gene expression. The study aim was to investigate whether these findings are applicable to other tumour types and to elucidate the mechanism by which induction of IE genes triggers apoptosis.

Methods: Apoptotic sensitivity to HDACi was determined for 50 cancer cell lines derived from CTCL, multiple myeloma and all major solid/haematological tumours. Apoptosis response was determined 72hr post drug treatment by measuring sub-G1 populations by PI/FACS analysis. Gene expression changes were determined by Q-RT-PCR. Gene knockdown was achieved by transient transfection of siRNAs using Lipofectamine or Amaxa Nucleofector.

Results: HDACi treatment induced IE gene expression in multiple tumour types. The magnitude of IE gene induction (Fos, Jun and Atf3) correlated positively with apoptosis induction. HDACi-induced IE gene expression was evident 2hr post treatment and sustained over 24hr, in contrast to the rapid and transient induction of these genes following growth factor treatment. Down-regulation of Jun and Atf3 attenuated HDACi-induced apoptosis in multiple cancer cells. Jun and Atf3 knockout MEFs displayed significantly reduced sensitivity to HDACi compared to matched wild-type cells. The negative regulators of apoptosis, Bcl-xL and c-FLIP, were identified as downstream transcriptional targets of Jun and Atf3 and their magnitude of repression correlated with HDACi sensitivity.
Conclusions/Significance: Sustained induction of IE genes is a consistent transcriptional response induced by HDACi in multiple tumour types. Jun and Atf3 induction are functionally important in HDACi-mediated apoptosis, acting via Bcl-xL and c-FLIP repression. This represents a novel transcriptional mechanism of apoptosis induction in response to a targeted therapy, which may facilitate biomarker discovery and the clinical use of these agents.

Cell motility regulation in prostate cancer: A biomarker and systems biology approach

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1. Department of Medicine (Austin Health), The University of Melbourne, Heidelberg, VIC, Australia
2. Queensland Facility for Advanced Bioinformatics, The University of Queensland, St. Lucia, Queensland, Australia

Prostate cancer (PCa) is one of the leading causes of cancer death in men. Our group has shown that a membrane-bound protein, CD151, predicts prognosis and is associated with migration and invasion, a key process in metastasis and cancer survival¹ ². The tetraspanin family member CD151 is associated with regulation of migration of normal and tumour cells via cell surface microdomain formation. Our aim is to focus on systems biology approach by examining protein-protein interaction networks involved with CD151.

Application of high-throughput technology has become one of the driving forces of molecular biology research. In a complicated process like PCa progression, identification and interpretation of differential gene expression profiles are necessary to gain insights into changes in protein complexes, networks and pathways. These insights will allow us to identify molecular signatures and master regulators of prostate cancer progression. We have analysed a set of whole genome microarray expression data from control siRNA knockdown (KD) PC-3 cells vs. CD151 siRNA KD cells, in order to identify differentially expressed genes linked to CD151. Significant changes were seen in 171 genes after CD151 KD vs. control KD PC-3 cells. We examined the protein interaction networks of the differentially expressed genes using Cytoscape³. We thus identified extended biological networks, which are modules of interconnected proteins that share functional connectivity based on known functional annotations and molecular interactions. Specifically, our analyses revealed a high level of connectivity surrounding genes involved in transcriptional regulation, microtubule-based movement and protein folding and complex formation. These findings will now enable us to assess regulation of cell motility in PCa and may enable identification of novel druggable targets.


“Sing me back home before I die” – Popular songs and existential connectedness at the end of life.

John Hedigan¹

1. Austin Health, Heidelberg, Vic, Australia

For younger patients facing cancer, acceptance of pending death can be a difficult transition. Many struggle with the realities of their prognosis and avoid discussion of death with family and clinicians. Singing popular songs that stimulate existential thinking can help patients to access the resilience needed to open up about these topics. After doing so, many connect to their lives in a deeper way and come to a place of acceptance.

This paper will present three case vignettes illustrating the planned use of lyrically meaningful songs to facilitate resilience, emotional expression and connectedness at the end of life. The planned use of these songs (parts of each will be sung and lyrically analysed) to facilitate resilience and connectedness will be explained, along with discussion of how to find and use songs that will assist patients in this way.
The importance of songs and context will be introduced, referring to the need to resonate with a patient's situation, and how certain songs may speak to them in a powerful way. Where a song may hold meaning for a patient in rehabilitation, the same song can feel very different for the patient at the end of life.

Bob Dylan once sang, “I know my song well before I start singing.” Similarly, RMTs should have a good understanding of the potential meanings within the songs they sing. Knowing a lyric well enough to see that one small phrase may have powerful potential for a patient can be a useful tool to facilitate connectedness. RMTs should develop repertoire that is useful for engaging patients existentially, with the aim of emotional catharsis and connectedness.

Investigation of the cancer-testis antigen ropporin as a melanoma immunotherapy target.

Christopher Hudson¹, Andreas Behren¹, Matthew Anaka¹, Claudia Freyer¹, Pu-Han Lo¹, Jonathan Cebon¹
1. Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Background: Malignant melanoma accounts for just a small percentage of all skin cancers but is responsible for the majority of deaths. While new classes of targeted therapeutics show promise in clinics, immunotherapeutic strategies have yet to overcome obstacles like the choice of antigen and adjuvant. Here we describe ropporin, a cancer-testis antigen, whose 97% homologous isoforms are ROPN1 and ROPN1B, and their antigenic potential; making ropporin a possible new target for immunotherapy in malignant melanoma.

Methods and results: RT-qPCR screening of a variety of normal tissues revealed minimal to no expression of ROPN1, except the immune-privileged testes. This was supported by the analysis of publically available gene-expression datasets. Various tumour samples showed very low (colon, lung, glioma, kidney prostate, testes) or mixed (breast) expression of ROPN1 by RT-qPCR. However, a large cohort of human melanoma cell lines and tumour samples showed high ROPN1 expression. IHC of melanoma cell lines and tumours confirmed ropporin protein expression.

When utilizing a dataset of clinical melanoma specimens to investigate ropporin expression during disease progression, it was shown to increase, and is highest during the metastatic growth phase and lymph node metastasis.

Moreover, a number of melanoma patient sera were subjected to a protein arrays to identify antibodies against a number of tumour antigens. Among the most immunogenic were the ropporin isoforms ROPN1 and ROPN1B.

Conclusion: Together, these observations suggest that ropporin is an immunogenic antigen, particularly for melanoma. Its correlation with disease progression indicates its targeting could afford disease management for advanced melanoma patients.

Intensified salvage chemotherapy and transplant conditioning in high risk relapsed or refractory aggressive NHL

Wojt Janowski¹, Andrew Grigg¹
1. Austin Health, Heidelberg, VIC, Australia

Introduction
Salvage chemotherapy (typically 2-3 cycles of rituximab, ifosfamide, carboplatin and etoposide: RICE) followed by autologous stem cell transplant (typically with BEAM conditioning) has been the standard of care for relapsed/refractory aggressive NHL. A significant subset of patients will not be cured with this approach, particularly those with a short duration of CR1 (<12 months) or primary refractory disease. While augmented ICE (intensified ifosfamide and etoposide) has been shown to improve salvage response in high-risk relapsed/refractory Hodgkin lymphoma, its potential role in NHL has not been established. Since 2011, eligible Austin patients have been offered salvage with two cycles of
augmented RICE followed by transplantation with pharmacokinetcially dose-adjusted IV busulphan and melphalan conditioning.

**Methods**
A retrospective analysis was performed of patients who received augmented RICE +/- autograft. Safety (but not efficacy) assessments included one patient treated for Hodgkin lymphoma.

**Results**
Eight patients received 15 cycles of augmented RICE (rituximab was not used in T cell lymphoma or Hodgkin lymphoma). Baseline characteristics are attached. Median duration of neutropenia (ANC<0.5) was 4 days, with count recovery between day 13 and 15. Two patients with extensive marrow involvement had delayed recovery. Toxicity was acceptable, with febrile neutropenia (5 cases) and reversible ifosfamide encephalopathy (2 cases) being the most significant. All patients recovered without sequelae. Nausea was significant but managed with a modified antiemetic regimen. All patients responded, with 4 achieving CR. Six patients collected stem cells and proceeded to autograft, with most patients experiencing significant mucositis requiring TPN and narcotic analgesia. Four patients remain in CR. Two have died of progressive lymphoma (median followup 6.5 months post-autograft). The non-transplanted patient remains in CR 6 months following further standard RICE.

**Conclusion**
Augmented RICE and bu-mel conditioning has promising activity in patients with high-risk relapsed/refractory aggressive NHL, with significant but acceptable toxicity. A prospective multi-institutional trial is planned.

<table>
<thead>
<tr>
<th>Baseline Characteristics (NHL only)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients:</strong></td>
</tr>
<tr>
<td>Median Age/Range (yrs)</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Median No. Prior Regimens</td>
</tr>
<tr>
<td>Stage at Relapse</td>
</tr>
<tr>
<td>1-2</td>
</tr>
<tr>
<td>3-4</td>
</tr>
<tr>
<td><strong>B symptoms</strong></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td><strong>Elevated LDH</strong></td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td><strong>Extraneal Disease Other than BM</strong></td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>ECOG 0-1</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td><strong>Disease</strong></td>
</tr>
<tr>
<td>'Standard' DLBCL</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>TCR-DLBCL</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Transformed Follicular</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>PTCL</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td><strong>Risk Factor</strong></td>
</tr>
<tr>
<td>Refractory to Induction</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Refractory to Salvage</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>Short CR1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>
The efficacy of brentuximab, a novel anti-CD30 antibody-drug conjugate, in relapsed or refractory Hodgkin lymphoma

Wojt Janowski1, Jason Butler2, Hugh Goodman3, Leanne Berkahn4, Matthew Ku5, Robin Filshie5, Philip Campbell5, Andrew Grigg1

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2. Royal Brisbane and Women's Hospital, Herston, QLD, Australia
3. Waikato Hospital, Hamilton, New Zealand
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5. St Vincent's Hospital Melbourne, Fitzroy, VIC, Australia
6. Geelong Hospital, Geelong, VIC, Australia

Introduction

Brentuximab vedotin is a novel antibody-drug conjugate directed against the CD30 antigen expressed on Reed-Sternberg cells, which in overseas trials has demonstrated efficacy in Hodgkin lymphoma (HL) relapsing post-autograft. This review examines the efficacy of brentuximab in Australasia when available under a compassionate access scheme in 2011 for relapsed/refractory HL.

Methods

Six centres in Australia and New Zealand participated. Patient records were retrospectively assessed by the contributing sites before central analysis.

Results

Sixteen patients with a median age at initial diagnosis of 25 years (range 17-55) received brentuximab at a median 38 months post-diagnosis (range 9-194). Disease status when starting brentuximab is shown below. 14 patients had undergone autologous stem cell transplantation.

<table>
<thead>
<tr>
<th>Disease Status</th>
<th>n (%)</th>
<th>Median Prior Treatments (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary refractory</td>
<td>7 (44%)</td>
<td>3 (2-5)</td>
</tr>
<tr>
<td>Refractory relapse</td>
<td>5 (31%)</td>
<td>6 (5-7)</td>
</tr>
<tr>
<td>Untreated relapse</td>
<td>3 (19%)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Untreated relapse, post-autograft</td>
<td>1 (6%)</td>
<td>5</td>
</tr>
</tbody>
</table>

Patients received the standard schedule of 1.8mg/kg q21 days for a median of 6 cycles (range 1-12). Two severe adverse reactions were reported (anaphylaxis, fatal respiratory infection in context of recent autograft). No other significant toxicities were seen.

Seven patients responded to treatment, with one achieving CR. A further six had stable disease. Median duration of best response including stable disease (censored for transplantation) was 3 months (range 2-9 months), with one patient maintaining a PR in the absence of transplantation.

Following brentuximab, 3 patients underwent allogeneic stem cell transplantation, all of whom achieved CR (sustained in two at 6 and 8 months post-transplant). Three received autografts, two remaining in CR at 1 and 11 months.

At a median follow up of 10 months (range 2-18) post-first brentuximab dose, 4 patients are alive in CR, 10 are alive with disease, 1 is deceased and 1 is lost to follow up.

Conclusion

A significant proportion of this cohort of very high-risk patients responded to brentuximab. While the response was temporary in most, 38% of patients were able to proceed to potentially curative transplantation. Further studies are evaluating brentuximab in combination with standard chemotherapy as initial therapy of HL.

Activation of TGF-beta signalling enhances epithelial-to-mesenchymal transition in acquired drug resistant metastatic melanoma

Aparna Jayachandran¹, Andreas Behren¹, Matthew Anaka¹, Pu-han Lo¹, Prashanth Prithviraj¹, Jonathan Cebon¹

1. Ludwig Institute for Cancer Research, Heidelberg, VIC, Australia

Background: Melanoma, the most aggressive form of skin cancer, is refractory to current treatment options. Overcoming drug resistance is a major challenge in treating melanoma. Emerging evidence suggests molecular association between epithelial-to-mesenchymal transition (EMT), the switching of cell states, and acquisition of drug-resistance in various cancers.

Objective: This study investigated whether human melanoma cell lines treated with three conventional chemotherapeutic agents with divergent mechanisms of action (5-fluorouracil, cisplatin and paclitaxel) induced EMT. We aimed to identify the signalling pathways regulating EMT in these cells.

Methods and Results: EMT gene-expression profiling lead to the identification of two subsets among seventy melanoma cell-lines: epithelial-like and mesenchymal-like. These cellular subsets exhibited differential expressions of EMT-associated transcription factors, indicative of the occurrence of EMT in melanoma. Epithelial-like cells were drug-sensitive, whereas mesenchymal-like were drug-resistant. Drug-resistant cells generated by short and long-term exposure of epithelial-like cells to drugs expressed high levels of mesenchymal markers including most of the EMT associated transcription factors. Furthermore, components of the TGF-beta pathway were highly expressed in the drug-resistant and mesenchymal melanoma cells. Thus epithelial-like cells may acquire drug-resistance by undergoing EMT. Subsequently, we identified TGF-beta1 as a potent inducer of EMT in melanoma cells. Gain and loss of function studies of the EMT associated transcription factors lead to the attenuation of functions associated with TGF-beta-induced-EMT and acquisition of drug-resistance.

Conclusion: These observations suggest that EMT is a major event controlling melanoma progression and inhibition/reversal of EMT may be a valid therapeutic option in melanoma.

Correlation of wtEGFR activation assessed by mAb806 binding and EGFR kinase mutations in Stage III pathological N2 NSCLC

Thomas John¹, Mun Sem Liew¹, Carmel Murone¹, Marzena Walkiewicz¹, Paul Mitchell¹, Hui Gan¹, Stephen Barnett², Prudence Russell³, Gavin Wright⁴, Andrew Scott¹

1. Ludwig Institute for Cancer Research, Melbourne, Australia
2. Department of Thoracic Surgery, Austin Health, Melbourne, Australia
3. Department of Anatomical Pathology, St Vincent’s Health, Melbourne, Australia
4. Department of Thoracic Surgery, St Vincent’s Health, Melbourne, Australia

Introduction: Epidermal-growth-factor receptor (EGFR) overexpression occurs with gene amplification and activating EGFR kinase mutations (EGFRmt). Monoclonal-antibody 806 (mAb806) is an anti-EGFR antibody targets tumours with EGFR mutation variant III and overexpression but not cells with low EGFR expression (wild type EGFR, wtEGFR) and normal tissues.¹ To characterise mAb806 binding, we correlated mAb806 expression with EGFRmt, EGFR immunohistochemical (IHC) expression and overall survival (OS).

Methods: Formalin fixed paraffin embedded tissues were stained using the mAb806 and EGFR antibody. A H-score was obtained based on staining intensity and percentage of cells stained. mAb806 H-scores 0-50 were classified as negative (mAb806-) and ≥50 as positive expression (mAb806+). EGFR H-scores were calculated to stratify patients into low (Dako score <200) and high (≥200, EGFR≥200) expressors.

Results: Of 107 patients with non-small cell lung carcinomas (NSCLC), 88% expressed EGFR (EGFR+), of which 26% patients were EGFR≥200 expressors. Thirty-seven percent were EGFR+ and mAb806+, while 18% were EGFR≥200 and mAb806+. Squamous cell
carcinomas (SqCC) were more likely to express mAb806+ than adenocarcinomas [55% vs 30%; \( p=0.0353 \)]. Of the 15 patients with activating EGFRmt, 14 were mAb806+, and 1 was mAb806- (\( p=0.0001 \)). Forty percent EGFRmt and 19% wtEGFR tumours were EGFR≥200 expressors (\( p=0.1895 \)). Of 107 patients, OS was similar in both mAb806+ and mAb806-cases. In patients with SqCC, mAb806+ was associated with significantly poorer survival than mAb806- tumours (HR 2.82, 95% CI 1.13-7.04; \( p=0.02 \)).

**Conclusions:** mAb806+ was associated with EGFRmt but was not prognostic in adenocarcinomas. mAb806+ in SqCC was associated with a poorer prognosis. Given its use as a therapeutic target and its potential role as a prognostic biomarker, further studies exploring the treatment efficacy of mAb806 in NSCLC are warranted.

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**Glyoxia: integrated analysis of glucose metabolism under hypoxia with \(^{18}\text{F}\)-Fluoromisonidazole (FMISO) and \(^{18}\text{F}\)-fluorodeoxyglucose (FDG) PET in high grade glioma patients.**

Antoine Leimgruber¹, Sze Ting Lee¹ ², Kevin Hickson¹, Hui Gan¹, Lawrence Cher¹, John Sachinidis¹, Graeme O’Keefe¹, Andrew M Scott¹ ²

1. Austin Health, Heidelberg, VIC, Australia
2. Ludwig Institute for Cancer Research, Heidelberg, Victoria, Australia

**Background**

Tumour hypoxia is a centerpiece of disease progression mechanisms, and hypoxia-resistant malignant cells can cripple radiotherapy strategies. Early identification of hypoxic regions at risk for recurrence are a necessity for tailored therapeutic strategies. Combined FDG with FMISO PET imaging allows representation of glucose metabolism under hypoxic conditions.

**Methods**

Glyoxia is a disease-independent fully automated software which offers qualitative and quantitative analyses of FDG and FMISO PET to study glucose metabolism under hypoxic conditions, with an analysis of hypoxic volume (HV), hypoxic glycolytic volume (HGV) and total glycolytic volume (TGV) with MRI coregistration. This system was applied to a prospective clinical trial of 10 high grade glioma patients with post-operative, pre-radiotherapy and early post-radiotherapy FDG and FMISO PET studies.

**Results**

Within this cohort, 15 out of 18 FMISO PET studies performed showed detectable hypoxia. Seven patients survived to complete post-radiotherapy studies. Post-radiotherapy hypoxic region detection failed in 1 patient. HGV increased in 3 patients, while HV changes were less prominent. These patients had a mean of 85 days progression-free interval and 416 days overall survival from diagnosis, versus 152 and 649 days respectively for the 3 other patients. HGV images were consistent with disease relapse topology.

**Conclusion**

The ability to decipher glucose metabolism in hypoxic tumours is a key tool in devising tailored therapies to cancer patients and better understanding tumour metabolism.
$^{11}$C-choline PET scan is more accurate than biopsy in assessment of localized prostate cancer planned for radical prostatectomy

Ian D Davis$^{1,2}$, Sze Ting Lee$^{1,2}$, Sylvia Gong$^1$, Lekshmy Shanker$^1$, David Clouston$^1$, Damien M Bolton$^1$, David Angus$^1$, David Webb$^1$, Nathan Lawrencechuk$^1$, TS Woon$^1$, Daryl Lim Joon$^1$, Joe H Chang$^1$, Stephen Esler$^1$, Richard O'Sullivan$^3$, Kunthi Pathmaraj$^1$, Henri Tochon-Danguy$^1$, Graeme O'Keefe$^1$, Andrew M Scott$^{1,2}$

$^1$Austin Health, Heidelberg, VIC, Australia
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$^3$Healthcare Imaging, Melbourne, Victoria, Australia

$^{11}$C-choline PET scanning is more accurate than biopsy in assessment of localized prostate cancer planned for radical prostatectomy

**Background**

Optimal outcomes for prostate cancer require selection of prostatectomy patients who have no extraprostatic disease. $^{18}$F-FDG (FDG) and $^{11}$C-choline (CHOL) PET in men planned for radical prostatectomy were assessed to determine the effects and accuracy of PET, and correlation with PSA, MRI, TRUS biopsy and prostatectomy specimens.

**Methods**

All men consented and underwent standard staging investigations, followed by FDG-PET and CHOL-PET. The urologist documented their treatment plans before and after the PET scans. The prostatectomy specimen was reconstructed and correlated with the TRUS and imaging results by sextant analysis.

**Results**

30 patients enrolled. Neither PET modality significantly affected decisions about surgery, but CHOL-PET was most sensitive & accurate, compared to pathology.

<table>
<thead>
<tr>
<th></th>
<th>TRUS</th>
<th>MRI</th>
<th>FDG</th>
<th>CHOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.76</td>
<td>0.45</td>
<td>0.09</td>
<td>0.83</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.56</td>
<td>0.83</td>
<td>0.86</td>
<td>0.44</td>
</tr>
<tr>
<td>False positive</td>
<td>0.44</td>
<td>0.17</td>
<td>0.14</td>
<td>0.56</td>
</tr>
<tr>
<td>False negative</td>
<td>0.24</td>
<td>0.55</td>
<td>0.91</td>
<td>0.17</td>
</tr>
<tr>
<td>PPV</td>
<td>0.87</td>
<td>0.87</td>
<td>0.72</td>
<td>0.86</td>
</tr>
<tr>
<td>NPV</td>
<td>0.36</td>
<td>0.38</td>
<td>0.19</td>
<td>0.39</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.72</td>
<td>0.56</td>
<td>0.24</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**Conclusions**

CHOL-PET was superior to TRUS and MRI, with greater sensitivity, accuracy and highest congruity with pathology, and highest positive predictive value.

Supported by grant 487916 through Cancer Australia, Prostate Cancer Foundation Australia, Australian Government Department of Health and Aging. IDD is supported by an NHMRC Practitioner Fellowship.
Maintaining & sustaining a supportive care service for patients with brain tumours.

Dianne M. Legge¹
1. Austin Health Cancer Services, Heidelberg, VIC, Australia

Austin Health together with the John Cummins Memorial Fund established the unique role of the Brain Tumour Support Officer in 2008. This tumour-streamed supportive care role has enabled patients with brain tumours and their families to be resourced, supported and assisted throughout their healthcare journey, from the point of diagnosis, through their treatment and beyond.

For the 12 months to July 2011, over 125 families have been assisted through individual and group interventions, with more than 71% accessing the Brain Tumour Support Officer on multiple occasions. The Brain Tumour Support Service (BTSS) focuses on four primary service strategies;

• Inform
• Resource
• Acknowledge
• Support

Support intervention is targeted towards specific points on the disease trajectory and offers a range of opportunities and services, so that individual support needs can be addressed.

In response to patient demand and feedback, the BTSS has expanded into areas of carer education, community forums and development of health professional support in rural and regional areas. During 2011, a number of information & education based activities have been conducted out of hours, culminating in an open public forum during brain tumour awareness week, which attracted both health professionals, and people affected by brain tumours. These programs have been highly successful, well attended and mark a departure from the traditional style of patient health education.

This poster will present recent program evaluations and highlight the multi-pronged approach taken in the delivery of brain tumour support at Austin Health. The presentation will also outline the benefits and challenges of maintaining and sustaining a unique tumour-specific supportive care model.

Addressing the unmet needs: the story of an information based program for people with Brain Tumours.

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In establishing the brain tumour support role at Austin Health, careful consideration was taken to address the unmet supportive care needs of patients, families and carers' of people with primary malignant brain tumours. As part of this process the Cancer Council Victoria “Living with Cancer Education Program “ model was considered as an appropriate vehicle to present information relevant to the needs of people affected by the diagnosis of a primary brain tumour. The “Living with Cancer Education Program” model has been in operation since 1985, and is designed as a collaboration between the CCV, healthcare centre or Cancer treatment centre and the community.

The Austin Health brain tumour specific program (LWCEP 4 BT) was piloted back in March 2009 as a single day module, with some minor changes to the standard program content, to target the needs of brain tumour patients. Whilst the initial feedback was positive, the long format of the day meant that some potential participants were discouraged from attending due to fatigue issues. The LWCEP 4 BT has developed in structure and content over this time and is now run regularly twice per year, over a 4 week period.
To date 105 people have participated in 6 Brain tumour specific programs run through the Austin. Over 95% of participants rated the program as "very Satisfactory" or "Satisfactory" and 100% of participants would recommend the LWCEP 4 BT to other people. The program content has been refined each time to include a wide range of topics identified by past and current participants which go some way to meeting the informational needs of patient & carers'.

This presentation will outline the development, structure and content of the LWCEP 4 BT, and look at some of the evaluation feedback from participants which has helped drive this support care program.

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TKTL1- a promising anti-tumour target in melanomas

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Enhanced glucose consumption is the most consistent hallmark of cancers. Unlike normal differentiated cells, 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 transketolase-like protein 1 (TKTL1) is a key enzyme in the process and is over-expressed in many human cancers. The strong correlation between high TKTL1 expression and poor patients’ survival as well as tumour progression has also been shown in different cancers.

We examined the expression pattern of TKTL1 in melanoma tumours, cell lines and a panel of normal human tissues and found it to be highly expressed in human testis but not other normal tissues. We detected TKTL1 at high levels in 40% of the melanoma tumours. IHC staining confirmed TKTL1 expression in tissue microarrays of melanoma patients. In order to investigate the functional role of this enzyme in melanoma, studies using small molecule TKTL1 inhibitor, siRNA and exogenous expression assays were performed on human melanoma cell lines. These functional studies indicate that TKTL1 enhances glucose utilization and lactose production in melanomas. We observed changes in TKTL1 levels also altered the proliferative and invasive abilities of melanomas. Furthermore, downstream glycolytic targets were compared between TKTL high and low expressing cells by microarray. This data provides new evidence for the role of TKTL1 in melanoma and indicates that TKTL1 is not only a tumor marker but also a good target for anticancer therapy.

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A study of the palliative prognostic (PaP) score in a comprehensive cancer centre

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Accurate prognostication is vital in the management of patients with cancer. The PaP score is a prognostic tool that uses clinical and laboratory parameters to classify patients with advanced cancer into three risk categories predictive of 30-day survival [1, 2]. The PaP score is calculated based on clinical prediction of survival (CPS), performance status, anorexia, dyspnoea, total white blood cell count and lymphocyte ratio [2]. Although the PaP score is extensively validated in inpatients, limited studies evaluate its use in outpatients. The CPS, which is commonly determined by a single clinician is poorly reproducible and tends to overestimate survival [3]. At our institution, the CPS is determined by a palliative care multidisciplinary team (MDT).

This is a retrospective observational study of the PaP score in a series of cancer patients referred to the Pain and Palliative Care Department of the Peter MacCallum Cancer Center (PMCC). The aims are to evaluate the clinical application of the PaP score in our patients, assess the accuracy of prognostication by a palliative care MDT and identify other factors that affect prognostication such as age, tumour type and setting of care.
644 patients referred from 1st January 2010 to 31st Dec 2011 excluding haematological and renal malignancies were included (421 inpatients; 223 outpatients; mean age 61 years). Data was sourced from the Pain and Palliative Care Database which routinely collects patient data including demographic information, episode information, PaP score and CPS predicted by a palliative care MDT.

Kaplan Meier estimates and log rank tests will compare survival among subgroups. Other factors affecting prognosis will be entered into a Cox regression analysis. Exploratory analyses will be carried out in outpatients and in patients who have had multiple PaP scores calculated at serial points in their disease trajectory. Statistical analysis of the data is underway and results pending.


Budd Chiari Syndrome (BCS): The Austin Experience

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Aim: BCS is a rare disorder, with annual incidence of 0.2-0.8 per million.¹ The few available studies report liver transplantation rates of 18%² to 42%³ and poor 5-year transplantation-free survival of 28%³ for primary BCS. We aimed to investigate the epidemiology, natural history and outcomes without transplantation of BCS patients at Austin Health.

Method: Retrospective study reviewing the records of all patients with primary BCS managed at Austin from 2002 to 2012. BCS secondary to local compression was excluded.

Results: Twenty-seven primary BCS patients were identified. Median age at diagnosis was 42 years (range 21-76), 59% were female. 23(85%) were at least one identifiable risk factor, the commonest being a myeloproliferative neoplasm (MPN, n=15). JAK-2 was positive in 7 of 12 MPN patients tested. Six patients had a delayed MPN diagnosis, with median time from BCS to MPN of 4 months (range 3-150). An underlying MPN cannot be excluded in five patients, who did not have JAK-2 testing or bone marrow biopsy. Other risk factors included hereditary/acquired thrombophilia (n=8) and the contraceptive pill (n=4), of whom 1 and 3 patients respectively also had MPN. All patients were anticoagulated with warfarin or low molecular weight heparin, with 7 major bleeding complications (variceal, intracranial). The primary intervention was transjugular intrahepatic portosystemic shunting (TIPS) in thirteen patients (48%) and angioplasty/stenting in eleven (41%). One patient had a splenorenal shunt. No patients required transplantation. At a median follow-up of 5 years, 18 patients have compensated liver disease, 2 have decompensated liver disease, 5 patients died (2 from BCS complications). 2 were lost to follow-up.

Conclusion: MPN is the commonest aetiological factor in BCS. This can be missed at diagnosis, and all patients should have JAK2 testing or bone marrow biopsy. TIPS or angioplasty/stenting, together with anticoagulation and treatment of any MPN, results in favourable long term transplantation-free outcomes (no patients required transplantation) and represents optimal standard of care.

2. Plessier et al, Aiming at Minimal Invasiveness as a Therapeutic Strategy for Budd-Chiari Syndrome, Hepatology 2006, 1308-1316
Quality of setting treatment goals and limitations and identification of need for palliative care for inpatients in an acute care hospital.

**Juli Moran**, Daryl Jones
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We conducted an observational study to determine whether patients in a Melbourne tertiary hospital were having treatment goals and limitations documented by the treating team, whether the need for palliative care was recognised and association of these factors with outcome.

After ethics approval we reviewed the records of patients in every ward (excluding ICU / CCU / psychiatry & paediatrics) over two weeks. Data collected included demographics, co-morbidities, treatment goals and limitations, need for referral to palliative care and outcomes.

We reviewed 287 records. 55% were male and the median age was 66.7 (range 18-100). Twenty three patients seemed suitable for referral to the Palliative Care Service, but only 9 (39%) had been referred to the service. Sixty per cent of patients had no goals of treatment.

Only 10 patients were asked if they had an advance care plan. According to hospital policy, 165/287 (57%) patients should have had a documented resuscitation plan, but 72 of these (44%) had no plan recorded. Eighteen per cent of patients were confused, and of these 60% had no record of goals being discussed with family. Nine per cent of patients died, 76% went home and 8% to residential care. Patients were more likely to die or go to residential care the longer they stayed in hospital and the greater their co-morbidities.

There is a need to improve setting and documenting treatment goals and limitations in our hospital, and to improve recognition of the need for palliative care.

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**18**F-FDG uptake in bowel in diabetic patients treated with metformin

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**Aim:** The aim of this study is to assess the relationship between metformin and bowel uptake of 18F-FDG in diabetic patients.

**Methods:** 18F-FDG PET/CT scans were performed in 50 non-insulin dependent diabetic patients on oral hypoglycaemic agents referred to our department, of whom 31 patients were on metformin. The scans were compared to a control group of 50 non-diabetic patients matched for body mass (BMI) and gender. The scans were analysed by two independent nuclear medicine physicians blinded to the clinical information. Bowel uptake was graded relative to liver on a 5 point scale.

**Results:** FDG uptake in bowel was significantly increased in diabetic patients on metformin compared to the non-diabetic patients (p < 0.0001). No significant difference in FDG bowel uptake was demonstrated between NIDDM patients on oral hypoglycaemics other than metformin and non-diabetic patients.

**Conclusion:** NIDDM patients treated with metformin demonstrate significantly increased FDG uptake in the bowel, which may increase the difficulty in detecting abdominal malignancy.
Targeting mutant K-Ras with novel siRNA-antibody conjugates for the treatment of colorectal cancer

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Despite significant improvements in treatment and diagnosis, colorectal cancer (CRC) still remains one of the leading causes of cancer related deaths in Australia and worldwide. More effective therapies for the treatment of this disease are clearly needed. K-Ras is an oncogenic protein that is mutated in ~50% of CRCs and is a key driver of tumorigenesis. In CRC 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 CRC therapies, but has evaded all attempts at direct therapeutic targeting so far. There is considerable interest and effort in developing specific and potent inhibitors of mutant K-Ras activity. The high sequence specificity of small interfering RNAs (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 CRC cells containing G12V, G12C, G12D and G13D mutations. We have also shown that treatment with K-Ras mutant specific DsiRNAs results in significant inhibition of proliferation and increase in apoptosis of CRC cells harbouring a mutation. This result is very promising given that CRC cells with K-Ras mutations are resistant to treatment with cetuximab. Importantly, we have shown that mutant specific DsiRNA does not significantly reduce the K-Ras expression levels in CRC cells containing wild-type K-Ras. To further develop these siRNAs as a therapeutic for use in patients, we have 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 therapeutic agents for the treatment of CRC.

Mechanism of Insulin-like Growth Factor (IGF) induced Epithelial-to-Mesenchymal Transition (EMT) in Melanoma Progression

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Background: Melanoma is a common and highly aggressive form of skin cancer with a high propensity to metastasise. Despite recent improvements in treatment, metastatic melanoma is nearly always fatal. The role of EMT in facilitating cancer metastasis, progression and recurrence in various cancers has been demonstrated. Growing evidence supports the role of EMT in melanoma progression. However, detailed mechanistic insights into how EMT impacts metastasis in melanoma are currently lacking.

Rationale: Since IGF signalling is elevated in invasive melanoma cells and has been implicated in promoting EMT in other cancers, it is conceivable that IGF may contribute to EMT in melanoma. The aim of this study is to identify and target key components of the IGF signalling pathway to inhibit or reverse the EMT process in melanoma cells.

Methods & Results: Microarray analysis identified components of IGF signalling pathway that are differentially expressed. Using qPCR, we confirmed differential expression of various components of IGF signalling pathway in invasive melanoma cell lines. We tested the influence of IGF ligands on the proliferative abilities of melanoma cells by MTS assay. Furthermore, we tested the invasive and migratory behaviour of melanoma cells post IGF treatment by subjecting them to Matrigel coated or uncoated Boyden chambers. We studied
the effect of IGF treatment on the expression of classical EMT markers. These studies indicate that IGF signalling plays a role in EMT, and suggest targeting this pathway may be a valid therapeutic option in melanoma.

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Maintaining Planned Patient Geometry Through the Utilization of Robust Nutritional Support Interventions

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2. Royal Marsden Hospital Trust & Institute of Cancer Research, Marsden, England

Purpose/Objective(s): Radiotherapy treatment planning plays an integral role in achieving improved clinical outcomes. Radiation can initiate mucosal reactions, resulting in dysphagia and subsequent malnutrition. Weight maintenance throughout radiotherapy presents a great challenge, to ensure spatial geometry of the patient is maintained to enable precision dose delivery. Furthermore, it provides a platform for an enhanced overall treatment tolerance. A robust nutritional program facilitates good weight management. This is of particular importance in patients where target geometry and geography prevent idealist avoidance of swallowing anatomy. A study of pre and post-treatment weight management was undertaken to determine the role of dosimetry and acute dysphagia in influencing this variable.

Material/Methods: Seventy-six patients with SCC of the head and neck were retrospectively analysed in this study. All were nutritionally managed throughout treatment. Dose recommendations (Dmean, Dmax and V50Gy) to the larynx were used. Patients were reviewed weekly for acute dysphagia toxicity (CTCAEv3). Weight at treatment commencement and finish was recorded.

Results: Patients with larynx V50Gy<27% delivered 65.1%(p=0.008) and 61.0%(p=0.001) grade 3 toxicity reduction at weeks 5 and 6 respectively when compared to those with larynx V50Gy>27%. Dmean<44Gy (n=38) delivered 64.7%(p=0.005) and 48.6%(p=0.006) at weeks 5 and 6 respectively when compared to those with Dmean>44Gy. Dmax<66Gy presented a 58.2% (p=0.002) reduction at week 6 compared to Dmax>66Gy.

No significant weight reduction was witnessed across any of the groups.

Conclusion: Treatment induced acute dysphagia may negatively impact quality of life throughout a course of head and neck irradiation. Adequate nutritional support throughout treatment facilitates good weight management, and is not necessarily dependent on dose to swallowing structures of the neck. This enables maintenance of planned patient geometry for precision dose delivery, whilst maintaining a higher likelihood of patient treatment tolerance.

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Evaluation of the use of D2-40 and CD31 immunohistochemical staining in identifying lymphovascular invasion in stage I colorectal cancer.

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Background: Recent evidence suggests that lymphovascular invasion (LVI) in stage I colorectal cancer (CRC) is associated with shorter disease-free and overall survival. Therefore accurate assessment of LVI may become important to identify high-risk patients who may benefit from systemic adjuvant therapy, whereas currently all stage I CRC is treated with surgery alone.

Aim: To evaluate difficulties of morphological interpretation in the recognition of LVI and the role of immunohistochemistry (IHC) for endothelial markers D2-40 and CD31 in the identification of LVI.

Methods: All stage I CRC reported at Austin Pathology in 2011 were reviewed. Each tumour was analysed for evidence of LVI by conventional H&E and by D2-40 and CD31 IHC staining, and categorised as negative, positive or suspicious.

Results: Of 29 carcinomas in the study, on routine H&E staining LVI was found in one case, and this was confirmed on IHC. 11 cases were suspicious for LVI on H&E, of these LVI was
confirmed in three and was negative in six by IHC. Two cases remained unresolved. LVI was not detected by IHC in any cases assessed as negative on H&E.

Conclusion: This study confirmed that it is difficult or impossible to make a reliable diagnosis of LVI on H&E alone due to factors such as retraction artefact, mucin extravasation, obscuring fibroinflammatory reaction and tumour budding. IHC was helpful in the clarification of LVI, with a pick up rate of 3/11 (27%) in cases assessed as suspicious for LVI on H&E and with LVI confidently excluded in 6/11 (55%) of these cases. IHC can potentially give a false positive result (pseudovascular staining), but no true LVI was detected in cases negative by H&E. The findings support the use of IHC as an adjunct to routine H&E analysis in confirming the presence of LVI in stage I colorectal cancer.

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Evaluation of an impedance-based probe to detect early cell death events

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The estimation of cell density and cell viability of mammalian cell lines in cell culture has traditionally been performed using the exclusion dye trypan blue that stains “dead” cells when their cell membrane is damaged. In large scale cell cultures using bioreactors this estimation is performed off-line. The online biomass probe is based on the principle that under the influence of an electric field between two electrodes, ions in suspension migrate toward the electrodes. The cell plasma membrane is non-conductive so that the cells with intact plasma membranes are polarized and act as tiny capacitors and it has been shown that capacitance increases as the cell concentration does. The measurement is based on the linear relationship between the permittivity difference ε₁ - ε₂ and the viable biomass concentration.

This study compares the data obtained using the biomass probe at different frequencies with measurement of rhodamine-123 and pan-caspase activation by flow cytometry for a number of mammalian cell lines.

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Characterisation of I-124 Radiolabelled anti-TAG72 Pegylated Diabody AVP0458

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3. Avipep Pty Ltd, Parkville, Victoria, Australia

Objectives: Diabodies (Avibody™ products) are single chain antibody fragments (scFv) that spontaneously self-associate at high yield into stable multivalent dimers. This study aimed to generate a unique diabody against TAG 72 antigen, an oncofetal antigen found on the surface of many epithelial cancers.

Methods: AVP0458, a diabody engineered from scFv domains of mouse monoclonal antibody CC49 is produced from E Coli expression and purification. Preclinical biodistribution and imaging study was conducted with iodine-124 radiolabelled PEG-AVP0458, in BALB/c nude mice bearing TAG-72 positive LS174T xenografts. In-vitro analyses confirmed retention of antigen-binding activity and serum stability.

Results: High invivo tumour uptake >52% was observed 24-48 hrs p.i with prolonged tumour retention. Normal tissues showed minimal 124I-PEG-AVP0458 uptake, consistent with blood pool activity (<3 %ID/g at 24 hrs p.i). Pharmacokinetic analysis calculated a serum half-life of T₁/₂ a = 6.7 hrs, T₁/₂ b=29.7 hrs. This compares with T½ β= 72.2 hr previously observed with the parent antibody, CC49, in the same LS174T human colon carcinoma xenograft model.
Specific tumour localisation of $^{124}$I-PEG-AVP-0458 was observed over 3 days by Positron Emission Tomography (PET) imaging.

Conclusions: $^{124}$I-PEG-AVP0458 shows optimal blood clearance, with high specific and prolonged tumour uptake *in vivo* and is an ideal radioimaging product for Phase I PET bioimaging clinical trial.

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The Effect of Microenvironmental Factors on the Biology of Primary Prostate Cancer Cells in Vitro

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Introduction

Prostate cancer (PC) affects 13,000 Australian males annually and is incurable once metastasised, the most common site being bone. Novel treatment strategies are required to target metastatic prostate cancer. The aim of this study was to examine the biological function of primary PC cells in response to defined and complex microenvironmental factors that are found within the prostate and/or the bone.

Materials and Methods

Primary PC epithelial cells were isolated from radical prostatectomy specimens using enzymatic digestion and EpCAM-mediated bead purification, and cultured in defined epithelial growth medium in vitro. The biological properties examined were: 1) adhesion to extracellular matrix proteins; 2) proliferation on extracellular matrix proteins; and 3) proliferation in response to exogenous factors.

Results

Primary PC epithelial cells bound to collagens and Matrigel but not to laminin or osteopontin. Immediately after isolation, their proliferation was increased when cultured on laminin, however this effect was not seen after passaging. Primary PC cell proliferation was stimulated by aFGF, conditioned medium from cancer-associated fibroblasts, and conditioned medium from osteoblasts, although these responses were not seen with all cancer specimens. Treatment with androgen, EGF, bFGF, or HGF alone had no effect. TGFβ and TNFα treatment consistently inhibited proliferation by a mechanism other than apoptosis.

Conclusions

The biology of primary PC epithelial cells is affected by specific soluble and extracellular matrix factors. Primary PC cells consistently adhere to collagens and Matrigel, however their rate of proliferation is not affected by these extracellular matrix proteins. Proliferation is consistently inhibited by TGFβ and TNFα, while aFGF and conditioned medium from cancer-associated fibroblasts and osteoblasts occasionally stimulate proliferation. These differences may indicate underlying differences in the biology of these cancers, which could have prognostic and/or predictive implications for PC patients.

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Aim:

The inadequate recording of patient medical treatment preferences on Advance Care Directives (ACDs) and medical treatment decisions as documented on the Resuscitation Plan (RP) was identified as a potential source of error within Radiation Oncology where patient information is sourced and recorded across dual clinical information systems: Mosaig locally and MedTrak/Cerner Systems hospital-wide. Hospital Policy and new national accreditation
standards outline the importance of timely identification of ACD and RP’s in the determination of the appropriate medical treatment plan for the acutely deteriorating patient.

**Methods:**
A prospective audit was conducted on patients receiving radiation therapy (RT) between 1/7/2011-30/11/2011 on the identification and recording of ACDs and RPs within the Mosaiq system compared with hospital-wide systems. MedTrak and Scanned Medical Records (SMR) were examined for existing alerts and valid documents respectively. ACDs audited included Medical Enduring Power of Attorney (MEPOA), Statement of Choices (SOC) and Refusal of Treatment Certificate (RTC).

**Results:**
99 patients, 39 inpatients and 60 outpatients were randomly selected. The below table summarises those ACDs/RPs valid at the time of RT and recorded within clinical systems.

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**Conclusion:**
Recording of ACDs and RPs within the local Mosaiq patient information system was poor. Deficiencies in this area highlighted the need for improved procedures and staff education in order to ensure optimal quality of care is delivered to patients. The results of this audit will inform local guideline development and implementation that will be assessed 12 months post-implementation for effectiveness.

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**Are Polymer fiducial markers better than the “Gold” standard?**

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**Introduction:** Implanted fiducial markers are often used as a soft tissue surrogate in radiotherapy, with gold the current material of choice. Gold however, produces image artifact on x-ray imaging, which may obscure the absolute position of the marker and the surrounding anatomy, affecting target delineation and treatment position verification. We assess a polymer-based fiducial marker in a tissue equivalent phantom recently released with claims of reduced x-ray artifact and comparable MRI visualization.

**Methods / Discussion:** A tissue equivalent phantom, a mix of gelatin, water and glycerol, was created with comparable x-ray and MRI characteristics to soft tissue. PolyMarkTM and GoldMarkTM fiducials were implanted within the full-scale phantom using a rigid template between 14 and 34mm apart. The phantom was scanned on CT and MRI to assess the relative value of each marker in radiotherapy planning imaging. Voxel grey values of the markers and surrounding phantom material on CT and MRI were analysed using ImageJ software.

Markers were further imaged with each treatment imaging modality available in our centre, including 2D kV and MV, and 3D cone beam CT (CBCT). Visibility was assessed on a scale from 1 to 4, where 1 was clearly visible, and 4 was not visible.

Polymer fiducials had visibly less artifact on CT and treatment imaging than gold and were comparable in their appearance on MRI. Grey value measurements found the polymer fiducial to produce a more discrete image than the gold. The polymer fiducials were easily visualized on CBCT, with less artifact than gold, and were visible on 2D kV imaging. Poor contrast resolution meant the polymer seeds were not visible on 2D MV imaging.

**Conclusion:** Polymer fiducial markers produce markedly less image artifact on x-ray imaging and are comparable to gold on MRI. They provide a potential improvement in radiotherapy target definition and treatment verification.
Patterns of use of perioperative chemotherapy in patients treated with radical cystectomy for urothelial bladder carcinoma.

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Introduction: Radical cystectomy (RC) for curative treatment of invasive urothelial cancer (UC) of the bladder is associated with high relapse rates, especially in patients with extravesical (pT3) and node positive (N+) disease. The aim of this study was to review perioperative chemotherapy (PC), shown to improve survival after RC, and its impact on oncological outcomes.

Methods: Eighty-eight patients underwent RC and/or PC between 2004 and 2011 were identified. Clinical & pathological data, recurrence and death were assessed by retrospective chart review.

Results: The median (range) age of the patients was 65 year (37-84), and 66 (75%) were males. Pathologic features included 84 (95%) UC (pure or mixed), 81 (92%) high grade tumours, pT-stage ≥T3 in 34 (38.6%) and pN+ in 10 (11.4%) patients. Twenty-five (28.4%) patients underwent PC, including 8 (9%) neoadjuvant and 21 (24%) adjuvant. There was a significant trend over the study period in the use of neoadjuvant but not adjuvant chemotherapy. Patients undergoing chemotherapy were more likely to be node positive (p<0.05) and had a trend toward higher T-stage (p=0.06). Twenty-four (27%) patients relapsed at a median interval (range) of 11 (1-83) months and 29 (33%) patients died, at a median interval (range) of 17 (0-84) months. Relapse free survival (RFS) and overall survival (OS) were comparable between chemotherapy and non-chemotherapy patients, but on multivariate analysis after adjusting for age, pT-stage and pN-stage, chemotherapy significantly impacted RFS (RR 0.34, p<0.05) but just failed to reach statistical significance for OS (RR 0.39, p=0.057).

Conclusions: There was a trend over the study period in increased use of neoadjuvant chemotherapy, but overall, PC remains under-utilized. Patients who received chemotherapy had poorer prognostic features, but had better RFS after adjusting for other factors.

Efficacy of sorafenib in advanced renal cell carcinoma (RCC) independent of prior treatment, histology or prognostic group

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Background
Multiple targeted therapies have become available for RCC over the past decade. Sorafenib is an orally active multikinase inhibitor which has been shown to improve PFS in low-risk & intermediate-risk status clear-cell RCC after previous systemic therapy.

Methods
Between May 2006 and December 2010, patients with advanced RCC with either clear cell or non-clear cell histopathology who had progressed on prior systemic chemotherapy or were treatment naïve, ECOG performance status 0-2, commenced treatment with sorafenib (400 mg twice daily continuously) through an expanded access clinical trial in two tertiary centres in Sydney and Melbourne. We report the pooled outcomes of these two studies.

Results
Total 47 patients with metastatic RCC were treated with sorafenib. The median PFS was 4.6 months with median follow up of 11.7 months. Thirty-eight patients died by the time of analysis. Overall one patient experienced complete response, nine patients (19%) had documented partial response (PR) & 27 patients (57%) had stable disease (SD) as the best
response. Eight (17%) had non-clear cell histopathology & five (10%) had sarcomatoid features. In non-clear cell histopathology cohort, 12.5% experienced PR & 50% had SD. Twenty-three (49%) patients were treatment naïve. Fourteen (30%) & 22 (47%) patients had high risk status according to MSKCC & Heng prognostic scores, respectively. Median PFS for poor prognostic patients was 4.5 & 2.8 months based on MSKCC & Heng scores, respectively. Sorafenib-related serious adverse events were seen in 10 patients. Hand-foot syndrome (53%), rash (47%), fatigue (42%), nausea (40%) and diarrhoea (32%) were the most common adverse events.

Conclusion
This study confirms the efficacy & tolerability of sorafenib in a different spectrum of advanced RCC patients including non-clear cell histology, poor prognostic status & as first line treatment.

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**Epigenetic silencing of the negative feedback regulator of mitogen-activated protein kinase (MAPK) signalling, DUSP5, in colorectal cancer**

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**Introduction**
Signalling through the extracellular signal-regulated protein kinase (ERK) pathway (MAPK/ERK pathway) is deregulated in 50% of all colorectal cancers (CRC) due to mutations in the proto-oncogenes braf or kras. The magnitude and duration of pathway signalling is tightly regulated by the induction of negative feedback regulators, which include the dual-specificity phosphatase (DUSP) family of MAPK phosphatases (MKP). Whether negative feedback regulators of the pathway need to be overcome in order to facilitate constitutive MAPK/ERK signalling in CRC, and the mechanisms by which this may occur are unknown. We postulate that one mechanism by which negative feedback regulators may be inactivated is through epigenetic silencing.

**Materials and Methods**
DUSP gene methylation status was determined in 39 CRC cell lines using Infinium Human-Methylation27 beadchips and was confirmed by bisulphite sequencing. Quantitative RT-PCR (qPCR) was performed to determine DUSP5 gene expression. RNAi-mediated knockdown was performed by transient transfection of siRNAs into cancer cells using Lipofectamine.

**Results and Discussion**
Methylation array-based screening of MKP gene family members in 39 CRC cell lines identified DUSP5 as a gene frequently methylated in colon cancer. To confirm this finding the methylation status of DUSP5 promoter CpG island was analysed by bisulphite-sequencing. Quantitative RT-PCR (qPCR) was performed to determine DUSP5 gene expression. RNAi-mediated knockdown was performed by transient transfection of siRNAs into cancer cells using Lipofectamine.

**Conclusion**
These results demonstrate that the negative feedback regulator of MAPK/ERK signalling, DUSP5, is epigenetically inactivated in colon cancer.
Histone deacetylase and proteasome inhibitors induce overlapping transcriptional responses and synergistically induce apoptosis in tumour cells.

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Background/Aim: Histone deacetylase inhibitors (HDACi) are a novel class of targeted anti-cancer therapeutics approved for treatment of cutaneous T-cell lymphoma (CTCL). We previously demonstrated that HDACi-induced apoptosis is mediated via the induction of ‘immediate early’ (IE) gene expression in cancer cells. Bioinformatics analysis indicated that proteasome inhibitors induced a similar transcriptional response to HDACi. Recently, combinations of HDACi and proteasome inhibitor treatments were shown to synergistically induce apoptosis, and led to improved progression-free survival in patients with multiple myeloma (MM) in a Phase III trial. We investigated whether the molecular basis for the activity of this combination is linked to enhanced induction of IE gene expression.

Methods: Tumour cells were treated with HDACi (SAHA, 0.5-5 µM) alone, proteasome inhibitor (Bortezomib, 1-50 nM) alone, or both agents in combination. Apoptosis was determined by PI staining and FACS analysis. Anti-proliferative effects of the drug treatments were determined by MTS assay. IE gene induction was determined by qRT-PCR. MAPK pathway activation and histone hyperacetylation was determined by western blot analysis.

Results: SAHA and Bortezomib combination treatment synergistically induced apoptosis and inhibited cell growth in cell lines derived from multiple tumour types, including CTCL, MM and colorectal cancer. In parallel, this drug combination markedly enhanced induction of multiple IE genes (FOS, JUN, ATF3) compared to single-agent treatment. IE gene induction by Bortezomib but not HDACi was mediated by activation of MAPK signalling pathways (JNK and P38), while HDACi-induced IE gene expression was associated with global increases in histone acetylation.

Conclusions: This study established a link between the induction of IE gene expression and synergistic induction of apoptosis in cancer cells treated with SAHA and Bortezomib. We demonstrated that HDACi and proteasome inhibitors induce IE genes via distinct mechanisms which could explain the synergistic anti-tumour activity of this drug combination.

Improving the quality use of antifungal agents in the haematology unit – are guidelines enough?

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Background: Updated antifungal prophylaxis guidelines in haematology patients at-risk of invasive fungal infection (IFI) were implemented in February 2011 by the Infectious Diseases (ID) and Haematology units, in-line with Australian recommendations and the changing patient group at Austin Health. Recommendations primarily involve the use of oral posaconazole and liposomal amphotericin (L-AmB) in high-risk leukaemia patients.

Aims: Ensure appropriate use of antifungals in the haematology unit and monitor compliance with guidelines
Reduce unit expenditure on unfunded posaconazole and L-AmB

Methods: Prospective monitoring of antifungal prophylaxis commenced in March 2011. Patients were identified and guideline compliance reviewed by the ID pharmacist.

Data collected included: demographics; diagnosis; antifungal; duration of neutropenia; if IFI was suspected/proven; presence of oral absorption issues; relevant drug interactions.

Expenditure on unfunded posaconazole and L-AmB before and after the intervention was compared.
Results:
86 patients received 137 courses of prophylactic antifungal agents between March 2011 and June 2012. 132 courses were identified as being compliant with the guidelines or had appropriate ID input (96%); only 5 courses were initiated contrary to guidelines. Unfunded posaconazole and L-AmB costs reduced from $785,700 (2010) to $208,052 (2011) despite increasing numbers of high-risk patients being treated over this same period (22 versus 27 AML patients) and new L-AmB prophylaxis costs. This was due to the reduced number of confirmed IFI cases from 6 in 2009/10 to 3 in subsequent years, less empiric L-AmB use and increased funded posaconazole dispensing.

Prospective monitoring identified means to optimise posaconazole absorption (minimising potent antacid coadministration; compliance with fat supplements). Posaconazole therapeutic drug monitoring was also introduced during this period to identify patients with poor absorption requiring alternative prophylaxis.

Conclusion:
Compliance with antifungal prophylaxis guidelines within the haematology unit is excellent. Prospective monitoring identified ways to optimise antifungal use and resulted in a reduction in the overall cost of antifungal agents despite a substantial increase in unit activity.

Spirometry in patients with stage IV non-small cell lung cancer (NSCLC)

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Symptom control is paramount in the palliative management of stage IV NSCLC. Once stage IV NSCLC is diagnosed, the focus tends to be palliation using chemo/radiotherapy. The treatment of co-existing COPD may be neglected resulting in suboptimal management of dyspnoea. We assessed the frequency of spirometry in patients with newly diagnosed stage IV NSCLC.

Records of 130 current/ex-smokers with stage IV NSCLC were reviewed. Symptoms at first consultation, co-morbidities, possible contributing factors to dyspnoea, use of bronchodilators as well as spirometry performed within six months prior or post first review were recorded. The GOLD staging system was used to document COPD severity.

Median age was 66.5 years (37-87) and 69% were male.

61/130 (46%) patients complained of dyspnoea. Of these, 75% (46/61) were not known to have prior COPD Only 14/46 (30%) were referred for spirometry, resulting in 10/14 (71%) being diagnosed with COPD (40% GOLD stage I, 20% stage II, 20% stage III, 10% stage IV). 28/46 patients had at least one other known factor potentially contributing to dyspnoea. Eighteen patients were breathless without a clear cause. However, only 6/18 (33%) were referred for spirometry and 4/6 patients (66%) had COPD.

The median pack years smoked was 37. Only 3/46 (6.5%) dyspnoeic patients were on bronchodilators at the time of first consultation as a result of spirometry in the context of their cancer diagnosis.

A minority of patients (30%) with stage IV NSCLC and dyspnoea without known COPD underwent spirometry; 71% had COPD when tested. COPD is a clear contributor to dyspnoea in this group of patients and warrants timely investigation and intervention. Patients with a smoking history should have spirometry during work up for stage IV NSCLC, regardless of other contributors to dyspnoea.
Differential processing of NY-ESO-1 by Dendritic Cells and Melanoma.

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Background: NY-ESO-1 is a cancer testis antigen, expressed in germ cells and upregulated in many human tumours. It is highly immunogenic and an attractive target for therapeutic cancer vaccination. 17 MHC class I epitopes associated with 11 haplotypes have been described. Degradation of cellular proteins by the proteasome is critical to the generation of MHC-associated peptides. The constitutive ‘standard’ proteasome (cP) and the IFN-γ-induced immunoproteasome (iP) differ in the use of three catalytic β subunits. In iP’s LMP-2,-7, MECL1 replace the β1, β5, β2 cP subunits, which alters production of MHC class I epitopes. Dendritic cells (DC) constitutively express iP’s, whereas melanoma iP expression is infrequent. The potential for a differential repertoire of epitopes produced between DC and tumour cells thus arises.

Method: We studied presentation of three NY-ESO-1 HLA-Cw3 restricted epitopes by both DC and melanoma cell lines. Using antigen specific T cell clones we assessed presentation of each epitope. We induced expression of iP’s in melanoma cells with IFNy, and demonstrated enhanced presentation of each epitope following processing through the iP. We further investigated presentation of these three epitopes by selective inhibition of cP/iP catalytic subunits (LMP7, LMP2 and β5) using small molecule inhibitors. In some cases we performed siRNA knockdown of catalytic subunits.

Conclusions: Enhanced surface presentation of all three NY-ESO-1 epitopes following iP processing was demonstrated. Data highlights a discrepancy in presentation of described immunodominant epitopes between DC and melanoma cells. Results may explain why previously observed vaccine-induced immune responses to NY-ESO-1 failed to result in objective clinical responses in patients. Awareness of how individual cancer epitopes are processed by melanoma cells is therefore critical to future development of therapeutic cancer vaccines.

The expression of calcitonin receptor detected in malignant cells of the brain tumour glioblastoma multiforme and functional properties in high grade glioma cell lines

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Aim: Previous studies established expression of calcitonin receptor (CTR) in brain tumours from a majority of patients with glioblastoma multiforme (GBM). The aim here is to describe the function of CTR in high grade glioma (HGG) cell lines that resemble brain tumour initiating cells (BTICs).

Methods and results: In GBM samples, tissues with complex glomeruloid structures surrounded by malignant cells were analyzed for CTR-ir using anti-human CTR antibodies generated against two separate epitopes of CTR. CTR-ir was associated predominantly with glial cells. Regions with CTR-ir cells were found in 12/14 GBM tumours (p<0.05). Using confocal microscopy CTR-ir cells that were also positive for glial fibrillary acidic protein, nestin and CD133, characteristic of malignant glioma cells and in particular BTICs. HGG cell lines were investigated for modified activities of second messenger systems in relation to CTR. The inverse agonist sCT(8-32) stimulated growth and survival (intracellular LDH levels) of HGG cell lines with a maximal effective concentration of approximately 40nM. Furthermore anti-CTR antibodies induced apoptosis (annexin V binding) in HGG cell lines in vitro.
Conclusion: The findings (i) that CTR was expressed by glioma cells and in particular BTICs of GBM in a majority of patient samples, (ii) that the ligands of CTR namely the inverse agonist sCT(8-32) stimulates growth and (iii) antibody against an external epitope induces apoptosis demonstrated the proof of principle that CTR might be a useful target for therapy. These results suggest further studies in vivo in mouse xenograft models of GBM to restrict tumour expansion.


DUSP5, a known negative feedback regulator of ERK/MAPK signalling, is induced by cellular stress response pathways

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Background/Aim: There are 3 major mitogen-activated protein kinase (MAPK) pathways in mammalian cells - ERK, JNK and p38, which control proliferation (ERK) and cellular stress responses (JNK and p38). The dual-specificity phosphatase family of proteins (DUSPs) are negative regulators of MAPK signalling. The aim of this project was to determine whether DUSP5, a known negative feedback regulator of the ERK/MAPK pathway, is activated by stress and whether it can act as a negative regulator of JNK/MAPK and p38/MAPK signalling.

Methods: LIM1215 colon cancer cell line were treated with 50ng/ml epidermal growth factor (EGF) to induce the ERK/MAPK pathway and with the proteasome inhibitor MG132 (500nM), the pyrimidine analogue 5FU (50uM), sorbitol (0.25M) and ultraviolet light (300J/m2) to activate the JNK/MAPK and p38/MAPK pathways. DUSP5 mRNA expression was measured using quantitative RTPCR and pathway activation was determined by western blotting.

Results: EGF treatment induced activation of ERK signalling in LIM1215 cells as evidenced by the rapid phosphorylation of ERK1/2, which was maximal 30 mins post-treatment. As expected, EGF treatment also resulted in a rapid induction of DUSP5 mRNA expression. Interestingly, treatment of cells with the stress inducing stimuli MG132, 5FU, sorbitol and UV, also resulted in induction of DUSP5 mRNA expression.

Conclusion: Consistent with previous reports, DUSP5 was induced following activation of ERK signalling in colon cancer cells. Interestingly, DUSP5 was also up-regulated by stress-inducing stimuli which lead to JNK and p38 signalling. These findings suggest DUSP5 may also play a role in regulation of the JNK and p38 MAPK pathways.

Efficacy and safety of bendamustine and obinutuzumab (GA101), a new generation CD20+ monoclonal antibody (MoAb), in untreated follicular lymphoma (FL).

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Background: FL is an indolent CD20+ lymphoma accounting for approximately 20-25% of new lymphomas. The addition of rituximab, a type I anti-CD20 MoAb, to chemotherapy has improved outcomes in FL, but most patients subsequently develop disease progression. GA101 is a novel type II glycoengineered anti-CD20 MoAb with increased antibody dependent cellular cytotoxicity (ADCC) and direct cell death compared with rituximab. Bendamustine, a new alkylating agent, has also shown better efficacy than standard chemotherapy in FL. As part of a larger multicentre international phase 1b study evaluating these two agents in first-line treatment of symptomatic FL, we report the Austin experience of efficacy and safety of this combination

Methods: GA101 was given day 1 (all cycles) and day 8 (cycle-1) with bendamustine (day 1-2) 4 weekly for 6 cycles followed by maintenance GA101 3 monthly in responding patients.
Results: Seven patients were screened from March–September 2011. One unrelated death occurred before treatment initiation. One participant withdrew consent halfway through treatment. The remaining 5 patients completed treatment, which was well tolerated with a low incidence of neutropenia, fever and nausea. No patient has evidence of active FL a median of 9 months after commencement of therapy. All 3 patients tested with PET/CT are in metabolic remission.

Conclusion: Bendamustine and GA101 is an effective and well tolerated treatment of FL. Patients are now prospectively enrolled in a randomised trial comparing rituximab with GA101 in combination with bendamustine.

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**Computed tomography relationship for thoracic paravertebral space**

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Introduction: Thoracic paravertebral blockade (PVB) is an effective regional analgesic technique, but has a risk of causing pneumothorax. We hypothesized that using a more lateral insertion point has a more superficial but consistent depth to the pleura, and that the distances between skin and transverse process (TP) at different points are directly related to the patient’s anthropomorphic indices.

Methods: After Human Research Ethics Committee approval, existing CT scans of 42 adult patients were retrospectively examined. Anthropomorphic data were correlated with each patient’s skin to TP, and skin to pleura depths at the level of the 4th thoracic vertebrae, at a point 25 mm lateral to the midline and again at the most lateral extent of the TP.

Results: The average distance from the midline to the most lateral tip of the TP was 32 mm. Measurements from the most lateral tip of the TP to the pleura were on average 9 mm smaller and less variable than the measurements at 25 mm form the midline. Measurements from skin to pleura, and skin to TP at 25 mm from midline, as well as measurements through the lateral tip of the TP, correlated strongly with anthropomorphic indices (R> 0.8). Prediction bands around their regression lines proved too broad to be clinically significant.

Conclusions: Compared to the traditional insertion point at 25 mm lateral to the midline, a more lateral insertion point has a shallower and less variable depth to the paravertebral space. Patient’s anthropomorphic indices are significantly correlated with paraspinous landmark depths relevant to PVB, but are too imprecise to provide clinically reliable predictions for insertion depth. The use of an insertion point up to 10 mm more lateral than the recommended landmark technique may improve the safety of paravertebral blockade.

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**The effect of perioperative ketamine on the risk of longer term postoperative pain**

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Aims: Most studies on postoperative pain management focus on acute postoperative pain (< 48 hours). Few examine longer term pain outcomes. N-methyl D-aspartate (NMDA) antagonists (ketamine, N₂O) block spinal sensitization mechanisms that lead to chronic pain. A 2 year follow up of 640 patients in the ENIGMA 1 trial found the incidence of severe (VAS>5) post-surgical pain was 9.2% and that the rate was halved after N₂O anaesthesia (Chan et al. Pain. 2011. 152(11): 2514-20). The potential for ketamine to protect against this debilitating complication is unclear.

Methods: A literature review was conducted using PubMed of all prospective randomized controlled studies examining the use of perioperative IV ketamine (intraoperative +/- up to 24 hours postoperative) to influence postoperative pain in comparison with either placebo or a standard of care regime not including an NMDA antagonist. Only those studies which included a measure of pain intensity at 5 or more days postoperatively were selected for final analysis.
Results: 5 studies were found which met the criteria. In 4 of these (total \( n = 330 \) patients), pain after 5 days was significantly less after perioperative IV ketamine. In 2 of these studies (\( n = 194 \)) the difference was still significant at 6 months. In the fifth study (\( n = 40 \)) only a borderline effect on pain intensity at 7 days was found and this failed to reach statistical significance.

Conclusion: The potential for NMDA antagonist drugs to influence important chronic pain outcomes in surgical patients has been obscured by a confused approach among published studies, including lack of awareness of the problem of chronic post-surgical pain, a narrow focus on acute pain management, a mechanistic obsession with “pre-emptive” versus perioperative administration, and confused and uninformative trial designs. Available data suggests that a large randomized trial of an IV NMDA receptor blocker to prevent chronic post-surgical pain is warranted.

Cognitive changes after saline or Plasmalyte infusion in healthy volunteers: a blinded, randomized, cross-over trial

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Background: In a study of plasma chemistry changes in healthy volunteers after infusion of saline or Hartmann’s solution\(^1\), participants reported subjective cognitive changes, particularly slower thinking, after saline. We tested the hypothesis that saline infusion would produce greater cognitive changes than Plasmalyte infusion.

Methods: With Ethics Committee approval, we conducted a randomized, cross-over, blinded study of healthy adult volunteers. On separate days participants were randomized to 30 ml/kg over one hour of either 0.9% saline or Plasmalyte. Plasma chemistry was tested on venous samples. As part of a battery of cognitive tests our primary end point was the reaction time index.

Results: We studied 25 participants. Plasma chloride was greater after saline, difference 5.4 mmol/L (95%CI: 4.1 to 6.6 mmol/L, \( P<0.001 \)) associated with greater metabolic acidosis: base-excess 2.5 mmol/L more negative (95%CI: 1.9 to 3.0 mmol/L more negative, \( P <0.001 \)). There were no important differences in reaction time index between the two arms of the study. After saline, the mean reaction time index was 411 (SD: 63) msec, and after Plasmalyte was 385 (SD: 55) msec: saline 9 msec slower (95 CI: 12 msec faster to 30 msec slower, \( P = 0.39 \)). None of the other cognitive and mood tests differed.

Conclusions: Despite significant differences in plasma chemistry, reaction times after saline did not differ from reaction times after Plasmalyte. Further, other measures of cognition did not differ. This finding was contrary to our hypothesis. We cannot exclude differences with Hartmann’s solution, however, cognitive differences associated with mild hyperchloremic metabolic acidosis seem unlikely

Look Both Ways Before Crossing the Subclavian Vein - An Ultrasound Comparison of Contralateral Vein Size

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Vein size and use of dynamic ultrasound guidance have been shown to be directly related to a reduction in insertion failure and complication rates during subclavian vein catheterisation. We hypothesised that contralateral subclavian vein sizes are significantly different within the same patient and that both sides should be scanned to distinguish the larger side before attempts at central venous catheter insertion are made. We also aimed to demonstrate the relationship of subject's anthropomorphic indices with vein size and contralateral vein size difference.

We examined fifty adult patients' bilateral subclavian veins by ultrasound. Forty-five patients (90%) of patients were right hand dominant. The mean proportional cross-sectional area difference between sides in individual patients was 59.7% (SEM: 9.2%), with absolute difference of 26.7 mm² (SEM: 2.8 mm²) at p<0.0001. There was no relationship between right hand dominance and ipsilateral subclavian vein size (p = 1.0), nor was there any clinically significant correlation between subject's anthropomorphic indices and subclavian vein size or contralateral vein size difference (largest Pearson’s r = 0.22).

We conclude that contralateral subclavian vein sizes within the same patient are significantly different and that the magnitude of contralateral difference or absolute subclavian vein size cannot be predicted by a subject’s anthropomorphic indices. Patients planned for subclavian central line insertion should have both sides examined by ultrasound to determine which side has the largest vessel.

Pharmacoeconomic evaluation of fluid administration to patients undergoing colonoscopy in a tertiary hospital: a prospective blinded observational study

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Introduction

The benefits and costs of fluid therapy for endoscopy are unclear. We hypothesised that routine fluid prescription would not affect periprocedural adverse events and would be associated with increased pharmacoeconomic costs.

Methods

With Ethics approval, we conducted a prospective, blinded observational study. Inclusion criteria: adult patients, undergoing elective colonoscopy receiving intraprocedural fluids. Outcomes: volume of fluid administered, size of intravenous cannulae, vasopressor use, periprocedural adverse events, procedure duration. Procedural anaesthetists were blinded to the study. Data was analysed by logistic regression techniques.

Results

We collected data on 289 consecutive patients. Median age: 48yrs (range 18-83), gender: 174 (60%) female; median duration of procedure: 24 minutes (range 12-48). Cannula size: 181 (63%) 22G or smaller. Median volume of fluid administered during colonoscopy: 300ml (range 0-1000ml). Fluid volume was most strongly associated with increasing cannula diameter (p=0.0001). There was no association between fluid volume administered and vasopressor use, periprocedural adverse events, or procedure duration. Currently, fluid therapy cost about $4.90 per patient: 1L crystalloid $1.18 (Baxter Healthcare®, Australia) and fluid delivery set $3.77 (BMDI TUTA® Healthcare, Australia). Our institution performs over
9000 endoscopic procedures annually with fluid therapy costing about $45,000/year

Conclusion:
Consistent with our hypotheses, we found no association with fluid volumes prescribed and procedural hypotension, other adverse events, or procedure duration. We think anaesthetists could question the clinical and pharmacoeconomic value of routinely administering intravenous fluids through smaller cannulae for endoscopy.

Pharmacoeconomic evaluation of anaesthetic volatile agent consumption in Victorian public hospitals

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Introduction: With increasing demand on healthcare resources we performed a cost identification analysis of patterns of volatile agent use. We hypothesised a practice change from the cheaper agents sevoflurane and isoflurane towards the more expensive desflurane.

Methods: The number of bottles of isoflurane, sevoflurane and desflurane used between 2005-2011 was obtained from IMS¹. Pharmacy departments of four teaching hospitals independently verified data. Annual consumer price index adjusted expenditure (Australian dollars) was calculated for each volatile agent.

Results: Data from 62 public hospitals was analysed. In 2005 the acquisition cost per bottle of isoflurane (250mL), sevoflurane (250mL) and desflurane (240mL) was $100, $339, and $180 respectively. In 2011 the same bottles cost $80, $147 and $235 respectively. Total number of bottles utilised from 2005 to 2011: isoflurane: 2,061 to 465; sevoflurane: 11,276 to 17,429; desflurane: 1,296 to 4,416. For the same period annual acquisition costs of isoflurane decreased from $251,084 to $37,913. Despite a 55% increase in sevoflurane usage, annual acquisition costs decreased from $4,587,076 to $2,589,815. In contrast, desflurane costs increased over three fold from $281,875 in 2005 to $1,044,894 in 2011. In 2005 consumption patterns of use for isoflurane: sevoflurane: desflurane was 16: 80: 4 changing to 3: 88: 9 in 2011.

Conclusion: There is a progressive shift from the cheaper agents isoflurane and sevoflurane to the more expensive agent desflurane. Choice of volatile agent has important pharmacoeconomics considerations.

1. IMS: a leading provider of information services for the healthcare industry covering markets in 100 countries around the world (www.imshealth.com)

Volume resuscitation and use of blood products during adult liver transplantation: a single centre retrospective observational study

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Background
While liver transplantation is traditionally associated with large volumes of intraoperative fluids, actual fluid volumes are rarely reported. Further, clinical approaches now aim to minimize use of donated blood products. We proposed that there would be large variation in fluid volumes infused.
Methods
With Ethics approval, we conducted a retrospective observational study of fluids used during liver transplantation recording all intraoperative fluids including crystalloids, colloids, cell saver blood, and Red Cross donor products: packed cells, fresh frozen plasma, cryoprecipitate, and platelets.

Results
We had data for 115 consecutive patients undergoing transplantation between 2008 and 2010. Ranked by total fluid volume infused, the lower 75% (87 patients) received a median of 7 L (Range: 3 to 14 L), with 1 L from cell savers (Range 0 to 3 L); while the top quarter (28 patients) received a median of 22 L of infused fluids (Range: 14 to 44 L), with 4 L from cell savers (Range 1 to 14 L). The lower 75% received a median volume of 1.5 units of donor red cells and no platelets or clotting factors. In contrast the upper 25% received a median of 5 units of packed cells, 4 units of fresh frozen plasma, 2.5 units of cryoprecipitate and 2 bags of platelets.

Conclusions
Fluid infusion for adults undergoing liver transplantation varied more than 10 fold. One in four patients undergoing liver transplantation required at least 14 L of fluid transfusion. With current practices many patients receive only small amounts of donor blood products.

Elevated Plasma High Sensitivity C-Reactive Protein and Endothelin are Associated with Decreased Flicker-light Induced Retinal Arteriolar Dilatation

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Introduction: C-Reactive protein (CRP) and endothelin (ET-1) are biomarkers of cardiovascular risk. Flicker-light induced retinal arteriolar dilatation (FI-RAD) is a measure of microvascular endothelial function. We sought to determine the relationship of FI-RAD with CRP and ET-1.

Methods: Patients with atherosclerosis risk factors were recruited (n=258). FI-RAD was measured using the Dynamic Vessel Analyzer (DVA) and expressed as percentage increase over baseline diameter. Plasma CRP and ET-1 level were measured. Linear regression analysis was used to determine the relationship.

Results: 46% had atherosclerosis risk factors without coronary artery disease (CAD), 30% had CAD and 24% had acute coronary syndromes (ACS). Mean age was 58±11 years with 68% male, 73% hypertensive, 78% with dyslipidaemia and 36% with diabetes. CRP was 8.3±14.5mg/L and ET-1 was 2.5±0.7pmol/L. Increased CRP and ET-1 was associated with decreased FI-RAD (β=-0.02%; 95%CI -0.03, -0.001; p=0.04) and (β=-0.44%; 95%CI -0.78, -0.1; p=0.01) respectively. After adjustment for age, gender, hypertension, blood pressure, BMI, dyslipidaemia, diabetes, cholesterol and glucose; the association between CRP and FI-RAD continued to be significant, however, the association between ET-1 and FI-RAD was no longer significant. In the subgroup with ACS; increased ET-1 was associated with decreased FI-RAD (β=-1.07%; 95%CI -2.1, -0.04; p=0.04), after adjustment for the mentioned variables.

Conclusion: In patients with and without CAD, elevated CRP was associated with attenuation of FI-RAD. The relationship between FI-RAD and ET-1 was only present among ACS patients. These data suggest that plasma markers of endothelial function and vascular inflammation are linked to retinal microvascular vasodilator function.
Plasma Endothelin and Adrenomedullin are Associated with Coronary Conduit and Microvascular Function.

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Introduction: Elevated endothelin (ET-1) is associated with cardiovascular disease, whereas elevated adrenomedullin (ADM) may result in protection from atherosclerosis. We sought to determine the relationship of ET-1 and ADM with coronary circulatory function.

Methods: Patients with chest pain syndromes having coronary angiography were recruited (n=32). Plasma ET-1 and ADM were measured. Coronary flow mediated dilatation (FMD), index of microcirculatory resistance (IMR) and coronary flow reserve (CFR) were measured. Pearson’s correlation and linear regression analysis were used to determine the relationship between plasma biomarkers and coronary measures.

Results: Mean age was 66±9 years (69% male) and 75% had hypertension, 84% had dyslipidaemia, 34% had diabetes, 13% were smokers and 66% had obstructive coronary disease. Correlation between plasma biomarkers and coronary function was significant between CFR and ADM (r=0.50; p=0.04), IMR and ET-1 (r=0.57; p<0.01) and between coronary FMD and ADM (r=0.62; p=0.01). For each 1pmol/L increase in ADM, CFR increased by 0.20 (95%CI 0.01, 0.40; p=0.04) and coronary FMD increased by 0.92% (95%CI 0.29, 1.55; p=0.01). For each 1pmol/L increase in ET-1, IMR increased by 7 units (95%CI 2.8, 10.5; p<0.01). After adjustment for age, gender, blood pressure, hypertension, diabetes, dyslipidaemia, BMI, serum glucose and cholesterol, the relationship between ADM and CFR was no longer significant, however, the association between ADM and coronary FMD (β=0.79; 95%CI 0.45,1.13; p<0.01), and between ET-1 and IMR (β=5.7; 95%CI 1.4,10.0; p=0.01) remained significant.

Conclusion: Significant independent relationships were found between plasma ADM and coronary conduit vessel function, and plasma ET-1 and coronary microvascular function.

Baseline Brachial Artery Diameter, Reactive Hyperaemia Velocity and Shear Rate Correlates with Retinal Vascular Calibre

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Introduction: Retinal arteriolar narrowing is a marker of coronary artery disease (CAD). Larger brachial artery baseline diameter (BLD), and reduced shear rate (SR) and reactive hyperaemia velocity (RHV) are associated with increased risk of CAD. However, the relationship between brachial artery diameter, SR, RHV and flow mediated dilatation (FMD) with retinal vessel calibre is not well studied.

Methods: Patients with atherosclerosis risk factors were recruited (n=119). Retinal photographs were acquired utilising a fundus camera and assessed using a computer-based standardised protocol. Retinal arteriolar and venular diameter was expressed as central retinal artery and vein equivalent (CRAE, CRVE). Ultrasound was used for brachial artery imaging and RHV and SR were calculated during forearm reactive hyperaemia. Pearson’s correlation and linear regression were used to assess relationships.

Results: Mean age was 55±12 years with 55% male. FMD was not significantly correlated with CRAE and CRVE (p=0.50). BLD was inversely correlated with CRAE and CRVE (r=-0.43; p=0.01 and r=-0.26; p=0.01 respectively). RHV was correlated with CRAE (r=0.21; p=0.03), and SR was correlated with both CRAE and CRVE (r=0.26; p= 0.01 and r=0.23; p=0.01 respectively). Each 1mm increase of BLD was associated with a 6.1 µm decrease in
CRAE (95%CI -9.7, -2.5; p=0.001) after adjustment for age, gender, blood pressure, BMI, diabetes, serum cholesterol and glucose.

Conclusion: In patients with vascular risk factors, a larger BLD was independently associated with narrower retinal arteriolar calibre. These data support the notion that brachial artery BLD may be a risk marker for vascular dysfunction.

Baseline Brachial Artery Diameter and Flow are Associated with Flow Mediated Dilatation and Brachial Artery Haemodynamics at Hyperaemia

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Introduction: Flow mediated dilatation (FMD) is a measure of endothelial dysfunction. Baseline brachial artery parameters are easier to obtain. We sought to determine the degree of association of baseline brachial artery diameter (BLD) and velocity (BLV) with hyperaemic measurements.

Methods: Patients with atherosclerosis risk factors (n=197) were recruited. Reactive hyperaemia was induced by 5 minutes forearm cuff inflation. Vascular ultrasound was used to determine reactive hyperaemia velocity (RHV), peripheral vascular resistance (PVR), shear rate (SR) and GTN-induced vasodilatation. Associations between measurements were assessed with Linear regression. ROC curve was used to determine optimal sensitivity and specificity of BLD for FMD <3%.

Results: Mean age was 58±12 years with 66% males. BLD was inversely associated with FMD (β=-0.78%;95%CI-1.13,-0.43;p<0.01), GTN-induced vasodilatation (β=-2.7%;95%CI-3.5,-1.9;p<0.01), RHV (β=-6.3cm/sec;95%CI-9.47,-3.20;p<0.01), SR (β=-109/sec;95%CI-132,-85;p=0.01) and PVR (β=0.14units;95%CI-0.17,-0.10;p<0.01). BLV was proportional to FMD (β=0.13%;95%CI 0.05,0.21;p<0.01), RHV (β=1.57cm/sec;95%CI 0.92,2.22;p<0.01) and SR (β=13.6/sec;95%CI 8.1,19.2;p<0.01) and inversely related to PVR (β=-0.01unit;95%CI-0.02,-0.002;p<0.01). A BLD of 4mm resulted in a sensitivity of 70% and specificity of 50% for FMD <3% (c-statistic 0.65).

Conclusion: Baseline brachial artery diameter and flow velocity were found to be associated with reactive hyperaemic measurements and FMD. Further studies are warranted to determine if baseline brachial artery parameters are sufficient to determine the presence of endothelial dysfunction.

Multiple Vascular Reactivity Measures Improves our ability to Predict Underlying Coronary Artery Disease

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Introduction: Dynamic retinal vascular assessment and peripheral endothelial tonometry are non-invasive measures of microcirculatory function. Correlation between these two techniques and whether these novel markers improve our ability to predict underlying coronary artery disease (CAD) is unknown.

Methods: Patients with and without CAD were recruited. Flicker Light induced Retinal Arteriolar Dilatation (FI-RAD) was measured as percentage increase over baseline diameter using the Dynamic Vessel Analyzer. Augmentation Index (AI) was assessed utilising the endothelial peripheral arterial tonometry (endoPAT) system. Baseline characteristics were analysed with Chi-square and t-test. Logistic regression was performed to determine predictors of CAD. Pearson’s correlation was used to assess the relationship between FI-
RAD and AI. The incremental value of FI-RAD and AI to predict CAD over cardiovascular risk score was assessed with ROC curves.

Results: Subjects with CAD (n=78) were older (63±9 vs 55±12 years; p<0.01), more likely to be male (82% vs 55%; p<0.01) and have dyslipidaemia (96% vs 74%; p<0.01) than subjects without CAD (n=119). FI-RAD was attenuated in CAD patients (1.5±1.5% vs 2.4±2.0%, mean±SD; p<0.01). AI was higher among CAD patients (21±18% vs 12±18%, mean±SD; p=0.01). Both were independent predictors of underlying CAD adjusted for age, gender, atherosclerotic risk factors and cardiovascular medications; OR 1.60; 95%CI 1.14, 2.25; p=0.01 for each 1% decrease in FI-RAD and OR 1.60; 95%CI 1.09, 2.34; p=0.02 for each 10% increase in AI. Correlation between FI-RAD and AI was significant (r = −0.24; p<0.01). The addition of AI to a model including FI-RAD as a predictor of underlying CAD increased the c-statistic to 0.66 (95%CI 0.59, 0.73; p<0.01) compared to 0.60 for cardiovascular risk score.

Conclusion: Increased AI and reduced FI-RAD independently predict the presence of CAD, indicating that these novel markers might be used to identify patients at risk. However, the incremental predictive value above traditional atherosclerosis risk factors was small.

Evaluation of Right Ventricular Systolic Function - A Comparison of 2D RV strain and 3-Dimensional Echocardiography with Cardiac Magnetic Resonance

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Background: Cardiac magnetic resonance (CMR) is the gold standard for assessment of right ventricular (RV) systolic function, but is limited in availability. RV strain and real time 3-dimensional echocardiography (RT3DE) are emerging technologies. We compared 2D RV strain, RT3DE and CMR for RV function assessment in a prospective cohort.

Method: Over a 13 month period, all clinical patients referred for CMR underwent echocardiography on the same day. Analysis of 2D RV strain and RV ejection fraction (EF) from RT3DE and CMR was performed offline, blinded to the results of other parameters. The inter- and intra-observer variability was 2.6% and 3.2% respectively.

Results: 60 patients were recruited (mean age 45; 60% male). Of 41 cases with adequate image quality for RT3DE RVEF analysis, 33 cases were analyzed for 2D RV strain (missing 2D data n=4; suboptimal image quality n=4). Mean 2D frame rate was 71±14 fps. Strong correlations were found between CMR RVEF and RV free wall longitudinal strain (RVFWSL r=0.58, p<0.001), RV septal longitudinal strain (RVSLSL r=0.68, p<0.001) and RV global longitudinal strain (RVGLS r=0.71, p<0.001). On linear regression analysis, RVGLS -20.95% correlated with CMR RVEF 50% (p<0.001). RV strain parameters also correlated significantly with RT3DE RVEF: RVFWSL (r=0.53, p<0.001); RVSLSL (r=0.52, p<0.001); RVGLS (r=0.62, p<0.001). There was good correlation in RVEF between RT3DE and CMR (r=0.66, p<0.001).

Conclusion: RV strain and RT3DE RVEF are strongly associated with CMR RVEF. 2D RV strain provides a rapid, simple assessment of RV function suitable for incorporation into routine clinical practice. The best correlation is found with global longitudinal strain.

Symptom/Rhythm Detection Using 7 Day Event Monitors

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AIM. To assess the usefulness of 7-day event monitoring in evaluating symptoms suggestive of cardiac arrhythmias when ECG and/or 24 hour Holter monitoring are non-diagnostic.

METHODS. All patients undergoing 7 day event monitoring from January 2011 to February 2012 were included in the analysis. Indications included: palpitations (70%); atrial
fibrillation/flutter (15%); pre-syncope (10%); syncope (2%). Prior ECG and/or 24 hour Holter monitors were non-diagnostic.

**RESULTS.** 84 patients were included (mean age 51 ± 17 years, 76% female). Referral sources were: cardiologists 86% (53% electrophysiologists); emergency physicians and general practitioners 14%. 360 events in total were recorded by 69 (82%) patients (maximum possible 15 per patient). Symptoms were: palpitations (55%); irregular heart beat (21%); pre-syncope (15%). 58% of patients had a defined symptom-related arrhythmia, including: atrial fibrillation/flutter (5%); supraventricular tachycardia > 10 seconds (6%); atrial runs < 10 beats (10%); ventricular ectopics (26%); atrial ectopics (35%); sinus pause > 3 seconds (1%); sinus tachycardia >120bpm at rest (10%). In the remaining 24% of symptomatic patients, sinus rhythm only was recorded.

**CONCLUSIONS.** 7 day event monitors detect arrhythmias in the majority of symptomatic patients when prior ECG and/or 24 hour Holter monitoring have been non-diagnostic.

### Measurement of left ventricular pressure and arterial pressure simultaneously in conscious animals using dual pressure telemeters

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We have recently purchased newly-released dual pressure telemeters (Data Sciences International product) capable of measuring arterial pressure (AP), left ventricular pressure (LVP), core temperature, ECG and locomotor activity simultaneously in a conscious animal. These telemeters are able to detect very subtle changes in these parameters and provide a more time and cost efficient approach to animal studies. Our aim was to implant dual pressure telemeters and monitor all variables in young rats. Seven week old male Sprague-Dawley rats were isoflurane-anaesthetised and artificially ventilated. A catheter was inserted into the left ventricle of the heart via a trans-diaphragmatic method for measurement of LVP. A second catheter was inserted into the abdominal aorta for AP measurement. Biopotential leads were attached to the animal for measurement of ECG, locomotor activity and core temperature. After a 2 week recovery period, animals are placed on receivers where data was collected and analysed using a new comprehensive program, PONEMAH. We successfully implanted telemeters in 7 animals and found that we were able to monitor all parameters over a 13 week period. We are interested in the aetiology of cardiovascular diseases in obesity. Left ventricular diastolic function is commonly used as a marker for cardiac diseases and may occur independently of AP and is not strongly linked to left ventricular geometry. Therefore, this equipment will enable comprehensive measurement of parameters in conscious animals that can be used to temporally track cardiac and other changes occurring in association with the development of hypertension in diet-induced obesity.

### Are Advanced Glycation End Products Associated Elevated Filling Pressures in Diabetes?

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**Background:** Pre-clinical diastolic dysfunction is common in diabetes. The parameter, E/e’, has emerged as a powerful variable in the assessment of diastolic function; E/e’ is the ratio of the early mitral inflow (E) to septal mitral annular velocity (e’) in diastole, and is a continuous variable that correlates with increased left ventricular (LV) filling pressure. In retrospective studies in diabetes, E/e’ >15 predicted mortality and heart failure (HF). As advanced glycation end products (AGEs) accelerate collagen crosslinking and contribute to myocardial stiffening,
this study investigated if serum and urinary AGEs are associated with elevated filling pressures in diabetes.

**Methods:** We recruited 137 patients with diabetes attending a diabetes clinic as part of a routine surveillance program. Patients with LV ejection fraction <50%, macrovascular disease and history of HF were excluded. A transthoracic echocardiogram was performed and elevated filling pressures defined as E/e´ >15. N-carboxymethyllysine AGE-modified proteins were measured in serum and urine using an ELISA. Data are presented as mean ±SD for parametric data and geometric mean (25, 75th quartile) for non-parametric data.

**Results:** Mean ±SD age was 61 ±13 years (58% male), BMI 31 ±6 kg/m², HbA1c 7.6 ±1%, median diabetes duration 14 (8,23) years and a low eGFR <60 ml/min/1.73m² occurred in 14%. Levels of serum AGEs (mean ±SD) and urinary AGEs (geometric mean (25, 75th quartile) are shown below. An elevated E/e’ was associated with higher serum AGEs after correcting for age, gender, BMI, eGFR and diabetes duration (P = 0.004).

**Conclusions:** This is the first study to show increased serum AGEs are associated with subclinical elevated filling pressures in patients with diabetes. Serum AGEs may be a useful biomarker of diastolic dysfunction and a potential therapeutic target for treatment with crosslink breakers.

<table>
<thead>
<tr>
<th>Serum AGEs (μmol/mol lysine)</th>
<th>E/e’&lt;15 (n = 108)</th>
<th>E/e’&gt;15 (n = 29)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>3.175 ±1278</td>
<td>4.114 ±1659</td>
<td>0.001</td>
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<tr>
<td>Urine AGEs (nmol/mol lysine)</td>
<td>2.8 (1.0, 7.9)</td>
<td>5.6 (1.1, 27.6)</td>
<td>0.073</td>
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Are Advanced Glycation End Products Associated With Macrovascular Disease in Type 2 Diabetes Mellitus?

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**Background:** Macrovascular disease remains a significant cause of morbidity and mortality in type 2 diabetes (T2DM). Advanced glycation end products (AGEs) are implicated in the pathogenesis and progression of diabetic renal complications, but their role in cardiovascular disease (CVD) is not clear. This study investigated whether AGEs are associated with macrovascular complications in T2DM.

**Methods:** We recruited 182 patients with T2DM attending a Diabetes Clinic. Serum N-carboxymethyllysine AGE-modified proteins were measured using an ELISA. Macrovascular disease was defined as documented coronary artery disease, cerebrovascular disease or peripheral vascular disease (n = 70).

**Results:** Patients with macrovascular disease were older (mean ±SD age 70 ±10 vs 65 ±11 years), more likely to be male (76 vs 55%), with dyslipidaemia (97 vs 81%) and impaired renal function (eGFR <60ml/min/1.73m², 43 vs 21%) (all P <0.05), compared to those without macrovascular disease. Systolic blood pressure, BMI, HbA1c, diabetes duration and smoking were similar in the 2 groups. AGEs were elevated in patients with macrovascular disease (geometric mean [25th, 75th percentile] 3662 [2508, 4027] vs 3183 [2664, 4616] μmol/mol/lysine, P = 0.036). In logistic regression analysis, age, male gender, dyslipidaemia and AGEs were associated with macrovascular disease. Serum AGEs were associated with a 7-fold increased risk of macrovascular disease (OR 7.3; 95%CI 1.1, 48.7; P = 0.041).

**Conclusions:** Elevated serum AGEs are strongly associated with macrovascular disease in T2DM. It is unknown if increased AGEs are a cause or a consequence of CVD, and whether drugs that target AGEs such as cross-link breakers represent a novel treatment strategy for CVD in diabetes.
Implant Electrical Characteristics Predict Response to Cardiac Resynchronisation Therapy

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2. Cardiology, Heartcare Victoria, Heidelberg, Victoria, Australia

**Aims:** The optimal site for left ventricular (LV) lead placement with cardiac resynchronization therapy (CRT) is uncertain. This study analysed intracardiac electrogram (EGM) characteristics at implant and the response rates (RR) to CRT.

**Methods:** 41 consecutive patients undergoing CRT were enrolled. Patients in sinus rhythm, with an Ejection Fraction (EF) < 35% and abnormal dysynchrony index (DI) were included. The RV lead was placed in the mid septum and the LV lead targeted to a delayed zone identified by echocardiogram. Intracardiac EGMs measured the intrinsic delay (Int RV-LV), the RV paced delay (RVp-LV) and LV paced delay (LVp-RV). The difference between the LVp-RV and RVp-LV was recorded as the delta LV. Response was defined as an improvement of EF > 10%, a reduction in LVEDD >15% and a symptomatic improvement of 1 NYHA class.

**Results:** Overall RR was 79%. The LV lead was placed in the target location in 91%.

The Int RV-LV was 101±14ms in the responders and 78±11ms in the non-responders. (p<0.05) An Int RV-LV > 100 had a RR of 87% and an Int RV-LV < 100 had a RR of 68%. The LVp-RV and RVp-LV did not differ significantly between the responders and the non-responders. A Delta LV > 70ms had a RR 56%; compared with a delta LV < 70ms RR 85%. There was no significant correlation between lead position, DI, QRS duration or EF and IEGM measurements.

**Conclusions:** Intracardiac EGMs measured at implant can predict response to CRT. This information may be used to determine optimal lead positions.

Left Ventricular Lead Reposition for Non-Response to Cardiac Resynchronisation Therapy.

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**Aims:** A significant proportion of patients are non-responders to cardiac resynchronization therapy (CRT). We hypothesized that repositioning the left ventricular (LV) lead could improve resynchronization and clinical parameters in non-responders.

**Methods:** We evaluated 7 non-responders to CRT, age 63+-9, with no improvement in ejection fraction (EF) left ventricular end diastolic diameter (LVEDD) or NYHA Class following CRT. All original LV leads were successfully implanted in targeted areas identified by pre-implant echocardiographic assessment. The LV lead reposition was based on non-response, lack of improvement in Dysynchrony Index (DI) and intracardiac electrograms (EGM) showing lack of intrinsic EGM separation <60ms or marked delay in LV paced to RV sensed EGM of > 180 ms.

**Results:** LV lead reposition took place 6 – 13 months following the original implant. Removal of the old lead was achieved by simple traction in all cases. Lead reposition was achieved in all patients with a new LV lead in the same or adjacent anatomical segment. There were no procedural complications.

The mean EF improved from 27%+-4 to 34%+-7 (p<0.01), the mean LVEDD reduced from 65+-7 to 59+-11 (p=ns) and the DI improved from 49+-11 to 39+-12 (p<0.05) following LV lead reposition. The intrinsic EGM improved from 48ms+-17 to 83ms+-16 (p<0.01) and the LV paced to RV sensed delay was <180ms for all repositioned LV leads. 4 patients were defined...
as echocardiographic responders and 5 patients reported a symptomatic improvement of > 1 NYHA class.

**Conclusions**: Repositioning of LV leads can improve echocardiographic parameters and symptoms in some non-responders to CRT.

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### Variation in Electrical and Mechanical Dyssynchrony with Multipolar Left Ventricular Leads.

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**Aims**: A proportion of patients fail to respond to cardiac resynchronization therapy (CRT). Multipolar left ventricular (LV) leads increase the anatomical area available for pacing. We hypothesize that there will be variation in electrical and mechanical dyssynchrony at different electrode positions, affecting potential for resynchronization.

**Methods**: 9 consecutive patients underwent CRT using St Jude Medical Quadripolar LV leads (electrode spacing 0mm, 20mm, 10mm, 17mm). Patients were assessed at least 6 weeks following implantation. Electrical parameters were assessed using intracardiac electrograms from right and left ventricular leads. Mechanical dyssynchrony was measured by echocardiography (GE, Vivid 9) using the dyssynchrony index (DI). Measurements were recorded during intrinsic rhythm to each of the 4 electrodes; and during LV-pacing from each of the 4 electrodes.

**Results**: One patient was excluded due to poor echocardiographic imaging. All patients had variations in electrical and echocardiographic parameters between different LV electrodes. Mean maximal intrinsic electrical difference between the 4 electrodes was 8ms (3-15ms). During LV-only pacing from each of the 4 electrodes, mean maximal electrical difference was 35ms (3-67ms) between the electrodes. During LV-only pacing from each of the 4 electrodes, maximal difference in DI in each patient between the 4 electrodes was 40ms (15-121ms). In 4 patients the electrode of maximal intrinsic electrical delay corresponded with the electrode with maximal LV-paced delays.

3 patients had correlation between electrical parameters and mechanical dyssynchrony.

**Conclusion**: Using multipolar leads, pacing from different LV sites can result in different electrical activation and echocardiographic measures of dyssynchrony, increasing options for resynchronization.

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### Cardio-renal Anaemia Syndrome is an Independent Risk Factor for Death in Patients with Heart Failure with Reduced Ejection Fraction.

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**Background**: Anaemia and chronic kidney disease (CKD) are common in patients with heart failure with reduced ejection fraction (HFREF) and have been associated with adverse outcomes. We analysed the impact of cardio-renal anaemia (CRA) syndrome, defined as anaemia (male <130g/L, female <120g/L) and greater than stage 3 CKD (eGFR <60mL/min/1.73m²), in outpatients with HFREF.

**Methods**: Consecutive patients with HFREF were prospectively enrolled between 2000-2005. Baseline clinical characteristics, pathology tests and medication use between those with and without CRA syndrome were compared. The primary endpoint of all-cause mortality was determined via clinical follow-up with cross reference to the National Death Index.

**Results**: 602 patients with a mean follow up of 2.5 ± 1.6 years were enrolled. CRA syndrome was present in 180 patients (30%). Patients with CRA syndrome had higher all-cause mortality 54% vs. 28% (p<0.01). They were: older (mean age 76 ± 11 vs. 67 ± 16 years, p<0.05), had higher rates of diabetes (32% vs. 23%, p<0.01) and ischaemic heart disease
Independent predictors of mortality were: presence of CRA syndrome (HR 1.9 [1.4-2.8] p<0.01), LV systolic dysfunction per grade (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 age –per year (HR 1.1 [1.0-1.1] p<0.01). CRA syndrome was independently associated with increasing age, high serum potassium and diabetes.

Conclusions: CRA syndrome is common in patients with HFREF and is an independent predictor of all-cause mortality.

The CTGF gene -945 G/C polymorphism is not associated with echocardiographically determined cardiac disease or chronic kidney disease in type 2 diabetes.

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Aim:
Connective tissue growth factor (CTGF) mediates tissue fibrosis and has been implicated in the cardiac and kidney complications of type 2 diabetes (T2DM). The CTGF -945 G/C promoter polymorphism is associated with susceptibility to systemic sclerosis, a disease characterised by tissue fibrosis. This study investigated the association of the CTGF -945 G/C polymorphism with echocardiographically determined cardiac disease (left ventricular hypertrophy (LVH), diastolic and systolic dysfunction) and chronic kidney disease (CKD) in T2DM.

Methods:
The CTGF -945 G/C promoter polymorphism was genotyped in 495 Caucasian subjects with T2DM. Cardiac structure and function were assessed by transthoracic echocardiography. CKD was defined as an estimated glomerular filtration rate of <60 ml/min/1.73m² and/or the presence of albuminuria.

Results:
The mean age ± SD of the cohort was 62 ± 14 years; BMI was 31 ± 6 kg/m², median diabetes duration was 11 years [25th, 75th interquartile range; 5, 18] and hypertension was present in 82% of the cohort. An abnormal echocardiogram was present in 73% of subjects; of these 8% had LVH alone, 74% had diastolic dysfunction, and 18% had systolic ± diastolic dysfunction. CKD was present in 42% of the study cohort. Both before and after adjustment for the covariates of age, gender, BMI, blood pressure and hypertension, the CTGF -945 G/C polymorphism was not associated with the cardiac complications of LVH, diastolic or systolic dysfunction, or with CKD.

Conclusion:
The CTGF -945 G/C polymorphism is not associated with echocardiographically determined cardiac disease or CKD in subjects with T2DM.
Echocardiographic cardiac disease in adults with type 1 diabetes

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Aim:
Patients with type 1 diabetes (T1DM) are at increased risk of cardiac death. This study assessed the prevalence and predictors of echocardiographically determined cardiac abnormalities in adults with T1DM.

Methods:
Cardiac structure and function were assessed by transthoracic echocardiography in 193 T1DM patients attending a Diabetes Clinic as part of a complication surveillance program. Predictors of an abnormal echocardiogram were identified using logistic regression analysis.

Results:
The mean (±SD) age was 44±15y and median diabetes duration, 18y [25th, 75th interquartile range; 9, 27]. An abnormal echocardiogram was present in 34%; of these 42% had left ventricular hypertrophy (LVH), 77% had diastolic dysfunction; and 6% had systolic ± diastolic dysfunction. Those with an abnormal echocardiogram were significantly older with a higher BMI, longer duration of diabetes, and were more likely to have hypertension, micro- and macro-vascular disease, and reduced creatinine clearance (all, p<0.05). The risk of an abnormal echocardiogram increased 13-fold in those aged >40y [odds ratio (OR) 13.6 (95% CIs, 2.7-68.5), p=0.002], 5-fold with diabetes duration >10y [OR 5.3 (1.1-24.8), p=0.03] and 3-fold if hypertension was present (p=0.047). Increased BMI (p=0.04) and reduced creatinine clearance (p=0.02) were also associated with the risk of an abnormal echocardiogram. Micro- and macro-vascular complications were not independent predictors of an abnormal echocardiogram.

Conclusion:
One third of patients with T1DM have an abnormal echocardiogram. It is known that LVH and systolic dysfunction are associated with adverse outcomes, but further studies are needed to determine the prognostic significance of diastolic dysfunction in this population.

Cardiac MRI: Indications and Clinical Utility- a Single Centre Experience

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Background: Cardiac MRI (CMR) is a gold standard investigation for non-invasive assessment of cardiac structure and function. CMR continues to evolve and advance as a useful tool in cardiac diagnostics. Its clinical use has been limited in Australia to date, but is increasing

Methods: We reviewed the 2-year data from Austin Health (February 2010 to February 2012) with respect to clinical indications, diagnostic utility and incidental findings.

Results: A total of 231 CMR scans were done over this period for clinical indications. The mean age of the patients was 49 +/- 16 years (Range 17 to 88 years). There were 59% male patients and 41% females. The source of referral was primarily from cardiologists (87%), followed by Cardiothoracic surgeons (5%), physicians (<1%) and other (7%).

Indications were cardiomyopathy (48%), primary arrhythmias (35%), Tissue characterization (34%), congenital/structural heart disease (14%), valvular heart disease (9%), tumours (8%)
and others (10%). Of the cardiomyopathy indications, 47% were for ARVD, 21% for hypertrophic cardiomyopathy (HCM) 21% for dilated cardiomyopathy and 11% for LV non-compaction.

Overall 229 scans were performed successfully (99.1%). For cardiomyopathy, the diagnostic yield was 5.7% for ARVD, 70% for HCM, 75% for LV non-compaction and 57% for DCM. For infiltrative cardiac involvement, there was 50% diagnostic yield for cardiac amyloidosis.

Incidental findings were present in 14% of CMR scans. These included pericardial and pleural effusion, hilar lymphadenopathy etc.

Conclusion: CMR is a valuable tool for assessment of cardiac structure and function with a high diagnostic yield.

Impact Of Renal Function In Patients With Multi-vessel Coronary Disease (MVD) On Long-Term Mortality Following Coronary Artery Bypass Grafting (CABG) Compared With Percutaneous Coronary Intervention (PCI)

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**Background:** Renal failure is an important predictor of mortality in patients with MVD who undergo coronary revascularisation. We sought to determine if the degree of renal impairment affects long-term mortality based on choice of revascularisation strategy.

**Method:** We analysed 7,841 patients with MVD undergoing either CABG (n=6,739) or PCI (n=1,102) between 2004 and 2008, enrolled in two large multi-centre Australian registries. Patients were assigned to three groups using stratified propensity matching based on their estimated glomerular filtration rate (eGFR) at baseline; ≥60mL/min/1.73m² (CABG=4,674 vs. PCI=839), 30-59mL/min/1.73m² (CABG=1,799 vs. PCI=226) and <30mL/min/1.73m² (CABG=266 vs. PCI=37) respectively. Shock, myocardial infarction (MI) <24 hours, previous CABG, valve surgery or PCI were exclusions. We compared Cox-proportional hazards-adjusted National Death Index-linked long-term mortality (mean 3.2 years).

**Results:** In patients with eGFR30-59 and ≥60mL/min/1.73m², there were more women, octogenarians and recent MI in the PCI group and a higher prevalence of cerebrovascular disease, peripheral vascular disease, prior heart failure and MI in the CABG group. Observed long-term mortality rates (CABG vs. PCI) were 4.8% vs. 4.3%, p=0.50, 11.3% vs. 17.3%, p=0.009, 19.9% vs. 40.5%, p=0.005 in the three strata, respectively. Following adjustment, patients with eGFR≥60mL/min/1.73m² had no significant difference in long-term mortality. However, with eGFR=60mL/min/1.73m², PCI was an independent predictor of long-term mortality (HR1.55, 95%CI1.07-2.25, p=0.02). For eGFR<30mL/min/1.73m², there was a trend towards higher mortality with PCI (HR1.80, 95%CI0.95-3.41, p=0.07).

**Conclusion:** In this stratified, propensity-matched study there was a long-term mortality hazard associated with PCI for patients with eGFR=30-59mL/min/1.73m².
Long term survival of elderly patients undergoing percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) complicated by cardiogenic shock (CS)

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Background: Few studies have reported on long term survival in elderly patients with ACS complicated by CS undergoing emergent PCI. We sought to assess long term survival of elderly patients (age ≥75 years) undergoing PCI for ACS complicated by CS in a contemporary multicentre PCI registry.

Methods: We analysed baseline characteristics, procedural, clinical outcomes and long term survival in 421 consecutive patients (299 patients<75 years and 122 patients≥75 years) presenting with ACS and CS who underwent PCI from the Melbourne Interventional Group registry from 2004-2010. Long-term follow-up was up to 7.2 years.

Results: The majority (87%) of patients with ACS and CS had ST-elevation myocardial infarction (MI). The elderly cohort had significantly more females, peripheral and cerebrovascular disease, impaired renal dysfunction, prior congestive cardiac failure (CCF) and prior MI (p<0.05 respectively). There was no significant difference in the proportion of patients who underwent PCI<6hrs from symptom onset (67% vs. 58%, p=0.2). Procedural success was less in the elderly (83% vs. 92%, p<0.01) with higher in-hospital mortality (48% vs. 36%, p<0.05). Overall mortality at 4.1 years was significantly higher in the elderly group (64 vs. 46%). Unsuccessful procedure, renal impairment, CCF and diabetes mellitus were independent predictors of long term mortality however age ≥75 was not a significant predictor (HR 1.3; 95% CI 0.9-1.8; p=0.10).

Conclusion: Elderly patients with ACS and CS have lower procedural success and higher in-hospital mortality compared to younger patients. However, long term survival can still be achieved in elderly patients with selective use of early revascularization.

A continuum of exercise rehabilitation in survivors of intensive care: A randomized trial

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Background: There is increasing evidence that survivors of ICU experience can experience long term physical impairments associated with their ICU admission resulting in poor health related quality of life[1]. Whilst several studies have shown short term improvement in physical function at hospital discharge [2, 3], no previous trials of ICU survivors have examined rehabilitation outcomes across the continuum of care from acute hospital to the community.
Aim: To investigate the effectiveness of an exercise rehabilitation program commencing during ICU admission and continuing into the outpatient setting compared with usual care on physical function and health related quality of life in survivors of ICU.

Methods: This was a single centre randomized trial. One hundred and fifty participants were stratified and randomized to receive usual care or intervention if they were in ICU ≥ 5 days and had no permanent neurological insult. The intervention group received intensive exercises in the ICU, on the ward and in outpatient setting. Participants were assessed at recruitment, ICU discharge, hospital discharge and 3, 6 and 12 months after ICU discharge. Physical function was evaluated using the 6 minute walk test (6MWT); timed up and go test and patient reported outcomes were measured with Short form 36 (SF36) version 2 and assessment of quality of life instrument (AQoL). Data were analysed using linear mixed models.

Results: The intervention group improved significantly in the rate of change of the six minute walk compared with usual care. Between-group differences in mean changes from baseline at 3 and 12 months were moderate and clinically significant. The TUG between group differences approached significance. There were no significant differences in HRQoL measures.

Conclusion: Rehabilitation that commenced in the ICU and continued through to an outpatient programme resulted in improved physical function for survivors of ICU.


Arterial carbon dioxide tension and survival to discharge home after non-traumatic cardiac arrest

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Introduction

The optimal arterial partial pressure of carbon dioxide (PaCO2) after cardiac arrest is unknown. The relationship between PaCO2 and outcome after non-traumatic cardiac arrest was examined. The primary outcome was survival to discharge home.

Subjects and setting

The Australian New-Zealand Intensive Care Society Adult Patient Database (ANZICS-APD) was used to identify patients with non-traumatic cardiac arrest from 125 intensive care units (ICUs) between 2000 and 2009. Patients were divided into 3 groups according to the most abnormal PaCO2 value obtained in the first 24 hours of intensive care admission. The three groups were hypercarbic (PaCO2 >45 mmHg); hypocarbic (PaCO2 <35 mmHg) and normocarbic (PaCO2 35-45). Institutional ethics committee approval was obtained.

Results

Within the study period, 12,108 patients were admitted to ICU after cardiac arrest. Of those, 4986 (41.1%) remained normocarbic (normocarbic group), 4785 (39.6%) presented at least one episode of hypercarbia (hypercarbic group) and 2337 (19.3%) one episode of hypocarbia (hypocarbic group).

In-hospital mortality was significantly lower in the normocarbic group (2335/4986 [53%]; P<0.0001) as compared with both hyper- (2880/4785 [60%]; P<0.0001) and hypocarbic groups (1437/2337 [61%]; P<0.0001). On multivariate logistic regression, patients within the hypercarbic group had a higher likelihood to be discharged home than those within the hypocarbic group (OR 1.35 (1.10-1.66) p=0.003) or the normocarbic group [OR 1.2. (1.03-1.41) p=0.002].
Conclusion
Hypo- or hypercarbia are common in the first 24 hours of ICU admission for non-traumatic cardiac arrest patients. After adjustment for potential cofounders, patients with at least one episode of hypercarbia were more likely to survive to discharge home than those with hypo- or normocarbia. Further evaluation of PaCO2, its management and impact on outcome after cardiac arrest is warranted.

Introduction of an N-acetylcysteine weight based dosing chart reduces prescription errors in the treatment of paracetamol poisoning
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Aim
Under or overdosing of N-acetylcysteine (NAC), when used to treat paracetamol toxicity, is associated with significant morbidity and mortality. This study evaluated the effect of a weight based dosing chart (WBDC) introduced to decrease NAC prescription errors.

Methods
We undertook a pre- and post-intervention trial in a single tertiary referral emergency department (ED). The intervention (the NAC WBDC) was introduced in January 2011 and publicised within the ED by posters and presentations at medical handovers and education sessions. ED staff were blinded to the study and not aware that use of the WBDC was to be evaluated. Data were collected using a retrospective explicit medical record review by a single investigator. The study endpoint was the proportion of NAC prescriptions with errors.

Results
The 81 and 42 patients enrolled in the pre- and post-intervention periods, respectively, did not differ in age, gender or weight (p>0.05). Post-intervention, there were significant reductions in prescription errors of fluid type/volume (50.6% versus 4.8%, p<0.001), NAC dosage (13.6% versus 0.0%, p=0.01) and infusion rate (11.1% versus 0.0%, p=0.03). The overall proportion of prescriptions with any errors also decreased (56.8% versus 14.3%, p<0.001). However, there were no improvements in the documentation of patient weight (65.4% versus 64.3%, respectively, p=0.90) or the proportion of incomplete prescriptions (4.9% versus 11.9%, p=0.16).

Conclusion
The introduction of a WBDC significantly reduces NAC prescription error rates. Its use is recommended in an attempt to improve the safety associated with NAC administration.

Development of a telemedicine model: The Victorian Stroke Telemedicine Project (VST)
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Aim: Telemedicine can facilitate evidence-based care for patients with stroke in rural hospitals. Telemedicine systems are not widely used for acute stroke care in Australia. We aimed to test the feasibility of a stroke telemedicine protocol in an initial pilot study.

Methods: A network of nine metro-based neurologists was on call 24/7 for one rural hospital 200 kms from Melbourne. Neurologists conducted telemedicine via laptops and wireless broadband. Structured feedback was obtained from neurologists and end-users (ED clinical staff) to refine the protocol focusing on clinical utility, technology issues, and stroke education. Stroke education included community awareness activities, and staff training in telemedicine. Telemedicine consultations were undertaken for patients with suspected stroke symptoms and who arrived within 4 hours of onset. Patient clinical data were collected and a 3 month outcome follow-up conducted.
Results: The protocol was initiated for 14 patients; 57% female, median age 70 years (range 29-87 years); 28% (n=4) received t-PA. There was one communication failure. Of the 13 consultations, 31% were full video-conferencing consultations (video/audio/brain imaging), and 69% were telephone only. Neurologists accessed CT scans in 9 cases (69%). Median door-to-consult time was 67 minutes (IQR 37-108). Length of consults ranged from 1–50 mins (median=17 mins). Information technology and clinical factors were reported as facilitating or hindering telemedicine use for the neurologists and ED clinical staff.

Conclusions: Overall the pilot telemedicine protocol was feasible and provided evidence to streamline processes and overcoming technology problems. The one year phase of the revised protocol is underway.

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Emergency department management of anaphylaxis – a good news story?

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Aim: To characterise anaphylaxis presentations to ED and describe current management in accordance with established guidelines.

Methods: Patients discharged from the ED of a major tertiary health service between June-December 2011 with a diagnosis of anaphylaxis were included. A retrospective audit was undertaken to collect demographics, presenting symptoms, potential triggers, pre-hospital and ED management. Key endpoints were the appropriateness of the diagnosis of anaphylaxis, use of adrenaline intramuscular first line, appropriateness of antihistamine, corticosteroid and adrenaline auto-injector prescription.

Results: 25 patients were included (48% were male, 67% were adults). The identified triggers included food (52%), insect sting (8%), medications (20%), latex (4%) and pollen (4%). All patients had clinical features of anaphylaxis – none solely had skin/mucous membrane involvement. Pre-hospital, 11 (44%) patients received intramuscular adrenaline and 5 (20%) patients had adrenaline auto-injector. Nine patients received adrenaline in ED – 8 received intramuscular doses and one received a nebuleulised dose. Sixteen (64%) patients received a corticosteroid in ED. Fourteen (56%) patients received an antihistamine; all had documented itch/urticaria; all doses were oral, except one intramuscular promethazine dose. Eight patients received promethazine, the remainder were non-sedating antihistamines. Fourteen of the 15 patients who received adrenaline auto-injector on discharge, had unavoidable triggers; one inappropriate prescription was for penicillin-induced anaphylaxis.

Conclusion: Overall ED management of anaphylaxis is concordant with guidelines. Areas for improvement are avoiding sedating antihistamines for skin-related symptoms and prescribing adrenaline auto-injector only for anaphylaxis due to unpredictable/unavoidable triggers.

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A clinical intervention trial of nurse-initiated analgesia for paediatric patients in the emergency department

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Objective: We aimed to evaluate the impact and safety of a nurse-initiated analgesia (NIA) intervention for paediatric patients in the emergency department (ED).

Methods: We undertook a pre- and post-intervention trial in a large, tertiary referral, mixed ED. The intervention comprised development and implementation of a comprehensive NIA Standing Order. In addition to paracetamol, which nurses could initiate pre-intervention, they could administer non-steroidal anti-inflammatory drugs, opioids and topical local anaesthetics prior to a doctor assessing the patient.

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All nurses were trained and credentialed prior to the intervention. Patients aged 5-17 years with a triage pain score of ≥4 (Wong-Baker or numerical rating scale) were eligible for enrolment. Parental satisfaction with overall ED pain management was measured using a 1-6 point ordinal scale (1=very unsatisfied, 6=very satisfied).

Results: Fifty one children were enrolled in both the pre- and post-intervention periods. Patient gender and mean age, weight and triage pain score did not differ between the groups (p>0.05). In the post-intervention period, significantly more patients received NIA (3.0% versus 43.9%, p<0.001) and the median time to analgesia was significantly reduced (57.6min versus 23.0min, p<0.01). At follow up 48 hours post-discharge, the proportion of parents who were very satisfied with their child’s pain management overall increased in the post-intervention period (47.1% versus 66.7%, p<0.05). No adverse events were observed during either period.

Conclusion: Nurse-initiated analgesia is safe, significantly reduces time to analgesia and is associated with high levels of parental satisfaction.

Near infrared spectroscopy can provide a surrogate of forearm blood flow

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Introduction
Estimation of forearm blood flow (FBF) may be an important method of assessing the circulation in shock states. Strain gauge plethysmography (SGP) is an accepted method to measure FBF but is too cumbersome in the intensive care unit (ICU). Near Infrared Spectroscopy of the thenar eminence (NIRSth) is a non-invasive technique to measure tissue oxygenation (StO2) which can be deployed in ICU.

Objectives
We aimed to test whether NIRSth derived changes in estimated tissue haemoglobin concentration (ΔTHC) after short-lived venous occlusion correlate with SGP measurement of FBF and to develop an equation to convert such NIRSth derived ΔTHC into FBF.

Methods
FBF was measured in 6 volunteers with SGP during short lived venous occlusion manoeuvres while simultaneously obtaining data on ΔTHC. Measurements were obtained during baseline and induced hyperaemia. We used correlation analysis (Pearson’s r, coefficient of determination r² and Spearman’s rho) to compare Δvolume (FBF-equivalent) (ml/dL/min) obtained by SGP and ΔTHC (g/dl/min) from NIRSth.

Results
We performed a total of 42 paired measurements. There was a strong positive correlation between NIRSth ΔTHC and SPG derived FBF across a range of blood flows (r=0.90, r²=0.82, rho=0.78). Using linear regression analysis, the equation FBF=12.668 X ΔTHC + 0.006 (95%CI for coefficient: 11.349-13.987, 95%CI for constant: -0.004-0.010) could be developed to predict FBF using NIRSth ΔTHC.

Conclusion
NIRSth can be used estimate FBF in man. Given its ability to also measure StO2 and vascular reactivity, NIRSth can provide a comprehensive assessment of the forearm circulation in man.
Review of Men with Minimal Trauma Fracture and Circulating Testosterone Levels

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Introduction: Androgen deficiency is a risk factor for osteoporosis in men. However, illness and acute fracture may lead to a reduction in circulating testosterone.

Methods: To explore this possibility, we conducted a case-control study of 240 men presenting to the Emergency Department (ED) with a radiologically confirmed minimal trauma fracture (MTF), and of 75 controls.

Results: Compared to controls, cases had lower total testosterone (TT, 7.1 vs 13.2 nmol/L, p < 0.0001) and calculated free testosterone (cFT, 113 vs 172 pmol/L, p < 0.01). Cases were older (74 vs 68 years, p < 0.001), had lower lumbar spine T-score (-0.6 vs 0.0, p = 0.04), femoral neck T-score (-1.7 vs -1.1, p < 0.0001), and renal function (eGFR 78 vs 82 ml/min, p= 0.02). There was no difference in BMI (27.4 vs 27.9 kg/m2), and 25OH-vitamin D (58 vs 64 nmol/L), p = n.s.. Lower TT remained associated with a higher fracture risk after adjustment for differences between groups including age and bone mineral density (OR 1.21 p< 0.0001). Of the cases, the 142 admitted to the hospital had lower TT than the 98 discharged from the ED (4.6 vs 10.3 nmol/L, p < 0.0001), and lower cFT (78 vs 151 pmol/L, p < 0.0001). There was also a difference in TT between cases discharged from ED and controls (10.3 vs 13.2 nmol/L p < 0.0001); but not in cFT (151 vs 172 pmol/L, p = n.s.). In the 34 cases with follow-up testosterone (median of 4 months after the initial testosterone), follow-up TT was 8.5 vs 5.1 nmol/L, and cFT was 127 vs 81 pmol/L, both significantly (p <0.001) higher compared to the initial testosterone.

Conclusions: The diagnosis of hypogonadism and appropriate commencement of androgen replacement in men is challenging. Neither should it be based on measurements following minimal trauma where, at least in part, deficits in serum testosterone may be effects of an acute, fracture-associated, stress response

Body composition and metabolic changes between two weeks and six months of stroke

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Loss of muscle mass and increased fat mass after stroke is thought to contribute to detrimental bone loss and insulin resistance. This study aims to determine skeletal, body composition and metabolic changes occurring within six months of stroke.

Method: Patients with medically stable hemispheric stroke from Austin Hospital, without history of stroke or known diabetes, and inability to walk within one week of stroke are included.

Outcome measurements: total body bone mineral density (BMD), total lean and fat mass using dual-energy x-ray absorptiometry (DXA); and glucose tolerance. Assessments are undertaken within two weeks, one month, three months and six months of stroke.
Results: Twenty-two baseline and 14 six month assessments have been undertaken. Median age of patients was 67.7 years (range 41.7 – 89.8), nine (40.9%) female. Body mass index (BMI) was 26.84 kg/m$^2$ (95% CI 24.80, 28.88). Mean six-month change in BMD was -0.023 g/cm$^2$ (95% CI -0.035, -0.012), n=12. Changes in total lean and fat mass were -477.83g (95% CI -1853.01, 897.3426) and -3610.33g (95% CI -6185.44, -1035.22) respectively. BMI at six months was 26.85 kg/m$^2$ (95% CI 24.80, 28.90), n=14.

Dysphagia prevented baseline glucose tolerance testing in eight participants. Glucose tolerance at six months was normal in ten participants and impaired in three. One participant declined blood testing.

Conclusion: Within six months of stroke, expected trends in loss of total body BMD and lean mass were observed. BMI was expected to reduce, but was maintained, and fat loss was greater than expected. However, sample size was small and individual results varied. Two year follow-up is planned for 35 participants to determine the emergence of insulin resistance and bone loss.

Bone, metabolic and body composition changes in men undergoing androgen deprivation therapy after 2 years follow up in a dedicated Men’s Health service.

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Androgen deprivation therapy (ADT), an effective treatment for prostate cancer, has been associated with accelerated bone loss, visceral fat gain, and insulin resistance.

Aim: To evaluate bone and metabolic outcomes in men with non-metastatic prostate cancer receiving ADT.

Methods: A prospective cohort study of men receiving long-term ADT (2007-2011) was performed. All men attended the Austin Health Men’s Health Clinic (MHC) and underwent standardised assessments according to current guidelines$^1$. Men were followed for 2 years and paired data was analysed using Student’s t test.

Results: 126 men were eligible for 2-year follow up. 95 men had data available for analysis (16 failed to attend, 6 deceased, 6 moved away from local area, 3 ADT ceased prior to 2 year follow up). Comparison of co-morbidities at baseline and 2 years are presented in tables 1 and 2 (stratified according to treatment for the co-morbidity at 2 years).
Non-diabetic patients had a significant rise in HbA1c associated with an increased prevalence of type 2 diabetes at 2 years. Despite increases in BMI and waist circumference, patients had a lowering of blood pressure, and improvement in lipid profile. Total hip BMD declined despite increases in vitamin D to optimal levels. BMD was maintained in those who received anti-osteoporosis therapies, whereas a decline was seen in those untreated over 2 years. **Conclusion:** ADT is associated with adverse effects on body composition and bone health. Active treatment of cardiovascular risk factors and osteoporosis according to current guidelines is effective in minimizing cardiovascular risk and maintaining bone density. Larger studies are needed to determine effects on cardiovascular outcomes and fracture prevention.


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**The performance of the Chronic Kidney Disease Epidemiology Collaboration (CKD- EPI) and Modification of Diet in Renal Disease (MDRD) formulas for estimating Glomerular Filtration Rate (eGFR) in Indigenous Australians with and without diabetes**

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Background: The CKD-EPI formula has been proposed as a more accurate marker of GFR than the MDRD formula. However, the best method for estimating GFR in Indigenous Australians with diabetes is still unclear.

Aims: To analyse the performance of CKD-EPI and MDRD formulas for estimating GFR (eGFR) in Indigenous Australians with or without diabetes.

Methods: Participants were Indigenous Australians with (n= 234) or without (n=345) type 2 diabetes (T2DM). A reference GFR measurement was obtained using the plasma disappearance of iohexol (mGFR) over 4 hours. Serum creatinine was measured by an enzymatic method. Performance was determined as bias (absolute difference), derived from mGFR-eGFR and accuracy (percentage of eGFR within 30% of mean mGFR).

Results: In the entire study population, the performance of the CKD-EPI formula was superior to the MDRD formula. However, in Indigenous Australians with diabetes, the CKD-EPI
formula underestimated mGFR to a greater extent ($p < 0.05$) and was less accurate ($p < 0.05$) than in those without diabetes.

Conclusion: In Indigenous Australians with diabetes, the CKD-EPI formula has a greater negative bias and is less accurate compared to those without diabetes. Nevertheless, the CKD-EPI equation outperforms the MDRD equation in all Indigenous Australians and remains the preferred equation to estimate GFR in this high risk population.

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Relationship between urinary sodium excretion and estimated glomerular filtration rate (eGFR) trajectories in type 2 diabetes.

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OBJECTIVE—Most guidelines recommend that patients with type 2 diabetes should reduce their intake of salt to improve blood pressure control. However, few studies have examined the relationship between dietary salt intake and renal function in patients with diabetes.

RESEARCH DESIGN AND METHODS—
Six hundred and seventeen participants attending a single diabetes clinic were followed in a prospective cohort study. Baseline characteristics were determined in 2000. From 2001 through 2008, the results of each participant’s serial 24h urine collections and eGFR were determined using the CKD-EPI formula. Sodium excretion was estimated from 24h urine collections (24hU-Na). A linear mixed model was used to identify predictors and to estimate the change of eGFR over time (as a linear variable).

RESULTS: There were a total of 2631 years of eGFR measurements throughout the follow up period in 443 survivors. The following predicted eGFR: sodium excretion (in quartiles), baseline eGFR, BMI, HbA1c, age, albumin excretion rate. In the alive participants (as a linear variable, interacting with the categorical UNa) there was no difference in the rates of decline of eGFR in each quartile ($p=0.50$, joint test of contrasts). Furthermore, there was no difference between the rates of decline of eGFR ($p=0.79$) in those participants who were deceased (n=174) according to quartiles of urinary sodium ($p=0.50$, joint test of contrasts, Table).

CONCLUSION: In this study of 443 patients over an 8 year follow up period, there was no demonstrable differential effect across quartiles of 24hUNa on the rate of decline of eGFR. Further exploration of the mortality effect of time change of 24hUNa would be best explored by a joint model of longitudinal and survival data.

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Trimester Specific Reference Intervals for Thyroid Function

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Using non-pregnancy reference intervals in pregnancy can be misleading. We aimed to establish trimester-specific reference intervals for thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) specific for Beckman Dxi 800 analytical system, a commonly used method for measuring thyroid function in Australia.

Healthy women attending Mercy Hospital for women (a tertiary maternity hospital in Victoria) for antenatal care were followed prospectively.
Normal reference intervals for serum TSH, fT4 and fT3 were determined at each trimester and post partum.

154 women were recruited into this study. After excluding women who had miscarriage, twin pregnancy and women who were thyroid peroxidase antibody positive, 131 women’s results were used for the reference interval determination. For trimester 1 (T1), trimester 2 (T2) and trimester 3 (T3), the median (2.5th 5th, 95th, 97.5th percentile ) TSH were 0.76 (0.02, 0.05, 2.37, 3.22), 1.16 (0.26, 0.43, 2.70, 3.34) and 1.33 (0.03, 0.34, 2.66, 3.34) mIU/L, respectively. Free T4 (mean±SD) was 10.7±2.4, 8.1±1.6, 7.6±1.5 pmol/L, respectively. Free T3 (mean±SD) was 4.8±0.5, 4.4±0.4, 4.3±0.4 pmol/L, respectively. In T2 and T3, 34.5% and 40.3% of the fT4 values respectively, fell below the manufacturer's quoted reference intervals.

The trimester specific TSH reference intervals in our cohort are very similar to those put forward by ATA 2011 Guidelines. However, guided by non-pregnancy associated reference intervals for fT4 levels, up to 40% of of pregnant women would be considered inappropriately as having abnormal thyroid function which may lead to confusion and potential mismanagement. This study highlights the need for establishment and use of pregnancy and trimester specific reference intervals for fT4 in addition to TSH.

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**Plasma disappearance of inulin as a marker of glomerular filtration rate in type 2 diabetes**

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**Background:**
Glomerular filtration rate (GFR) provides a valuable indicator of renal function. The gold standard for measuring GFR is inulin clearance using constant infusion and timed urine collections. However, this is a cumbersome procedure.

**Aims:**
To compare inulin-GFR with 99mTc-diethylene-triamine-penta-acetic acid (99mTc-DTPA)-GFR using single injection plasma disappearance rates in patients with type 2 diabetes, and with GFR estimates using MDRD and CKD-Epi equations.

**Methods:**
We compared GFR measured by different methods in 26 patients with type 2 diabetes. Plasma 99mTc-DTPA and inulin clearance were determined by the slope intercept method modified according to the Brochner-Mortensen equation for a one compartment model. After a bolus intravenous injection of 5g inulin, blood was sample at 20, 60, 120, 165, 210 and 240 minutes. Serum inulin concentration was measured by HPLC and clearance was also calculated using two sampling times as described previously.

**Results:**
Mean 99mTc-DTPA-GFR was 78.8mL/min/1.73m² with a range from 22 to 149mL/min/1.73m². There were 21 combinations possible using 2 blood sampling times after inulin injection to calculate GFR. Table 1 illustrates bias, accuracy and precision of GFR with accuracy >70% when compared with 99mTc-DTPA-GFR. GFR calculated using samples at 20 and 120 minutes after inulin injection yielded the best result, with accuracy of 72% and bias of 2.4mL/min/1.73m², and this improved in the subgroup with GFR >60mL/min/1.73m². Accuracy with 2-point inulin GFR was comparable to inulin clearance calculated with slope-intercept method using all timepoints. CKD-Epi performed best overall, however, results were similar to inulin-GFR once GFR was >60mL/min/1.73m².

**Conclusion:**
Single injection inulin-GFR provides an efficient and simple alternative to radioisotopic methods in diabetic patients. Calculating GFR using samples 20 & 120 minutes after inulin injection gave comparable results with that of 99mTc-DTPA-GFR, particularly for GFR above 60mL/min/1.73m².
Vitamin D requirements during sunlight deprivation

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During a 12-month Antarctic expedition, prolonged sunlight deprivation results in vitamin D deficiency and may lead to bone loss unless exogenous sources of vitamin D are provided (1). We observed that vitamin D deficiency (25(OH)D <50nmol/L) was prevented when expeditioners were provided with 50,000IU vitamin D3 alternate months. Current recommendations suggest that 25(OH)D levels >75nmol/L denote adequacy (2). We conducted a double-blind randomised trial and based on changes to 25(OH)D levels in 110 expeditioners assigned to a monthly, bi-monthly or a single dose of 50,000IU vitamin D3 prior to departure, the following recommendations are proposed to ensure vitamin D adequacy is maintained using 25(OH)D levels prior to departure as a benchmark for supplementation (3).

For those with 25(OH)D >100nmol/L at baseline, a single 50,000 IU dose prior to departure likely maintains sufficiency. For those with baseline levels between 75-100nmol/L, a bi-monthly dose would be recommended, and for those with baseline 25(OH)D <50nmol/L, a monthly dose would be necessary. It is recommended that 25(OH)D levels are measured prior to departure, the appropriate supplementation dose administered, and levels remeasured on return from Antarctica to ensure adequacy was maintained. Monthly supplements can be easily administered during routine monthly medical examinations, and helps ensure good compliance, which may be compromised with daily therapies. No adverse events were reported.

The above protocol helped ensure vitamin D sufficiency was maintained during expeditions, and may have application to others with limited sunlight exposure such as those in aged-care.

(1) Iuliano-Burns et al. Osteoporosis Int. 2009
(2) Bischoff-Ferrari. Best Practic Res Clin Rheumatol, 2009
(3) Iuliano-Burns et al. Osteoporosis Int. 2012

The deleterious effects of fat in C2C12 muscle cells are associated with an impaired mitochondrial stress response

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The unfolded protein response (UPR) prevents the accumulation of deleterious unfolded protein aggregates, mainly in the endoplasmic reticulum. However mitochondria form another compartment with protein-folding function, and hence there is also a specific mitochondrial UPR (UPRmt). Studies in C.elegans have shown that in response to mitochondrial protein misfolding CLPP-1 conveys a signal to the cytoplasm resulting in UBL-5 and DVE-1 forming a complex that can up-regulate the expression of mitochondrial chaperone genes. UBL-5 therefore appears to have a key role in the response to mitochondrial stress. However the importance of UBL-5 in protecting mammalian cells exposed to stresses such as excess nutrient availability has not been previously investigated.

To determine whether excess nutrients can affect the UPRmt we incubated C2C12 myotubes with either 20 mM glucose or 0.75 mM palmitate for 18 hrs and assessed the levels of Clpp and Ubl5 mRNA transcripts as well as cell viability. We found that both high glucose and palmitate reduced cellular ATP levels, and that this was associated with increased Clpp expression indicative of ‘mitochondrial stress’. In the high glucose condition this mitochondrial stress signal was accompanied by an increase in Ubl5 expression, and under these circumstances there was no change in cell viability. In contrast the palmitate incubation did not increase Ubl5 gene expression and this resulted in decreased cell viability.

Therefore when Ubl5 expression is increased in response to mitochondrial stress, muscle cells are protected against cell death, whereas a lack of a Ubl5 response is associated reduced cell viability.
Relationship between serum aldosterone levels and urinary sodium excretion in patients with diabetes in the presence and absence of renin angiotensin aldosterone blockade

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4. Professorial Fellow, The University of Melbourne, Melbourne, Australia
5. Director of Endocrinology, St Vincent’s Health, Melbourne, Australia
6. Endocrinology Consultant, Austin Health, Melbourne, Australia
7. Research Fellow, The University of Melbourne, Melbourne, Australia
8. Research Fellow, Menzies School of Public Health, Melbourne, Australia

Background: Current guidelines recommend people with hypertension and diabetes to reduce salt intake[1]. In a previous study in patients with type 2 diabetes, we have shown that low 24-hour urinary sodium excretion (mmol/24h,24hUNa), is associated with increased mortality[2]. In the general population, low salt intake is associated with increased plasma renin activity(PRA) and serum aldosterone concentration[3]. The current study investigated the relationship between 24hUNa, PRA, aldosterone and brain natriuretic peptide(BNP) in patients with diabetes.

Methods: In a cross-sectional study, clinical characteristics, 24hUNa, PRA, aldosterone, and BNP were recorded in 329 consecutive patients attending diabetes clinics at Austin Health. A subgroup of 46 patients was not taking medications which interfere with renin-angiotensin-aldosterone-system(RAAS). The relationship between 24hUNa with aldosterone, PRA and BNP was examined by Pearson correlation. A linear regression model which included PRA and 24hUNa was generated to predict aldosterone levels.

Results: Mean age was 64 years, 60% were males, mean HbA1c was 7.7±1.3%(mean±SD), and 77% had type 2 diabetes. In total study group, there was a negative correlation between serum aldosterone and 24hUNa(r= -0.14, p=0.04). This relationship was most pronounced in the subgroup of patients not taking RAAS-interfering agents(r= -0.36, p=0.02). Using linear regression model, PRA and 24hUNa significantly predicted aldosterone levels in people not taking RAAS-modulating medications. However, this relationship was not seen in subjects receiving RAAS-modulating medications(F=6.8, p=0.002). There was also no significant correlation between aldosterone and 24h urinary potassium excretion or serum potassium.

Conclusions: The relationship between 24hUNa and aldosterone was masked in people with diabetes taking RAAS-modifying medications. This study highlights the difficulties in further delineating the mechanisms linking low urinary salt excretion and increased mortality in people with diabetes.


Non-DNA binding-dependent androgen receptor pathway in fat

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Our androgen receptor knockout (ARΔZF2) mouse model has an exon 3 deletion of the AR. This deletion abolishes DNA binding-dependent AR actions, but non-DNA binding-dependent actions are retained. ARΔZF2 male mice have increased subcutaneous and renal fat mass compared to wildtype (WT) males, but decreased total body mass. To further investigate the non-DNA binding-dependent pathway in fat, we performed orchidectomy in ARΔZF2 male mice,
to remove all endogenous androgens, and then treated mice with non-aromatisable dihydrotestosterone (DHT) or control implants for 10 weeks. Differences between control orchidectomised and DHT-treated orchidectomised ARΔZF2 males must arise through non-DNA binding-dependent AR pathways. We validated our orchidectomy surgery by showing that in orchidectomised WT males, seminal vesicles completely regressed and kidney mass was decreased by 33% compared to sham-operated males (p<0.001). DHT treatment of orchidectomised WT males increased kidney mass to 13% above sham (p<0.001), suggesting a slightly supraphysiological androgen delivery. There was a mean difference of 13% in subcutaneous fat mass and 18% in renal fat mass in DHT-treated orchidectomised ARΔZF2 males compared to orchidectomised ARΔZF2 controls (n≥20/group), but these were not significant and the study was underpowered to determine if this difference was significant. IL-6 gene expression, known to be repressed by the non-DNA binding-dependent AR pathway, was not different between the two groups, however Western analyses in subcutaneous fat showed that ERK phosphorylation was increased by 87% in DHT-treated orchidectomised ARΔZF2 males compared to orchidectomised controls (p<0.05). This data demonstrates a molecular role of non-DNA binding-dependent AR signalling in subcutaneous fat.

## AR replacement specifically in mineralising osteoblasts of androgen receptor knockout mice partially restores trabecular bone but does not restore bone size

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3. Bone and Mineral Research Unit, Portland Veterans Affairs Medical Center, Oregon, USA

Androgens are essential for skeletal growth and bone accrual during puberty and bone maintenance post-puberty in males. We have shown that global knockout of the androgen receptor (AR) in mice (ARKO) results in reduced bone size, cortical thickness and volume compared to control males. To determine the relative contribution of androgens in proliferating (pOBL) versus mineralizing (mOBL) osteoblasts, we replaced the AR in ARKOs at either the a) proliferative (pOBLAR:ARKOs) or b) mineralisation (mOBLAR:ARKOs) stage of osteoblast development.

Femurs of mOBLAR:ARKO replacement and control mice were analysed by uCT at 6 and 12 weeks of age (n=7-15/group). Replacement of the AR in mineralizing osteoblasts did not restore bone size or thickness, as measured by periosteal circumference and cortical thickness, respectively. Trabecular bone was partially restored in adult mOBLAR:ARKO replacement mice with increases in trabecular number (P<0.05), while BV/TV was unaffected.

These data are consistent with the hypothesis that androgen action to increase bone size and cortical thickness during growth is mediated via the AR in proliferating osteoblasts, while androgen action in mineralizing osteoblasts plays a partial role in maintaining trabecular bone in adult mice. This hypothesis is being further tested in global ARKO mice in which the AR has been specifically replaced in proliferating osteoblasts (pOBLAR:ARKOs).
Nocturnal Dipping of Mean Arterial Blood (MAP) Pressure is Related to Sodium Intake in Type 2 Diabetes and Abolished by morning angiotensin II receptor blockade (ARB)

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AIMS (1) To assess the relationship between nocturnal dipping of blood pressure (ND), urinary sodium excretion (24h UNa) in hypertensive patients with type 2 diabetes and habitually high (sodium excretion >200 mmol/24 h) or low (sodium excretion <100 mmol/24 h) salt intake. (2) To determine if the relationship between ND and 24h UNa is influenced by morning ARB with or without salt supplementation.

METHODS: 28 Patients received 4 weeks of ARB (telmisartan, 40mg mane) and in a double-blind randomised fashion, sodium chloride (NaCl, 100 mmol/24 h) or placebo capsules in addition to their habitual salt intake during the last 2 weeks of telmisartan therapy. The protocol was repeated with NaCl and placebo capsules administered in reverse order to allow each participant to act as his or her own control. At 0, 4 and 18 weeks, 24 h ambulatory blood pressure (ABP) and 24 h urine collections were performed. The ND (%) was calculated as = ((MAP day - MAP night)/MAPDay) x 100, where MAPDay and MAPNight are daytime and night time values of MAP.

RESULTS

Correlation between ND of MAP (mmHg) and 24h UNa:

<table>
<thead>
<tr>
<th></th>
<th>ND (mean ± SEM)</th>
<th>r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10.41 ± 1.09</td>
<td>0.478</td>
<td>0.0234</td>
</tr>
<tr>
<td>telmisartan</td>
<td>6.98 ± 1.39</td>
<td>0.095</td>
<td>0.628</td>
</tr>
<tr>
<td>telmisartan + NaCl</td>
<td>5.99 ± 1.32</td>
<td>0.479</td>
<td>0.0099</td>
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There was a significant correlation between the 24h UNa and the ND before the commencement of telmisartan which disappeared during telmisartan therapy but was restored with NaCl supplementation.

CONCLUSION

In type 2 diabetes, the degree of ND correlates with urinary sodium excretion and suggests that dietary salt intake increases blood pressure to a greater extent during the day than night. This correlation is abolished by telmisartan administered in the morning. This study raises the possibility that evening administration of telmisartan may restore ND in subjects with low dietary salt intake.
Implications of Fc-engineering to a humanised anti-LeX antibody on receptor binding and cellular effector function

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The interaction between the immunoglobulin G (IgG) Fc region and Fcγ receptors (FcγRs) is the primary mechanism linking antibody-mediated immune responses with cellular effector functions. Enhancement of this interaction through amino acid variation and glycoengineering has provided a way to enhance immune effector functions. The majority of this research has been aimed at enhancement of interactions with FcγRIIIa, which is the key mediator in natural killer (NK) cell antibody-dependent cell-mediated cytotoxicity (ADCC). But as NK cells are poor infiltrators of solid tumours, engineering for additional receptors and their linked effector functions is warranted. A previous study has shown that mutant antibodies with increased affinity for FcγRIIa relative to FcγRIIb have enhanced macrophage phagocytosis of antibody-coated tumour cells. This is mirrored by clinical data showing improved survival in patients possessing the R131 polymorphic FcγRIIa variant, which has higher affinity for IgG class used in therapy. Here, we describe a set of novel Fc variants through amino acid engineering with altered FcγR binding affinities. These novel variants were first determined by in silico methods from crystal structures and then produced in a mammalian expression system for further analysis. Preliminary ADCC and binding affinity data for each of the variants is described.

Dissecting the Cross-presentation Pathway in cutaneous HSV Infection Using a Novel Transgenic Mouse Model

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CD8+ T cells are the main effector cells in the adaptive immune system that eliminate pathogen infected cells. During the initial infection by a pathogen, naïve CD8+ T cells are “primed” by recognizing specific antigenic peptide/MHC class I complexes expressed by professional antigen presenting cells (APCs), such as DCs1. DCs may become directly infected leading to direct-priming of CD8+ T cells; or they may uptake pathogen-derived antigen of other infected cells, process such antigen and present their peptides to CD8+ T cell (a process termed cross-priming). Although DCs are known as the key controllers of CD8+ T cell immunity little is known about their actual contribution to direct- and cross-priming2. Understanding how DCs regulate such T cell activation is central to our capacity to favourably manipulate the immune system for optimal anti-viral responses and anti-tumour immunity. To determine the antigen presentation pathway in a physiological viral infection setting, we utilize a well established cutaneous HSV infectious model3 in conjunction with a novel Cre reporter transgenic mouse strain named ELOFIR: NY-ESO-1 (a cancer/testis antigen) flanked by IoxP sites, followed by the antigen of interest, mOVA/FLAG and IRES/DsRed. The infection model employed utilizes a recombinant HSV-Cre virus as a source of Cre Recombinase. It is anticipated that HSV-Cre infection leads to the conditional elimination of NY-ESO-1 and switches on expression of the mOVA/FLAG and DsRed in infected cells, but not in the cells that uptaken cell debris of infected cells. the novel mouse model will enable us for the first time to accurately identify HSV infected APCs in vivo or ex vivo, both functionally and phenotypically, and allow us to differentiate cross- vs direct-presentation under physiological conditions.

Sex steroid receptor signalling and immune function

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Background and Aim: Immune function differs between the sexes but the mechanism for this is not known. Sex steroid receptors have been found to be expressed on various immune cell subtypes, including “non-genomic” membrane bound receptors. The biological function of these molecules and their direct effects on immune function have not been explored. We aimed to determine the biological effects and mode of action of sex steroid receptor signalling on immune cell function. Methods: Genomic androgen receptor (AR) signalling on T cells was assessed using qRT-PCR. Readout genes were known to be AR-dependent in other cell types and expressed in T cells. Effector function of flu-specific T cells was measured by intracellular staining for interferon gamma (IFNγ) following antigen presentation in the context of AR activation or blockade. Non-genomic signalling was screened using a calcium flux assay to indicate activation of unconventional pathways in response to AR activation or blockade. Results: Up regulation of AR regulated genes in response to DHT was observed in LNCaP but not in flu specific T cell clones, suggesting lack of genomic AR signalling in T cells. Preliminary calcium flux experiments suggest both early and delayed effects of DHT signalling implying possible activation of non-genomic pathways but these results remain to be confirmed. Conclusion: These results indicate that the AR signalling pathway in T cells may be at least in part non-genomic but these results need further confirmation. Comparable studies for estrogen receptor signalling are ongoing.

An analysis of patient satisfaction with the 'Better Backs @ Austin' physiotherapy back rehabilitation programme

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Aim Patient satisfaction is a patient-focused indicator of healthcare quality and is associated with treatment adherence. Back education and therapeutic exercise are evidence-based components of back rehabilitation that underpin the ‘Better Backs @ Austin’ programme, a twice weekly 8 week programme with longitudinal continuity of physiotherapy instruction and supervision. Since patient satisfaction and compliance with exercise therapy are linked, evaluation of patient satisfaction is important to inform content and delivery of future programmes.

Method ‘Better Backs @ Austin’ participants who had completed a minimum of 12/16 programme sessions returned anonymous patient satisfaction questionnaires evaluating 10 domains of service delivery. Satisfaction was rated on a 0-10 scale (0=extremely dissatisfied and 10=extremely satisfied) for each domain; 7 internal subscales (patient-therapist interactions) and 3 external subscales (non-therapist issues e.g. gymnasium environment) were assessed and compared. Results were analysed as appropriate.

Results Seventeen questionnaires were analysed and overall mean patient satisfaction across all 10 domains was 9.5±0.2. Highest scoring domains were for internal factors: educational lectures and exercise instruction 9.8±0.5, availability of the physiotherapist 9.8±0.5, overall benefit from attending the programme 9.6±0.8. Scores for internal subscales (9.5±0.9) were not significantly different from external subscales (9.5±0.7, p=0.6) indicating that, in contrast to other studies, Austin Health scored equally well for non-therapist domains. Qualitative comments were positive (e.g. best physiotherapy management, marvellous service, beneficial, professional, excellent instructor and explanations, useful exercise sheets), with parking the exception (car park full, expensive to park). Longitudinal continuity of
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care has been associated with high patient satisfaction with outpatient physiotherapy and our results support this finding.

**Conclusion** Results indicate high patient satisfaction with the ‘Better Backs @ Austin’ programme and suggest this is valuable for group back rehabilitation in physiotherapy outpatients.


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**The physiological role of calcitonin via the calcitonin receptor to protect the maternal skeleton during pregnancy and lactation in mice.**

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Calcitonin is well documented to potently inhibit osteoclasts, the cells responsible for bone resorption. The lack of any obvious pathophysiology in individuals either deficient in, or with excess levels of serum calcitonin, however, has led to much debate as to whether calcitonin has a physiological role. We have previously shown a physiological role for calcitonin, acting via the CTR on osteoclasts, to protect against calcium stress, such as induced hypercalcemia [1]. We now hypothesise that the inhibitory role of calcitonin via the CTR is also important to protect the maternal skeleton against excessive resorption during pregnancy and lactation.

To determine if calcitonin acts via the CTR to limit bone loss during pregnancy, we generated two CTR knockout mouse models, a global CTRKO and an osteoclast (OCL)-specific CTRKO. Female global-CTRKOs, OCL-CTRKO and control mice were time mated at 8 weeks of age and sacrificed at either 18 days (pregnancy) or 39 days (lactation) post-mating.

Preliminary mCT analysis of the distal femur shows no significant difference in cortical or trabecular bone in the global CTRKOs compared to controls during pregnancy or lactation. However, mean trabecular bone values are reduced by 44% in global-CTRKOs vs controls during lactation (3.61 vs 5.22 respectively, n=9-4), and this is associated with increased bone resorption as measured by increased bone cathepsin K expression (p=0.03, n=6-10). These data are consistent with our hypothesis that the CTR acts to protect the maternal skeleton from excessive resorption during lactation. Further analysis of the OCL-specific CTRKOs will allow us to determine if this increased bone resorption is predominantly due to the loss of CTR activity in osteoclasts.

1. Davey RA et al. The calcitonin receptor plays a physiological role to protect against hypercalcemia in mice JBMR 23(8):1182-93, 2008.

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**Audit of native joint septic arthritis at a single Melbourne hospital over five years**

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**Aim:**

To evaluate the clinical, epidemiological and laboratory details of patients presenting to a single Melbourne Tertiary Hospital with native joint septic arthritis (defined by criteria specified previously by Newman[1]) over a five year period.

**Method:**
A retrospective chart review was completed of all patients admitted to the Austin Hospital Melbourne with native joint septic arthritis between 01/01/2007 to 01/01/2012. Cases were identified from (1) hospital discharge summaries (2) the Austin Hospital orthopaedic department’s electronic database of cases and referrals (3) The Austin Hospital Microbiology Laboratory database of all aspirate and cultures performed in the study period.

Results:
51 cases of native joint septic arthritis were identified. The mean age was 53, and 32 cases (63%) were male. The mortality rate was 6%.
Of the 51 cases 35 (70%) had a synovial fluid aspirate performed, and of these patients 24% had a positive gram stain, and 37% grew a pathogenic organism. A synovial fluid cell count of > 50,000 was only seen in 29% of patients.
The three most commonly affected joints were the knee (51%), wrist (13%) and shoulder (10%). The most common organisms isolated were *Staphylococcus aureus* (41%) and *Streptococcus* (12%).
A number of variables were found to be associated with length of stay, time to theatre and also the probability of isolating a pathogen.

Conclusions:
Septic arthritis is a rare condition but has a high morbidity and mortality. In our cohort traditional risk factors such as positive gram stain on aspirate and elevated cell count had poor predictive value for the diagnosis.

Objectives- To improve efficiency within the Outreach service to allow for the increased time required to ensure adequate care of our MND patients while maintaining the level of service currently provided to the whole of our client group.

Method- The following changes in practice, for our entire client group, were developed and implemented:

- A more streamlined ventilator servicing process
- Reducing the potential for client/carer error, extra carer education, and the need for ongoing intervention from Outreach staff by providing a second same model ventilator
- Biannual education workshops for care providers
- The sourcing of an external provider for ventilator external battery equipment who can also provide 24/7 support

Outcome- The MND/NIV group now benefit from:

- Increased ability for Outreach home visit to address NIV and PEG issues
- Increased availability in the office to address phone enquires
- Increased access to relevant education for carers of those living with NIV with flow-on effect of continuity of care and improved communication between care providers
- Increased access to professional assistance should battery equipment issues occur

Nurses' attitudes to working with older people in acute care

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Introduction: There is little evidence that factors influencing attitudes to older persons have been investigated (Moyle 2003). Changes to service delivery models within acute health have meant that there has been an increase in Day of Surgery Admissions (DOSA) and a decrease in length of stay. Therefore, in-patient populations in acute care are generally older, with greater acuity and increasing co-morbidities. Australia expects an increase from 4.3 million older persons in 2021 to around 6.8 million in 2051 (Australian Bureau of Statistics (ABS, 2008). Aim: The aim of this study is to build on existing research (Courtney, Tong and Walsh 2000; McLafferty 2005; Wray and McCall 2007; Poole 2009), with a particular focus on immersion in practice and the impact of experience through immersion in practice on nurses' attitudes to working with older people. Methods: This study is ethnographic and utilized individual semi-structured interviews with 15 nurses. Four nurses agreed to be interviewed again to more fully explore their experiences of working with older people. The interviews were transcribed verbatim and thematically analysed. Analysis continued until saturation when no further themes were emerging from the data. Findings: Three themes emerged from the analysis: Role-modelling, Dependence and Relationality. These themes are consistent with the existing literature and have been used to inform a professional development program for nurses.

Conclusions: Immersion in clinical practice and socially constructed reflection impact nurses' attitudes to working with older people in acute care.

The themes have informed the design of a professional development program that utilises a reflective practice paradigm. I have called the program Achieving Relational Reflection: improving care of older people.
Health outcomes for STEMI patients undergoing a primary percutaneous coronary intervention in one metropolitan hospital

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\textbf{BACKGROUND} Timely treatment for ST-segment elevation myocardial infarction (STEMI) is critical to patient outcomes. This study compared door to balloon times (DTBT) as hospital processes changed to optimise timely treatment at one metropolitan cardiac catheterisation laboratory.

\textbf{METHOD} A five-year retrospective review on all patients who received coronary intervention for STEMI was conducted. During the study there were two process changes. Comparisons were made of baseline characteristics, clinical variables and long-term outcomes for DTBT greater or less than 90 minutes, and between process change groups. ANOVA and Chi-square were calculated where appropriate. Logistic regression identified predictors of DTBT<90 minutes. Data analysis: SPSS V19.

\textbf{RESULTS} 470 STEMI patients underwent immediate coronary intervention. Median DTBT time in minutes (') improved with each process change (109' vs. 79' vs. 70'; p<0.001). Those receiving timely treatment (DTBT <90 minutes) were younger (p<0.04), male (p<0.04), presented via ambulance (p<0.001) during business hours (p<0.001). Timely treatment was associated with a lower Thrombolysis In Myocardial Infarction (TIMI) risk score (p<0.01), lower in-hospital mortality (3.7% vs. 10.8%; p=0.0002), lower 365-day mortality (3.7% vs. 15.3%; p=0.001) and receiving phase 1 and 2 cardiac rehabilitation (p<0.01). Length of stay, unplanned representation and readmission were similar (p=NS).

\textbf{CONCLUSION} This study reports DTBT for a centre that introduced process changes resulting in improved time to treatment for STEMI patients. Earlier treatment was associated with better short and long-term survival. Further work is required to explore the disparity between age, gender and time to treatment.

A retrospective comparative study of acute cardiac care in a level two metropolitan and regional hospital

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Guidelines for the management of acute coronary syndrome exist to optimise health outcomes. Little evidence reports comparisons between regional and metropolitan health facilities.

\textit{Aim} i) identify the profile of the regional and metropolitan acute cardiac patient; ii) identify the documented acute cardiac management in both settings and iii) measure unplanned cardiac representation and readmission rates six months following the index admission.

\textit{Method} A retrospective study of the first 100 patients admitted to one regional or metropolitan Level Two public hospital in 2011 was conducted. Files were identified in chronological order based on an ICD-10 code of I21 (AMI). Comparisons were made of baseline characteristics, clinical variables and health outcomes to six months. T- tests, Chi-square and Mann-Whitney U tests were calculated where appropriate. Data analysis: SPSS V20.

\textit{Results} There were 182 files that met the inclusion criteria, 86% had a documented discharge diagnosis of NSTEMI and 14% STEMI. There were no statistically significant differences between locations by marital status, residence, mode of transport to the Emergency
Departments. Metropolitan subjects were statistically significantly different when compared by gender (p=0.04), country of birth (p<0.001), with more modifiable cardiac risk factors: diabetes (p<0.01), hypercholesterolemia (p<0.01), smoking status (p<0.01), obesity (p<0.001). Regional patients reported a history of CVD (p<0.001), skin disease (p=0.04). Metropolitan subjects were managed in a dedicated Coronary Care Unit (p<0.001), received phase one (p<0.001), phase two (p<0.01) but not phase three (p<0.001) cardiac rehabilitation. No differences were identified between locations six months post index admission by unplanned ED presentation (p=0.14) or admission (p=0.12). Conclusions Differences exist in the characteristics and clinical management. Representation and readmission rates six months from the index admission are not statistically significantly different.

A comparison of mean door to needle for thrombolysis as compared to mean door to balloon time for primary PCI in the first 12 months of a new cardiac cath lab

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Background Survival following ST segment Elevation Myocardial Infarction (STEMI) can depend on rapid access to treatment. Fibrinolysis was the standard of care; this has been replaced with Primary Percutaneous Coronary Intervention (PPCI). National guidelines report a door to balloon (DTB) time < 90 minutes is best practice. International guidelines report that this needs to be achieved with 75% of the presenting population. Aim We sought to compare the DTB times in the first year of a new PPCI service compared with historical door-to-needle (DTN) times in that same hospital of all patients who presented to the Emergency Department (ED) with a STEMI. Methodology A retrospective review of all patients who presented with STEMI in a 12 month calendar year were compared with the first 12 months of prospective data on all who presented to ED with a STEMI who underwent a PPCI. Parametric and non-parametric tests were used as appropriate. Analysis: SPSS V19. Results Ninety seven patients met the study inclusion criteria. Sixty nine patients underwent fibrinolysis and 28 patients underwent PPCI in the first year. Independent t-tests identified no statistically significant differences in all those who presented to the ED with a STEMI and were treated with either thrombolysis in 2006 or PCI in 2007 with respect to Age: t(95)= -0.59, p=0.55; gender: t(42)=1.14, p= 0.26 or; place of residence: t(90)= -0.478, p=0.63. Time to treatment for each strategy was statistically significantly different: DTN 36 minutes, DTB 53 minutes, p<0.01. A secondary analysis identified no change in DTN time by seasonal presentation p=0.52 to the ED. Conclusion Results suggest that the DTB times were well within the accepted time frames for improving outcomes compared with fibrinolysis. A new clinical service can meet the benchmark in timely treatment from the outset.

Benefit finding in cancer: enabling factors and health outcomes

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Background: The ongoing challenges faced by those living with cancer have prompted psycho-oncology research to more closely examine the association between effective coping
strategies and health outcomes. Emerging research is revealing that the use of positive coping strategies may be beneficial to people living with and beyond cancer treatment. Benefit finding (BF) is a cognitively-mediated, positive-oriented coping strategy believed to influence psychological and physical health outcomes.

Aims/Method: The Review summarises the current state of knowledge about; 1] factors that influence the capacity to use BF in cancer caused adversity and, 2] the associated health benefits. Studies were identified through an electronic multi data-base (CINAHL, Medline, PsychINFO, and Embase. 1990-2012) search strategy, using key search words ‘benefit find*’, ‘find* benefit’, ‘perceived benefit’, cope*, ‘positive emotions’, and ‘neoplasms’. Published studies in English that used a validated BF measurement tool with adult cancer populations formed the basis of the Review (n=10).

Results: Positive associations between social support (between-person factor) (n=5/10) and BF, and optimism (within-person factor) (n=5/10) and BF were consistently reported. The relationship between depression and BF (n=6/10) was reported as a negative correlation (n=3/6 - more BF less depression), no association (n=2/6), and a positive association (n=1/6 - more BF higher depression). Increased use of BF was associated with a reduction in stress-related bio-markers (n=2/10).

Conclusion: Differences of homogeneity, measurement tools, sample sizes, and time line in cancer trajectory have likely contributed to the inconsistent association between depression and BF. Further research is required to determine whether psychological and/or physical health outcomes are most impacted by BF.

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The introduction of a Nurse Practitioner model to acute inpatient care setting.

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This poster describes the development and implementation of the nurse practitioner role within the Department of Neurosurgery acute care setting in Melbourne Australia.

Background. Neurosurgical activity within the acute care ward has been increasing. Public health networks around Australia have introduced a variety of initiatives to address the growing concern of timely access for specialised care. One such initiative has been the introduction of nurse practitioners

Methods. Through investigation of literature, local practices and national and international practice was performed

Results

• Endorsement of the neurosurgery NP (first in state of Victoria)
• Expansion of scope of practice to include more complex patient groups & procedures
• Further NPs models developed & implemented at Austin Health

Conclusions. The implementation of the NP & now NP role within the Department of Neurosurgery at Austin health has been proven to be a success for patients and staff alike

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An evaluation of a structured learning program as a component of the clinical practicum in undergraduate nurse education: a repeated measures analysis

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Background: A previous publication had reported a reduction in anxiety and increased self efficacy following participation in a 3-day structured learning programme for final year
Bachelor of Nursing (BN) students undertaking an acute care clinical placement at Austin Health.

Aim: To examine whether the benefits of the 3 day structured learning program are maintained until completion of the clinical practicum.

Method: A repeated measures design was used. The intervention comprised the 3-day program on starting the clinical practicum. A questionnaire included the anxiety subscale of The Hospital Anxiety & Depression Scale (The HAD) and the General Self-Efficacy Scale (GSES-12). The questionnaire was completed day one, completion of the 3-day program (Time Two) and completion of placement on day 18 (Time Three). Parametric and non-parametric tests were used as appropriate. Data analysis: SPSS V19.

Results: Questionnaires were collated at baseline (n=116), Time 2 (n=90) and Time 3 (n=32). There was a statistically significant effect in reducing anxiety and this was maintained over time: F(2,42)=22.89, p<0.001. There was a statistically significant effect in increasing self efficacy and this was maintained over time: F(2,30)=25.40, p<0.001.

Conclusion: This is the first report that we are aware of that has measured final year BN student’s report of both anxiety and self efficacy over repeated measures of time. Students continue to benefit from a structured learning programme and the benefit of the intervention is sustained for the clinical placement duration.

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ISBAR (VITALSS)- Creating patient safety through improved nursing handover

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Background
Communication errors occurring during nursing shift-to-shift handovers threaten patient safety. Implementing a structured handover process reduces communication errors between nursing shifts. To improve clinical handover the ISBAR(VITALSS) clinical handover process was introduced at Austin Health.

Aim
This study sought to evaluate the implementation of ISBAR(VITALSS) as the nursing shift-to-shift handover process.

Method
On one acute ward, the ISBAR(VITALSS) nursing handover process was introduced over a two-month period. Educational material and training sessions for nursing staff based on the Australian Commission for Safety and Quality in Health Care (ACSQHC) guidelines for used to assist implementation. A pre and post implementation audit of ISBAR(VITALSS) using a ‘care plan’ and ‘medication chart’ audit and staff satisfaction survey was performed.

Results
Pre-implementation revealed a low rate of accuracy and completion of patient care plans and a high percentage of missed medication doses. In the post-implementation period the accuracy and completion of patient care plans was almost 100% and there was a 27% reduction in missed medication doses. Staff survey findings revealed that the process of the handover had improved dramatically since the introduction of the ISBAR(VITALSS) process and was now the preferred method of nursing handover.

Conclusion
ISBAR(VITALSS) brought a standardised approach to nursing shift handover at a single ward at Austin Health. Introduction of ISBAR(VITALSS) saw an improvement in the maintenance of nursing care plans and a reduction in missed medication doses. Importantly, nursing staff valued the ISBAR(VITALSS) process that provided a structure and logical sequence to handover. Organisation wide implementation of ISBAR(VITALSS) for Austin Health is planned for July, 2012.
The addition of partially hydrolysed guar gum (PHGG) prevents diarrhoea in critically ill patients receiving enteral nutrition

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Aim/Background: Diarrhoea is common in critically ill patients receiving enteral nutrition. Resultant interruptions in enteral feeding may lead to increased length of hospital stay and/or malnutrition. This pilot study aims to determine if PHGG (dietary fibre) added to formula reduces the incidence and severity of diarrhoea experienced in enterally fed ICU patients.

Methods: Ten participants admitted to Austin Health ICU, being enterally fed for at least four days were randomised to receive PHGG (21g/day) or control (21g maltodextrin) via addition to their enteral formula. The incidence and severity of diarrhoea and the use of aperients was determined. Consistency of bowel movements and incidence of gastrointestinal symptoms were also recorded.

Results: Four (80%) patients in the control and three (60%) in the treatment group experienced diarrhoea. There was a significant reduction in the frequency of loose stool days in the treatment group as compared to the control group (54.8% vs. 78.8%; P=0.018) with greater use of aperients in the control group. In addition a trend was shown towards increased frequency of formed stool in the treatment group. Four patients in the control group suffered peri-anal excoriation whilst no patients in the treatment group experienced this complication (P=0.048).

Discussion / Conclusions: Participants that received PHGG experienced less diarrhoea than those in the control group. In addition there were less diarrhoea related complications and use of aperients in the intervention group. This study was limited by low numbers and recruitment is ongoing in order to confirm this effect.

Consuming two additional serves of dairy food a day significantly improves energy and nutrient intakes in ambulatory aged care residents: A feasibility study

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Low-level aged care residents are at high risk of malnutrition. Oral supplements and fortified foods are used to overcome malnutrition in the elderly but require special preparation and administration by staff, over and above standard food and beverages served. We proposed that increasing current dairy food intake in residents from two to four serves per day would improve energy and nutrient intakes and prevent malnutrition. This was a prospective intervention study involving 68 residents (78% female, mean age 86.5 years) in 2 low-level aged care facilities in Melbourne, Australia. Menus were modified to include at least two additional serves of dairy food per day. Mean macro- and micro-nutrient intakes before and after intervention were recorded using observed intake (food served minus waste) and comparisons in intakes made using paired t-tests. Following intervention, daily increases in mean energy intake (900kJ, p<0.001), protein intake (+25g, p<0.0001), proportion of energy from protein (+4%, p<0.0001) and proportion of estimated energy requirements (+18%, p<0.0001) were observed, while proportion of energy from fat decreased (-3%, p<0.0001).

Increases in mean daily micronutrient intakes were observed for numerous nutrients including calcium (+679mg, p<0.0001), vitamin D (+1.4mg, p<0.0001), phosphorus (+550mg, p<0.0001), and zinc (+2.8mg, p<0.0001), with recommended intake levels achieved on the higher dairy diet. Mean sodium intakes remained unchanged. Two additional serves of dairy food can significantly improve nutrient intake in aged care residents and its ease of provision makes it a viable option to potentially prevent malnutrition.
Meeting the Nutritional Needs of Elderly Residents in Aged-Care: Are We Doing Enough?

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Institutionalized elderly are at high risk of malnutrition, including low-level aged-care residents who can self-feed. We used comprehensive dietary intake assessments to determine the nutritional adequacy of food served to residents and if food waste contributed to insufficient nutrient intakes. This cross sectional study involved 199 residents (mean age 86.7yrs, 76% females) from 18 low-level aged-care facilities. Dietary data was ascertained using 3-6 day weighed food records. Foods were categorized into main food groups (grains, fruit, vegetables, meats, dairy and ‘extra’) and quantified based on recommended serving sizes. Chi squared test were used to determine sex differences in proportion of residents below recommended intake levels. Residents were provided with sufficient serves of fruit (>2) and meats (>1), but not dairy (<3), vegetables (<5) and grain foods (women only, <4), and excess serves of ‘extra’ foods (>2). Mean dietary intakes did not meet recommendations for calcium, zinc, magnesium, potassium, folate and dietary fibre with many residents not meeting energy and protein requirements. Sodium intake was 4-5 times higher than recommended, and sugars consumed in excess. Food waste resulted in men not consuming recommended serves of grain foods and contributed to mean intakes of women not achieving recommended levels for iron and phosphorus. ‘Extra’ foods contributed substantially to energy intake but provided few of the required nutrients. Substituting some ‘extra’ foods for serves of dairy, vegetables and wholegrain foods would improve the nutritional quality of foods, without altering food volume, so is feasible to improve nutritional status in elderly aged-care residents.

Evaluation of protected mealtime strategies at lunchtime on a subacute ward

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Aim: Protected meal times is a NHS initiative and this concept has been used on the oncology wards to improve meal access and potentially reduce malnutrition. It was theorised that successful implementation on other wards would require individualised approaches. We aimed to identify specific barriers for patients on a subacute ward accessing meals and develop strategies for improvement.

Method and Results: Baseline: Redesign methodology including observation of patients (n=34) at lunchtime, value stream mapping and root cause analysis was used to determine specific barriers to food access. Main barriers observed prior to meal commencement were: patients returning late from therapy, difficulty opening packaging and interruptions by staff. During meal consumption, the most prominent issues were: difficulty opening packaging, waiting for assistance and interruptions by staff and visitors. Implementation: Staff, patients and visitors were educated regarding protected mealtimes, packaging was replaced with easier to open alternatives. Timing for lunch delivery was deferred to enable patients at therapy to return on time. Evaluation: Observation (n=19) was repeated and the number of patients experiencing delays prior to meals reduced from 62% to 11%, the maximum delay reduced from 46 minutes to 7 minutes. During consumption of lunch, interruptions reduced from 76% to 28% of patients with the maximum interruption time decreasing from 30 minutes to 7 minutes.

Conclusion: Protected meal time strategies have been shown to improve a patient’s access to meals. Current work is being done to evaluate barriers on other subacute wards across Austin Health and develop individual ward strategies to address meal access.
**Saliva composition and upper gastro-intestinal symptoms in chronic renal disease**

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Aim: To determine any associations between changes in salivary composition and upper gastrointestinal (GI) symptoms in chronic renal failure patients.

Background: Many chronic kidney disease (CKD) patients experience uraemic symptoms including dry mouth, taste changes, nausea, vomiting and dry retching. Saliva is composed of a number of active compounds that play vital roles in taste stimulation. Salivary composition differs in CKD and whether these changes affect uraemic symptoms is unknown.

Methods: Thirty CKD patients (24 males, 6 females, age 69.7 ± 14.2yrs, glomerular filtration rate <25mL/min) were recruited from the Austin Hospital outpatient renal clinic. A saliva sample was collected to determine biochemical composition. A symptom questionnaire regarding taste changes and upper GI symptoms experienced was completed.

Results: Only 3 (10%) CKD patients reported no upper GI symptoms while 63% complained of a dry mouth, 56% had a change in taste, 30% complained of nausea and 20% vomited or dry retched. Saliva bicarbonate concentration was inversely related to both dry mouth (p<0.003) and dry retching (p<0.01). An elevated level of saliva calcium and a dry mouth also reached significance (p<0.01). Nausea was reported with higher saliva sodium levels (p<0.03) and a higher saliva sodium/potassium ratio (p<0.02). Forty-three percent of patients indicated their symptoms contributed to decreased food intake.

Conclusions: This study provides evidence that active compounds are present in the salivary fluid and can impact upper GI symptoms in CKD. In particular lower saliva concentrations of bicarbonate are associated with dry mouth and retching. Higher saliva calcium levels also related to a dry mouth, while higher sodium levels and a greater sodium/potassium ratio were associated with nausea.

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**Appetite After Weight Loss by Two Different Energy Restricted Diets.**

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Introduction: Failure to maintain weight loss is likely due to a series of co-ordinated physiological adaptations designed to encourage weight regain. This project aimed to determine the impact of the rate of weight loss on appetite, leptin, insulin, ghrelin and glucose levels.

Methods: Initial baseline testing was performed then all 200 participants were randomised at a 1:1 ratio to:

A. Rapid Weight Loss Diet (RD)-average daily intake ~3,500-4,000Kj.
B. Gradual Weight Loss Diet (GD)- average daily intake~8,000-9,000Kj.

Following the weight loss intervention participants who lost 15% of their body weight underwent measurements as per baseline visit.

Results: Of the 200 participants (51 male and 153 female) average age 50±1 years, and initial BMI 35.3 ± 0.3 m/kg². Following the intervention, 78.4% of the RD achieved the goal of 15% weight loss compared to 52.4% in the GD (p<0.001). No differences in the change in fasting glucose (p=0.10), leptin (p=0.15), ghrelin (p=0.24) and insulin (p=0.31) were seen between the RD and GD. Fasting desire to eat (DTE) (p=0.018), hunger (p=0.003) and prospective food consumption (PFC) (p=0.02) increased following the GD. In contrast, fasting DTE and hunger did not change (p=0.85, p=0.34; respectively), and PFC decreased (p=0.075) following the RD.
Conclusion: This study suggests that slow weight loss results in greater feelings of hunger, DTE and PFC than rapid loss. Rate of weight loss does not influence appetite regulating hormones/nutrients.

References:

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Post-operative night splinting for twelve weeks is no better than six weeks in Dupuytren’s Disease- A Prospective randomised trial

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Purpose:
Controversy surrounds the use of postoperative splinting following surgery for Dupuytren’s disease. Many believe splinting prevents early recurrence of contracture, however much of the information about its value as a therapeutic tool is based upon empirical evidence. Further controversy exists on the duration of splinting. We investigated the duration of static night splinting on recurrence of flexion contracture and range of motion following fasciectomy.

Methods:
This prospective randomised controlled trial was undertaken at Austin Health. Patients with Dupuytren’s contracture had fasciectomy. Following 1-2 weeks in a volar plaster slab applied in theatre, subjects were randomised into two groups of static night extension splinting for either 6 or 12 weeks postoperatively.

Three hundred and four patients with 463 affected MCP joints and 312 affected PIP joints were randomized. Range of motion measurements have been taken pre-operatively, then at the following time points postoperatively: 1-2 weeks, 3 weeks, 6 weeks, 12 weeks, 18 weeks, 24 weeks and 1 year.

Results & Conclusions:
We found that night splinting for 12 weeks following surgery had no extra benefits than night splinting for 6 weeks. No significant differences were found between the two groups in terms of achieving and maintaining joint extension or regaining joint flexion after surgery. As the joint was not violated in the operative procedure, it was suggestive that postoperative night splinting during the fibroblastic phase of wound healing was sufficient to prevent early recurrent joint deformity due to scar contracture. Importantly, the use of night splinting did not adversely affect the ability to regain active joint flexion as MCP, PIP and DIP joint flexion were regained to preoperative levels during the 18-week postoperative follow-up period in both groups.

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Using meaningful occupation as an intervention approach to support the contemporary model of palliative care at Austin Health

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“Palliative care is an approach that aims to improve quality of life (QOL) for patients and their carers facing the problems associated with a life threatening illness” (WHO, 2012). Until now, palliative care has been seen to largely focussed on medical management of specific symptoms, with little or no consideration given to the patient’s occupational identity and goals. Occupational Therapy (OT) is a profession whose core philosophy is grounded in occupational participation (Keesing & Rosenwax, 2011). OT’s have the skills and expertise to incorporate an individual’s functional goals into their treatment plan, thereby assisting people to participate in personally meaningful occupations, within the limits of their illness and physical capacity.

This poster illustrates how personally meaningful occupational participation has been used to support a contemporary model of palliative care at Austin Health, resulting in better patient
outcomes and improved QOL for patients and their carers. This poster also intends to challenge current thinking on the role of OT within palliative care and looks to how this profession can be expanded to incorporate client-centred goal setting within contemporary palliative care practice.

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Lumbosacral radicular syndrome: a novel clinical decision-making model to optimise referral to spinal surgery following a trial of physiotherapy.

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Aim: First-time referrals to neurosurgical clinics are often suboptimal and long waiting lists compromise patient care. Improved primary care reduces waiting times and improves specificity of surgical referrals¹. We have designed a novel clinical decision-making model for primary care management of lumbosacral radicular syndrome (LRS). Comprehensive physiotherapy baseline and follow-up assessments (including clinical examination, pain and function measures, radiology) are compared to identify cases requiring surgical referral. This pilot study aimed to test the clinical utility of pathways within the model.

Method: Utility of the model was assessed in a case series of patients with LRS (n= 21) referred for a trial of physiotherapy (guideline recommendation prior to referral to neurosurgical outpatients by general practitioners)¹. After patient-specific physiotherapy intervention was trialled², clinical examination, pain and function outcomes (baseline and follow-up) were compared to distinguish recovery from failed conservative management. Deterioration in pain (numerical scale 0-10) and function scores (Oswestry Disability Index - ODI; Roland-Morris Questionnaire - RMQ) potentiated referral for spinal surgery.

Results: Eleven patients recovered with physiotherapy alone and 10 proceeded to surgery. There were no significant differences between the surgery group (SG) and the non-surgery group (NSG) for age (SG 55.5±17.8; NSG 50.5±14.2, p=0.48) or baseline scores: pain (p=0.68), ODI (p=0.78), RMQ (p=0.72). Significant differences were noted between the SG and the NSG after physiotherapy intervention (pain: p=0.001; ODI: p=0.004; RMQ: p=0.0002). Patients also improved significantly after surgery (pain: p=0.007; RMQ: p=0.04).

Conclusion: Results suggest physiotherapy alone is efficient for primary care, non-surgical treatment of patients with LRS. A key advantage of the model is appropriate patient disposition and improved specificity of referral for spinal surgery³, without compromising sensitivity of patient selection.

Quantifying physical activity levels of survivors of intensive care: A prospective observational study

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Background: Promotion of increased physical activity is advocated for survivors of an ICU admission to improve physical function and health related quality of life [1].

Aim: The primary aims of this study were: to measure free-living physical activity levels and correlate measures with scores on a self-reported activity questionnaire. The secondary aim was to explore factors associated with physical activity levels.

Method: Nested within a larger RCT, participants were block randomised to measure free-living physical activity levels. At 2 months following ICU discharge, included participants wore an accelerometer for 7 days during waking hours. At the end of the 7 days monitoring, participants were interviewed using the Physical Activity Scale for the Elderly (PASE) questionnaire. Factors associated with physical activity were explored using regression analysis.

Results: The ICU survivors were inactive when objectively measured at 2 months following ICU discharge. Participants spent an average of 90% of the time inactive and only 3% of the time walking. Only 37% of the sample spent 30 minutes or more per day in the locomotion category defined as more than 20 steps in a row. Activity reported using the PASE questionnaire was lower than that reported in adults who were healthy. The PASE scores correlated only fairly with activity measured by steps per day. The presence of co-morbidities explained one third of the variance in physical activity levels.

Conclusions: Survivors of an ICU admission greater than 5 days demonstrated high levels of inactivity for prolonged periods at 2 months following ICU discharge, and the majority did not meet international recommendations regarding physical activity. Presence of co-morbidity appears to be an influential factor associated with activity levels.

A new physical function test for use in ICU: Validity and responsiveness of the PFIT-s

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Background: Early exercise intervention aims to improve morbidity and outcomes for ICU survivors [1]. Conventional exercise tests are often impossible to perform in the ICU due to patient weakness and environmental limitations

Aim: To improve the utility of the physical function in ICU test (PFIT) [2] by deriving an interval total score (PFIT-s) and to test its clinimetric properties.

Method: This was a nested study recruiting 150 participants who performed the PFIT at ICU admission and discharge. Original test components were modified using principle component analysis. Rasch analysis was performed to examine the unidimensionality of the PFIT and an interval score derived from performance on the original modified PFIT. Correlations were performed to test validity and multiple regression analyses to predict predictive ability. Responsiveness was assessed using the effect size index (ESI) and the minimally clinically important difference (MCID) was calculated.

Results: The bilateral component of the original PFIT was removed. Unidimensionality of the admission and discharge PFIT-s was confirmed. The PFIT-s demonstrated moderate
convergent validity with the timed up and go test ($r=0.60$), the six minute walk test ($r=0.41$) and Medical research Council (MRC) sum score ($\rhoo=0.49$). The ESI of the PFIT-s was 0.82 and the MCID was 1.5 points (interval scale ranging 0-10). Higher admission PFIT-s was predictive of the development of ICU acquired weakness (MRC sum score ≤48): increased likelihood of discharge home; reduced likelihood of discharge to inpatient rehabilitation and reduced acute hospital length of stay.

Conclusions: The PFIT-s is a safe and inexpensive test of physical function with high clinical utility. It is valid, responsive to change and predictive of key outcomes. We recommend that the PFIT-s be adopted to test physical function in ICU.


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**Is Physiotherapy effective for children and adolescents with Complex Regional Pain Syndrome?**

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**Background and Aims:** Physiotherapy is recommended in clinical guidelines as a treatment for CRPS-1 [1]. While evidence exists to support the use of physiotherapy in adults [2], it is not clear which techniques are the most effective in children and adolescents. This study synthesised current research evidence to provide physiotherapy treatment recommendations and to identify areas for further investigation.

**Methods:** Nine electronic databases were searched for quantitative studies which evaluated the effect of physiotherapy on children and adolescents with CRPS-1. The methodological quality of the studies was independently evaluated by two researchers using the McMaster Critical Review Form. Data were extracted regarding the study design, participant characteristics, physiotherapy technique and its effectiveness. Results were synthesised narratively.

**Results:** The search strategy identified 303 articles; 12 met inclusion criteria. The ‘stand-alone’ value of physiotherapy could not be determined as physiotherapy was prescribed with psychological and medical interventions. Moreover, physiotherapy treatments varied between studies and were often not described to allow replication in the clinical setting. There is low volume, poor to fair quality evidence which suggests that physiotherapy prescribed with other interventions may lead to short-term improvement in signs and symptoms or functional ability in children with CRPS-1.

**Conclusion:** There is a paucity of high quality evidence on the effectiveness of physiotherapy for children and adolescents with CRPS-1. Future studies should evaluate a package of care, which includes physiotherapy, investigate the effects of physiotherapy treatments with proven effectiveness in adults with CRPS-1 and provide details of the physiotherapy intervention to allow replication in clinical practice.

This systematic review was published in the Clinical Journal of Pain, January 2012, and is available online at www.clinicalpain.com

The clinimetric properties of the Radboud Skills Questionnaire and the Human Activity Profile for adult upper limb Complex Regional Pain Syndrome.

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Aims: CRPS-1 is a painful, disabling condition that occurs in up to 7% of upper limb fractures. Chronicity is common, despite intensive clinical management. Effective clinical management is challenged by the lack of reliable and valid tools that measure the impact of CRPS-1 and the effect of treatments not only on impairments but also activity limitations and participation restrictions. The aim of this study was to investigate the clinimetric properties of the Radboud Skills Questionnaire (RASQ) [1] and the Human Activity Profile (HAP) [2] in a group of adults with upper limb CRPS-1. Methods: All participants were diagnosed with CRPS-1 in an upper limb according to IASP revised diagnostic criteria [3]. The English language version of the RASQ, the HAP and a participant feedback questionnaire were completed by twenty participants on two occasions, two weeks apart, to evaluate the face and content validity, reliability, interpretability and feasibility of the two questionnaires. Participants were predominantly female (75%), with a mean age of 53.7 (7.0) years and mean duration of CRPS-1 of 33.1 (29.7) months. Results: The RASQ and HAP have adequate face and content validity. The Adjusted Activity Score (AAS) on the HAP was found to have superior test-retest reliability (ICC2,1=0.96) to the Maximum Activity Score on the HAP and all sub-scores on the RASQ. The interpretability, measured by the Minimum Detectable Change favoured the AAS on the HAP. Feasibility was greater for the HAP. Conclusion: Based on face, content validity, test-retest reliability, interpretability and feasibility, the HAP was the most appropriate questionnaire to evaluate activity limitation and participation restriction in Australian adults with upper limb CRPS-1. The number of subscores on the RASQ and the lack of a valid aggregate score diminished its clinimetric properties.

1. Oerlemans et al, 2000 Disability and Rehabilitation 20: 233-245
the patient’s discharge from ICU. This was compared with actual 12 month patient outcomes measured in the larger trial.³

Results
Physicians predictions for mortality had the highest sensitivity & specificity (95% CI) 83% (78-91%), 96% (36-100%), followed by the physiotherapists 50% (12-88%), 100% (89-100%). Physicians were more accurate than physiotherapists in predicting future HRQoL (p = 0.04). Physicians, physiotherapists & nurses were comparable in their predictions of who would return home with specificity of (95% CI) of 100% (CI 84-100%), 96% (CI 79-99%) & 94% (CI 73-99) respectively. There was moderate correlation between the Functional Comorbidity Index & the Physical Component Score of the Short-Form 36 V2 (rho = -0.60).

Conclusions
Intensivists are well placed to make assessments of future functional capacity, HRQoL & mortality of survivors. Whilst intensivists, nurses & physiotherapists were all good at predicting who would return home, physiotherapists were best at predicting who would survive.


The incidence of benign paroxysmal positional vertigo (BPPV) in patients admitted to the Acquired Brain Injury (ABI) Unit.

Ro Packer¹

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Aims: BPPV is the most common cause of vertigo in adults that, if detected, can be treated quickly and with good outcomes. Head trauma has been described as a cause of BPPV, however, little has been recorded regarding the incidence in inpatient ABI patients. Aims of this review were to:

1. Determine the incidence and type of BPPV in patients admitted to the ABI unit.

Method: A prospective audit of all patients admitted over one year. Specific details regarding the type of BPPV, treatment techniques and response to treatment were collated.

Results: Data from 89 patients were collated. Forty-eight percent of all patients reported dizziness at some point during their inpatient stay with 40.6% of this group then clinically diagnosed with BPPV. All cases of BPPV were related to patients with traumatic brain injury with 17.8% of all patients admitted in this diagnostic group affected. Fifty-four percent of patients with BPPV had bilateral symptoms. Thirty percent of the patients treated had a resolution of symptoms after a single treatment while 70% required multiple treatment sessions.

Conclusion: The incidence of BPPV in this cohort of patients was far higher than an incidence of 2.4% that has been suggested for the general adult population. The results highlight BPPV as a potential cause of dizziness in inpatients in the traumatic ABI population. While multiple interventions were often required the overall outcome of treatment was good with cessation of all symptoms.
Falls in the first month following discharge from rehabilitation.

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Aim
There is limited data on falls rates for people being discharged home following inpatient rehabilitation. One study found that 30% of people with stroke fall within two weeks of discharge,1 indicating that the risk of falling may be increased in the immediate post-discharge period. The purpose of this study is to quantify i) the proportion of people who fall and ii) falls rate in the month immediately following discharge home in people who have received inpatient rehabilitation.

Method
One hundred and thirty three participants being discharged home following inpatient rehabilitation at Austin Health will be recruited. Participants will be contacted by a physiotherapist one month post-discharge to determine whether they have fallen during this time period.

Results
Data collection will be complete by the end of August. The proportion of fallers and the rate of falling over the one month period will be calculated.

Conclusions and implications for practice
People who have undergone inpatient rehabilitation are likely to be at increased risk of falls. There is evidence that individualised, multifactorial interventions may reduce falls in older people living in the community, 2 however it is not clear whether these strategies are effective in people being discharged from inpatient rehabilitation programs. This study will provide baseline data about how frequently falls occur immediately following discharge, and provide an insight into the size and nature of the problem. The results of this study could provide an impetus for changes in clinical practice that reduce the risk of falls in the community.

References

Do patients face delays resuming their usual medications after surgery?

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Aim
To determine whether elective surgery patients experience delays in recommencing their regular medications post-operatively.

Method
A retrospective chart audit was conducted at a major Melbourne metropolitan hospital. All patients admitted on the day of surgery in February 2012 were included.

Data collection included: number of regular medications taken pre-operatively, time of arrival on post-operative ward, date regular medications were charted and whether there was a delay in their administration, whether patients were on medications known to be problematic in the post-operative period such as warfarin or chronic opioids and whether the patient had attended pre-admission clinic.
Results

301 patients were identified; 95 were excluded (63 were not on regular medications, 31 were admitted to ICU and were unable to take oral medications, and the records for 1 patient were unavailable).

Of the remaining 206 patients, 9 (4.4%) and 23 (11.2%) were taking warfarin and chronic opioids, respectively. 69 (33.5%) patients missed some or all of their usual medications in the 24-hours post-surgery. 156 of the 206 (76%) patients were seen in pre-admission clinic. Of these, 63 of the 69 (91.3%) patients who had their medications charted in pre-admission clinic experienced no delay in administration of their medications post-operatively compared to 49 of 87 (56.3%) patients who did not have their chart written in clinic (difference in %=35.0%, p<0.001). Half of the patients not seen in pre-admission clinic missed some or all of their medications, post-surgery.

Conclusion

One third of elective surgery patients experienced a delay in resuming their usual medications post-operatively. Patients were least likely to experience delays in administration of their usual medications post-operatively if they were seen and had a medication chart written in pre-admission clinic. This baseline data will be used to evaluate the impact of increased pharmacist involvement in the pre-admission process.

Observation and response chart – facilitating early identification of deteriorating patients

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Background

Early identification of deteriorating patients remains a key area for improvement in Australian hospitals. The National Consensus Statement and Standard 9 of the National Safety and Quality Health Service Standards call for the introduction of track and trigger observation response and response chart (ORC). The ORC incorporates Urgent Clinical Review (UCR) criteria to prevent patients reaching MET criteria. In 2010 the Austin Health ‘track and trigger’ ORC was successfully trialled on 5 wards (6W, 5E, 7E, 7W and 8W).

Aim

To evaluate impact and clinical response to implementation of the Austin Health ‘track and trigger’ observation and response chart.

Method

Four ORC’s were developed to accommodate differences in emergency responses. Ward based project teams were established to lead the ORC introduction, to provide education on the clinical importance of abnormal physiology, use of the ORC, and discuss a case study and escalation actions in response to abnormal vital signs. From 23 wards, 10 patient observation charts were audited pre and 1 month post ORC introduction. Data collected included frequency of recorded vital signs, modification to MET criteria and responses to abnormal vital signs.

Results

Post ORC implementation findings identified improved compliance with the documented frequency of observations being performed. Documentation of all vital signs increased, especially for respiratory rate. Introduction of the UCR identified UCR being reached in 25% of audited cases with UCR criteria modified in 41% of cases. Although there was a 2% increase in the number of MET calls there was a corresponding 13% increase in the number of modified MET criteria.

Conclusion

Introduction of the Austin Health ORC has been associated with substantial improvement of vital sign documentation, and a subsequent increase in MET calls. There is need to improve rates of review when UCR criteria are fulfilled, and to understand clinicians attitudes towards the ORC, the escalation protocol, and MET activation process.
Ascitic Taps in a major quaternary liver transplant unit – is best practice safe enough?

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Background
Ascitic taps are perceived as relatively low risk that can be safely performed by any medical staff. However the safety of this procedure is poorly documented.

Aim
This study aims to document the current practice of ascitic taps at Austin Health, and compare against "best practice" techniques and complication rate.

Methodology
1. 12 months of ICD code “30406-00 Abdominal paracentesis” procedures for:
   a. Unique identifier
   b. Unit/ location
   c. Patient factors (age, sex, principle diagnosis)
   d. Outcome
2. Targeted clinical case review patients deceased within the same admission as procedure.

Results
691 procedures performed over 12 months, in 191 individual patients. 554/691 male patients (80.2%). 516/691 elective procedures (74.7%), with 436/691 (63%) day procedures, 249 (36%) overnight. The top 3 units were: 283/691 (40.9%) Gastroenterology/ Hepatology, 230/691 (33.3%) Liver Transplant unit, and 51/691 (7.4%) Oncology. The most procedures performed on an individual patient was 48. 153/691 (22.1%) ultrasound-guided.

Mortality: 4 deaths directly related to procedure(5.7 per 1000 procedures) compared to 1.6-3.9 per 1000 proceduresii

Morbidity: Ascitic leak 4pts, Severe abdominal pain 1pt

Other findings: poor documentation of technique, no single organizational policy, only 4 of 51 procedures had consent form.

Discussion
Ascitic taps are performed in significant volumes at Austin Health, mostly in Gastroenterological/ Liver Transplant units. Practice seems consistent with literature, however, the mortality rate is higher, and likely reflects the higher risk population.

Recommendations: (1) a single standardised policy for credentialing/ supervision/ technique for ascitic taps (2) consent essential (3) use of ultrasound guidance as the preferred model (4) centralised mechanism for auditing ascitic taps.


Up-to-date – ascitic tap complications, accessed 27 July 2012
Are clinical registries actually used? The level of medical staff engagement in Clinical Registries, and integration in formal clinical governance structures within a major tertiary teaching hospital

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Aim:
To document the level of medical staff engagement in Clinical Registries, and the level of integration into formal clinical governance structures within the organisation.

Methodology:
(1) Semi-structured interviews with Medical Leadership roles within Austin Health in August 2011, against benchmarked list of Registries (31 in total) from the Centre for Research Excellence in Patient Safety.
(2) Review of 12 months of minutes (September 2010 to August 2011) of formal Clinical Governance Committees for Registry data

Results:
Austin Health Medical Units contribute in full to all but one of the benchmarked list of relevant Registries. A further 15 opportunities for external benchmarking of patient outcomes were identified, including Department of Health initiatives and unit initiated benchmarking amongst individual clinicians from outside the health service. The Registries encompassed a broad range of surgical, medical, critical care, subacute and psychiatry specialties.

Registry data is integrated on a monthly basis in the Infection Control Committee, and Surgical Audit and Review Committee, and four ad-hoc presentations of Registries data was presented at Board Committee. However, there is a lack of systematic integration of Registries data into the Clinical Governance framework more broadly.

Conclusions and recommendations:
This study demonstrates not only a very high level of medical staff engagement in Clinical Registries, and potential opportunities for future clinical registry development, there is generally a lack of systematic integration of Clinical Registries data into the clinical governance framework beyond individual unit level.

Examining deterioration in the period prior to Medical Emergency Team review in a teaching hospital –A Cluster Review

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Background
Medical Emergency Teams (MET) have been established for more than a decade. With increasing focus on early identification and intervention of clinical deterioration, we examined for deterioration in the period prior to MET-calls.

Aim
To explore predictors of MET-calls, and evidence of delayed detection/ recognition/ escalating prior to MET activation.

Methodology
1. Audit of 12 months (August 2010– July 2011) of prospectively collected MET-call data
2. Randomly selected retrospective targeted case review (40)
Results:
1296 MET-calls in 12 months. Top wards: Surgical Ward-A 193 calls (14.9%), Surgical Ward-B 173 calls (13.3%), Medical Ward-A 125 (9.6%).
Top units bed-days adjusted: Urology (9.93/1000bd), Liver Transplant/ Hepatobiliary (9.26/1000bd), Haematology (8.64/1000bd), however General Medicine has most gross numbers at 274 (21.26%). Top Diagnostic-Related-Groups: Pneumonia-unspecified (32 calls), Congestive heart failure (23), Sepsis (23). MET-call triggers consistent with previous studies.

During MET: 735 received oxygen (56.7%), only 330 (25.5%) received any medication, ICU-level intervention: 9 intubation, 20 manual ventilation, 65 CPAP/BIPAP. 1109 (85.5%) patients remained on the ward, and 103 (7.9%) transferred to Intensive Care. 360 (27.7%) MET-calls in the setting of end-of-life issues.
Case reviews: 14 cases delayed activation by 1 hour, half with no documented escalation. Variable level of unit involvement in MET-call. 23 cases involved new problems unrelated to admission diagnosis.

Discussion and Conclusions:
Although complex surgical units have higher numbers of MET-calls, difficult to predict as over half involved new problems. Approximately one quarter of calls have sub-optimal observations and/or escalation of care. Recommendations: early detection/prevention of clinical deterioration, particularly in complex surgical patients; end-of-life issues; and further analysis of factors to reduce pre-MET deterioration.

Understanding Medical Emergency Responses in a Sub-acute Setting: a Cluster Review

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Background
Medical Emergency Teams (MET) have been established for more than a decade in acute health services, however it was unclear the nature and issues surrounding the Medical Emergency Response (MER) in the sub-acute setting.

Aim:
To assess the characteristics and outcomes of 12 months of MER-calls at Subacute campus

Methodology:
Retrospective analysis of 70 randomly selected MER calls from Mar 2011- Feb (total 182), using targeted audit tool for:
1. patient characteristics/ location/ward
2. clinical reason for escalation
3. proportion of patients that required transfer to acute facility
4. documentation of advanced care planning
5. mortality cases reviewed by Palliative Care

Results
Median age 81; 40/70 male (57%), 61/70 (87%) independent living at home prior to admission. Most occurred in the acute aged-care ward (27/70, 38.6%), 14/70 and 12/70 respectively in the two aged-care/rehabilitation wards. 43 of 70 (61%) between 8am-5pm. Top triggers consistent with acute literature. 28/70 (40%) in the first 48hrs of admission. Outcome: 33/70 (47%) remained on ward, 26/70 (37%) transferred to acute ward, 8/70 (11.4%) transferred to Intensive Care. 30/70 discharged home. 21 deceased in same admissions (4 same day as MER). 47/70 no documented escalation prior to MER. 56/70 had some element...
of limitations-of-treatment documented prior to MER. Mortality audit: 10/21 (50%) appropriate for MER, 18/21 (85%) clear documented limitations-of-treatment. Of those transferred, 10/21 (50%) deemed appropriate for transfer.

Discussion and Conclusions:
MER in sub-acute setting peaked within first 48 hours of admission, mostly in acute aged-care wards, often requiring transfer for higher level of acute ward management, necessitating seamless transfer mechanisms to an acute ward facility. The mortality rate was 30%, however 50% of deaths were deemed not appropriate for MER in light of documented limitations of treatment orders.

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**Understanding Medical Staff Perceptions of Patient Safety: A 3 year experience of Patient Safety Culture Survey in a major tertiary teaching hospital in Australia**

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**Background**
Internationally, perceptions of patient safety have been shown to vary across differing health professional groups. However, the perceptions of medical staff regarding patient safety culture within Australia are yet to be documented.

**Aim**
To document medical staff perceptions of the elements of Patient Safety within a major tertiary teaching hospital.

**Methodology**

**Results:**
Medical Staff response rate: 140 (9.3%) for 2010, 201 (13.4%) for 2011 and 98 (6.5%) 2012 (total approximately 1500 medical staff). Response rate proportionately distributed across specialties. On 3-yr average, 68.2% were Senior Medical Staff, 23.4% Registrars and 8.3% HMOs. Strengths identified: “Teamwork within units” rated positively by 82%, “communication openness” rated positively by 73%. Weakness identified: “Hospital handoffs and transitions” rated positive by 18%, “Staffing” rated positively by 36%, and “Frequency of events reported” rated positively by 36%. Medical staff rated 10 of 12 safety dimensions lower than the organization, with the largest difference in “Management support for patient safety” at 51% vs 64%. Only “Teamwork within units” (82% vs 80%), and “communication openness” (73% vs 71%) rated as higher, which is consistent with key international studies. Medical staff were also provided the opportunity for feedback on strategies to improve patient safety.

**Conclusions and Recommendations:**
This study documents an Australian experience of patient safety perceptions of medical staff and have been utilized to guide improvement strategies.

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**Physician Orders for Life Sustaining Treatment forms within an acute care setting.**

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**Aim:** There are only a limited number of studies on the content and completion of Physician Orders for Life Sustaining Treatment (POLST) forms. This study specifically examined the use of POLST forms within an acute hospital setting.

**Method:** We audited 3191 randomly selected Resuscitation Plans, a locally developed form of POLST, completed at Austin Health during 2011. Matching UR numbers resulted in 2163
individual patients (48.6% Male; 64% aged 75+), of whom 654 had multiple plans during the audit period. The most recent plan for each patient was examined for content.

Results: Plans were most commonly completed by Registrars (1423, 65.8%), and 99.9% of plans were signed. The majority of plans indicated orders for treatment limitation (1630, 75.4%), and these patients were significantly older than patients with an order for full treatment (p<.001). Information on the decision making process was completed in 1518 cases (70.2%). Of these, 63% had evidence that the patient/family had been involved in, or were informed of the decision. There was a significant association between limitation of treatment and evidence of family/patient involvement in the decision making process (p=.001).

Thirty-eight percent of patients with multiple plans had orders in the most recent plan for less-aggressive treatment, compared to the prior plan. A further 51% had no changes to treatment orders over consecutive plans.

Conclusion: The use of POLST forms in an acute care environment is dynamic and complex. Evidence of patient/family involvement in the decision making process should increase as local advance care planning programs progress.

The Australian Stroke Clinical Registry: contributing to the quality of care and treatment outcomes in stroke

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Aim

Variations in the quality of hospital care may contribute to poor health outcomes in people with stroke. We aimed to use the Australian Stroke Clinical Registry (AuSCR) to better understand the variations in hospital care by providing prospective, systematic data on patient characteristics, clinical processes and outcomes following admissions for stroke.

Method

Since 2009, the AuSCR has been used to collect a minimum data set on stroke admissions across Australia, including Austin Health. Descriptive data obtained in 2011 are presented.

Results

In 2011, 16 hospitals contributed data for 2593 stroke episodes (Austin Health n= 383) and 56% of all eligible registrants completed a 3 month follow-up (n=1215; Austin Health n=125). The mean age of patients was 73 years; 47% female and 72% Australian-born. Approximately, two thirds (67%) of patients had an ischaemic stroke, 14% intracerebral haemorrhage, 17% transient ischaemic attack, and in 3% stroke type was undetermined. The median length of stay was 7 days. Most episodes (77%) resulted in management on a stroke unit (Austin Health 96%). Among ischaemic patients, 14% received intravenous thrombolysis. Discharge on anti-hypertensive agents occurred in 77% of episodes. At follow-up, 33% of patients discharged from hospital were living at home with support and 45% without support. With respect to registrants’ quality of life at 90-180 days post-stroke, there were no problems regarding: mobility for 50%; self-care for 66%; usual activities for 40%; pain/discomfort for 52%; and, anxiety/depression for 52% of the respondents.

Conclusion

AuSCR provides valuable information on stroke care at an aggregate level, as well as for individual hospitals to promote quality assurance.
Advance care planning training and implementation in residential aged care facilities

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Aim: Published literature suggests that advance care planning (ACP) activity in aged-care homes is inconsistent and often of poor quality. This study specifically examined the effectiveness of formal training and implementation of ACP in Victorian Residential Aged Care Facilities (RACFs).

Method: The Respecting Patient Choices (RPC) Program piloted a training and implementation model in 19 RACFs in 2010-11. The model required selected RACF nurses and management staff to attend 3 workshops over a 6-month period and complete pre and post-workshop tasks. The workshops included didactic and experiential teaching, discussions and role plays. Model evaluation included ACP documentation audits, in addition to pre and post implementation surveys to examine aged-care home ACP policies and practices, and staff knowledge, attitudes and behaviours around ACP.

Selected Results:

Conclusion: The available data suggests that the RPC model of ACP in aged-care homes succeeded in training staff and implementing ACP.

Application of the RCPath UK workload guidelines to an Australian histopathology laboratory context – Phase 1

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A shortage of pathologists exists worldwide, with Anatomical Pathology particularly affected due to its labour-intensive nature. Despite recognition of this shortage and its potential effects on quality, in Australia there are currently no benchmarks for safe workloads and staffing in common use.

Instituting a system to accurately measure workloads will assist in:
• Ensuring equity of workload distribution between pathologists
• Ensuring that staffing levels relative to workload are maintained at appropriate levels for quality reporting and patient safety
• Providing appropriate evidence to justify requests for workforce EFT increases.

The Royal College of Pathologists (RCPath) UK recently published Guidelines on staffing and workload for histopathology and cytopathology departments1. These Guidelines include a points rating system for cases based on complexity and suspected diagnosis, and a recommendation for the points total safely achievable per four-hour reporting session.

Given similarities between the practice of histopathology in Australia and the UK, it was anticipated that the RCPath points system could be implemented at Austin Anatomical Pathology. In the first phase of this process, points ratings were assigned retrospectively to all...
specimens reported over June 2012. The average daily points total was determined for each pathologist.

The average daily points ranged from 60 to 105 and the mean per pathologist was 76. The RCPath recommendation for safe reporting levels is 72 points. Assuming that the points system provides an accurate measure of workload, this indicates that the staffing level over June 2012 was reasonable but that there was inequity in the daily case allocation.


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**Filling the knowledge void- the BioGrid facilitated solution**

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**Introduction:** To compete on an international level, a collaborative approach to research in Australia is crucial to ensure efficiency, sustainability and relevance of research activity. BioGrid Australia operates a federated data linkage and integration infrastructure using the web, enabling research on patient specific information in a privacy protected manner. Data are multiple types (clinical, treatment, genomic, image, biospecimen and outcome), across a range of diseases (oncological, neurological, endocrine and respiratory) across Australia.

**Method:** BioGrid successfully implements technology and processes allowing researchers to efficiently extract data from multiple sources, without compromising data security and privacy. Data are connected to BioGrid from each institution's source database. Each site uses the technology, applications design and platforms of their choice, ensuring sites can maintain their existing source systems. BioGrid manages all aspects of the data acquisition process according to the researchers needs; including project review, ethics submissions, data provision and data analysis.

**Results:** BioGrid has over 25 members; including Austin Health, other health services, universities and medical research institutes. The linkage technology has enabled over 80 research publications. Linking data from multiple institutions, has yielded research projects with sample sizes greater than 10,000 allowing Australian research to compete on an international scale.

**Conclusion:** The Australian research capabilities and resultant impact on health outcomes is underutilised. By integrating and linking our health information we can improve our outcomes and performance and advance our research capacity as a world leader. BioGrid Australia provides one such solution to the Australian health research needs.

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**Enhancing Stroke Care in Victorian Hospitals: Evidence from the Victorian Stroke Clinical Network**

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**Background and aims:** In 2007, the Stroke Care Strategy for Victoria was developed. The Victorian Stroke Clinical Network (VSCN) was then established and Stroke Network Facilitators (SNFs) employed in eight health services to initiate several priority recommendations (e.g. new stroke units and thrombolysis services) over a three year period. We aimed to describe the perceptions of clinicians working within these health services on the benefits of the SNF appointments and provide evidence of changes in stroke care.

**Methods:** Mixed method design using: i) semi-structured clinician surveys; ii) a focus group with selected clinicians two to eight months after the SNF appointment finished at their site;
and c) comparison of 2007-09 and 2011 medical record audit data. Descriptive statistics for categorical data. Thematic analysis used for open text and focus group responses.

Results: 121 clinicians completed the survey and 57 participated in 8 focus groups. Most clinicians were from acute wards (40%) and 84% were nurses/allied health. Overall, 65% of clinicians reported that SNFs achieved ‘all’ or ‘most’ of the objectives of the role, and 95% believed that the changes facilitated by the SNF and VSCN had improved stroke care at their health service. Audit data included 600 patients for 2007-09 and 387 for 2011. Patient access to stroke units (2007-09 53% versus 2011 86%) and thrombolysis (2007-09 2% versus 2011 9%) significantly improved.

Conclusions: Use of local SNFs was perceived to have enhanced stroke care. These findings were substantiated using patient audit data.

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Biobanking to Support Cancer Research
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The Victorian Cancer Biobank (Biobank) is a tissue banking facility which provides high-quality cancer biospecimens with clinical data to researchers in the academic and commercial sectors. The Biobank was established as a Consortium utilizing Victorian Government funding through the Victorian Cancer Agency and is built on the expertise of four founding member tissue banks, located at Austin Health, Melbourne Health, Southern Health and Peter MacCallum Cancer Centre.

The Biobank currently operates as a multi-site ‘hub and spokes’ collection model, combined with a centralized application process. This allows for a large-scale collection of biospecimens and a streamlined access for researchers. Patients consent to donate surgical tissue not required for diagnosis, as well as blood and clinical data to be used in future unspecified research. From October 2006 – December 2011, more than 17,000 patients have donated tissue.

Centrally managed quality control and standard operating procedures are used at all sites to ensure that uniform, high-quality biospecimens and clinical data are provided. This is particularly important for dispatching high-quality samples to cancer researchers which may lead to a better understanding of cancer biology and development of therapeutics. More than 24,000 aliquots of samples have been distributed to researchers since 2007 and over 30 papers have been published acknowledging the role of the Biobank.

A centralized web-based informatics system is being developed which will allow researchers to preview availability, view associated de-identified data and apply for biospecimens on-line. The system will also support distribution, inventory management and cost recovery.

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Impact of electronic prescribing (Cerner Millenium) for discharge prescriptions across a major metropolitan health service.
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Aim: To evaluate the impact of electronic prescribing for discharge prescriptions, on medication safety and staff work-flow.

Methods: A multifaceted pre- and post-implementation study evaluated the impact of electronic prescribing (Cerner Millenium) on: • Error prone abbreviation (EPA) use • Accuracy of medication regimens listed on medical discharge summaries • Frequency and nature of pharmacist interventions on patients’ discharge medication regimens • Time and motion studies of ward pharmacists and emergency physicians
Completeness of PBS prescriptions for submission to Medicare Australia.

Results: Key findings were:

- Moderate/high risk EPA use declined from 53 to 1 EPA/1000 prescriptions (p<0.001).
- Moderate/high risk discrepancies for regular medications on the medical discharge summary declined from 87 to 27/1000 at 6-months (p<0.001). Remaining discrepancies were due to manual changes to the printed discharge prescription not being updated on the electronic version.
- Pharmacist intervention rate for clinical issues did not significantly decline.
- Ward pharmacist time spent on discharge related issues approximately doubled at 2- and 6-months post-implementation.
- No significant change in emergency physician time spent on tasks undertaken using Cerner Millenium.
- Significant reduction in information that needed to be corrected/add to prescriptions to ensure reimbursement by Medicare Australia.

Conclusions: Implementation of electronic prescribing for discharge prescriptions has been associated with improvements in some aspects of safety and completeness of prescriptions. Ongoing monitoring will occur as electronic prescribing is rolled out to include in-patient prescribing.

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How do clinical staff manage oral medications when patients have restrictions on oral intake?

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Aim: To determine how clinical staff manage regular oral medications when patients have restrictions on oral intake.

Method: A survey was conducted online and consisted of 4 scenarios involving a patient who was either ‘fasting’, day 1 post surgery, ‘nil by mouth’, or had a nasogastric feeding tube. Staff had the option to give, withhold, cease, contact someone or change formulation before giving each medication. The patient’s medications included:

- Aspirin (Cartia) 100 mg mane
- Gliclazide (Diamicron MR) 60 mg mane
- Atorvastatin (Liptor) 40 mg mane
- Metoprolol (Betaloc) 50 mg bd
- Levodopa/Carbidopa (Sinemet CR 200/50) 1 tds
- Ginkgo 7500 complex 1 mane

A sample of 320 staff (10%) was sought.

Results: 622 (19%) clinical staff participated. When ‘fasting’, staff would ‘give’ metoprolol (65%) and levodopa (68%) but not aspirin (70%), gliclazide (63%), atorvastatin (50%) and ginkgo (65%). The majority of staff would administer all medications day 1 post surgery and a small proportion chose to ‘give’ the medications (metoprolol 14%, levodopa 12%, aspirin 8%, gliclazide 5%, atorvastatin 11%, ginkgo 3%) when ‘nil by mouth’. For the nasogastric feeding tube scenario, the consensus was to ‘give’ the medications, including gliclazide (40%) and levodopa (46%) via the tube.

Discussion & conclusion: There is some confusion around the management of oral medications when patients have restrictions on oral intake e.g., withholding atorvastatin when ‘fasting’, giving oral medications when ‘nil by mouth’, and giving modified/controlled release gliclazide and levodopa via the nasogastric feeding tube. A draft policy is being piloted to address/clarify these issues.
The Effectiveness of Employing Exclusive Advance Care Planning (ACP) Clinicians

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Aim: ACP improves end-of-life care and patient/family satisfaction. Nevertheless, health professionals struggle to include ACP in their busy work schedule in a tertiary teaching hospital environment. In response, since 2008, the Respecting Patient Choices Program, Austin Health has employed nurses to facilitate ACP discussions.

Method: A prospective audit of the activity of ACP Clinician activity was conducted between 1/1/2010-31/12/2011 and included patient demographics, referral characteristics, ACP activity and outcomes.

Results: 1580 patients (median age 76 (18-102) were referred. Their primary admission diagnoses was cancer 23%, renal 18%, respiratory 12%, cardiac 10% or other 43%. Prior to referral 11% had a substitute decision maker (SDM) and 6% had an Advance Care Directive (ACD). 1463/1580 (93%) referred patients were seen by an ACP. Of these 32% of all patients completed new ACD documentation: SDM alone (26%) while 28% either/both a Statement of Choices or Refusal of Treatment ACD. When patients completed ACD’s, the time spent per patient was longer (median 73 versus 47 minutes) and the total visits were more (median 2 versus 1). When patients completed an ACD, outlining treatment preferences, 46% expressed a wish to not receive cardiopulmonary resuscitation, 41% did not want any Life Prolonging Treatment, 43% only wanted treatment if a patient-defined reasonable outcome expected.

Conclusion: Employment of ACP clinicians is an effective method of providing ACP and enabling patients to appoint a SDM and formally document their treatment preferences on an ACD as desired.

Creating Accessible Medication Information for Inpatients with a Range of Communication Difficulties

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This project provides an example of effective interdisciplinary team work and shows that simple adaptations to information for people with communication difficulties can make a difference. The aim of the project was to support individuals with a range of communication difficulties to better understand their prescribed medications through development and use of a simplified, picture-based medication list. This project was carried out in a neurological inpatient rehabilitation setting.

A database of simplified medication information was developed using best-practice investigations according to current literature. Participants’ understanding of their medications was evaluated before and after routine pharmacy education, and pharmacy education implementing the simplified medication list. It was found that the use of simplified, picture-based medication lists was beneficial for patients with a range of communication difficulties. The feedback from participants provides insight into the experiences of people with communication difficulties in a hospital setting.

Further research into the practical implementation of accessible medication information in the hospital system is needed.
Macrophage polarisation in primary lung cancer

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Macrophages are part of the tumour microenvironment and have been shown to play a major part in promoting and/or suppressing tumour growth and metastasis. Aim: To identify how the macrophage phenotypes M1 (classically activated macrophage phenotype, known to be associated more with tumour regression) and M2 (alternative activated macrophage phenotype, known to be associated more with tumour progression) are affected by the local and systemic microenvironment of patients with primary lung cancer. Methods: Alveolar macrophages (AMs) were isolated from patients with primary lung cancer (n=8) and non-cancer control patients (n=9) and analysed for phenotypic differences by flow cytometry.

Results: M2 markers (CD163, CD36, CD150, CD195) were more highly expressed (p < 0.05) compared to M1 markers (CD253, HLA-DR, IP-10) in patients with primary lung cancer. In addition, surface expression of myeloid markers CD11b and CD71 was shown to be increased in patients with primary lung cancer. Discussion: The increased surface expression of myeloid markers suggests that lung tumours have the ability to alter AM functions and may also play a role in changing the polarising conditions of AM phenotypes. This study has the potential to contribute to the identification of new biomarkers for primary lung cancer and may assist in the development of more effective anti-tumour treatments in the future.

The rate of obstruction and restriction varies depending on whether FVC or VC is used in the interpretation of spirometry.

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Introduction: ERS/ATS guidelines for spirometry interpretation suggest using FEV1/VC rather than FEV1/FVC for identifying airflow obstruction. Also, in clinical practice a reduced VC is interpreted as suggesting a restrictive ventilatory defect. We aimed to evaluate the impact of using FVC compared with VC in interpretation of spirometry. Methods: Spirometric data containing both FVC and VC over 11 years was analysed. The rate of airflow obstruction was assessed separately using FEV1/FVC below the lower limit of normal (LLN) and FEV1/VC below the LLN. True airflow obstruction was defined as FEV1/the largest of FVC and VC (LVC) below the LLN. The rate of spirometric restriction was determined separately using FVC below the LLN and VC below the LLN and compared with true spirometric restriction (defined as LVC below the LLN). Results: 16,800 spirometric results were analysed with the median VC being 60 (inter-quartile range -50 to +190) ml higher than FVC. Using FEV1/VC resulted in significantly higher rates of airflow obstruction (30%) compared with FEV1/FVC (25%). The sensitivity of using VC in detecting obstruction was 94% compared with 79% for FVC. Using LVC to define spirometric restriction, FVC incorrectly identified 21% compared with 8% for VC. In a small subset of subjects with lung volume measurements (n=714) VC had a lower false positive rate than FVC (63% vs 68%). Conclusions: Spirometric classification of obstruction and restriction is altered by the choice of vital capacity in a significant number of cases. These results have implications for clinical diagnosis and prevalence estimates of lung diseases in epidemiological studies, and highlight the utility of VC measurements in routine spirometry.
A normal cardiopulmonary exercise test excludes a systolic pulmonary artery pressure of greater than 40mmHg as measured by echocardiography.

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Rationale: The diagnosis of pulmonary hypertension (PHT) by cardiopulmonary exercise test (CPX) is difficult, particularly if the test is non-invasive. In patients being investigated for dyspnoea, the exclusion of pulmonary hypertension as a potential cause may be useful. This study was performed to determine if a normal CPX excludes an elevated systolic pulmonary artery pressure (sPAP) as measured by echocardiography. Methods: The cardiology and respiratory medicine databases were interrogated to identify patients who had a CPX and echocardiogram within 6 months of each other. The sPAP, estimated by tricuspid regurgitant jet velocity (plus right atrial pressure) on echocardiography, was compared with the CPX results. An estimated sPAP above 40mmHg was considered abnormal. Results: 252 patients were identified. The estimated sPAP was less than 40mmHg in 222 patients, 40-60mmHg in 25 patients and greater than 60mmHg in 5 patients. A total of 30 of these CPXs were reported as completely normal. When analysed as a continuous variable, sPAP showed significant inverse correlations with VO₂max, Wmax and end-tidal CO₂ at anaerobic threshold. When analysed as a nominal variable (sPAP <40mmHg or ≥40mmHg), there was a significant association with arterial desaturation (greater than 3% fall during exercise). In patients where the CPX was reported as normal, the median (IQR) sPAP was 22.5 (19-30) mmHg and no sPAP was greater than 40mmHg. Discussion: These results show that elevation of sPAP is associated with important abnormalities on CPX such as reduced VO₂max and arterial desaturation. These abnormalities however may be due to the presence of pathologies other than PHT. These results also suggest that a normal CPX excludes pulmonary hypertension as defined by a sPAP of greater than 40mmHg on echocardiography. In patients being investigated for breathlessness a normal CPX may eliminate the need to measure sPAP by echocardiography.

Treatment of obstructive sleep apnea with a novel hypoglossal nerve stimulation system: Single center experience with recruitment, screening and enrollment

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Introduction: Continuous Positive Airway Pressure (CPAP) is effective in treating obstructive sleep apnoea. However, CPAP is often poorly tolerated with poor patient usage. Thus an issue for clinicians is providing alternative, effective treatments. In feasibility studies, stimulation of the hypoglossal nerve (HGN) has shown promise as an effective treatment. We report our experience in assessing participants for a randomised controlled trial of a novel HGN stimulation (HGN®, Apnex Medical, Inc) system.

Methods: Potential participants were identified from the clinic patient population at Austin Health, a newspaper article and direct referrals from sleep physicians. Research volunteers were pre-screened for history of CPAP failure/intolerance and body mass index ≤35 kg/m². Those who chose to progress were then further evaluated via a complete medical history and physical examination, in-lab overnight polysomnography and nasendoscopy.
Results: Since September 2011, our research centre has contacted 220 patients from our database of patients and 890 people telephoned us following a small informative article in a tabloid newspaper in December 2011. Of those from the CPAP database, 100% were eligible at pre-screening, 20% were interested in receiving further information and 1% were implanted. Of those who responded to the newspaper article, 69% were eligible at pre-screening, 69% were interested in receiving further information and to date 2% have been implanted.

Conclusions: At this centre, there was a high degree of interest in participating in this clinical trial with over 1000 individuals evaluated for participation. Many potential participants who initiated contact with our research office were still using CPAP for 2 – 4 hours per night, but very keen to try an alternative form of therapy. These individuals were not enrolled in the study. To date we have consented and completed screening for 44 participants of whom 17 have passed screening and progressed to implantation of the HGNS system.

Pathogenesis of obstructive sleep apnea in quadriplegia

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Obstructive sleep apnea (OSA) is estimated to be two to five times higher in patients with quadriplegia than in able-bodied (AB) individuals. In order to better understand the causes of OSA in quadriplegia we investigate the upper airway (UA) function in quadriplegia.

High UA resistance can predispose to OSA. In people with quadriplegia the spinal sympathetic circuits lose tonic control and induce vascular engorgement of the airway. This causes the nasal (and possibly pharyngeal) mucosa to thicken, which likely increases the UA resistance. Other factors might also increase the UA resistance in this population.

We hypothesized that nasal (Rna) and pharyngeal (Rph) resistance will be 1) elevated in patients with quadriplegia and OSA (Quad-OSA) compared to AB individuals with and without OSA, and 2) reduced to AB levels with phenylephrine (restores vasoconstriction of the blood vessels).

AB individuals and participants with quadriplegia both with and without OSA are recruited. Subjects are instrumented with epiglottic and choanal pressure catheters, nasal mask and pneumotachograph. All measurements are performed supine during wakefulness. Rna and Rph (at a flow rate of 200mL/s), are determined for 10 minutes, before and after application of decongestant (0.5ml of phenylephrine 0.5%).

Six participants Quad-OSA, and one AB control without OSA have been studied to date. The Quad-OSA seem to have elevated Rna at baseline (2.61, 3.03, 3.61, 6.26, 11.05 and 21.14, compared to 2.44 in the AB), which is decreased to AB levels after phenylephrine, Rna=1.06 on average, and Rna=0.72 in the AB.

Although preliminary, these data suggest that after quadriplegia the Rna is particularly elevated. The recovery of Rna to AB levels after phenylephrine suggests that this elevation is due to the increased parasympathetic activity in quadriplegia. The results to date, highlight the high Rna and Rph as potential risk factors for OSA in quadriplegia.
How many people who are ventilator dependent can really be transitioned to direct diaphragm pacing?

Mark Howard¹, Nicole Sheers¹, Doug Brown¹, Andrew Nunn¹, Mehrdad Nikfarjam¹, Richard Macdonell¹, Linda Rautela¹, David Berlowitz¹

Introduction: Direct diaphragm pacing (DDP) is an alternative to long-term mechanical ventilation in selected patients. In 2009, the VRSS at Austin Health aimed to reduce ventilator dependence by transitioning suitable ventilator dependent patients from 24 hour invasive ventilation to periods of DDP. We report the outcomes of our previously described selection processes and the longer term usage by those who were implanted.

Methods: Audit of consecutive ventilator dependent patients who were implanted with DDP.

Results: The VRSS manages over 650 patients requiring long-term mechanical ventilation throughout Victoria. Of these, 7.3% are ventilator dependent (greater than 16 hours/day). Seventeen patients have a tracheostomy and require 24 hour ventilation. Of the ventilator dependent patients screened, two had pre-existing phrenic nerve pacing units and were excluded. Ten patients with high cervical spinal cord or brain stem injury were further assessed as detailed previously (1,2). Three patients did not have intact phrenic nerves and thus were not suitable for DDP. Seven patients with brainstem or spinal cord injury (C2 or above) proceeded to implantation (mean age 34.9 years, mean time since injury 7.6 years). No surgical or peri-operative complications were observed. The median tidal volume on DDP increased from 230 mls immediately following surgery to 580 mls following diaphragm training (p=0.02, Z=-2.3). The time that DDP could be utilised ranged from 1 minute to 2.5 hours initially to 2.5 to 24 hours at follow-up after training. Time capable of using DDP was greater than the patient’s usual time (median hours 16 versus 10 hours). Five out of seven patients routinely used DDP for ventilation at follow-up, with median use 12 hours per day.

Conclusions: DDP has been implemented in seven long-term ventilator dependent patients in Victoria. The amount of time patients routinely use DDP is highly variable and further research is required to understand the determinants of longer-term, community usage.

Direct Diaphragm Pacing - Predicting Success

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Introduction: Diaphragm pacing is an alternative to long term mechanical ventilation. We evaluated predictors of post-operative success in ventilator dependent patients with high cervical spinal cord injury or central hypoventilation.

Methods: Patients referred for direct diaphragm pacing (2009-2011) were evaluated pre-operatively with: cervical spine MRI (injury level and degree of damage at C3-C5); transcutaneous phrenic nerve conduction studies (NCS); and simultaneous fluoroscopy to assess diaphragm movement. Results were compared to the intra-operative ability to stimulate the diaphragm and long term ventilation outcomes.

Results: Eight ventilator dependent patients with high cervical spinal cord or brain stem injury were assessed (mean age 36.4 years (SD 6.7)), time since injury 8.9 years (range 0.6 to 26.3)). Six proceeded to laparoscopy and five had implantation of the direct diaphragmatic pacing system. Three of these had moderate to severe cervical cord damage at the C3-5 level, whilst the other three were normal or mildly damaged. All of those with normal or mild cervical spine damage and two of those with moderate to severe damage had normal phrenic NCS with diaphragm movement on screening on one or both sides. The results from the phrenic NCS and diaphragm screening were closely related to the ability to stimulate the diaphragm intra-operatively (χ² = 7.2, p = 0.03 and χ² = 15.1, p < 0.01 respectively).
However, there were individual cases when diaphragmatic movement was evident on diaphragm screening during pre-operative phrenic nerve stimulation whilst the NCS suggested impaired function.

Conclusion: Normal cervical spine imaging at the C3-C5 level and preserved diaphragm muscle function on fluoroscopy during pre-operative phrenic NCS predicted successful stimulation of the diaphragm at surgery.

**Does having a large ventilatory response to arousal (VRA) predispose to subsequent low upper airway dilator muscle activity and upper airway collapse?**

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Arousals from sleep occur frequently in Obstructive Sleep Apnea (OSA) and have been proposed to perpetuate obstruction via hyperventilation, hypocapnia and upper airway dilator muscle hypotonia on the return to sleep. The magnitude of the ventilatory response to arousal (VRA) varies between individuals and the proposed sequence of events may only occur in individuals with large VRA. Therefore, the aims of this study are to 1) assess the variability of the VRA and 2) compare dilator muscle activity changes between individuals with large and small VRA.

**Methods:** 38 healthy individuals were instrumented with electrodes for sleep staging, a nasal mask with pneumotachograph and intramuscular dilator muscle (genioglossus, GG) electrodes. End tidal CO2 was determined from the mask. Auditory tones (40–100dB, 0.5s 1000Hz) were played during stable sleep to induce brief arousals.

**Results:** Adequate data were obtained in 21 subjects to date. The peak VRA ranged from 7% to 58% above the pre-arousal level of ventilation (median 32%). Physiologic data were compared between 4 subjects with large VRA (>50% increase in ventilation) and 5 subjects with small VRA (<25% increase in ventilation). By design, post arousal ventilation was significantly different between groups (9.4±0.8 vs 6.6±0.3 L/min), but no other variable differed significantly. GG activity was not reduced below baseline following the return to sleep in either group.

**Conclusions:** These preliminary data suggest that reduced GG activity does not occur on the return to sleep following arousal, even in individuals with a large VRA.
**Exercise prescription from 6 minute walk test achieves the suggested training intensity in interstitial lung disease.**

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**Aim:** The recommended exercise training intensity for patients in pulmonary rehabilitation is 60% of peak oxygen uptake (VO2peak) measured during cardiopulmonary exercise testing (CPET). However in clinical practice, exercise prescription is often based on the 6-minute walk test (6MWT). Our aim was to investigate whether prescription of walking speed from the 6MWT provides a training intensity within the recommended range.

**Methods:** Participants with interstitial lung disease (ILD) underwent CPET and 6MWT in random order. Peak oxygen uptake (VO2) was measured with a portable metabolic cart. Participants then underwent 10 minutes of treadmill walking at 80% of 6MWT speed. Peak VO2 recorded during treadmill walking was compared to that obtained during CPET.

**Results:** Eleven participants (6 idiopathic pulmonary fibrosis) were included, with mean (SD) TLCO 49(13)% predicted and 6-minute walk distance (6MWD) 481(99) metres. Peak VO2 during 6MWT was 95(15)% of that recorded on CPET (range 73-116%). During treadmill walking, VO2 ranged from 67% to 116% of that recorded on CPET.

**Conclusions:** In ILD, prescribing walking exercise intensity at 80% of 6MWT speed achieves a training intensity within the recommended range for pulmonary rehabilitation.

**Dentist outreach model for supply of an oral appliance in patients with quadriplegia and obstructive sleep apnoea.**

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**Background:** We are currently undertaking a study to examine the feasibility of a mandibular advancement device (MAd) for treating OSA in people with quadriplegia. Travel to hospital or a dentist’s rooms is often difficult and time consuming for people with quadriplegia. We are therefore trialling an outreach model to minimise participants' travel time.

**Methods:** Our dentist has assembled a home-visit kit that allows him to undertake the usual procedures required for creation of a custom-made MAd. Each participant is visited four times by our dentist and a dental assistant. At the first visit a dental history and examination is carried out and dental impressions are made. The custom-made device is set-up at 60-72% of the total range of protrusion and is delivered at the second visit. At the third visit participants and their carers are taught how to advance the device. Participants are contacted weekly for adverse event and adherence monitoring. The final visit occurs at the conclusion of titration to ensure that maximal comfortable mandibular advancement is achieved.

**Results and Discussion:** We have provided a MAd for six participants. Geographical range is limited to Melbourne and suburbs with travel time to each participant ranging from 20 minutes to 70 minutes. The first visit has taken 50-60 minutes whereas the three subsequent visits have taken 15 to 30 minutes. Two participants have completed titration; one within 8
weeks of device delivery and the other within 10 weeks. The four remaining participants are in the titration phase. There have been no discontinuations and all participants have been very positive regarding the outreach model.

This project is proudly supported by the Transport Accident Commission.

Exercise training is beneficial in non-CF bronchiectasis - a multi-centre, randomised controlled trial

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Aim: To determine whether exercise training improves exercise capacity and health-related quality of life (HRQOL) in people with non-cystic fibrosis (CF) bronchiectasis.

Methods: Participants with non-CF bronchiectasis with a modified Medical Research Council (MMRC) dyspnoea score ≥1 were randomly allocated to receive 8 weeks of supervised exercise training or twice weekly telephone support. Exercise capacity, using the incremental shuttle walk distance (ISWD) and 6-minute walk distance (6MWD), and HRQOL were measured using the Chronic Respiratory Questionnaire, Leicester Cough Questionnaire and Hospital Anxiety and Depression Scale by a blinded assessor at baseline, 6 and 12 months following intervention.

Results: Eighty-five participants, aged (mean ([SD]) 64 (13) years, FEV₁ 74(22)% predicted and median MMRC score of 1 (IQR 3) were included. Of those in the exercise training group (n = 42), 35 (83%) completed the program. There was a greater magnitude of change in the ISWD (mean difference 62 m, 95% CI 24 to 101 m) and the 6MWD (mean difference 41 m, 95% CI 19 to 63 m) in the exercise training group compared to the control group. Exercise training significantly reduced dyspnoea (p=0.009) and fatigue (p=0.01) but there was no difference in cough-related QOL or mood between groups. At 6 and 12 months following intervention, there were no differences between groups for any outcome variable.

Conclusions: Exercise training in non-CF bronchiectasis improves exercise capacity and symptoms of dyspnoea and fatigue, but these benefits are not sustained at 12 months following intervention.

Riding the Wave of Research Administration in a Small Institute

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The Institute for Breathing and Sleep is a small health and medical research organisation located at Austin Health. It employs one part-time research administrator, a situation that presents a mixture of opportunities (peaks) and challenges (troughs).
Short term effects of 3 different modes of non-invasive ventilation (NIV) in stable obesity hypoventilation syndrome.

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Introduction: Obesity hypoventilation syndrome (OHS) results in increased morbidity, mortality and health resource utilization. The optimal treatment for patients with stable OHS remains unclear. This project evaluated the impact of three treatment modalities on short term outcomes.

Methods: Patients with stable OHS who were currently successfully treated with domiciliary NIV were recruited from Austin Health. Subjects underwent a single night of testing on each of three different modes of NIV (CPAP, Bilevel S and Bilevel ST). The order of treatment was randomized and an identical device was used to deliver each ventilatory mode (ResMed VPAP Quicknav). Testing nights were separated by 2 weeks. Subjects undertook polysomnography on each treatment and subjective assessments of sleepiness and treatment preference. Subjects and scientists were blinded to treatment modes. Differences between outcome measures for the three treatments were assessed using repeated measures ANOVA.

Results: 11 subjects completed the study (age 57.8 yr, BMI 50.6 kg/m2, FVC 72.5% predicted). Morning PaCO2 was significantly lower on Bilevel ST and S compared to CPAP (PaCO2 41.0, 40.6 and 46.7 mmHg respectively, F 7.28 p= 0.01) with changes in PaCO2 of –6.5, -6.3 and +2.4 mmHg (F 7.40 p=0.01). There was no significant difference in PaO2 or the proportion of the night with SpO2 < 90%. There was a trend towards improved sleep efficiency on Bilevel ventilation (71%, 66% and 62% for Bilevel ST, Bilevel S and CPAP). 66% of subjects preferred Bilevel S, 33% Bilevel ST and none preferred CPAP.

Conclusion: There was better control of hypoventilation on both forms of bilevel support compared to CPAP after one night of treatment. There was a preference for Bilevel S with no subject preferring CPAP. A long-term study is required to determine whether the acute benefits identified herein translate into clinically important outcomes.

Performance on the paced serial addition task (PASAT) in people with acute quadriplegia and obstructive sleep apnoea.

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Obstructive sleep apnoea (OSA) has effects on daytime functioning, including memory and attention deficits. The prevalence of sleep apnoea is high in acute quadriplegia, and our group is leading a randomised controlled trial examining the effect of OSA treatment in acute quadriplegia on neurocognitive performance. This paper outlines the relationships between initial OSA severity and performance on the PASAT, a sensitive measure of information processing and attention.

69 acute patients (64 male), aged 18-69 years (M=44.64, SD=15.84) underwent a sleep study and neuropsychological testing. Patients with an apnoea hypopnoea index (AHI) of less than 10 were excluded prior to neuropsychological testing.

A wide range of sleep apnoea severity was observed, with an AHI range of 11.2 - 107 events per hour of sleep (M=39.9, SD=21.95). Performance on the PASAT also varied widely with scores ranging from 1-180 (M=93.42, SD=48.58) of a possible 180. Patients were grouped based on AHI, and PASAT performance was analysed between groups using two tailed, unequal variance t-tests.
Patients in the mild AHI group performed best on the PASAT, with both moderate and severe AHI groups displaying significantly poorer performance. Increased severity in sleep apnoea is related to poorer performance in information processing and attention, which has important implications for daytime functioning and rehabilitation outcome.

The optimal technique for removal of upper airway foreign bodies: a repeated-measures, cross-over trial in a porcine model

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Objective:
Anterior chest thrusts (with the victim sitting, thrusts applied to the lower sternum towards a supported spine) are recommended by the Australian Resuscitation Council for clearing upper airway obstruction by a foreign body. However, due to a lack of evidence, lateral chest thrusts (with the victim lying on their side) are no longer recommended. We compared anterior and lateral chest thrusts and the Heimlich manoeuvre in the generation of airway pressures.

Methods:
This was a repeated-measures, cross-over, clinical trial of eight anaesthetised, intubated, adults pigs. For each animal, ten trials of each technique were undertaken with the upper airway obstructed. A chest/abdominal pressure transducer, a pneumotachograph and an intra-oesophageal balloon catheter recorded peak chest/abdominal thrust, peak expiratory airway and pleural pressures, respectively.

Results:
The mean (SD) thrust pressures generated for the anterior, lateral and Heimlich techniques were 120.9 (11.0), 135.2 (20.0), and 142.4 (27.3) cmH2O, respectively (p<0.0001). The mean (SD) peak expiratory airway pressures were 6.5 (3.0), 18.0 (5.5) and 13.8 (6.7) cmH2O, respectively (p<0.0001). The mean (SD) peak expiratory pleural pressures were 5.4 (2.7), 13.5 (6.2) and 10.3 (8.5) cmH2O, respectively (p<0.0001). At autopsy, no rib, intra-abdominal or intra-thoracic injury was observed.

Conclusion:
The lateral chest thrust and Heimlich techniques generated significantly greater airway and pleural pressures than the currently recommended anterior thrust technique. The findings support the re-introduction of the lateral chest thrust technique for clearing upper airway obstruction by a foreign body, especially as the Heimlich technique has been associated with organ damage.
Shift work affects mood and sleepiness, but not performance.

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Aim: Shift work results in disturbed sleep and decreased sleep duration and is thought to be related to increased work and road-related accidents. This study investigated affective and neuropsychological functioning and driving simulator performance in shift workers compared to a control group.

Methods: Shift worker (n=41) and control (n=40) participants completed a driving simulator task (30min), the Psychomotor Vigilance Task (PVT) and two Oxford Sleep Latency Resistance Tasks (Osler). The Optalert™ Drowsiness System, which records eyelid movements, objectively measured sleepiness during each task. Three mood questionnaires and four neuropsychological tasks were completed: Beck Depression Inventory (BDI), State-Trait Anxiety Scale (STAI), Profile of Mood States (POMS), Logical Memory, Trails A & B, Digit Span and Stroop. Participants completed Epworth Sleepiness Scales (ESS) and provided information on working schedules. Shift workers attended the day after the end of their night shift schedule, with at least 24 hours’ recovery.

Results: Shift workers worked longer shifts (p<0.001), had less sleep on work nights (p<0.001) and were significantly sleepier on the ESS (p<0.001). There were no differences in performance on the driving simulator, Osler and PVT tasks. Maximum drowsiness scores on the Optalert were greater in shift workers for both Osler tasks (Osler 1: p<0.01; Osler 2: p<0.005), but not for driving simulator or PVT tasks. The shift workers scored significantly higher on the BDI (p<0.01) and were more fatigued (p<0.001) and less vigorous (p<0.005) than controls on the STAI or across any of the neuropsychological tasks.

Conclusion: Shift work schedules affect fatigue, mood and feelings of sleepiness but do not impact upon shift workers’ performance on a range of driving, psychomotor and neuropsychological tasks outside of their work environment.

Maternal Sleep-Disordered Breathing and Foetal Outcomes

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Aim: Symptoms of sleep-disordered breathing are thought to be more common during pregnancy. However, there is little data on the foetal outcomes of confirmed obstructive sleep apnoea (OSA) in pregnancy. This study aims to determine if OSA during pregnancy is associated with acute and chronic foetal compromise.

Methods: At 37 weeks’ gestation, a sleep study with synchronised foetal heart rate monitoring (CTG) was performed, and cord blood was collected at delivery. Foetal growth trajectory across the third trimester was determined by performing serial ultrasound examinations. Either a fall in customised centile of ≥ 30% from 32 weeks to birth or confirmed foetal growth restriction at birth (<10th centile) was considered significant.

Results: Forty-one women completed the study, ten of whom had confirmed OSA (AHI ≥ 5/hr).
Acute compromise: one case of an abnormal CTG occurred in a woman with OSA and a growth-restricted foetus. In most women with OSA, no foetal heart rate abnormalities were detected, despite significant maternal oxygen desaturation.

Chronic compromise: Among women with OSA, 50% were found to have impaired foetal growth, compared with 19% of controls (p = .07). Insulin growth factor-I (IGF-I), a key endocrine regulator of foetal growth, was significantly decreased in infants of mothers with OSA compared to BMI-matched controls (p = .03). A corresponding increase in the insulin like growth factor binding proteins 1 and 2 (IGF-BP1 and IGF-BP2) was also observed (IGF-BP1 – p = .004; IGF-BP2 – p = .06).

Conclusions: Sleep-disordered breathing during pregnancy may be associated with acute and chronic foetal compromise, even in otherwise uncomplicated pregnancies. Further study with larger numbers is needed to confirm these results. If a link between OSA and increased foetal risk can be established, effective treatment of OSA with continuous positive airway pressure (CPAP) may be able to attenuate adverse perinatal outcomes.

Spirometry during hospital admission for patients with an acute exacerbation of COPD

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Background and objective: The role of spirometry in the management of acute exacerbations of chronic obstructive pulmonary disease is unclear. The objectives of this study are to assess the feasibility and clinical benefits of performing spirometry during admission for patients experiencing such exacerbations.

Methods: Patients admitted to hospital with a clinical diagnosis of acute exacerbation of chronic obstructive pulmonary disease performed spirometry daily until discharge. Treating doctors completed a survey regarding the clinical benefits of having spirometry results made available to them.

Results: 17 of the 20 participants were able to perform at least one acceptable spirometry test during their admission, 15 within the first three days of admission. No statistically or clinically significant changes in lung function were observed between admission and discharge. Results of the survey suggest doctors like to have spirometry results available but management is unchanged.

Conclusions: It is possible for sick patients to perform accurate and reliable spirometry during acute exacerbations of chronic obstructive pulmonary disease, and doctors indicated a desire to have such results made available. An inpatient admission can be an opportune time to obtain otherwise unavailable lung function data from patients with chronic obstructive pulmonary disease, but it does not affect patient management.
Relative laterality of dominant and non-dominant hand sensory function: an fMRI study

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Background and Aim

Cerebral laterality of function can be an important consideration in diagnosis and management of many neurological conditions including stroke and epilepsy. However the relative degree of cerebral laterality of sensory functional representation of the dominant side of the body, compared to the non-dominant side, is still relatively unexplored. We addressed this issue by performing a functional magnetic resonance imaging (fMRI) study of controlled tactile stimulation of the fingertips of the left or right hand of healthy subjects.

Methods

Normative data from 13 right-handed healthy subjects (Age 23 - 79 years; mean±SD = 61.7±12.3 years, 6 males) were analysed. Each subject performed a sensory fMRI study for each hand, involving a standardised sensory stimulus via a tactile stimulation device consisting of a plastic texture grating, of set spatial interval, presented to the 2nd, 3rd and 4th digits of the hand [1]. Image pre-processing and statistical parametric mapping was performed using SPM8 and iBrain™. An adaptive and largely threshold independent method of objectively determining laterality [2] was adapted for this study to permit statistical comparison of laterality of activity associated with dominant compared to non-dominant-hand stimuli.

Results

All subjects exhibited contralateral dominance of activity in primary somatosensory cortex (SI) for left and right hand stimuli. Subjects tended to exhibit contralateral dominance in secondary somatosensory cortex (SII) for stimuli of their dominant hand. However the situation was mixed for stimulation of the non-dominant hand, with subjects exhibiting laterality ranging from strongly contralateral through to strongly ipsilateral. The group difference between the left and right-hand laterality of SII was statistically significant (P=0.034).

Conclusions

In the clinical context, one should beware of assuming the laterality of SII for sensory function of the non-dominant hand.


Vagal afferent responses to gut hormones are altered in obesity: Implications for the aetiology of hypertension

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We have previously demonstrated that gut hormones are involved in cardiovascular regulation via a vagal afferent mechanism that involves a central reflex response in the medulla. These hormones elicit a sympathoinhibitory response in the renal and splanchnic nerves accompanied by regional vasodilator responses. However, in obesity, these
responses are blunted, and we hypothesize that this is due to aberrant vagal afferent signaling. Therefore the aim of the current study is to determine whether the subdiaphragmatic vagal nerve responses to these hormones are altered in obesity.

Thirty-two outbred male Sprague-Dawley rats (n=32) were placed either on a medium high fat diet (MHFD, n=24) or a low fat diet (LFD, n=8) for a 13 week period. Rats were subsequently placed under isoflurane anaesthesia and tracheostomised for artificial ventilation. The brachial and carotid arteries were cannulated for measurement of HR and AP, and arterial infusion of CCK, respectively. The subdiaphragmatic vagus nerve was isolated and placed onto bipolar silver wire electrodes to measure the effects of CCK infusion (0.1-4µg/kg). For analysis, MHFD rats were stratified into obesity prone (OP; n=8) or obesity resistant (OR; n=8) post-hoc, depending on whether their weight gain fell into the upper or lower tertile, respectively. OP animals had greater weight gains compared to OR or LFD rats (P<0.001 for both). AP was elevated in OP (126.0 ± 3.6) compared to LFD (94.6 ± 6.1; P<0.01) or OR (102.5 ± 5.0; P<0.05). There was no significant difference in subdiaphragmatic vagal responsiveness to CCK between OP, OR or LFD animals (P > 0.05 for all). These findings therefore suggest that altered reflex sympathetic and vasodilator responses in OP animals may result from defective central rather than peripheral signaling mechanisms.

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Using embryonic stem cells to provide human neurons for drug screening

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Background: Over 500 therapeutic agents have shown protection against ischaemic injury in in vivo animal models of stroke; however, this efficacy is yet to be successfully translated in humans. While stem cell based therapies could be of benefit, an alternative use of stem cells is to create an in vitro screening system in which human embryonic stem cells (hESCs) differentiated into neurons are used to test candidate drugs. The aims of this study were to differentiate hESC lines into neurons, develop a model of ischaemic injury and test potential therapeutic agents.

Methods: Human ESCs were differentiated into neurons in the presence of bone morphogenic inhibitor protein, Noggin. The mature neurons were maintained for 11 days prior to the induction of injury. Two injury models were used: Oxygen glucose deprivation (OGD) and oxidative stress. Three potential therapeutic agents (hypothermia, melatonin, and NXY-059) were tested at various concentrations and cell death was quantified using a lactate dehydrogenase assay.

Results: Hypothermia and Melatonin were shown to be neuroprotective following four hours of OGD injury, reducing cell death by 28% and 44.2% respectively, compared to injury control. This was also seen following four hours of oxidative stress with a reduction of 33.6% (Hypothermia) and 37.8% (100µM Melatonin) compared to injury control. NXY-059 however had no effect on neuronal cell survival in either of the injury models.

Conclusion: These results demonstrate that hESCs have the potential to be a useful model for future drug screening. Identifying neuroprotective agents that work in such human in vitro systems may bridge the gap between animal studies and clinical trials thereby addressing the current translational failure.
Tonic Seizures of Lennox Gastaut Syndrome: ictal SPECT shows a cortico-pontine network

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Aim: To reveal cerebral activity changes during tonic seizures of Lennox-Gastaut syndrome (LGS), by comparing ictal and interictal single photon emission computed tomography (SPECT).

Background: LGS is a severe epilepsy phenotype with diverse aetiologies. Characteristic electro-clinical features include tonic seizures.

Method: From the Comprehensive Epilepsy Programmes of Austin Health and Royal Children Hospital, Melbourne (Jan 2001 - Dec 2011) we identified LGS patients with: 1) interictal SSW and GPFA; 2) tonic seizures on video-EEG monitoring (electrodecrement, low voltage fast activity, then high voltage SSW); 3) ictal SPECT. After global intensity normalisation, we compared ictal and interictal SPECT studies across the group (SPM8), thresholded at p< 0.05 (uncorrected), extent k>125 voxels.

Results: We identified ten ictal-interictal SPECT pairs from 7 patients (20.1 ± 14.5 yrs). Median tonic seizure duration was 10 seconds (range 6-29). Injection latency (from offset of ictal fast activity) was -8 to 48 seconds. In the early injection group (<10 seconds; 3 studies) hyperperfusion occurred over bilateral frontal and parietal association cortex, with a significant cluster of SPECT activation in pons (p<0.05 corrected FWE), and reduced perfusion in primary sensori-motor and visual areas. In the late injection group, hyperperfusion was seen over cerebellum and bilateral parietal cortices.

Conclusion: Tonic seizures appear to result from activity in a cortico-recticular network, containing bilateral frontal and parietal association areas, and the pontine brainstem reticular formation. This may explain why tonic seizures of LGS share similar, axial motor predominant clinical features, despite aetiologies that include cortical lesions of varied type and location.

Lennox Gastaut Syndrome: a secondary network epilepsy

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Aim: To reveal the contribution of lesions to the interictal activity of Lennox-Gastaut Syndrome (LGS) using EEG-fMRI with time course analysis.

Background: LGS is a severe epilepsy phenotype with characteristic interictal EEG features of slow spike-and wave (SSW) and generalised paroxysmal fast activity (GPFA) implying common cerebral networks are involved. However, cortical lesions of varying type and location can cause the phenotype.

Method: Clinical - Six subjects (32–42 yrs), with intractable epilepsy of ‘Lennox-Gastaut Phenotype’ and: 1) interictal SSW and GPFA; 2) tonic seizures; 3) structural MRI lesion.

EEG-fMRI - Up to 60 minutes of 3T fMRI; (TR 3.2 sec); Event-related analysis (SPM8 and iBrain) of fMRI change, associated with EEG detected SSW and GPFA, combined in a second-level group analysis. We explored the time course of fMRI change in key regions of interest, including the attentional and REST networks, primary cortical areas and the lesion.
Results: GPFA – Increased fMRI activity in frontal and parietal association cortex, thalamus, pons. Simultaneous fMRI increases in ‘attentional’ and ‘REST’ networks, a highly unusual pattern.
SSW – Increased fMRI activity in association cortex and thalamus, reductions in posterior cingulate, precuneus, primary cortical areas. Pre-event rise in fMRI activity across the network, followed by prominent post-event reduction.
Lesion – fMRI increases with both GPFA and SSW.
Conclusion: The epileptiform activity of LGS recruits association cortical areas, pons and thalamus. Increased fMRI activity was present in lesions during generalised epileptiform activity. The three subjects (2, 3 and 5) who proceeded to lesionectomy are >1 year seizure free.

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AVERT: Ongoing phase III, multicentre, international trial of very early rehabilitation after stroke

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Background: Early and frequent out of bed activity (mobilisation) starting within <24 hours of stroke, may be an important component of effective stroke unit care. Within a multi-centre, single blind, randomised controlled trial, we hypothesise that early and frequent mobilisation will reduce death and disability and be cost effective.

Methods: Medically stable patients within 24 hrs of stroke are included. Patients with severe pre-morbid disability and co-morbidities are excluded. Randomisation is stratified by site and stroke severity. Intervention is delivered by a nurse/physiotherapist, commences within 24 hours and continues for a maximum of 14 days. Control group patients receive standard care. Primary outcome is modified Rankin Scale at 3 months. Sample size is 2104 patients (n=1052 per group). Analyses will be intention to treat.

Trial status: 49 hospitals are participating in Australia, New Zealand, Malaysia, Singapore and the United Kingdom. At 30 July 2012, 1340 patients (5.6% of all strokes) have been recruited. The main exclusion reason is that patients are admitted > 24 hours after stroke (40.3%). Mean age of participants is 70.5 (12.9) years with 47% having moderate-severe stroke. 1251 patients have completed 3 month follow up with 5 drop outs.

Conclusion: The Data Monitoring Committee has met 8 times and no safety issues have been identified. We aim to complete recruitment in December 2014.

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Genetic Epilepsy with Febrile Seizures Plus: the full spectrum encompasses GGE and focal epilepsies

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Aim: GEFS+ (generalised epilepsy with febrile seizures plus) is a familial epilepsy syndrome characterized by phenotypic heterogeneity that we described in 1997. The GEFS+ spectrum includes Febrile Seizures (FS), Febrile Seizures Plus (FS+), FS/FS+ with generalised and
focal seizures, and a range of epileptic encephalopathies including Dravet syndrome and epilepsy with myoclonic-atactic seizures. GEFS+ is infrequently associated with mutations of sodium channel and GABA receptor subunit genes; in addition, susceptibility genes have been found in smaller families. We studied 31 new families and analysed the phenotypic spectrum in these families and our previously published families.

**Methods:** We performed detailed electro-clinical phenotyping on all available affected family members and reviewed EEG and neuroimaging studies. We report the phenotypic findings on 408 affected individuals in a total of 60 families and compare this data to other GEFS+ studies.

**Results:** New phenotypes in GEFS+ families included focal seizures without preceding FS (16/408, 4%), classical genetic generalised epilepsies (22/408, 5%) most commonly childhood absence epilepsy, and afebrile generalised tonic-clonic seizures (9/408, 2%). FS remains the most frequent phenotype in GEFS+ (178/408, 44%) followed by FS+ (110/408, 27%). Large GEFS+ families are suggestive of autosomal dominant inheritance. Many smaller families exist where the inheritance pattern is more suggestive of complex inheritance.

**Conclusions:** Initially GEFS+ was conceived as comprising generalized epilepsy phenotypes and identified through large autosomal dominant families. Our data, show that the spectrum of phenotypes within GEFS+ is greater than originally conceived. Focal epilepsies and classical GGE are seen in GEFS+ families. We propose that GEFS+ be renamed “genetic epilepsy with febrile seizures plus” in view of the number of individuals with focal epilepsies. The overlap between GEFS+ and the classical GGE is considerably greater than first thought and suggests that the two major groups of generalised epilepsies have shared genetic determinants.

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**Fusing PET and MRI data using super-resolution track-weighted imaging**

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PET is arguably unique in its ability to provide molecular information about the brain by using a number of specific radioligands. For example, \(^{11}\)C-DASB is a radioligand having high affinity for serotonin transporters, which regulates sleep and wake, mood, feeding and other essential aspects of normal daily function. However, a major limitation of PET is related to its relatively low spatial resolution. For this reason, PET studies are commonly performed and interpreted in combination with co-registered high-resolution MRI or CT data, and therefore dual PET-CT and PET-MRI systems are becoming increasingly popular. However, in these studies the information is commonly fused by a simple image overlay. The MRI technique of super-resolution track-weighted imaging (TWI) was recently proposed as a generalized framework to extend the principles of super-resolution track-density imaging (TDI). In TWI, the information from whole-brain diffusion fibre-tracking (the so-called tractogram) is combined with a reference image, to generate a super-resolution track-weighted version of that image. In this study, we apply the TWI formalism to PET data to generate a super-resolution track-weighted (TW) PET map. We illustrate the methodology using ultra-high field 7T MRI data, and \(^{11}\)C-DASB data from a high-resolution research tomograph PET system coupled to the MRI via a shuttle bed with sub-millimetre accuracy. This approach is shown to produce high-quality maps, with very high resolution, where the intensity represents a track-weighted version of the PET image values. These maps therefore encode the molecular information from PET and display the super-resolution characteristics of TWI. They should therefore have important applications in neurology and neuroscience.
Imaging neuroplasticity of touch after stroke: training-facilitated changes following intervention.

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Background and Aim

Little is known about neural correlates of training-facilitated recovery after stroke, particularly following loss of body sensations. We aimed to characterise changes in location of brain activation associated with clinically-effective touch discrimination training in stroke patients with sensory loss following lesions of cortical or subcortical somatosensory regions. We predicted return to more 'normal' activity with training-facilitated recovery, and involvement of attention and visual regions targeted in training.

Methods

Twelve stroke patients, 7 with lesions involving primary (SI) and/or secondary (SII) somatosensory cortices and 5 with subcortical lesions involving the thalamus were studied. Clinical and MRI testing occurred at 6 months post-stroke, and following 6 weeks of touch discrimination training. Touch discrimination was quantified using the Tactile Discrimination Test. Whole-brain functional MRI studies employed a dynamic touch discrimination stimulus to the fingertips via a block design. A full-factorial, random-effects analysis comparing activation pre- and post-training, and across lesion location (cortical, subcortical) was conducted.

Results

Improvements in touch discrimination were of a similar magnitude to previous clinical trials. Those with subcortical lesions showed distributed activation pre-intervention (left supramarginal and right angular gyri) with return to left ipsilesional SI and trend in contralesional SII and ipsilesional insula post training: regions activated in our age-matched healthy controls. Those with cortical lesions did not show common activation pre- or post-training. They did however show common change over time involving ipsilesional precuneus. This change was greater than for subcortical lesions. Trends were noted in right contralesional middle frontal gyrus, cuneus and fusiform gyrus.

Conclusions

Improvement in touch discrimination coincided with return to a more normal pattern of cortical sensory activation post-intervention with subcortical lesions, while those with cortical lesions showed common change over time, in ipsilesional precuneus. Different patterns of change were observed despite a common training protocol and similar levels of sensory impairment and recovery.

Familial patterns of BOLD activation during EEG with functional MRI

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Aim

EEG-fMRI studies of genetic generalized epilepsy (GGE) have identified a consistent pattern of blood oxygen level dependent (BOLD) signal change in group studies. However, BOLD change may vary between individuals and the reasons for this are not completely understood. GGE displays complex inheritance patterns and is likely to involve multiple genetic elements.
In this study we explored whether genetic factors may contribute to inter-subject variability in BOLD change during EEG-fMRI by studying twins and siblings with GGE.

**Method:**
Three sibling pairs (including one monozygotic twin pair) with GGE were recruited through the EEG departments of the Austin Hospital and the Royal Children’s Hospital in Melbourne. A standard event related EEG-fMRI was performed on each subject. A Pearson’s correlation coefficient was calculated comparing all voxels between each sibling pair to quantify the degree of similarity. As a comparator, three subjects with GGE, who each had two EEG-fMRI studies of similar duration, were studied.

**Results:**
The Pearson’s correlation coefficient for the three subjects studied twice was 0.43-0.77. Monozyotic twins with juvenile absence epilepsy had a similarly high correlation of 0.58. A non-twin sibling pair with same syndrome (eyelid myoclonia with absence) had a correlation of 0.4, whereas the sibling pair with different syndromes had the lowest correlation of 0.23.

**Conclusion:**
Our findings demonstrate that greater phenotypic and genotypic similarity leads to a stronger correlation in BOLD change across the brain. This suggests that genetic factors may contribute to the pattern of BOLD change seen with event related analysis of epileptiform events.

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**Nodular heterotopia and absence seizures: fMRI evidence of active nodule involvement.**

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**Aim:** In this study we used EEG-fMRI to investigate whether peri-ventricular nodular heterotopia (PNH) has a role in seizure generation in individuals with absence seizures (AS) and co-existent PNH.

**Method:** Two subjects with PNH were identified in a cohort of subjects with AS. We performed event related EEG-fMRI of the patients typical ictal and interictal events as well as performing functional connectivity (FC) seeded from the PNH to answer this question.

**Results:** Both subjects demonstrated event related BOLD change in the “core” absence network. Subject 1 also displayed event related BOLD increase in the nodules while FC analysis demonstrated connectivity between the nodules and the thalami and striatum bilaterally. The second subject did not display event related BOLD in the PNH but FC analysis demonstrated strong connections between the PNH and the parietal cortex.

**Conclusion:** This study demonstrates that the peri-ventricular nodules can show connectivity to the absence network in individuals with AS and may be involved in seizure generation. It suggests that the network associated with absence seizures may be driven by a focal abnormality.
Chronic Cerebrospinal Venous Insufficiency (CCSVI) is not more prevalent in CIS or mild Multiple Sclerosis: A sonographer-blinded case control study.

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We designed a prospective case-control study of patients with clinically isolated syndrome (CIS) and relapsing-remitting MS (RRMS) with expanded disability status score (EDSS) ≤2, and age-and-sex-matched healthy controls, to test the hypothesis that Chronic Cerebrospinal Venous Insufficiency (CCSVI) is more prevalent in patients with CIS or mild MS. All subjects were examined using a Siemens Antares duplex ultrasound machine. The internal jugular, vertebral, and intracranial veins were studied in supine and sitting postures. The sonographer was blind to clinical status. Ultrasound parameters included those proposed by Zamboni, and the presence of CCSVI was defined as ≥2 Zamboni criteria.

Seventy patient-control pairs were recruited, with 11 males and 59 females in each group. Only one case, a control subject, satisfied the Zamboni definition of CCSVI. However, 19 subjects and 13 controls had Zamboni criteria abnormalities, the difference explained by an increased prevalence of IJV stenosis defined as cross-sectional area <0.3cm². This difference disappeared with a more rigorous stenosis definition. Further analysis revealed an abnormal IJV valve in 7 patients and one control.

Our findings indicate that CCSVI does not have a causal role in MS. However, an apparent increase in IJV valve abnormalities in MS patients warrants further investigation.

The Montreal cognitive assessment is valid in stroke but so is the mini-mental state examination

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Background and aims

Cognitive screening tools that are valid in stroke are needed. The Montreal Cognitive Assessment (MoCA) has been proposed as a more sensitive measure of cognitive impairment than the Mini-Mental State Examination (MMSE). We aimed to examine performance of the MoCA and MMSE against a full neuropsychological battery after stroke.

Methods

Patients with confirmed stroke completed 2 separate testing sessions at 3 months post-stroke. The MMSE and MoCA, each scored out of 30 and taking approximately 10 minutes, were administered in session 1. In session 2, a neuropsychological battery was administered. Patients were classified as cognitively impaired if they scored >1 SD below the mean on 2 or more of the 6 cognitive domains (visuospatial, attention, executive, memory, language, neglect).

Results

Sixty patients participated in the study [mean age 72.1 years (SD=13.9), mean education 10.5 years (SD=3.9), mean NIHSS stroke severity 5.8 (SD=4.0)]. The MoCA yielded lower scores (mean = 20.0, SD = 5.4; median = 21, IQR = 17-24) than the MMSE (mean = 24.2, SD = 4.5; median = 26, IQR = 22-27). MMSE data were more skewed towards ceiling than MoCA data (skewness = -1.09 versus -0.73). Thirty-nine patients (65%) were classified as cognitively impaired according to the neuropsychological testing. Against this classification, the area
under the receiver-operator curve for the MoCA was slightly higher than that for the MMSE (0.87 versus 0.84). At their optimal cut-offs, the MoCA had better sensitivity than the MMSE (0.92 versus 0.82) but poorer specificity (0.67 versus 0.76).

Conclusions
The MoCA is a valid screening tool for post-stroke cognitive impairment, and is typically more sensitive but less specific than the MMSE. Contrary to the prevailing view, the MMSE also exhibited acceptable validity in this setting.

Recruitment to trials of late thrombolysis: lessons from the EXTEND study.

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Background
To increase the percentage of acute stroke patients benefiting from thrombolysis, the utility of expanding the time window of treatment beyond 4.5 hours after stroke onset needs to be investigated.

Aim
We aimed to identify the target population and potential difficulties of recruitment for trials investigating thrombolysis beyond 4.5 hours.

Methods
An existing trial paradigm, Extending the time for Thrombolysis in Emergency Neurological Deficits (EXTEND), a multicentre randomized controlled trial testing the efficacy of thrombolytic therapy in patients with clinically significant ischaemic penumbra between 4.5 to 9 hours after stroke onset, was used as a model of the trial type.

Data from all stroke patients admitted to Austin Health, over a one year period were retrospectively analysed. Patients’ case notes were examined to determine potential trial eligibility.

Results
Of 556 patients with stroke assessed during the study period, 95 (17%) presented during the EXTEND time window. Sixty seven of these (70.5%) were wake-up strokes (WUS) and 28 (%) arrived between 4.5 and 9 hours after symptom onset. At least one exclusion criterion was found for 78 (82%) of them. Hence, during the 12 month review period, only 17 (3%) patients arrived within an appropriate time frame for the study without any exclusion criteria.

Most of these (13) arrived outside routine MRI hours. The number of patients recruited would have increased by more than 3 times if imaging had been available 24/7.

Conclusion
A significant proportion of ischaemic stroke patients present between 4.5 and 9 hours after stroke onset. The majority are wake-up strokes. Since the most common cause of the failure to potentially recruit to a trial of thrombolysis was arrival outside routine hours, 24/7 imaging may be a critical factor in the management of this important cohort of patients.
GLUT1 deficiency: detection of large deletions in the SLC2A1 gene using MLPA.

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Aim

Glucose transporter 1 (GLUT1), encoded by SLC2A1, is the facilitative glucose transporter across the blood-brain barrier. Mutations in SLC2A1 are found in the GLUT1-deficiency encephalopathy, a severe autosomal-dominant metabolic encephalopathy with refractory infantile-onset seizures, complex motor disorder, cognitive impairment and microcephaly. GLUT1-deficiency due to mutations of SLC2A1 is an increasingly recognized cause of genetic generalised epilepsy.

DNAs from patients with potential GLUT1-deficiency are usually screened for mutations by conventional Sanger sequencing. Although this approach will detect most mutations, deletions and duplications might be missed. To assess the prevalence and type of deletions and duplications in the SLC2A1 gene, we have performed Multiplex Ligation-dependent Probe Amplification (MLPA) on 115 patients with possible GLUT1-deficiency.

Methods

Patients for the analysis were selected based on their clinical presentation. MLPA was performed using the P138 SLC2A1 kit (MRC-Holland). Comparative Genomic Hybridization (CGH) was done using Illumina’s 720K tiled array chips.

Results

No duplications were detected, but SLC2A1 gene deletions were found in 2 patients. The MLPA results suggested that all 10 SLC2A1 coding regions (exons) were deleted in one patient. CGH confirmed this observation and indicated a deletion of approximately 55kb in one allele. MLPA analysis of the parental DNAs showed that none of the parents carried the deletion. As expected from the clinical information and genetic knowledge, the deletion is a de novo mutation.

In the other patient the deletion included exons 3 and 4. The maximum length of this deletion is 13kb.

Conclusion

Deletions in the SLC2A1 gene are relatively common in and should be screened for when suspecting GLUT1 deficiency in a patient. MLPA is a cost-effective and rapid method for such investigations. It has direct clinical relevance as the ketogenic diet is indicated in cases with GLUT1 deficiency.

White Matter fibre Tractography: Why we need to move beyond DTI

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Introduction:

Neurosurgical planning requires biologically accurate mapping of white matter pathways that support eloquent cortical regions in order to determine safe margins of resection. The majority of white matter fibre tractography software packages available to clinicians rely on a fundamentally flawed framework to generate fibre orientations from diffusion-weighted data, namely Diffusion Tensor Imaging (DTI) [1]. This work provides the first extensive and systematic exploration of the practical limitations of DTI-based tractography and investigates
whether the higher-order tractography model Constrained Spherical Deconvolution (CSD) [2], provides a reasonable solution to these problems, within a clinically feasible timeframe.

Methods:
DTI- and CSD-based tractography was performed to visualise the corticospinal pathways in 10 patients with concomitant risk of neurological deficit following neurosurgical resection, and 45 normal control subjects.

Results:
In all cases, DTI-based tractography methods substantially underestimated the extent of tracks connecting to the sensorimotor cortex. In contrast, the CSD-based tractography method consistently produced the biologically expected fan-shaped configuration of tracks. In the clinical cases, where tractography was performed to visualise the corticospinal pathways in patients with concomitant risk of neurological deficit following neurosurgical resection, the CSD-based and DTI-based tractography methodologies indicated very different apparent safe margins of resection; the Constrained Spherical Deconvolution-based method identified corticospinal tracts extending to the entire sensorimotor cortex, whilst the Tensor-based method only identified a narrow subset of tracts extending medially to the vertex.

Discussion:
This comprehensive study shows that the most widely used clinical tractography method (DTI-based tractography) results in systematically unreliable and clinically misleading information. The higher-order tractography model, using the same diffusion weighted data, clearly demonstrates fibre tracts more accurately, providing improved estimates of safety margins, which are crucial for cortical surgical procedures.


A retrospective review of the ambulatory blood pressure patterns in subgroups of spinal cord injured patients

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Introduction: Patients with spinal cord injury (SCI) frequently experience disabling orthostatic hypotension and autonomic dysreflexia, which limit independence. Ambulatory blood pressure monitoring (ABPM) is advantageous over conventional BP measurement as it is able to evaluate diurnal BP patterns in the setting of variable BP as seen in spinal cord injury (SCI). Patients with high SCI have been noted to lose the nocturnal dip in BP and to have nocturnal polyuria.

Methods: A retrospective study was conducted on medical records of patients with traumatic SCI who were referred for ABPM. Results of ABPM and nocturnal urine production were compared between groups of patients classified according to level, completeness and chronicity of SCI. Patients with night:day systolic BP <90% were classified as dippers, 90-100% as non-dippers and >100% as reversed-dippers.

Results: Patients (45 quadriplegic, 9 paraplegic) were predominantly males (92.6%) of average age 40.8±2.5 years (mean±SEM). Mean 24-hour BP was 111.1±1.4/65.0±1.2 mmHg, with a mean night:day systolic BP of 101.7±1.3%; 56% were reversed-dippers. There was a greater proportion of reversed-dippers in quadriplegics than paraplegics (62% vs 22%, p=0.027), complete than incomplete (73.9% vs 38.9%, p=0.02), and acute than chronic (73% vs 40%, p=0.03) SCI. Quadriplegic patients with higher nocturnal urine production tended to be reversed-dippers (80% vs 30%, p=0.034) and have acute SCI (90% vs 43%, p=0.036).

Conclusions: In our referred population, patients with acute, complete and quadriplegic injuries tended to have the greatest reversal of diurnal BP variation and nocturnal urine production. Further study of ambulatory BP patterns and urine production in SCI may be useful in characterising the course of BP patterns after SCI, and its effects on patients.
Alternating Hemiplegia of Childhood is caused by De Novo Mutations in ATP1A3

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Aim
To identify the genetic basis and phenotypic features of alternating hemiplegia of childhood (AHC) in Australian and Danish patients.

Background
AHC is a rare infantile onset disorder characterised by recurrent hemiplegic episodes and progressive neurological deterioration including developmental slowing, episodes of dystonia and choreoathetosis. A large international consortium studied patients with AHC to identify the molecular basis of this devastating disorder.

Method
Patients with AHC from Australia and Denmark were recruited and phenotyping performed. Whole exome sequencing of seven patients with AHC was performed. Parental DNA was studied to determine the inheritance of variants of interest. The gene identified was then Sanger sequenced in 98 patients with AHC including the Australian and Danish patients.

Results
Whole exome analysis identified de novo mutations in ATP1A3 encoding the sodium-potassium (Na⁺/K⁺) ATPase a3 subunit in all seven of the original probands. In the remaining 98 patients, including our seven cases, sequencing identified ATP1A3 mutations in 75 patients. This included our patients with AHC including six missense and one splice site mutations. For our seven patients, alternating hemiplegia episodes had an infantile onset and all have experienced full body episodes, eye movement abnormalities, gross motor delay and intellectual disability. Five patients showed ataxia, one showed chorea, four showed dystonia and six developed epilepsy.

Conclusion
De novo ATP1A3 mutations account for 74% of patients with AHC including all seven of the Australian and Danish cohort. Sequencing is now a diagnostic test for AHC.

Mutations in FOXP2 cause childhood apraxia of speech: report of a novel intragenic deletion in an Australian patient

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Developmental speech and language problems are a heterogeneous group of disorders that can prove difficult to manage clinically. In most cases the etiology is unknown, although in a minority genetic variants have been implicated or shown to confer susceptibility. The first
specific subtype to be directly linked to a molecular genetic defect was childhood apraxia of speech (CAS) due to mutation in the FOXP2 gene at the SPCH1 locus. We completed recruitment of eight Australian families where the proband had a primary diagnosis of motor speech disorder. Affected family members were phenotyped using a comprehensive assessment battery that comprised measures of speech, oromotor function, language, literacy skills and cognition. We screened the coding regions of the FOXP2 gene in all eight probands and identified novel variants in two. We then determined the inheritance pattern and segregation of the variant in the probands' families. Here we describe these variants including the first reported small, intragenic deletion of the FOXP2 gene. Our results support the current evidence that FOXP2 mutation is a well-defined cause of CAS, and extend the genotype-phenotype spectrum of FOXP2-related speech and language disorders.

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Neural pathways mediating the counter-regulatory response to hypoglycaemia

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Here, it is hypothesized that ventral medullary presympathetic neurons relay the efferent information to the adrenal medulla in response to hypoglycaemia (i.e. counter-regulatory response), but do not sense the changes in glycaemia. The aim of the present study was to determine whether or not glucoprivation of presympathetic neurons in the rostral ventrolateral medulla (RVLM) increases the sympathetic discharge to the adrenal gland. Electrophysiological experiments were conducted in anaesthetized (urethane, 1.2 g/kg i.v.), paralysed (pancuronium bromide, 1 mg/kg i.v.), and artificially ventilated male Sprague-Dawley rats (N=7). Local glucopenia, induced by bilateral microinjections of 2-Deoxy-D-glucose (2DG; 15 ng/50 nl) into the RVLM, did not change adrenal sympathetic nerve activity (101±1% vs 84±14%) or blood glucose (6.4±0.1 vs 6.9±0.1 mmol/ml). On the other hand, subsequent intravenous infusion of 2DG (300 mg/kg i.v.) increased adrenal sympathetic nerve activity (101±1% vs 183±8%) and blood glucose (6.4±0.1 vs 13.5±0.4 mmol/ml). The results indicate that RVLM presympathetic neurons are not glucose sensitive, although they contribute to the efferent pathways involved in the counter-regulatory response. Supported by the NHMRC.

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Changes in activity levels in the first month after stroke

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Aim: In this pilot study participant activity was monitored prospectively over a single day on two separate occasions. Method: Individuals with confirmed stroke greater than 18 years and less than 15 days post-stroke at time of recruitment were eligible. Activity was measured from 8 a.m. to 5 p.m. using an accelerometer. The first day was scheduled within 14 days of stroke and the within four weeks post-stroke. We looked at the following activity categories number of transitions, time spent lying, sitting and dynamic activity. We were also interested in whether the device was comfortable to wear. Results: 16 individuals were included in this study with a median age of 79.5 (interquartile range 28.3). According to the NIHSS score, 56% of the participants had mild, 31% had moderate and 13% had severe stroke. The follow-up location included rehabilitation facilities and at home. At baseline a median of 2% per day was spent in dynamic activity, most time was spent sitting. There were no significant changes in number of transitions, time spent in dynamic activity and lying and sitting. The majority of participants stated that wearing the device was comfortable. Conclusion: Activity levels are
similar to activity levels early after stroke in other studies. Activity levels are low at an acute
stroke ward and do not significantly change within the first month. Using an accelerometer is
a feasible method to measure activity levels at an acute stroke setting and a rehabilitation
facility.

Comparison of cortical thickness measurement methods in a stroke
population
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Background: Accurate cortical thickness measurement is important for the imaging study of
neurodegenerative diseases. Cortical thickness measurement can be broadly classified as
surface-based and voxel-based. There is considerable variability in cortical thickness
measurements using different methods, even in similar patient populations, and there is no
formal agreement on what constitutes the best cortical thickness measurement method. Most
related study were based on dementia populations, and minimal data in stroke patients have
been published.

Aims: This study aims to compare the accuracy of different methods to demonstrate the
optimal method of estimating longitudinal cortical thickness changes in a stroke population.

Methods: Stroke patients presenting with first-ever acute middle cerebral artery stroke were
scanned in the MRI, studied within 2 hours and serially over 3 months. We compared the 2
hour and 3 month scans with independently acquired control images, also taken 3 months
apart. Control patients were age-matched and free from significant psychiatric and
neurological disease. Cortical thickness was separately measured by FreeSurfer, Laplacian
method and Registration method.

Results: Ten control participants (5 men, mean age=67.2 years) and twelve stroke patients (9
men, 7 left-hemispheric, mean age=65.1 years, 10 subcortical, 2 cortical) were included.
There was no significant change either in mean cortical thickness (p=0.68 using FreeSurfer,
p=0.36 using Laplacian and p=0.85 using registration) or change percentage (0.2%, 0.8% and
1.3%, respectively). In patients group, significant changes over the time were found using
FreeSurfer (1.7%±±, p=0.04) and Laplacian (-2.8%± p=0.02) methods. The registration
method detected a small decrease in thickness but this was not significant (-2.8%±, p=0.33).

Conclusion: In terms of longitudinal analysis for both patients and controls, these methods
perform equally well. Compared with Laplacian and Registration methods, the surface-based
method provides more accurate results in patients with stroke.

Nocturia in Multiple Sclerosis: A Pilot Study
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Background
Nocturia is a common symptom in multiple sclerosis (MS). One possible mechanism is that
autonomic dysfunction blunts the normal overnight reduction in blood pressure (BP),
increasing nocturnal urine production. This research was aimed at studying patterns of
nocturia in MS patients and its relationship to nocturnal blood pressure (BP) and heart rate
(HR).

Method
Consecutive ambulatory MS patients were screened for nocturia before being asked to
participate in the study. Urine production was measured by self-completed diary, and 24-hour
ambulatory BP and HR were measured by ambulatory recording (CardXplore). Results were
compared with sex matched controls of similar age (age 41±14.4 years).
Results
Amongst 30 ambulatory MS patients, 18 (60%) reported nocturia of >1/night, and 10 consented to the study. There were 5 females and 5 males, aged 43.1±9.3 yrs (mean ± SD), with EDSS ranging from 2-5. Patients who self-reported nocturia did have demonstrable increased nocturnal urinary frequency. Compared to controls, the study group had more frequent nocturia (1.6 vs. 0.1, \( p=0.008 \)), and passed both a larger proportion (36.3% vs. 28.2%, \( p=0.009 \)) and total volume (656 vs 414ml, \( p=0.04 \)) of urine at night. MS patients also had smaller urine volumes both during day (149 vs 210ml, \( p=0.05 \)) and night (246 vs 410ml, \( p=0.01 \)). Ambulatory BP and HR did not differ between the study group and controls.

Conclusion
Nocturia in ambulatory MS patients occurs in the context of higher nocturnal urine production. This does not appear to be related to higher BP and HR overnight.

Prevalence of rapid atrial fibrillation in stroke patients and its impact on management and prognosis

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Aim: to assess the prevalence of atrial fibrillation with rapid (>100 bpm) ventricular response (‘rapid AF’) in stroke patients and to establish its influence on their in-hospital mortality and morbidity. While it is known that atrial fibrillation is common in stroke patients and predicts poor outcome, the impact of rapid atrial fibrillation has not been previously reported.\(^1\)^2\(^3\)

Method: retrospective audit study. Medical records of all patients admitted to the Austin Hospital with a confirmed diagnosis of stroke in the period between 23/09/11 to 31/12/11, were analysed (total of 128 patients). 4 groups of patients were compared (rapid atrial fibrillation, controlled atrial fibrillation, sinus tachycardia and sinus rhythm), depending on the presence of a particular rhythm at any point during the admission. Logistic regression analysis for potential confounders was performed using STATA 12.

Results: atrial fibrillation was found to be more prevalent than historical reports (38% of patients vs. 13.6%-31%), while rapid AF was also common (16%). Importantly, when compared to sinus rhythm and adjusted for stroke severity and age, rapid AF was found to be strongly associated with increased in-hospital mortality (23.8%, OR = 5.45, \( p\)-value = 0.042, cf. 7.3% in sinus rhythm) and increased number of MET calls and ICU admissions (33.3%, \( p\)-value 0.004, cf. 5.45% in sinus rhythm). While mortality and morbidity were highest in the rapid AF group, the differences between rapid and controlled AF were not statistically significant.

Conclusion: Rapid AF was found to be a common co-morbidity in stroke patients that indicates worse prognosis and demands increased allocation of hospital resources. Further studies are warranted to determine which strategies can prevent rapid AF in stroke patients and management approaches that can improve outcomes.

Ambulatory Physiological Monitoring of Patients with Spinal Cord Injury using a Bluetooth Body Area Sensor Network with a mobile phone web interface measuring a range of parameters including Post Activity Cuffless Blood Pressure

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A fully wireless ambulatory multi-parameter monitoring system has been developed and trialled successfully on those with spinal cord injury. It is fully integrated, lightweight and wearable with low power consumption allowing for continuous monitoring in various real-time environments or epoch measurements for extended monitoring durations sending to a website.

Wireless sensor node technology is employed for signal conditioning, data acquisition, embedded digital signal processing and power management with a minimized form factor of 35x10mm and a weight of 20 grams.

Sensor node communication is implemented using Bluetooth to form a Body Area Network (BAN) with a Bluetooth enabled mobile phone is used as a data access point eliminating cumbersome wires between sensors. Chest sensors have also been woven into a tshirt.

This enables the wireless monitoring of a mobile SCI patient in the field to detect physiological stress related parameters such as heart rate, heart rate variability, skin conductance, body/environmental temperature, respiration rate and depth, activity (accelerometers and gyro’s) and now blood pressure.

Importantly blood pressure can now be estimated non-invasively and continuously through photoplethysmography (PPG) and ECG implemented within the above body area network (BAN) and validated at 2 non-intrusive locations (arm and leg) on paraplegic spinal cord injured (SCI) patients undergoing rehabilitation.

The prototype system will be demonstrated with real time data.

Keywords- Ambulatory monitoring, multi-parameter, stress, Monitoring Photoplethysmography; Body Area Network; Rehabilitation; Spinal Cord Injury; Blood Pressure

1. MONITORING SPINAL CORD PATIENTS DURING ACTIVITY USING A DATALOGGER, Technology and Disability 17, (2005) 1-7, Nunn et al
Unusual Mendelian Inheritance Unveiled by New Technology

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**Background:** Although many common human diseases, like epilepsy, are believed to be due to multiple genes (complex inheritance), there is increasing recognition for the role of Mendelian genetics in these disorders. Consanguineous families have proven powerful for solving recessive disorders, especially since the recent development of high-density SNP chips. We describe a consanguineous Turkish family where analysis using SNP technology identified a dominantly inherited Mendelian locus, despite the *a priori* assumption of a recessive disease model.

**Methods:** Fourteen family members (twelve affected and two unaffected) were chosen for SNP genotyping using the Illumina HumanOmniExpress BeadChip array. Parametric and non-parametric linkage analyses were performed using MORGAN. The parametric disease model used, where A is a healthy allele and a (P(a)=0.001) is the disease allele, was: P(affected | AA) = 0.0001; P(affected | Aa) = 0.5; P(affected | aa) = 0.999.

**Results:** A single large region (~40Mb) on chromosome 5 was identified, where all twelve affected family members shared one (inherited maternally or paternally) or two (inherited bi-parentally) copies of the same putative disease haplotype originating from a common ancestor. A single copy of the haplotype was also found in one unaffected family member, suggesting incomplete penetrance. No additional phenotypic variability was evident between individuals homozygous versus heterozygous for the disease haplotype.

**Conclusion:** We have shown that inbreeding can enrich for disease alleles resulting in dominant inheritance and that mutations in the heterozygous versus homozygous state may result in identical phenotypes. Most importantly, we have localized the putative disease allele with the opportunity now for novel gene discovery in a common form of epilepsy.

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Apparent Fibre Density: A novel MRI approach that identifies specific white matter tracts affected in Alzheimer's Disease

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**Diffusion MRI (DWI) studies have previously identified changes to white matter in Alzheimer’s Disease (AD).** These studies have investigated voxel-average quantitative measures derived from diffusion tensor imaging, with no fibre-bundle specificity in voxels containing multiple distinct fibre bundles. However, recent evidence suggests 90% of white matter voxels contain two or more fibre bundles. Here we investigate AD and mild cognitive impairment (MCI) using a new, more advanced diffusion MRI measure called Apparent Fibre Density (AFD) [1], which is a measure that can be interpreted as being proportional to the intra-axonal volume of axons oriented along a particular direction [1].

DWIs were acquired from 39 AD, 34 MCI and 91 healthy volunteers (Australian Imaging Biomarkers & Lifestyle cohort). Whole-brain AFD comparisons between the AD and healthy group were performed as in [1]. Within white matter fibre bundles that were identified as
having a significant AFD decrease in AD, we performed a subsequent ROI analysis to investigate MCI in comparison to healthy and AD subjects.

Significant decreases in AFD were observed in AD compared to healthy subjects in locations and orientations corresponding to the cingulum, uncinate, anterior commissure, corpus callosum, internal capsule, and superior longitudinal fasciculus. ROI analysis identified decreased AFD in all above listed structures except the internal capsule when comparing AD vs MCI, but only in the left cingulum, uncinate, and CST when comparing MCI vs healthy subjects.

We demonstrated extensive AFD decreases in AD vs healthy subjects in bundles known to be involved in language and memory. These were more extensive than have been reported using previous DWI metrics. Most of the affected regions were also different between MCI vs AD, with some regions also significant in MCI vs healthy. AFD provides improved specificity by identifying which particular white matter tract is affected even in regions with multiple fibre bundles.


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Evolution of ischemic damage over 6 months after stroke in the rat.
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INTRODUCTION: Infarct volume is the most common outcome of experimental stroke studies, yet is often only assessed acutely. This study aims to document the development of histological damage over 6 months post stroke.

METHODS: 132 Spontaneously Hypertensive Rats underwent thread occlusion MCAo for 90 minutes or sham, with stroke animals randomised to recovery time: 1, 3, 7, 14, 21, 28 days, 12 and 24 weeks (n≥11 per group). Damage was delineated on H&E stained sections.

RESULTS: 90 minute MCAo resulted in a medium sized cortical and striatal infarct. Acute damage was characterised by infarction of the striatum and cortex, with oedema peaking at 12.7% at 3 days. Oedema resolved by 7 days. Atrophy of the ipsilateral hemisphere was evident from 28 days. Macrophages and other infiltrating cells packed the area of infarct from 7 days, facilitating clearance of damaged tissue to leave a fluid filled cavity, which grew from 14 days. Whilst the volume of damage changed over time, an equivalent proportion of tissue was lost at all time points (26±7-34±12% of contralateral hemisphere).

CONCLUSION: Damage progressed from a necrotic infarct to a fluid filled cyst over time, with hemispheric size changing in relation to the type of damage. Variability in the volume of damage may be due to individual differences in the rate of clean up of the infarct. Examining the development of behavioural and histological damage to chronic timepoints is an important step in both understanding stroke and bringing animal models closer to the clinical situation.

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Clinical genetic study of the epilepsy-aphasia spectrum
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Purpose: To characterize the frequency and nature of the family history of seizures in probands with epilepsy falling within the epilepsy aphasia spectrum (EAS) in order to understand the genetic architecture of this group of disorders.
Methods: Patients with epileptic encephalopathy with continuous spike-and-wave during sleep (ECSWS), Landau-Kleffner syndrome (LKS), atypical benign partial epilepsy (ABPE) and intermediate epilepsy-aphasia disorders (IEAD) were recruited. All affected and available unaffected relatives up to three degrees of relatedness underwent phenotyping using a validated seizure questionnaire. Pedigrees were constructed for all families. The proportion of affected relatives according to each degree of relatedness was calculated. The epilepsy phenotypes in close relatives were analysed. The data were compared to the families of probands with benign childhood epilepsy with centrotemporal spikes (BECTS) using the same methodology.

Key findings: 31 probands, including 5 ECSWS, 3 LKS, 1 ABPE and 22 IEAD, were recruited. The mean age of seizure onset was 3.9 (range 0.5-7) years. A male predominance 21/31 (68%) was seen. 16/31 (51.6%) had a positive family history of seizures. Among 1254 relatives, 30 (2.4%) had a history of seizures: 13 (10.2%) of 128 first degree relatives, 5 (1.7%) of 291 second degree relatives, and 12 (1.4%) of 835 third degree relatives. There is no difference compared to relatives of BECTS. Of 19 relatives with epilepsy, 4 had BECTS, 4 epilepsies with focal seizures of unknown cause, 3 IEAD, and 7 unclassified. One had genetic generalized epilepsy.

Significance: The frequencies of seizures in relatives of probands with EAS suggest that the underlying genetic influence of EAS is consistent with complex inheritance and similar to BECTS. The phenotypic pattern observed in the affected relatives comprised predominantly febrile seizures and focal seizures. These findings suggest that a shared genetic predisposition to focal epilepsies underpin the epilepsy aphasia spectrum.

Phenotypic spectrum of PRRT2 mutations

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Aim: To examine the role of PRRT2 mutations in families with Benign familial infantile epilepsy (BFIE) and the associated syndrome infantile convulsions and choreoathetosis (ICCA); and to investigate the phenotypic spectrum of PRRT2 by studying patients with sporadic benign infantile seizures and non-BFIE familial infantile seizures.

Background: BFIE is an autosomal dominant, self-limited seizure disorder characterized by afebrile seizures beginning at about 6 months of age. In ICCA syndrome, infantile seizures and the childhood or adolescent-onset movement disorder, paroxysmal kinesigenic dyskinesia (PKD), co-occur. Mutations of the gene PRRT2, encoding the proline-rich transmembrane protein 2 gene, have been identified in families with PKD.
Methods: Sixty-seven families or individuals with benign infantile or neonatal-onset seizures underwent detailed phenotyping and PRRT2 sequencing. The familial segregation of mutations identified in probands was studied.

Results: Mutations in PRRT2 were identified in 18/23 families with BFIE and 10/11 families with ICCA. Two probands with no family history of infantile seizures or PKD had de novo PRRT2 mutations. Febrile seizures with or without afebrile seizures were observed in some families with PRRT2 mutations.

Conclusions: PRRT2 is the gene for BFIE and ICCA as well as PKD. Mutations are present in approximately 80% of BFIE and 90% of ICCA families, but are not a common cause of other forms of infantile epilepsy. De novo mutations of PRRT2 can cause sporadic benign infantile seizures. Seizures with fever may occur in BFIE causing difficulty distinguishing BFIE from febrile seizures and febrile seizures plus in small families. Mutations in PRRT2 cause both epilepsy and a movement disorder, and elicit pleiotropy in both age of expression (infancy versus later childhood) and anatomical substrate (cortex versus basal ganglia).


SIFT: Spherical-deconvolution Informed Filtering of Tractograms

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AIM

Diffusion MRI allows the structural connectivity of the whole brain (the ‘tractogram’) to be estimated in vivo non-invasively using streamlines tractography. The biological accuracy and interpretability of these data is however limited by the inherent biases associated with the reconstruction. We propose a method to retrospectively improve the accuracy of these reconstructions through selective removal of streamlines.

METHOD

Simulations have shown that the amplitude of the Fibre Orientation Distribution estimated using spherical deconvolution is proportional to the intra-cellular volume of the underlying axons aligned in the corresponding orientation. We model streamlines as bundles of fibres of some cross-sectional area; each streamline therefore contributes to the intra-cellular volume per unit length. Streamlines are selectively filtered from the reconstruction in a manner that improves correspondence between the intra-cellular volume as estimated by the tractogram, and the amplitude of the Fibre Orientation Distribution, in each image voxel.

RESULTS

Tractograms processed by this algorithm show a marked reduction in known reconstruction biases, such as the artificially dense reconstruction of long bundles (due to their large seeding volume) and the inadequate reconstruction of superficial white matter pathways. After filtering, streamline coverage of the white matter is relatively homogeneous, even in regions of complex fibre architecture; yet pathways with more dense fibre density (such as the cortico-spinal tract and optic radiations) are reconstructed as such.

CONCLUSION

Our method improves the correspondence between a streamlines reconstruction and the underlying white matter, and associates a biologically-meaningful interpretation to the connections it provides. Emerging analysis techniques in diffusion MRI that aim to characterise and compare the structural connectivity of the brain should benefit from the improved accuracy and interpretability of the reconstructions filtered using this method.
What are the main reasons for exclusion from an early rehabilitation trial (AVERT)?

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Background and Aims: A Very Early Rehabilitation Trial (AVERT) is an ongoing multi-centre international single-blind randomised controlled trial in stroke rehabilitation. Very early mobilisation represents a promisingly simple intervention with wider applicability than most drug interventions. However even with such a generalisable intervention recruitment has been slower than predicted. Therefore we aimed to analyse the main reasons for non-recruitment into AVERT and characteristics of those recruited versus non-recruited.

Methods: Design: Ongoing multicentre, international, Phase III RCT using nurse/physiotherapist clinicians as recruiters. Population: The inclusion and exclusion criteria for this study are broad with no upper age limit, no limit on stroke severity and people with haemorrhagic stroke are eligible for inclusion. Screening Logs: Centres regularly submit information on all patients admitted with stroke. Logs include age, gender, stroke type, stroke severity and reason/s that they were not recruit. Recruiters are able to select more than 1 reason.

Results: 20,000 patients at 46 centres were screened from July 2006 to December 2012 with 1159 recruited. The overall recruitment rate is currently 6.2% (range: 1.5 – 17.5%). Stroke severity and type were similar between those recruited and those not. Recruited patients however were more likely to be male. Women were more likely to present with premorbid disability, be more medically unwell and arrive after the 24 hour recruitment window. They were also more likely to be missed by the recruiter.

Conclusions: Patients recruited to AVERT are representative of the usual stroke population. The most common reason for non-recruitment is delay in getting to hospital (39%), similar to previous research. Women are more likely to delay getting to hospital. Therefore, public health programs of stroke symptom awareness targeting women may be worthwhile. A >6% recruitment rate is better than many acute stroke trials however we continue to explore strategies to improve patient recruitment.

Prior cognitive state systematically alters patterns of resting state functional connectivity

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Functional magnetic resonance imaging based resting state functional connectivity (FC) analysis, used to identify brain regions with shared variance in their temporal timecourses, is increasingly used in basic and clinical neuroscience. Previous work has suggested that FC is dependent upon the cognitive task engaged in immediately prior to collection of resting state data (Waite et al, 2005, Human Brain Mapping, 24:59). We sought to explore this possibility further, as any bias introduced by prior cognitive state could propagate through to influence the results of group FC analyses collapsed across different rest conditions. Twenty five healthy volunteers were scanned continuously for 27:00 minutes, alternating between periods of extended rest and block design task according to the following sequence: rest1, task1, rest2, task2, rest3. The two tasks were a language task and a motor task, with task order randomized across subjects. Four language and four motor seeds (5mm sphere) were selected based on the activation tasks. FC analyses seeded from each of these seeds were conducted on each block of rest data (rest1, rest2, rest3). Repeated measures t-tests comparing, across subjects, FC maps from each rest block were performed using SPM8.
Individual and group level connectivity changes were common, especially when the initial rest period was compared with post-task rest periods, and tended to occur in regions of weak to moderate connectivity with the seed. These findings indicate that prior cognitive state can induce systematic changes in FC networks, and that large group studies should endeavor to collect rest data under comparable conditions. Given the timescale of these changes, they also raise the question of the degree to which FC changes observed in neurological and psychiatric disease are due to “organic” and/or “psychological” factors.

MRtrix: tractography in crossing fibre regions

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Diffusion tensor imaging (DTI) has rapidly become established as the method of choice for the non-invasive study of brain white matter and its architecture, due to its unique ability to estimate the direction of the fibres, from which it becomes possible to infer long-range connectivity using so-called fibre-tracking or tractography algorithms. However, it is now well-known within the imaging community that the assumptions underlying DTI are flawed, since it can only characterise a single fibre orientation within each pixel. It has recently been shown that given the typical pixel size of 2.5 mm, crossing fibres can be detected in ~90% of the white matter. For these reasons, a number of alternative, higher-order models have been developed that can capture this type of information. Amongst these, our constrained spherical deconvolution (CSD) method has been shown to be robust, efficient and applicable to clinical realistic protocols.

While a large number of freely available software packages exist to perform DTI processing, relatively few packages are available to perform these higher-order analyses. To address this issue, we have made our MRtrix software package freely available to the community under an open-source license, to enable clinicians and neuroscientists to perform cutting-edge tractography analyses in a manner robust to crossing fibre effects. It provides the tools to estimate the fibre orientations using CSD, a probabilistic fibre-tracking algorithm, and a highly interactive viewing tool to display the results. We anticipate that the availability of this type of software will enable researchers to move beyond the flawed DTI model and hence obtain more reliable results, with obvious benefits for applications such as neurosurgical planning.

START-PrePARE – Prediction and Prevention to Achieve Optimal Recovery Endpoints after stroke: Study rationale and protocol.

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Background: Stroke and depression have two of the highest Burden of Disease rankings. About one third of stroke survivors experience depression. Depression is associated with worse outcome, more cognitive deficits, poorer functional and rehabilitation outcome, reduced quality of life and reduced participation in previous life activities. Yet, post-stroke depression is under-diagnosed. Moreover, good predictors of depression that could be used to identify ‘at risk’ patients early as part of the clinical care pathway currently do not exist. Further, we rarely measure the stroke survivor’s participation in domestic, social and leisure domains when they have returned home. The ability to predict the impact of neurological impairment and factors
such as depression and cognitive impairment on participation is critical for discharge planning, patient education and planning of health resources.

**Aim:** Our aim is to identify predictors of depression based on imaging and functional outcome (in particular participation), which may be used in clinical management to aid early diagnosis, prevention and more targeted interventions.

**Methods:** A longitudinal cohort of 100 stroke survivors will be investigated for functional and structural changes in putative brain regions associated with depression, and for functional outcome including cognition and participation. Participants will be recruited into the START-PrePARE study from selected study sites in metropolitan Melbourne. Stroke survivors will be investigated at Day 3-7, 3 months and 12 months for depression and 3 and 12 months for changes in the brain and functional outcome including cognition and participation. We will also monitor factors such as stroke severity, diet and lifestyle that may have an influence. The impact of depression on stroke outcomes and participation in previous life activities will be quantified using the Activity Card Sort.

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**Effects of central orexin 1 receptor blockade on locus coeruleus neurons with lateral hypothalamic input**

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The locus coeruleus (LC) receives a major orexinergic input and is densely packed with orexin 1 receptors (OX-1-R). Here we examined the effects of central administration of an OX-1-R antagonist on lateral hypothalamic (LH) stimulation-induced activation of LC neurons. All experiments were conducted using male Sprague Dawley rats under initial isoflurane (1.7-1.9%) anaesthesia followed by urethane (1.3-1.4 g/kg, i.v.). Electrical stimulation of the LH (0.5 Hz, twin 0.5 ms pulses, 3 ms interpulse interval, 300uA) evoked excitatory responses in all LC neurons studied (n = 24) as judged by construction of peri-stimulus time histograms. LC neurons responded to LH stimulation with an onset latency of 6.5±0.4 ms and a peak latency of 15.3±0.8 ms. Intracerebroventricular administration of the OX-1-R antagonist SB334867A (10 nmol) but not vehicle inhibited LH stimulation-induced activation of LC neurons by 58±14% (n = 6 neurons). Abolition of LH stimulation-induced excitatory responses in LC neurons occasionally (n=3/6) revealed constant latency antidromic responses verified by positive collision tests. These findings indicate that the LC receives a robust excitatory input which is mediated, at least in part, by orexin acting at OX-1-Rs. Furthermore, some LC neurons that receive an orexinergic input also project to the LH region. This pathway may be important for modulation of vigilance and arousal during hypoglycaemia. Supported by the Austin Hospital Medical Research Foundation.

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**In vivo tau imaging in Alzheimer's disease and other dementias**

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**Background:** Definitive diagnosis of Alzheimer’s disease (AD) and non-AD tauopathies still relies on post-mortem examination. These diseases are often difficult to differentiate clinically due to overlapping phenotypes, especially at early stages. In vivo imaging with PET will allow new insights into tau deposition, facilitating research into causes, diagnosis and treatment of tauopathies. We have characterized 18F-THK523 (THK523), a novel tau imaging ligand...
developed at Tohoku University in Sendai, Japan, assessing its selectivity and specificity for tau pathology both in vitro and in vivo.

**Methods:** Apart from acute toxicity microdose evaluation and *in vitro* receptor binding screen providing direct evidence of a lack of pharmacological activity or toxicity, preclinical evaluation included *in vitro* binding studies, autoradiography (ARG) and histofluorescence (HF) analysis of human hippocampal sections, and *in vivo* microPET studies in tau and APP/PS1 transgenic mice. To date, 18 human participants underwent both THK523 and PiB PET studies.

**Results:** THK523 binds with higher affinity to recombinant tau compared with Aβ fibrils. ARG and HF analysis of human hippocampal sections demonstrated THK523 co-localized with immunoreactive tau pathology, but failed to highlight Aβ plaques. MicroPET studies revealed higher brain retention of THK523 in tau transgenic mice compared with their wild-type littermates or APP/PS1 mice. Initial human PET studies comparing THK523 and PiB have shown that THK523 does not bind to Aβ in AD. Significantly higher THK523 retention was observed in the parietal, temporal and hippocampus, following the known regional distribution of tau deposits in AD. The hippocampal retention correlated with hippocampal atrophy and cognitive parameters.

**Conclusions:** Preliminary PET human studies suggest that THK523 does not bind to Aβ, presenting higher and appropriate regional distribution in AD patients. Further studies, assessing non-AD tauopathies such as Progressive Supranuclear Palsy and Corticobasal Syndrome, are underway to confirm these initial findings.

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**Kinetics of Aβ deposition: 20-year interval between normality and fully developed Alzheimer's disease**

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**Background:** In this study, we used longitudinal data from the AIBL cohort to calculate the rates of Aβ deposition in healthy controls (HC), Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD) patients.

**Methods:** One hundred and ninety five participants -142 HC; 36 MCI; and 17 AD- were evaluated at enrolment and 18 and 36 months later. On each visit, participants underwent neuropsychological examination, a MRI and PiB PET scan. A SUVR threshold of 1.5 was used to categorize high (+) and low (-) Aβ burden. Rates of change for Aβ deposition, MRI volumetrics and cognitive decline were derived from the slope of the regression plots over 3-5 years and used in the analysis. Only 158/195 participants, those with a positive (>0.0) rate of Aβ deposition, were used to calculate the dynamics of Aβ over time.

**Results:** Aβ deposition follows sigmoidal kinetics where it takes about 14 years to go from the mean SUVR of 1.17 observed in HC, to reach the 1.5 SUVR threshold. It then takes another 20 years to reach the mean SUVR of 2.35 observed in fully developed AD (average increase 2.9%/yr). As disease progresses, the rates of Aβ deposition start to slow down, although not completely reaching a plateau. While the rates of Aβ deposition, grey matter atrophy and memory decline were significantly faster in HC+ ad MCI+ compared to HC- and MCI-, the rates of hippocampal atrophy did not reach significance. In AD, the rates of grey matter atrophy and cognitive decline were similar to those in HC+ and MCI+, but the overall rates of Aβ deposition were slightly slower.

**Conclusions:** Aβ deposition is a slow and protracted process that extends for more than two decades. High Aβ deposition is associated with faster grey matter atrophy and faster cognitive decline.
Relation between rates of Aβ deposition, ApoE genotype and cognition: results from a 3-5 year longitudinal study

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Background: Longitudinal evaluation of ageing individuals is providing insight into the different factors leading to Alzheimer's disease (AD). In this study, we used longitudinal data from the AIBL cohort, to provide a better understanding of the relationship between Aβ deposition and cognition in the development of AD.

Methods: One hundred and nineteen participants -74 elderly healthy controls (HC); 29 Mild Cognitive Impairment (MCI) subjects; and 16 mild AD patients - were evaluated at enrolment and 18 and 36 months later. On each visit, participants underwent neuropsychological examination, a MRI and a PiB PET scan. Rates of change for Aβ deposition, grey matter atrophy and cognitive decline were derived from the slope of the regression plots over 3-5 years and used in the regression analysis.

Results: Aβ deposition increases with higher Aβ burden, but a gradual slow down is observed in individuals with the highest Aβ burden, as well as in AD patients as disease progresses. Aβ deposition and memory decline were significantly faster in PiB+ vs PiB- HC and MCI. ApoE e4 genotype was associated with faster Aβ deposition, cognitive decline and hippocampal atrophy in HC, but only with Aβ deposition in MCI. No association with ApoE genotype was observed in the AD group. The rate of cognitive decline was inversely associated with rate of Aβ deposition in all groups.

Conclusions: Aβ deposition is associated with cognitive decline even in asymptomatic healthy controls. This supports the theory that Aβ deposition plays a fundamental role in the development of AD and suggests that to be effective, anti-Aβ therapy may need to be given early in the course of the disease, perhaps even before symptoms appear.

Understanding the mechanisms responsible for the hypotensive effects of ghrelin

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Recent evidence suggests that ghrelin, an orexigenic peptide secreted from the stomach, is involved in cardiovascular function. Although, peripheral administration of ghrelin or ghrelin receptor agonists lowers blood pressure, intrathecal administration increases blood pressure

AIM: As the exact mechanism of action by which ghrelin exerts these effects is yet to be elucidated, the aim of this study was to investigate possible mechanisms, focusing on the peripheral site of action. We hypothesized that ghrelin decreases blood pressure via actions on vagal afferents, to induce a reflex inhibitory response in efferent sympathetic nerves, thereby withdrawing vasomotor tone to promote vasodilation.

METHODS: In anaesthetised male Sprague-Dawley rats (n=13), the jugular vein and carotid artery were cannulated for intravenous administration of drugs and for arterial pressure (AP) measurement, respectively. Splanchnic sympathetic nerve discharge (SSND) was recorded and responses to vehicle (saline) or ghrelin (10µg/kg) administration were monitored before and after bilateral cervical vagotomy.
RESULTS: Ghrelin significantly inhibited SSND (−6.2±2.9%) and AP (−4.91±1.2 mm Hg) compared to saline treatment \((P < 0.05\) for both), but these responses were not affected by vagotomy.

CONCLUSION: Peripheral ghrelin administration is likely to induce a decrease in AP via inhibition of sympathetic nerve discharge although this occurs independently of the vagus nerve. One possibility is that ghrelin may induce its reflex sympathoinhibitory responses by accessing hindbrain regions involved in cardiovascular regulation via circumventricular organs such as the area postrema.

Quantification of track-weighted imaging (TWI): characterisation of within-subject reproducibility and between-subject variability

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The diffusion magnetic resonance imaging (MRI) post-processing technique of whole-brain fibre-tracking exploits the anisotropic diffusion properties of tissue to trace the path of white matter fibre bundles throughout the brain parenchyma. Whole-brain tractography does not require expert knowledge concerning region of interest placement, or a priori information regarding which white matter fibre bundles to interrogate.

In the recently proposed Track-Weighted Imaging (TWI) technique (1,2), the whole-brain fibre tracking data (also known as the ‘tractogram’) can be combined with a reference image (a map of some other property of the brain tissue, such as mean diffusivity, or fractional diffusion anisotropy), to generate a super-resolution track-weighted version of that reference image. TWI therefore enables a unique visualisation of whole-brain connectivity by integrating the anatomical pathways (from the tractogram) with properties of white matter fibre bundles (from the reference image).

The novel image contrasts of these TWI methods may provide important new quantitative measures for clinical studies, particularly for voxel-based analysis. However, before they can be used reliably to generate quantitative measures, it is important to characterise the within-subject intra-session and inter-session reproducibility, and between-subject variability. In this work we have assessed the reproducibility and variability of a number of different TWI maps over multiple normal subjects. We demonstrate their potential for clinical application, and provide guidelines for required sample sizes and observable effect sizes.


The Therapeutic Potential of Neuroepithelial Cells after Rat Spinal Cord Injury

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Mammalian spinal cord injury is a neurological condition that results in the loss of both sensory and motor function within the central nervous system (CNS) as damaged cells (neurons) within the spinal cord are unable to regenerate. Recent research suggests that, in the rat, early embryonic spinal cord cells known as Neuroepithelial cells (NE) may have the potential to stimulate damaged corticospinal tract (CST) axons and encourage growth in the adult spinal cord after injury. Objective: To examine the growth promoting potential of NE cells at two embryonic (E) time points, on CST axons after adult rat spinal cord injury. Methods: Fischer (F344) rat NE cells were dissected from E10.5 and E11.5 embryos, dissociated and implanted into an adult female F344 rat spinal cord hemi-section injury \((n=8)\). Six weeks post...
Injury rats were injected with Biotin Dextran, to label CST axons. Two weeks after tracing, rats were sacrificed and tissue immunohistochemistry performed for Avidin Peroxidase. Total axonal numbers were analysed including axonal/cellular interactions. Results: Implanted NE cells generate both scarring and neural cell pockets within the implant site after spinal cord injury. Increased CST sprouting was observed within E11.5 NE cell implants but not E10.5 NE cell implants, with a significant increase in CST axonal number observed in E11.5 implants vs. controls. Sprouting axonal varicosities were most associated with the presence of mature neurons, as opposed to the presence of both microglia/macrophages and blood vessels. Conclusions: In E11.5 NE cell implanted rats, regenerating CST axons grow significantly further into the implant. However while neuronal rich regions vigorously stimulate growth, areas of scar tissue appear to form barriers preventing the passage of axons. Thus reducing the degree of scar formation may increase axonal growth within NE cell implants after rat spinal cord injury.

Incidence and Associations of Lobar Microbleeds: Results from the Australian Imaging, Biomarkers and Lifestyle Study of Ageing (AIBL)

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Background. Cerebral microbleeds are a frequent finding with Susceptibility-Weighted MRI (SWI) in individuals with stroke, intracerebral haemorrhage, Alzheimer’s Disease (AD) and cognitively-normal older controls, particularly in people with biomarkers for cerebral β-amyloid (Aβ). However, there is no literature to date on incidence of CMB relative to Aβ-imaging, as with 11C-PiB PET (PiB).

We assessed the baseline prevalence and annualized incidence of Lobar CMB (LMB) according to PiB findings in cohort ranging from cognitively-normal elderly (NC) through Mild Cognitive Impairment (MCI) to Alzheimer’s Disease dementia (AD), assessed over 3 years.

Methods. 174 participants were studied from the Australian Imaging, Biomarkers and Lifestyle Study of Ageing (AIBL) with 3T SWI MRI and 11C-PiB PET, of whom 120 had follow-up imaging. PiB binding was dichotomized as previously published (PiB+/−). Logistic regression analyses were performed relating baseline and incident LMB with PiB+/− status, presence of infarcts, white matter hyperintensity volume (WMH), vascular risk factors, ApolipoproteinEε4 carrier status, age and gender.

Results. LMB were present in 27% of NC, 41% of MCI and 45% of AD patients. The prevalence of LMB was 52% in PiB+NC and 15% in PiB− NC. The mean incidence rate of LMB was 0.52 LMB/year in PiB+NC and 0.10 LMB/year in PiB-NC (p=0.002).

In NC, baseline LMB were associated with age and PiB+ status, and in MCI/AD patients, with male gender and WMH volume. Incident LMB were associated with PiB+ and presence of baseline lacunes in NC, while for AD/ MCI patients, they were associated with baseline LMB and WMH volume.

Conclusion. Individuals with higher amounts of cerebral Aβ (PiB+) have fivefold higher incidence of LMB compared with those with low Aβ. Incidence of lobar microbleeds is associated with Aβ-burden, baseline LMB and also markers of small vessel disease (lacunes and WMH).