1st Scientific Meeting 2012

including abstracts of projects funded by
the Venerable Yen Pei - NKF Research Fund

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## CONTENTS

**Forewords**

*Prof Tan Chorh Chuan*  
Guest of Honour, NKF 1st Scientific Meeting  
President, National University of Singapore  

*Mr Gerard Ee*  
Chairman, National Kidney Foundation  

*Mrs Eunice Tay*  
CEO, National Kidney Foundation  

**The Venerable Yen Pei-NKF Research Fund – An Overview**  

**Scientific Programme**  

**Order of Abstract Presentation (Oral)**  

**Poster Presentations**  

**Plenary Lectures**

*The NKF Community Dialysis Programme*  
– Present Status and Future Plans  
A/Prof Evan Lee, Senior Director, Clinical, National Kidney Foundation  

*The NKF Peritoneal Dialysis Programme*  
Dr Marjorie Foo, Medical Director, Peritoneal Dialysis Programme, Singapore General Hospital  

*The Automated Wearable Artifical Kidney (AWAK)*  
Prof David Lee, Professor Emeritus of Medicine, David Geffen School of Medicine at UCLA  

**Abstracts**

Oral  
Poster  

**Projects**

funded by the Venerable Yen Pei-NKF Research Fund  

**Acknowledgements**  

**Executive Committees**

NKF Research Fund  
NKF 1st Scientific Meeting Organising Committee
FOREWORD

Prof Tan Chorh Chuan
President, National University of Singapore

It is a pleasure and honour for me to contribute a short message to these Proceedings, which together with the NKF 1st Scientific meeting, mark a significant milestone for NKF and renal research in Singapore.

Since the year 2000, Singapore has made great progress in developing and growing the biomedical sciences sector. Central to these efforts, has been a substantial investment in biomedical sciences research, and in more recent years, in translational and clinical research. This reflects and recognises the crucial role of R&D in accelerating Singapore’s transition into an economy and society that is based on knowledge and innovation. In tandem, the importance of research has also been embraced by the Ministry of Health as a key element contributing to good, affordable medical care and enhanced health outcomes.

It is heartening to see that the integral value of research has gained wider and growing acceptance in healthcare institutions and among the community of doctors and health professionals. I believe this is critical for a number of reasons. Firstly, there are significant ethnic differences in disease susceptibility, behavior and response to treatment. It is only through our research that we can discover and develop treatments and management approaches that best serve our local patients. Secondly, given our rapidly ageing population and growing burden of chronic disease, we need to develop innovative new approaches and modes of health promotion and care delivery that will prevent the onset of severe illness and manage chronic conditions in a more patient-centric yet cost-effective way. Thirdly, our research can make a contribution to addressing the daunting healthcare challenges which confront Asia, while helping Singapore to continue to play a role as a regional medical hub.

The NKF Scientific meeting, these Proceedings and the evident strong support of the entire Nephrology community, augur well for the future of kidney medicine in Singapore. Faced as we are with the prospect of rising kidney failure rates from the current high burden of diabetes and hypertension, it will be excellent and relevant research and its application that will help provide us with the innovative approaches and new solutions that we need for the longer-term.

FOREWORD

Mr Gerard Ee
Chairman, National Kidney Foundation

When Dr Khoo Oon Teik founded the National Kidney Foundation (NKF) in 1969, one of the fundamental aims he set out to do was to encourage and promote research in all aspects of kidney diseases. NKF today has not lost sight of this. We are committed to improving the understanding of kidney diseases, its causes, treatment and prevention through research.

There was one person who shared the NKF's ideals, the late Venerable Yen Pei, the leader of the Singapore Buddhist Welfare Services (SBWS), until his parting in 1996. He showed, with the support of the Temple’s devotees, his commitment towards relieving the plight of kidney patients.

The NKF is indeed grateful to SBWS for establishing the Venerable Yen Pei - NKF Research Fund that supports kidney-related research in both basic and clinical science. Venerable Kuan Yan, the successor to Venerable Yen Pei and current President of SBWS, strongly shares her mentor's aspirations to help humanity, in particular, the needy in society.

You will be pleased to know that so far, 74 grants have been awarded through this Fund to Principal Investigators & Scientists to carry out research.

NKF’s 1st Scientific Meeting will showcase the research work and findings of the completed projects supported by this Fund. We are extremely honoured to have Professor Tan Chorh Chuan, President of the National University of Singapore, grace this meeting as our esteemed Guest-of-Honour.

With the continued support of SBWS and our healthcare partners, all of us can be confident that this vital investment for the future of kidney health will continue to yield returns through the relentless work of all the researchers.
FOREWORD

Eunice Tay (Mrs)
Chief Executive Officer, National Kidney Foundation

In terms of morbidity, mortality, and economic costs, End Stage Renal Disease (ESRD) is one of the most serious chronic diseases. NKF strongly believes that understanding the mechanisms of kidney diseases through quality research is critical for developing innovative treatments and for prevention.

The NKF 1st Scientific Meeting serves as a platform for researchers and healthcare professionals to share and exchange ideas and encourage discussions to advance renal care, as well as present their research publications and data. This includes principal investigators, doctors and staff of NKF and their affiliates.

NKF supports a wide spectrum of projects and studies relating to kidney diseases, dialysis and transplantation through the Venerable Yen Pei - NKF Research Fund. All the research we fund is carefully reviewed by the NKF’s Research Committee, helmed by Professor Woo Keng Thye, Emeritus Consultant and Advisor, Department of Renal Medicine, Singapore General Hospital. The Committee ensures good stewardship and accountability for the way the Fund is disbursed.

I am happy to say that the research work done through this Fund by professionals in the renal community has been significant, insightful and of good quality.

It is important that NKF continues to work in partnership with the Restructured Hospitals, National Centres, other healthcare institutions, research funders and professional associations to support and advance renal research in Singapore.

We look forward to your continued support.

THE VENERABLE YEN PEI - NKF RESEARCH FUND

The Venerable Yen Pei - NKF Research Fund was set up to encourage and promote research in all aspects of kidney and kidney related diseases.

The late Venerable Yen Pei, leader of the Singapore Buddhist Welfare Services (SBWS) shared the vision and mission of the NKF in research, and with the support of the temple’s devotees, showed his commitment towards relieving the plight of kidney patients.

An NKF Research Committee, helmed by Professor Woo Keng Thye, Emeritus Consultant and Advisor, Department of Renal Medicine, Singapore General Hospital was set up to administer the Fund to ensure good stewardship and accountability. The selected areas of research are basic, translational and clinical science projects in the renal and renal-related diseases.

To date, 74 grants have been awarded through this Fund, of which, 2 are NKF research projects.
<table>
<thead>
<tr>
<th>TIME</th>
<th>PROGRAMME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1100-1200</td>
<td>Registration</td>
</tr>
<tr>
<td>1200-1210</td>
<td>Welcome &amp; Opening Address</td>
</tr>
<tr>
<td></td>
<td>- Mr Gerard Ee, Chairman, NKF</td>
</tr>
<tr>
<td>1210-1220</td>
<td>Presentation of Token of Appreciation to Venerable Kuan Yan</td>
</tr>
<tr>
<td></td>
<td>- Mrs Eunice Tay, CEO, NKF</td>
</tr>
<tr>
<td>1220-1230</td>
<td>Address by Guest-of-Honour</td>
</tr>
<tr>
<td></td>
<td>- Prof Tan Chorh Chuan, President, NUS</td>
</tr>
<tr>
<td>1230-1300</td>
<td>Lecture I</td>
</tr>
<tr>
<td></td>
<td>The NKF Community Dialysis Programme</td>
</tr>
<tr>
<td></td>
<td>- Present Status And Future Plans</td>
</tr>
<tr>
<td></td>
<td>- A/Prof Evan Lee, Senior Director, Clinical, NKF</td>
</tr>
<tr>
<td>1300-1400</td>
<td>Lunch - Viewing of Posters &amp; Exhibition by Sponsors</td>
</tr>
<tr>
<td>1400-1540</td>
<td>Oral Presentations</td>
</tr>
<tr>
<td>1540-1610</td>
<td>Break - Viewing of Posters &amp; Exhibition by Sponsors</td>
</tr>
<tr>
<td>1610-1625</td>
<td>Lecture II</td>
</tr>
<tr>
<td></td>
<td>The NKF Peritoneal Dialysis Programme</td>
</tr>
<tr>
<td></td>
<td>- Dr Marjorie Foo, Medical Director, Peritoneal Dialysis Programme, Singapore General Hospital</td>
</tr>
<tr>
<td>1625-1640</td>
<td>Lecture III</td>
</tr>
<tr>
<td></td>
<td>A Peritoneal Dialysis-Based Automated Wearable Artificial Kidney (Awak)</td>
</tr>
<tr>
<td></td>
<td>- Prof David Lee, Professor Emeritus of Medicine, David Geffen School of Medicine at UCLA</td>
</tr>
<tr>
<td>1640-1700</td>
<td>Presentation of Prizes &amp; Tokens of Appreciation</td>
</tr>
<tr>
<td>1700-1715</td>
<td>Closing Address</td>
</tr>
<tr>
<td></td>
<td>- A/Prof Evan Lee, Senior Director, Clinical, NKF</td>
</tr>
<tr>
<td>Time</td>
<td>Title</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>1400</td>
<td>Adipocytokine Zinc Alpha-2 Glycoprotein (ZAG) as Novel Urinary Biomarker for Normo-Albuminuric Diabetic Nephropathy</td>
</tr>
<tr>
<td>1410</td>
<td>Responses to Metabolically-triggered Inflammation Impair Renal Epithelial Immunity and Increase Susceptibility to Infection</td>
</tr>
<tr>
<td>1420</td>
<td>Genomic Expression and Single-Nucleotide Polymorphism Profiling Discriminates Chromophobe Renal Cell Carcinoma and Renal Oncocytoma</td>
</tr>
<tr>
<td>1430</td>
<td>Microrna Regulators for Aquaporins 1, 2 And 4 Expressed in the Kidney</td>
</tr>
<tr>
<td>1440</td>
<td>Decreased Immune Cell Intracellular Adenosine Triphosphate (ATP) Levels in Pediatric Renal Transplant Recipients with Post-Transplant Lymphoproliferative Disease and BK Viremia</td>
</tr>
<tr>
<td>1450</td>
<td>Protein Intake in a Multi-Ethnic Asian Population of Chronic Kidney Disease and Healthy Participants</td>
</tr>
<tr>
<td>1500</td>
<td>Prospective, Randomised Controlled Trial of Cutting Balloon Angioplasty (CBA) vs High Pressure Balloon Angioplasty (HPBA) in Dialysis Arterio-Venous Graft (AVG) and Arterio-Venous Fistula (AVF) Stenosis Resistant to Conventional Percutaneous Transluminal Angioplasty (PTA)</td>
</tr>
<tr>
<td>1510</td>
<td>Total Body Water Measurements by Bioimpedance Analysis Reflect Volume Changes Better than Extracellular Fluid Measurements in Haemodialysis</td>
</tr>
<tr>
<td>1520</td>
<td>A Large-Scale Survey of the Singapore Public on the Awareness of the Human Organ Transplant Act and it’s Relationship to Altruistic Behavior</td>
</tr>
<tr>
<td>1530</td>
<td>Ambulatory Diastolic Blood Pressure Predicts Microalbuminuria in Children and Adolescents with Diabetes Mellitus</td>
</tr>
<tr>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1 Side Population in Renal Cell Carcinoma Cell Line is Enriched</td>
<td>Dr Ong Choon Kiat, Division of Medical Sciences, National Cancer Centre</td>
</tr>
<tr>
<td>with Drug Resistant, but not Tumor Initiating Cells</td>
<td></td>
</tr>
<tr>
<td>2 Endotoxin-Tolerance Monocyte Profile in Minimal Change</td>
<td>Ms Chang-Yien Chan, Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore</td>
</tr>
<tr>
<td>Nephrotic Syndrome (MCNS): Role in Increased Susceptibility to</td>
<td></td>
</tr>
<tr>
<td>Bacterial Infections</td>
<td></td>
</tr>
<tr>
<td>3 Successful Renal Transplant from a Donor with TRPC6 Mutation</td>
<td>Dr Ng Kar Hui, Shaw-NKF-NUH Children’s Kidney Centre, University Children’s Medical Institute, National University Health System and Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore</td>
</tr>
<tr>
<td>may be Explained by Interactions between TRPC6 and Nephrin Single</td>
<td></td>
</tr>
<tr>
<td>Nucleotide Polymorphisms</td>
<td></td>
</tr>
<tr>
<td>4 Urine Svcam-1 And Sicam-1 Levels are Elevated in Lupus Nephritis</td>
<td>Dr Howe Hwee Siew, Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital</td>
</tr>
<tr>
<td>5 Towards a Microfluidic Renal Cell Supplement</td>
<td>Dr Partha Roy, Department of Bioengineering, National University of Singapore</td>
</tr>
<tr>
<td>6 Renal Regulation of Glucose Homeostasis by the Nuclear Receptor</td>
<td>Mr Dulesh N Peiris, Departments of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore and Cancer Science Institute of Singapore, National University of Singapore</td>
</tr>
<tr>
<td>HNF4A in Kidney Disease</td>
<td></td>
</tr>
<tr>
<td>Control of Fasting Hyperglycemia and Fatty Acid Metabolism in</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus by the Nuclear Receptor HNF4A</td>
<td></td>
</tr>
<tr>
<td>Increased GATA3 Expression and Th2 Cytokine Profile in DEC1-</td>
<td></td>
</tr>
<tr>
<td>Transfected Jurkat Cells: A Potential Mechanism of Action Of DEC1 in</td>
<td></td>
</tr>
<tr>
<td>the Pathogenesis of Minimal Change Nephrotic Syndrome</td>
<td></td>
</tr>
<tr>
<td>ERK-Inhibitor AZD6244 Enhances the Anti-Tumour Activity of Sorafenib</td>
<td>Ms Sim Mei Yi, Department of Urology, Singapore General Hospital</td>
</tr>
<tr>
<td>in a Xenograft Model of Human Renal Cell Carcinoma (RCC)</td>
<td></td>
</tr>
<tr>
<td>Pathogenesis of Hypercholesterolemia in Minimal Change</td>
<td></td>
</tr>
<tr>
<td>Nephrotic Syndrome (MCNS)</td>
<td></td>
</tr>
<tr>
<td>9 Pathogenesis of Hypercholesterolemia in Minimal Change</td>
<td></td>
</tr>
</tbody>
</table>
CLINICAL RESEARCH

10 Indians may have Lower Glomerular Filtration Rates which is Unrelated to Protein Intake
Mr Toh Qi Chun, Department of Medicine, Division of Nephrology, Yong Loo Lin School of Medicine, National University of Singapore

11 Nutritional Assessments are Valid in Asians but Total Protein Intake per Ideal Body Weight is Valid Only in Chronic Kidney Disease Patients
Ms Chow Pek Yee, Department of Medicine, Division of Nephrology, National University Health System

12 Sodium Restriction Should Be Emphasized in Chronic Kidney Disease Stages 1 to 3
Ms Chow Pek Yee, Department of Medicine, Division of Nephrology, National University Health System

13 Prevalence of Prehypertension/Hypertension in an Asian Pediatric Population
Dr Isaac Liu, Department of Paediatrics, National University Health System

14 Knowledge of Chronic Kidney Disease Among Primary Care Patients in Singapore
Dr Ong Siew Chin, Centre for Health Services Research, Singapore Health Services

15 Effects of Demographic Factors on Living Kidney Donation in Singapore
Mr Ow Yong Lai Meng, Department of Medical Social Services, Singapore General Hospital

16 Health-related Quality of Life (HRQOL) of Patients on Kidney Transplant Waiting List and Factors Impact on Them
Dr Ong Siew Chin, Centre for Health Services Research, Singapore Health Services

17 Health-related Quality Of Life of Patients on Kidney Transplant Waiting List versus Post-Transplant Patients
Dr Ong Siew Chin, Centre for Health Services Research, Singapore Health Services

18 Role of Blood Pressure Monitoring in Predicting Left Ventricular Hypertrophy and Arteriopathy
Dr Ng Kar Hui Ng, Shaw-NKF-NUH Children’s Kidney Centre, University Children’s Medical Institute, National University Health System and Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore

19 Is 24-Hr Urinary Metabolic Evaluation in Urolithiasis Useful in the Singapore Context?
Dr Tan Teck Wei, Department of Urology, Tan Tock Seng Hospital

20 Urine Biomarkers in Paediatric Kidney Disease
Dr Vidyadhar Mali, Department of Paediatric Surgery, National University Hospital
THE NKF COMMUNITY DIALYSIS PROGRAMME: PRESENT STATUS AND FUTURE PLANS

Assoc Prof Evan Lee
Senior Director, Clinical, National Kidney Foundation

The National Kidney Foundation (NKF), Singapore was founded in 1969 by Prof Khoo Oon Teik. It was founded primarily in response to the needs of the growing population of patients with renal failure who were financially needy. In 1975, it started a self dependency haemodialysis unit at Alexandra Hospital and in 1982 a haemodialysis unit was opened in the Kwong Wai Shiu Hospital.

In 1987, it opened the first of it’s community-based haemodialysis units in Toa Payoh. Over the next 24 years the NKF established 26 community dialysis centres in various communities in Singapore, most of them in public housing precincts. Currently, there are 24 centres operating. The aim was to provide haemodialysis facilities for patients that were near to their homes so as to reduce traveling time and to encourage patients to return to work and be rehabilitated as the majority of the patients were relatively young with very little comorbidities.

This programme grew steadily and the current prevalent patient population is 2398 at the end of June 2011. The average annual patient growth over the last 5 years has been 84.4 patients.

Patients enter the programme through application. Once having passed a means test, they are assessed medically for suitability for placement in a community-based centre. Once found suitable they are offered places for treatment. Since 2008 however, the NKF started to provide portable funding for patients considered too frail for the NKF community haemodialysis centres. There were, at the end on June 2011, 119 patients on this programme.

In 2010, 372 patients were assessed. Forty six percent were aged above 60 years. Twenty one percent were wheelchair-bound and a further 14% needed help in ambulating. Fifty six percent was considered clinically dependent in need of special care because of their comorbidities. Ninety two percent were hypertensive, 68% were diabetic, 54% had heart disease and 9% had peripheral vascular disease. Thirty two percent were malnourished with a subjective global assessment score of less than 4.

Twenty one applicants were considered too frail to be treated at NKF community-based centres

The current NKF patient coming on to the program is therefore not only financially needy but elderly and very likely to have many concurrent medical problems like diabetes, cardiovascular disease and malnutrition. It therefore became evident that for patients to be able to cope with their renal failure, they needed help with more than just a haemodialysis centre near their homes. They needed help with coping with various psychosocial needs, getting to and from the centres, and increased supervision and monitoring during their dialysis treatments.

There are therefore plans to 1) increase the monitoring of psychosocial needs in collaboration with other social agencies to broaden the support platform for these patients, 2) increasing nutritional surveillance and support, 3) identifying frail patients for increased monitoring and care during dialysis, 4) increasing the collaboration with restructured hospitals so as to manage the increasingly complex care of the patients and 5) increasing the support for peritoneal dialysis – a home based therapy, so as to reduce the reliance on haemodialysis.
THE NKF PERITONEAL DIALYSIS PROGRAMME

Dr Marjorie Foo
Medical Director, Peritoneal Dialysis Programme
Singapore General Hospital

The PD Program in Singapore is the most challenging and rewarding programme to manage; it is now into its third decade. The first PD programme was started in the Singapore General Hospital (SGH) in 1980; the programme looked after patients ranging from 5 yrs to 78 yrs. Over the next thirty years the programme, with its ups and downs, has grown in SGH to be one of the largest in the Asia-Pacific region with a population of about 500 patients. Technique and expertise improved with time and with the maturity of the programme, the National University Hospital started its own paediatric programme dedicated to children with kidney disease. The children’s PD programme is also one of the largest in the region. The PD programme has grown from patient care to research and training of fellows in the region.

The programme suffered a dip in 2003 due to SARS when catheter insertions were not possible, but since then, the programme had grown consistently, backed by subsidies from both voluntary welfare organisations (VWO) and the government.

In 2001, there was a government subsidy programme encouraging the uptake of PD and this led to a surge in the PD numbers; maximizing on the opportunity, VWOs also started to offer subsidies for PD patients. These were namely NKF in 2001 and Kidney Dialysis Foundation (KDF) in 2003.

The “old” NKF started out with the idea of a “neighborhood” PD programme that was linked to specific haemodialysis (HD) centres. It was initiated with the idea that patients be dealt with “locally”, rather than go to hospital for all problems. The idea, ideal as it may have seemed, was fraught with problems; one being the “ownership” of the programme between NKF and the Restructured Hospitals (RH). The “old” NKF Programme intended not just to provide subsidies, but also clinical care; however, due to a lack of PD clinicians and PD nursing manpower, the program was not sustainable. One of the many reasons of non-sustainability arose from multiple protocols from different RHs, lack of standardization of procedures and training issues. Last but not least, the “tender” policy for dialysis systems at that time resulted in inconvenience to patients as they had to change their dialysis system in order to get an NKF subsidy. This resulted in an adverse clinical outcome with regards to infection. The NKF PD programme came almost to a halt and the KDF PD program started to grow.

The New NKF resurrected the PD programme in 2006 with a new framework that excluded clinical care, leaving it to the RHs; the NKF subsidy covers only the dialysate package. Partnering closely with the RHs, NKF was able to boost the PD programme. They have developed an efficient way of patient assessment for subsidy without the need of medical reports. This has facilitated less interim haemodialysis and has indirectly boosted PD.

As part of the policy to support PD nationwide, NKF has helped to educate patients and the general population with regards to Renal Replacement Therapy (RRT), disseminating knowledge about dialysis and informed choices.

The success of NKF as a RRT provider of HD for the last 40 years has also been its Achilles heel when it comes to educating about PD. NKF in Singapore is synonymous with haemodialysis; however NKF has managed to gradually changed the landscape of RRT in Singapore by providing easy access to information, allowing Singaporeans to make well informed choices. Another significant milestone in the PD programme was the institution of a “Home Visit” programme in August 2011, providing a more holistic care to NKF PD patients. The home visit programme was well received and has facilitated direct referrals to relevant Social Welfare Departments for continuity of care.

The SGH PD programme constitutes about 60% of the NKF PD programme. This talk will elucidate the clinical outcomes and trends of patients in SGH who have been receiving the NKF PD subsidy.
A PERITONEAL DIALYSIS-BASED AUTOMATED WEARABLE ARTIFICIAL KIDNEY (AWAK)

David B. N. Lee
Professor Emeritus of Medicine, David Geffen School of Medicine at UCLA; Chief Scientist, AWAK Technologies, Singapore and Burbank, California, USA

Work on wearable kidneys has evolved around the technology of hemodialysis or hemofiltration, which calls for continuous anticoagulation of the extracorporeal circulation and are encumbered with potential immunologic and non-immunologic complications of continuous blood–artificial membrane interactions. A peritoneal-based automated wearable artificial kidney (AWAK) requires no extracorporeal circulation and is therefore “bloodless.” Because AWAK is designed to continuously regenerate and reuse the spent dialysate in perpetuity, it is also “waterless.” A sorbent-based assembly regenerates both the aqueous and the protein components (AqC and PrC) of the spent dialysate, producing a novel, autologous protein containing dialysate. The regenerated AqC has the same composition as the commercially available peritoneal dialysate, but contains bicarbonate instead of lactate and has a more physiological pH. The regenerated PrC is recycled back into the peritoneal cavity, thereby ameliorating or eliminating protein loss. Depending on the steady-state protein concentrations that can be achieved (under the condition of continuous dialysate regeneration and recycling), the PrC also has the potential of both augmenting ultrafiltration (through its oncotic pressure) and mediating the removal of protein-bound toxins. Additional sorbents can be incorporated into AWAK for the removal of middle molecular weight uremic toxins. At a regeneration rate of 4 L/h, AWAK can provide a dialysate flow of up to 96 L/day (8–12 times the current rate). Round-the-clock dialysis and ultrafiltration is expected to provide steady-state metabolic and fluid balance regulation, thereby eliminating the “shocks” of abrupt changes in these parameters that characterize the current dialytic modalities. Dialysis-on-the-go, made possible by AWAK’s “wearability” and automation, frees end-stage renal failure patients from the servitude that is demanded by the current dialytic regimentations.
Adipocytokine Zinc Alpha-2 Glycoprotein (ZAG) as Novel Urinary Biomarker for Normo-Albuminuric Diabetic Nephropathy

Su Chi LIM\textsuperscript{1}, Wan Ching TOY\textsuperscript{2}, Melvin WONG\textsuperscript{2}, Lee Ying YEOH\textsuperscript{1}, Cherine Tan MF\textsuperscript{3}, Dawn LAU\textsuperscript{2}, Clara Tan\textsuperscript{2}, Tavintharan SUBRAMANIAM\textsuperscript{1}, Chee Fang SUM\textsuperscript{1}

\textsuperscript{1}Department of Medicine, Khoo Teck Puat Hospital, Singapore; \textsuperscript{2}Clinical Research Unit, Khoo Teck Puat Hospital, Singapore; \textsuperscript{3}School of Chemical and Life Sciences, Nanyang Polytechnic, Singapore

Introduction:
Albuminuria detection is the recommended screening method for Diabetic Nephropathy (DN). However, a sizeable proportion (up to 55\%)\textsuperscript{1} of DN individuals with impaired GFR (<60mls/min/1.73m\textsuperscript{2}) are non-albuminuric (i.e. ACR<30mg/g). These individuals, at risk of ESRD and cardiovascular disease, cannot be identified by present standard albuminuria screening. It is also unclear whether non-albuminuric DN shares similar pathogenic mechanisms with classical proteinuric DN. Therefore, we aim to search for (and validate using different laboratory method) novel urinary peptide biomarkers for non-albuminuric DN. The identity of this peptide may also shed light on the pathogenesis of non-albuminuric DN.

Methods:
We studied three groups (N=6 per group) of males with type 2 diabetes: (1) normal renal function; (2) classical DN (urinary albumin-creatinine ratio, ACR>1000mg/g and glomerular filtration rate (eGFR)<60mls/min/1.73m\textsuperscript{2}) and (3) non-albuminuric DN (eGFR<60mls/min/1.73m\textsuperscript{2} and ACR<30mg/g). To control for confounding, study subjects were one-for-one matched according to gender (all male), ethnicity (all Chinese), smoking status (all non-smokers) and frequency matched for age.

We employed 2-dimensional fluorescence differential gel electrophoresis (2D DIGE), which allowed for multiplexing of samples from the three groups, minimizing gel-to-gel variation. A total of five 2D DIGE gels were run. Each of the first three gels included a pair of samples from the following three groups as described above. Specifically, first gel consisted of samples from group 1 and 2, second from group 2 and 3, third from group 3 and 1. The fourth and fifth gels were technical repeats for second and third gels respectively (given our primary focus on group 3) to ensure the reproducibility.

Identity of peptide spots was ascertained by Matrix Assisted Laser Desorption/Ionization time-of-flight mass spectrometry (MS) peptide identification. For peptide specificity, follow-up Western Blot validation was performed.

Results:
Sixty protein spots were differentially abundant between the non- and macro-albuminuric subjects (>2.5 fold, p<0.05). After stringent Bonferroni’s correction for multiple testing (i.e. 0.05/60=0.00083), two up-regulated spots were considered borderline significant in the urine of non-albuminuric subjects, α1-microglobulin (up-regulated 4.73 fold, P=0.00087) which had previously been reported in Singaporean diabetic adults across a spectrum of renal function. Given its non-discriminative, ubiquitous presence in the urine of diabetic individuals, we decided to follow up on the next most interesting spot, Human Zinc-α2-glycoprotein (ZAG) (up-regulated 3.44 fold, P=0.0026), a novel adipose-cytokine associated with glomerular injury. The relative abundance (arbitrary unit ± standard error) of urinary ZAG among the groups was- group 1: 12.7±1.5, group 2: 1.5±0.3, group 3: 6.6±1.3 (P trend 0.001). Post-hoc pair-wise comparison- group 1 vs. 2: P<0.001; group 1 vs. 3: P=0.009 and group 2 vs. 3: P=0.019. The identity of ZAG was confirmed (and quantified) by Western Blot in an independent male cohort of group 2 vs group 3.

To validate our preliminary observation, we replicated the 2D DIGE and Western Blot experiments in female subjects grouped according to the same criteria. ZAG was similarly upregulated in group 3 (P trend 0.06). Western Blot showed a consistent 2.6 fold increase in ZAG protein in group 3.

Conclusions:
ZAG, a novel adipocytokine, may be a urinary biomarker for non-albuminuric variant of DN.
**Responses to Metabolically-triggered Inflammation Impair Renal Epithelial Immunity and Increase Susceptibility to Infection**

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**Scientific Abstract:**

Diabetes mellitus is characterized by chronic inflammation and an increased risk of common and opportunistic infections, particularly of tissues/organs exposed to the external environment. While hyperglycemia is now recognized as an independent risk and prognostic factor, especially for nosocomial infections, the causal molecular mechanisms that affect immune cells and their functions are unclear. In this study, we hypothesized that, besides systemic immune cells, epithelial immunity mediated by resident parenchymal cells also contributes significantly to tissue-localized infections in diabetes. We show, by transcript and protein analyses, signatures of glucose-induced tissue damage, chronic inflammation, oxidative stress, and dysregulated expression of multiple inflammation- and immunity-related molecules in non-nephropathic diabetic kidneys compared to kidneys of non-diabetic subjects. Abnormal signaling pathways involving cytokines and their receptors, G-protein coupled receptors (GPCR), protein kinase C isoforms (PKCs), mitogen-activated protein kinases (MAPKs), nuclear factor-κB (NFκB) and toll-like receptors (TLRs) were also evident. These perturbations were accompanied by overexpression of negative regulators of NFκB, TLR and other proinflammatory pathways e.g. A20, SOCS1, IRAK-M, IκBα, Triad3A, Tollip, SIGIRR and ST2L. Anti-inflammatory and immunomodulatory molecules e.g. IL-10, IL-4 and TSLP that favor TH2 responses were also strongly induced. These molecular indicators of immune dysfunction led us to detect the cryptic presence of bacteria and cytomegalovirus in more than one-third of diabetic kidneys but none in non-diabetic kidneys. Similar signaling abnormalities could be induced in primary human renal tubular epithelial (but not mesangial) cell cultures exposed to high glucose, proinflammatory cytokines and methylglyoxal, and were reversed by combined pharmacological treatment with an antioxidant and a PKC inhibitor. Our results suggest that diabetes impairs epithelial immunity as a consequence of inappropriately activated negative counter-regulatory responses that are physiological protective mechanisms against chronic inflammation. The immune abnormalities and cryptic renal infections described here may be novel mechanisms that drive the development of diabetic nephropathy.

**Lay Summary:**

Diabetes has long been associated with increased opportunistic infections in organs such as lung, urinary tract and skin. However, the causative role of diabetes is undetermined and the mechanism involved is incompletely understood. Here, we demonstrate that normal kidneys of patients with diabetes, in comparison with those of non-diabetics, show molecular signatures of heightened pro-inflammatory responses and oxidative stress, and paradoxical impaired immunity associated with potent immunosuppressive cytokine environment. These results are further supported by the detection of bacterial and cytomegalovirus DNA in majority of the diabetic kidneys examined. Cytomegalovirus is frequently detected in transplanted kidney of an immunosuppressed host, and is associated with tissue senescence and renal failure. We used human primary cell cultures to validate the in vivo results and to investigate the role of diabetes in immune suppression. We demonstrate that high glucose and diabetes-associated metabolite and cytokines induced prolonged inflammation and delayed the immune responses of renal epithelial cells that cover and protect the kidney surface, when exposed to molecular mimicry of pathogens. These perturbations can be reversed with pharmacological blockade of pro-inflammatory signaling and oxidative stress. This work therefore contributes to the understanding of frequent infections in diabetes, paving the way to therapeutic prevention strategies.
Genomic Expression and Single-Nucleotide Polymorphism Profiling Discriminates Chromophobe Renal Cell Carcinoma and Renal Oncocytoma

National Cancer Centre

Introduction:
Chromophobe renal cell carcinoma (chRCC) and renal oncocytoma are two distinct but closely related entities with strong morphologic and genetic similarities. While chRCC is a malignant tumor, oncocytoma is usually regarded as a benign entity. Hence, the overlapping characteristics are best explained by a common cellular or histiogenic origin, and the biologic differences between chRCC and oncocytoma are therefore of considerable interest in terms of carcinogenesis and clinical management. We have previously reported that the hypoxia-regulated gene EGLN2 is dysregulated in chromophobe RCC, suggesting a common underlying dysregulation of hypoxia response in RCC, regardless of subtype.

Method:
Gene expression profiling using the Affymetrix HGU133Plus2 platform was applied on chRCC (n = 15) and oncocytoma specimens (n = 15). Supervised analysis was applied to identify a discriminatory gene signature, as well as differentially expressed genes. High throughput single-nucleotide polymorphism (SNP) genotyping was performed on independent samples (n = 14) using Affymetrix GeneChip Mapping 100 K arrays to assess correlation between expression and gene copy number. Immunohistochemical validation was performed in an independent set of tumors.

Results:
A novel 14 probe-set signature was developed to classify the tumors internally with 93% accuracy, and this was successfully validated on an external data-set with 94% accuracy. Pathway analysis highlighted clinically relevant dysregulated pathways of c-erbB2 and mammalian target of rapamycin (mTOR) signaling in chRCC, but no significant differences in p-AKT or extracellular HER2 expression were identified on immunohistochemistry. Loss of chromosome 1p, reflected in both cytogenetic and expression analysis, is common to both entities, implying this may be an early event in histogenesis. Multiple regional areas of cytogenetic alterations and corresponding expression biases differentiating the two entities were identified. Parafibromin, aquaporin 6, and synaptogyrin 3 were novel immunohistochemical markers effectively discriminating the two pathologic entities.

Conclusion:
Gene expression profiles, high-throughput SNP genotyping, and pathway analysis effectively distinguish chRCC from oncocytoma, with expression profiling suggesting potential dysregulation of c-erbB2 and mTOR signaling. We have generated a novel transcript predictor that is able to discriminate between the two entities accurately, and which has been validated both in an internal and an independent data-set, implying generalizability. A cytogenetic alteration, loss of chromosome 1p, common to renal oncocytoma and chRCC has been identified, providing the opportunities for identifying novel tumor suppressor genes and we have identified a series of immunohistochemical markers that are clinically useful in discriminating chRCC and oncocytoma.
Microrna Regulators for Aquaporins 1, 2 and 4 Expressed in the Kidney

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Introduction:
Diffusion of water molecules across the lipid bilayer is an uninhibited yet slow process. However it is deemed insufficient for cells such as the kidney epithelia which require high water permeability. Integral membrane proteins known as aquaporins (AQPs), form transmembrane water channels which play critical roles in controlling water flow into and out of cells. The human body expresses 13 AQP isoforms (AQPs 1-13) of which 8 (AQPs 1, 2, 3, 4, 6, 7, 8, 11) are expressed in the kidneys. Individuals with kidney AQP deficiencies have been shown to develop pathological conditions ranging from polyuria to nephrogenic diabetes insipidus to polycystic kidneys. All experimental evidence highlight the important roles for AQPs in renal diseases and hence modulating the expression of these proteins would provide valuable information to understand of the molecular mechanisms related to kidney diseases.

Method:
This study attempts to identify natural endogenous modulators of gene expression (microRNAs) which could be used to regulate AQP expression. This study focuses on three classical AQPs; AQP1, AQP2 and AQP4 that are highly expressed in kidneys. Using bioinformatic in silico predictions, microRNA profiling, quantitative PCR, cloning and luciferase reporter assays, microRNAs targeting these three genes were identified and validated.

Results:
Based on both bioinformatic search and experimental data (microRNA profiling), we identified 10 microRNAs (miR-16, -17-5p, -103, -125b -146a, -181a, -197, -210, -320a and -498) that were highly expressed of kidney cell line (ATCC HTB47). The predicted interaction sites (microRNA:mRNA) of the microRNAs were mapped to the 3'UTR region of their AQP gene targets. Target prediction analysis showed that miR-146a, -103 and -320a target AQP1, miR-125b, -146a, -181a, -197 and -498 target AQP2 while miR-16, -17-5p, -125b, -210 and -320a target AQP4. Experimental data from validation studies showed that miR-146a and -103 serve as modulators of AQP1, miR-197 as a regulator of AQP2 while miR-16 and 17-5p could be used to regulate AQP4. Furthermore miR-125b was seen as a common modulator of AQP2 and AQP4 while miR-320a was able to modulate the expression of both AQP1 and AQP4.

Conclusion:
The results obtained thus far suggest that AQP gene expression could be regulated by endogenous regulators. The microRNAs identified in this study allow for the independent regulation of each of the three classical AQPs as well as co-regulation. While this study highlights the novel finding that microRNAs could modulate AQP expression, further investigations are needed to determine the potential applications of these microRNAs in kidney diseases.
Decreased Immune Cell Intracellular Adenosine Triphosphate (ATP) Levels In Pediatric Renal Transplant Recipients with Post-Transplant Lymphoproliferative Disease and BK Viremia

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Introduction:
Renal transplant recipients are on life-long immunosuppressants to prevent allograft rejection, and hence are at increased risk of complications like opportunistic infections and malignancies. The Cylex®ImmuKnow™ assay provides a direct assessment of the immune function, aiding in the monitoring of the immune status of immunosuppressed individuals. The study aims to establish immune response targets in pediatric renal transplant patients without complications, as compared to i) healthy controls, ii) those with biopsy-proven rejections, iii) recipients with post-transplant lymphoproliferative disease (PTLD) and iv) recipients with BK viremia, with the eventual objective of providing guidance on dosage of immunosuppressant use.

Method:
A total of 38 pediatric renal transplant recipients and 2 healthy subjects have been recruited so far in this study. Sample blood was obtained from these study subjects, of which 250μL of anticoagulated whole blood incubated for 15–18 hours with PHA in a 37°C, 5% CO₂ incubator, in a 96-well microtiter plate. CD4+ cells were then positively selected within the microwells using magnetic particles coated with anti-human CD4 monoclonal antibodies (Dynabeads, Dynal, Oslo, Norway) and a strong magnet (Cylex Magnet tray 1050, Cylex Inc., Columbia, MD, USA) and then lyzed to release intracellular ATP. Released ATP was measured using luciferin/luciferase and a luminometer (Berthold, Knoxville, TN or Turner Biosystems, Sunnyvale, CA, USA), in nanograms per millilitre (ng/ml). Statistical analysis was performed using Student’s t-test to compare mean ATP values between BK viremic and non-BK viremic patients and between patients with PTLD compared to patient without PTLD. Receiver-operating characteristic (ROC) curve analysis was used to determine the ATP threshold that would predict BK viremia.

Result:
Amongst the 38 pediatric renal transplant recipients, 15(39%) had biopsy-proven rejections, 4(11%) had PTLD and 2(5%) had BK viremia. Mean intracellular adenosine triphosphate (ATP) levels, as measured using the Cylex®ImmuKnow™ assay, in transplant recipients with biopsy-proven rejection (430±206 ng/ml) was similar as compared to transplant recipients with no evidence of rejection (432±225 ng/ml). A significantly lower mean ATP level was observed in patients with PTLD as compared to transplant recipients without PTLD (239±105 ng/ml vs 466±213 ng/ml, p<0.001). Comparing transplant recipients with and without BK viremia, mean ATP level was significantly lower during episodes of BK viremia as compared to episodes without BK viremia (213.8±51.6 ng/ml vs 463.2±197.4, respectively, p<0.001). Using ROC curve analysis, the area under the ROC curve was 0.93 (95% CI: 0.85 -1.00), and using a threshold ATP level <256.5 ng/ml gave a sensitivity of 80%, specificity of 87.5% for BK viremia. An ATP level <256.5 ng/ml also yielded a positive likelihood ratio of 6.4 (95% CI: 2.9-14.0). Our results also suggest that adjustment of immunosuppression therapy towards a target ATP level >256.5 ng/ml may reduce the risk of developing BK viremia. The mean intracellular ATP in healthy controls was 401±137 ng/ml, similar to transplant recipients.

Conclusion:
Our study results suggest over-immunosuppression as a contributing factor of BK viremia and PTLD, supporting the notion of decreasing immunosuppressants in PTLD and BK viremic patients. Further prospective studies are, however, required to validate the above findings.
Protein Intake in a Multi-Ethnic Asian Population of Chronic Kidney Disease and Healthy Participants

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Introduction:
Different clinical practice guidelines recommend different levels of dietary protein intake in pre-dialysis chronic kidney disease (CKD) patients (1, 2). It is unknown how effectively these recommendations perform in a multi-ethnic Asian population, with its varied diets and cultural beliefs. We assess the profile of protein intake in a multi-ethnic Asian population.

Methods:
We analyzed the 24-hour urine collections of the Asian Kidney Disease Study (3) and the Singapore Kidney Function Study to estimate total protein intake (TPI; g/day) (4): 6.25×urine urea nitrogen + 30×actual body weight (ABW). We calculated ideal body weight (IDW; kg) (5): 22.99×height^2 (m). We used standard statistical tests where appropriate, and linear regression to assess associations of continuous variables with protein intake, via JMP 7.0.1.

Results:
There were 232 stable chronic kidney disease patients (3) and 103 healthy participants with mean age 53.5 ±15.1 year; comprising of 51% male, 38.5% Chinese, 29.6% Malay, 23.6% Indian, and 8.4% Others, and 35.5% diabetics.

Table 1 Mean protein intake

<table>
<thead>
<tr>
<th></th>
<th>SKFS1* (103)</th>
<th>AKDS* (232)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPI (g/day)</td>
<td>58.89±18.42</td>
<td>53.64±19.39</td>
<td>0.0206</td>
</tr>
<tr>
<td>TPI-IDW (g/kg/day)</td>
<td>0.97±0.28</td>
<td>0.91±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>TPI-ABW (g/kg/day)</td>
<td>0.91±0.27</td>
<td>0.77±0.26</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Overall, TPI is different by ethnicity (p = 0.0028), diabetic status (p = 0.0038), and is lower in women (p <0.001). It is positively correlated with height, ABW, BMI, GFR (all p <0.001), and serum albumin (p = 0.0032).

When normalized to ideal body weight, TPI-IDW (g/kg/day) is similar in healthy and CKD participants. TPI-IDW in CKD patients was lower in women (p = 0.0110) and decreased with age (p = 0.0407), but increased with ABW, BMI, GFR (all p <0.001), and serum albumin (p = 0.0141).

By NKF KDOQI guidelines, 29/232 (12.5%) of CKD patients with GFR <25 (in mL/min/1.73 m2) had TPI-IDW of <0.6g/kg/day (1). By CARI guidelines, 76.3% (177/232) of CKD patients had TPI-IDW >0.75 g/kg/day (2). By American Dietetic Association guidelines, 34.7% (44/127) of CKD patients with GFR <50 had TPI-IDW between 0.6 to 0.8 g/kg/day. Only 1/6 non-diabetic CKD patients with GFR <20 had a protein intake of between 0.3-0.5 g/kg/day. 21.9% (25/114) of diabetic CKD patients, had protein intake between 0.8-0.9 g/kg/day.
Prospective, Randomised Controlled Trial of Cutting Balloon Angioplasty (CBA) vs High Pressure Balloon Angioplasty (HPBA) in Dialysis Arterio-Venous Graft (AVG) and Arterio-Venous Fistula (AVF) Stenosis Resistant to Conventional Percutaneous Transluminal Angioplasty (PTA)

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Introduction:
To compare the efficacy and safety of high pressure balloon angioplasty (HPBA) versus cutting balloon angioplasty (CBA) in patients with dialysis AVG/AVF stenoses resistant to conventional percutaneous balloon angioplasty (PTA).

Method:
Patients with dysfunctional, stenotic dialysis AVFs/AVGs without central venous stenoses were enrolled and randomized to receive HPBA or CBA if conventional PTA was suboptimal, defined as residual stenosis of >30%. Primary end point was angiographic patency of AVF/AVG at 6 months. Secondary end points included technical success, procedural complication rate, 30 day mortality and 6 months secondary patency rate.

Results:
Between October 2008 and July 2011, 826 patients were enrolled into the study with 745 patients achieving good results following conventional PTA. Of the remaining 81 patients (9.8%) with suboptimal results following conventional PTA, 42 and 39 patients were randomized to HBPA and CBA arms respectively.

Primary patency rates at 6 months were 46.1%, 28.9%, 41.7% for conventional PTA, HPBA and CBA groups respectively.

Secondary patency rates at 6 months were 96%, 84.2%, 91.7% for conventional PTA, HPBA and CBA groups respectively.

The differences in 6 month primary and secondary patency rates between conventional PTA vs HPBA, conventional PTA vs CBA and HPBA vs CBA were all not statistically significant.

There was a significant complication of venous perforation following CBA which was successfully managed with prolonged balloon inflation. The 30 day mortality rate was 0.01%.

Conclusion:
Conventional PTA is effective treatment in the majority of patients (90.2%) with dysfunctional AVF/AVG. There is no statistically significant difference in 6 month primary/secondary patency rates between HPBA vs CBA in the treatment of resistant AVF/AVG stenoses following suboptimal conventional PTA. The patency rates of conventional PTA are also comparable to HPBA and CBA.

The routine use of HBP or cutting balloon as first line treatment for stenotic AVF/AVG is not justifiable especially since HBP is 2X and CB is 4X more expensive than a conventional balloon.

For resistant stenoses which responded poorly to conventional PTA, there appears to be a trend favoring CBA over HPBA as the 2nd line treatment in terms of primary patency. The statistical insignificant difference may be due to small sample size.
Total Body Water Measurements by Bioimpedance Analysis Reflect Volume Changes Better Than Extracellular Fluid Measurements in Haemodialysis

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Introduction:
Bioimpedance analysis (BIA) has been widely utilised for more objective body water measurement during haemodialysis, in end-stage kidney disease (ESKD) patients. However, the agreement between BIA obtained measures, with actual volume changes during haemodialysis, has not been thoroughly evaluated.

Method:
Using a whole-body multifrequency BIA machine (Bodystat® Quadscan 4000), we conducted a prospective observational study in hospitalised adult ESKD patients, and evaluated the agreement between pre- and post-haemodialysis BIA obtained extracellular volume (ECV) and total body water (TBW) changes, with ultrafiltration (UF) volumes and weight changes respectively.

Results:
Forty-four prevalent and nineteen incident haemodialysis patients were recruited, with data obtained from ninety-one haemodialysis sessions. Mean age was 54.9 years (+/- 11.6 years), 54.0% were males, and mean left ventricular ejection fraction was 54.8% (+/- 13.5%). Mean BIA obtained ECV, TBW, and intracellular volumes (ICV) were statistically different before and after dialysis (p<0.001), but not mean ECV/ICV or ECV/TBW ratios. Using Bland-Altman analysis, ECV changes pre- and post-dialysis, underestimated UF volumes, with average bias of -1.20L, with only 93.4% of data points within limits of agreement (-2.60L, 0.21L). In comparison, TBW changes estimated the weight changes with average bias of -0.41L, and 97.8% of data points lie within the limits of agreement (-3.54L, 2.71L), but the range of agreement was wide. Pre- and post-dialysis ECV/TBW and ECV/ICV ratio differences do not correlate with UF volumes or weight changes.

Conclusion:
Whole-body multifrequency BIA measured TBW change has better agreement with actual volume change in haemodialysis, as compared to ECV change.
A Large-Scale Survey of the Singapore Public on the Awareness of the Human Organ Transplant Act and its Relationship to Altruistic Behavior

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Introduction:
The Human Organ Transplant Act (HOTA) in Singapore allows for the removal of heart, liver, kidneys and corneas for the purpose of transplantation, from Singaporean citizens or permanent residents (PRs) who died in hospital. This is provided that they were (1) at least 21 years old, (2) did not opt out from HOTA during life and (3) of sound mind. Singapore is also the only country that includes Muslims in a presumed consent law. All Singaporeans citizens and PRs who turn 21 years of age will receive a letter from the Ministry of Health (MOH) to inform them about HOTA and the option to opt out. The MOH also informs the general public twice a year on HOTA through the media in the 4 official languages of Singapore. A cross-sectional survey of the Singapore public was performed to determine awareness of HOTA and whether it was positively related to altruistic behavior.

Method:
The study population consisted of 1384 of 1520 individuals (91% response rate) who visited 3 government primary care medical centres and completed a self-administered questionnaire. This questionnaire consisted of 34 questions to collect data on demographic characteristics, baseline knowledge of chronic kidney disease, attitudes and perceptions on living kidney donor transplantation and awareness of HOTA. Two questions were asked to assess respondents’ awareness of HOTA (AWA) and the option to opt out (OPT). Another two questions were asked to assess knowledge on which organs were recoverable (ORG) and who was included under HOTA (INC). Another 2 questions were asked to determine participation in altruistic behavior.

Results:
The mean age of respondents was 48.8 ± 15 years. Majority were Chinese (71.8%), men (50.3%) and received high school or lesser education (73.4%). These characteristics were comparable to those seen in the general Singapore population. For AWA, 32.8% and 46.5% reported being “familiar” and “somewhat familiar” of HOTA. Correct responses was seen in 68%, 57.2% and 43.5% for OPT, ORG and INC respectively. Multivariate logistic regression analysis revealed positive predictors were income more than SD$2000/month (for AWA and OPT), higher than high school education (for AWA, ORG and OPT) and Islam religion (for AWA and INC) (all p<0.05). Age > 60 years and chronic illness were other positive predictors for AWA and INC respectively (p<0.05). For altruistic behavior, 26.1% and 30.4% have donated blood or performed voluntary charity work. Univariate regression analysis showed an association of such altruistic behavior to familiarity with HOTA (OR=1.15; p<0.001) and the association persisted after adjusting for demographic characteristics (OR=1.13; p<0.001).

Conclusion:
This survey revealed that 79.3% of respondents were aware of HOTA but the knowledge levels for specific aspects of HOTA was lower. Other public measures such as inclusion of HOTA in the education curriculum of Singapore schools, road-shows in suburbia and putting the option to opt out during processing of identity cards or driving licenses may increase awareness and knowledge levels on HOTA.
Ambulatory Diastolic Blood Pressure Predicts Microalbuminuria in Children and Adolescents with Diabetes Mellitus

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Introduction:
Hypertension is a major risk factor for diabetic nephropathy (DN). Ambulatory blood pressure monitoring (ABPM) can underscore mild BP modifications that are not evident when assessed by a ‘once-off’ clinic BP measurement. Early DN with microalbuminuria (MA) has been associated with abnormal ambulatory blood pressure (ABP) measurements in adults. However, few studies have been performed in children and adolescents, especially among those with type 2 diabetes mellitus (DM). We aimed to study if ABP parameters predict microalbuminuria in children, adolescents and young adults with diabetes mellitus (DM).

Method:
Twenty-four-hour ABP, clinic BP and 24h urine albumin excretion (UAE) were studied in 47 pediatric DM (23 Type 1; 24 Type 2) patients. The median (range) age and duration of diabetes were 17.7 (8.6-24.9) and 6.5 (0-17.0) years respectively. Urine albumin and creatinine were measured from the 24h urine collection, and albumin:creatinine ratio (ACR) calculated. Mean systolic BP (SBP) and diastolic BP (DBP) for 24h, wake and sleep time periods were obtained. From these, BP loads (percentage of BP recordings exceeding 95th percentile of normative values), BP indices (mean BP divided by 95th percentile of normative values) and nocturnal BP dipping (percentage fall in mean sleep BP with respect to mean wake BP) were calculated. Hypertension was defined as BP load >30% and/or BP index ≥1. Nocturnal BP dipping <10% was defined as abnormal. Due to multicollinearity among ABP parameters, principal component analysis was performed to create four independent ABP factors (SBP load, DBP load, SBP index, DBP index) each for 24h, wake and sleep periods. Three multivariate linear regression models were then separately created for each time period using the respective sets of ABP factors, together with age, body mass index, type and duration of DM, low-density-lipoprotein cholesterol, average HbA1c, HbA1c variability and serum uric acid as predictors of urine ACR.

Results:
Four out of 47 (8.5%) patients had MA (UAE≥30mg/day). ABPM diagnosed hypertension in 24 patients (5 with 24h hypertension, 4 with wake hypertension, 15 with sleep hypertension) which were not diagnosed by clinic BP. Thirty (63.8%) patients had abnormal SBP and/or DBP nocturnal dipping. However, all patients with abnormal nocturnal BP were normoalbuminuric (NA) patients. Using a Mann-Whitney U test, wake DBP load was higher in MA patients than NA patients (median (range) MA 10.3% (5.9%-35.7%) vs NA 0% (0%-39.1%); p=0.013). In the 24h regression model, 24h DBP load (B=1.98, 95% CI 0.94 - 3.03, p=0.001) and 24h DBP index (B=1.32, 95% CI 0.34 - 2.30, p=0.011) were found to predict ACR. In the wake regression model, wake DBP load (B=1.804, 95% CI 0.66-2.94, p=0.003) was found to predict ACR. No associations were found between sleep ABP parameters and ACR.

Conclusion:
Abnormal ABP is present among DM patients with NA. ABPM is more useful than clinic BP measurements in the diagnosis and management of hypertension in pediatric patients with DM. Abnormal DBP parameters appear to predict MA among young diabetic patients and aggressive control of BP may prevent DN.
**BASIC SCIENCE**

**Side Population in Renal Cell Carcinoma Cell Line is Enriched with Drug Resistant, but not Tumor Initiating Cells**

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Several lines of evidences suggested the presence of tumor initiating cells in renal cell carcinoma (RCC) and side population (SP) may be enriched with these “cancer stem cells (CSC)”. Herein we demonstrated the presence of side population in 10 RCC cell lines ranging from 0.19 – 36.1%. Lineage tracing experiments showed that the SP can be enriched through sequential sorting and can differentiate into non-SP (NSP) which constitutes the bulk of the cells. The NSP can also repopulate the SP in ACHN cells. Interestingly, the presence of NSP seems to prevent SP from differentiating into NSP. The SP cells have about 15% higher proliferation rate and 50% greater colony forming efficiency which are reported characteristics of CSC. SP expresses higher level of cMyc, but lower level of CD105 as compared to the NSP. On the contrary, there is no difference in tumorigenic potential between SP and NSP using both correlation and in vivo studies. However, we verified that SP are 10% more resistance to mTOR inhibitor, RAD001 which coincide with higher expression of drug resistance membrane transporters ABCB1 and ABCG2 in SP cells. Treatment with RAD001 increases the amount of SP; concomitantly up regulate ABCB1 and ABCC1 significantly. Taken together, Hoechst dye exclusion did not define tumor initiating cells, but is a valuable technique for identifying drug resistance cells in RCC.

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**Endotoxin-Tolerance Monocyte Profile in Minimal Change Nephrotic Syndrome (MCNS): Role in Increased Susceptibility to Bacterial Infections**

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**Introduction:**
MCNS is often complicated by bacterial infections, contributing significantly to morbidity. We have previously shown upregulation of lymphocyte interleukin (IL)-13 gene expression during nephrotic relapses, associated with downregulation of pro-inflammatory cytokines, IL-8 and tumor necrosis factor (TNF)-α, in lipopolysaccharide (LPS)-stimulated monocytes, and decreased monocyte CD14 expression, suggestive of an IL-13-induced anti-inflammatory effect. This study aimed to identify the ‘gene signature’ in monocytes from MCNS patients, in both remission and relapse, in order to explain the increased susceptibility to infections during relapses. Findings from the human MCNS patient were subsequently validated against monocytes isolated from our IL-13 overexpression rat model.

**Method:**
Monocyte subset surface marker expression and intracellular cytokine production following LPS stimulation were performed using flow cytometry. Monocytes isolated from MCNS patients using MACS-monocyte Isolation Kit II, were cultured for 4 hours with and without LPS. Monocyte RNA from 7 patients in relapse and remission were hybridized into Illumina Human Ref-8 chips. Gene ontology (GO) and pathway analysis were carried out using Ingenuity®.

Recombinant rat IL-13 gene was transfected into Wistar rat quadriceps by in-vivo electroporation weekly till sacrifice at day 72. Monocyte CD43 surface expression and LPS-stimulated intracellular IL-10 and TNF-α production were measured by flow cytometry. CD62L and CX3CR1 gene expression levels in splenic macrophage from 5 controls and 15 IL-13 overexpression rats were measured by quantitative real-time PCR, and standardized against the housekeeping gene, GAPDH.

Statistical analyses were done using non-parametric Mann-Whitney test and Wilcoxon signed rank test for paired data.
**Results:**
Paired analysis showed that MCNS relapse patients had higher percentage of CD14+CD16+ ("anti-inflammatory") monocytes (p<0.05), increased monocyte CX3CR1 expression (anti-inflammatory chemokine receptor) (p<0.04), and decreased production of IL-6 (p<0.02) and TNF-α (p<0.02), compared to remission. Transcription profile of unstimulated monocytes from patients with MCNS in relapse revealed upregulation of genes involved in TRIF pathway and interferon-induction (IRF4, IRF7, IFI6, IFI27, IFI35, IFI44, SERPING1, Mx1, OAS1, OAS2, OAS3, OASL, CXCL9, CXCL10). However, following LPS stimulation, genes usually responsive to LPS (CD86, IL-6, TNF-α) were downregulated.

Consistent with the higher proportion of anti-inflammatory monocyte population in MCNS patients in relapse, the percentage of CD43+ macrophage was significantly higher in IL-13 overexpression rats compared to controls (48.4±2.92% vs 33.0±2.89%, p=0.003). Gene expression levels of CD62L and CX3CR1 were significantly lower in IL-13 overexpression rats (0.362±0.129 vs 0.707±0.190, p=0.045; and 0.484±0.107 vs 1.478±0.492, p=0.035 respectively). Following LPS stimulation, intracellular production of TNF-α was significantly lower in IL-13 overexpression rats compared to controls (41.4±4.12% vs 61.8±4.87%, p=0.005).

**Conclusion:**
Our results demonstrated the bipolar nature of monocytes in MCNS patients following infection-triggered relapse, with the presence of an inflammatory gene signature in-vivo, and a refractory profile following LPS stimulation, indicative of an endotoxin anergic state. Similarly, microarray analysis of monocyte transcriptome in MCNS patients in relapse demonstrated an upregulation of IFN-inducible genes in the TRIF pathway, consistent with an endotoxin-tolerance monocyte profile. This could account for the increased susceptibility to bacterial infections seen in MCNS patients in relapse. This finding was further validated in our IL-13 overexpression rat model of MCNS.

**TIR-domain-containing adapter-inducing interferon-β (TRIF)**
Method:
Human TRPC6 and Nephrin cDNAs were cloned into expression vectors pEGFP-C1 and pIRES2 to obtain eGFP-TRPC6 fusion gene and pIRES-Nephrin, respectively. The respective gene variants were inserted using site-directed mutagenesis. HEK293 cells were transfected in various combinations of TRPC6WT/TRPC6R68W and Nephrin WT/Nephrin SNPs. TRPC6 currents were studied using patch-clamp electrophysiology. The effects of nephrin SNPs on nephrin protein expression were analyzed by Western Blotting. Two-tailed Student’s t tests and ANOVA followed by Bonferroni’s t test were performed.

Results:
Cells expressing TRPC6R68W, compared to cells with TRPC6WT, exhibited higher mean inward (-540.9±40.51pA/pF versus -338.4±32.17pA/pF, p=0.0004) and outward (1065±113.9pA/pF vs 619.5±49.36pA/pF, p=0.0009) currents, indicating that R68W is a gain-of-function mutation. Cells with NephrinWT had lower mean TRPC6 WT or TRPC6 R68W currents than cells without nephrin (TRPC6WT: 21.78±pA/pF vs 35.34±4.86pA/pF, p=0.019 and TRPC6R68W: 30.25±2.75pA/pF vs 48.45±4.60pA/pF, p=0.0064), indicating that NephrinWT inhibits TRPC6 WT and TRPC6 R68W currents. Cells with homozygous 294C>T or 2289C>T SNPs have higher TRPC6 WT currents compared to those with NephrinWT (36.48±5.41pA/pF or 29.24±2.27pA/pF vs 22.74±2.18pA/pF, p=0.0219 and p=0.015 respectively). This was similarly shown for TRPC6R68W (46.81±3.74pA/pF or 43.72±4.88pA/pF vs 35.48±3.97pA/pF, p=0.0168 and p=0.2749 respectively). These results indicate that nephrin SNPs have decreased ability to inhibit TRPC6 WT and TRPC6 R68W currents compared to NephrinWT.

Conclusion:
Nephrin SNPs restricts TRPC6 activity more than NephrinWT, probably through decreased protein expression, and can resultanty affect phenotype. Asymptomatic family members with TRPC6 mutations can be potential renal transplant donors if such interactions are first identified.
Urine Svcam-1 and Sicam-1 Levels are Elevated in Lupus Nephritis

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Introduction:
Lupus nephritis (LN) affects a substantial proportion of systemic lupus erythematosus (SLE) patients, resulting in significant morbidity and mortality. Although renal biopsy is the most accurate assessment modality, it is invasive and not practical for clinical use in the regular monitoring of LN. Intercellular and vascular cellular adhesion molecules (ICAM-1, VCAM-1), membrane proteins necessary for anchoring leukocytes to the vessel wall and well-established markers of endothelial dysfunction, are over-expressed by both infiltrating leukocytes and resident renal cells in diseased kidneys. We sought to evaluate the relationship of urine levels of soluble cellular adhesion molecules sVCAM-1 and sICAM-1 in systemic lupus erythematosus (SLE) patients with or without lupus nephritis, and to explore their correlation with renal disease activity.

Method:
Paired serum and urine samples of 121 Asian SLE patients, and urine samples of 19 normal healthy controls were collected. Demographic data, disease activity and damage scores, and selected laboratory parameters including levels of anti-dsDNA antibody, complement C3, C4, and creatinine were captured. Renal disease activity was scored with renal SLAM-R (rSLAM-R). Serum and urine sVCAM-1 and sICAM-1 levels were assayed by ELISA.

Results:
Urinary sVCAM-1 (3.10±3.29 ng/mg Cr vs. 0.301±0.215 ng/mg Cr, \(P<0.001\)) and sICAM-1 (1.12±1.51 ng/mg Cr vs 0.330±0.255 ng/mg Cr, \(P<0.05\)) levels were elevated in SLE patients compared to controls. In the 33 (27.3\%) of 121 SLE patients who had active LN, significantly higher levels of urine sVCAM were found in correlation with rSLAM-R. In addition, significantly more patients with active LN had detectable levels of urine sICAM-1, but no correlation with renal activity was observed. Patients with active LN, had more active disease and significantly higher (SLICC/ACR Damage Index) SDI scores. The majority of patients did not suffer from significant renal damage as scored by SDI except for 3 patients, 2 of whom had active LN. No correlation was found between these serum and urine molecules and levels of traditional disease activity markers, including anti-dsDNA antibody and complement C3 and C4.

Conclusion:
Urinary sVCAM-1 may serve as a potential biomarker for early diagnosis of lupus nephritis as levels correlated with even mild abnormalities of the urine sediment. In addition, both urine sVCAM-1 and sICAM-1 levels may be useful in identifying patients at risk of lupus nephritis. However, additional longitudinal studies will be required to validate their usefulness in detecting the onset, reflecting the change in severity of inflammation and/or damage and treatment response.
Towards a Microfluidic Renal Cell Supplement

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Introduction:
It is now well established that the kidneys, like the liver, are highly active in metabolizing drugs. Certain biotransformations are faster in the kidney than in the liver. Current hemodialysis techniques are unable to provide specific cell mediated functions leading to severe complications in patients on long term dialysis. This has provided an impetus for the development of a bioartificial kidney (BAK). Despite the heterogeneity of the nephrons, proximal tubule cells are preferred as sole constituents of BAK due to their functional diversity. The proximal epithelial cell line LLC-PK₁, derived from the Hampshire pig, has been well characterized. We have studied phase II sulfate conjugating activity and directed transport of a monolayer of LLC-PK₁ cells.

Method:
Two separate experimental methods have been adopted for our study. The method for evaluating the phase II detoxification function involved the establishment of a monolayer of cells adhered to the bottom surface of microchannels. The cells were subjected to perfusion at different flow rates with buffer containing sodium sulfate and the substrate, harmol. Transient sulfation activity was studied at fixed flow rate and approach to steady state sulfation was monitored at variable flow rates. The microchannel eluent was subjected to HPLC-fluorimetric analysis to quantify the harmol sulfate produced. In the other method, aimed at quantifying directed transport, monolayers of LLC-PK₁ were cultured on hyaluronic acid (HA) coated nitrocellulose membranes. An ultrafiltration membrane and the HA membrane with confluent cell monolayer was sandwiched between two polydimethoxysiloxane (PDMS) chambers. Buffer containing the transport molecule (uric acid or phenol red) was placed in either chamber to establish an apical or basolateral directed net transport of the molecule. The PDMS device was subjected to gentle shaking to eliminate concentration gradients and aliquots were periodically sampled from the appropriate chamber. The collected samples were subjected to HPLC analysis to quantify the net amount of molecule transported across the monolayer of cells.

Results:
The study of sulfation of harmol yielded the following experimental observations: (i) a slow attainment of steady state sulfation, (ii) variation in steady state sulfation rate with fluid flow velocity or Péclet number (165 < Pe < 250), and (iii) differences in steady state sulfation rate with increasing or decreasing fluid velocity giving rise to a ‘hysteresis’ curve. Elution of harmol sulfate with increasing flow velocity is explained by mathematical modeling as a dilution effect. Intracellular processes are identified as the cause of decreased sulfation rate with decreasing fluid velocity.

Transport of molecules across the membrane-cell construct provided: (i) estimates of the membrane permeabilities by fitting model to data, (ii) confirmation of model prediction when cell monolayer is disrupted resulting in loss of polarization, and (iii) qualitative agreement of model with literature data. Additionally, phenol red transport is proposed as a rapid method to confirm the formation of a polarized monolayer.

Conclusion:
This study is a step towards our overall objective of developing a design paradigm and experimental methods to construct a microfluidic renal cell supplement that provides important cell mediated functions.
Renal Regulation of Glucose Homeostasis by the Nuclear Receptor HNF4A

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Aim:
To determine the role of HNF4A in regulating proximal tubule gluconeogenesis and renal contribution to blood glucose levels.

Hepatocyte nuclear factor 4 alpha (HNF4A, NR2A1) is an orphan nuclear receptor critical for development and function of the liver, pancreas and intestine. HNF4A helps regulate glucose metabolism by modulating insulin secretion and the expression of key gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase in the liver. Loss-of-function mutations of HNF4A or its response element in target promoters result in Maturity-Onset Diabetes of the Young Type I (MODY I) in humans.

Liver HNF4A regulates fasting glucose production via expression of the key gluconeogenic enzyme PEPCK. In the kidney, expression of HNF4A is strikingly restricted to the proximal tubule, the nephron segment that reabsorbs filtered glucose, performs PEPCK-mediated gluconeogenesis and metabolises insulin. Indeed, the proximal tubule produces up to 20% of circulating glucose in humans, and accounts for up to two-thirds of renal insulin clearance. It is not known if proximal tubule HNF4A controls renal gluconeogenesis and insulin breakdown in the fasting or hyperglycemic state.

Our data showed that VLCAD, an enzyme responsible for metabolism of Very Long Chain fatty acids is expressed in the proximal tubule epithelial cells of normal humans and diabetic nephropathy patients and in diabetic ob/ob mice as determined by immunohistochemical staining of renal biopsies and tissue respectively. Cloning and sequence analysis of the VLCAD promoter and 5’ UTR identified three putative HNF4A response elements at position -950bp, 332bp and 370bp relative to the transcription start site. Deletion analysis and point mutagenesis of transcriptionally essential nucleotides in the +370bp response element abrogated transactivation by HNF4A in cellular reporter assays, suggesting that the +370bp response element mediates HNF4-driven VLCAD expression.

Our data provide preliminary evidence to suggest that HNF4A regulates VLCAD-mediated fatty acid metabolism in the proximal tubule to provide substrates for renal gluconeogenesis. We will validate this with metabolic studies in a Tet inducible proximal tubule specific HNF4A knockout mouse model.
Expression of the Proximal Tubule-Specific Nuclear Receptor HNF4A in Kidney Disease

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Aim: To determine if expression of the proximal tubule-specific transcription factor HNF4A is altered in kidney diseases that involve proximal tubule injury, proliferation and regeneration, and thereby to suggest a role of HNF4A in the pathogenesis and pathophysiology of these diseases.

Kidney diseases that are associated with proximal tubule injury, proliferation and regeneration include acute tubular necrosis, interstitial nephritis, acute allograft rejection, chronic allograft nephropathy and hypertensive nephrosclerosis. In these conditions, failure of tubular repair and regeneration is often associated with interstitial fibrosis and renal impairment.

Hepatocyte Nuclear Factor 4 alpha (HNF4A, NR2A1) is an orphan nuclear receptor transcription factor that regulates normal development of the kidney, liver, pancreas and gastrointestinal tract. Studies in cellular and animal models, coupled with human cancer expression data, suggest that HNF4A represses cellular proliferation and promotes terminal differentiation.

In the kidney, HNF4A is expressed only in the proximal tubule. This strikingly restricted expression strongly suggests that HNF4A regulates proximal tubule-specific functions, such as proximal tubule proliferation and regeneration in response to injury.

We postulated that renal injury alters HNF4A expression and target gene readouts to impact proximal tubule proliferation and regeneration.

Our data showed increased HNF4A expression in human renal biopsy tissue in acute tubular necrosis, acute allograft rejection, IFTA, IgA and diabetic nephropathy and interestingly also in minimal change disease, as determined by immunohistochemical staining and computer algorithmic double blind scoring of renal biopsy tissue.

Our data provide preliminary evidence to suggest that HNF4A is upregulated in kidney diseases with a tubular component and raise the prospect of pharmacological regulation of HNF4A targets in the treatment of these conditions. We are creating a Tet inducible proximal tubule specific HNF4A knockout mouse model as a tool to identify HNF4A targets and probe HNF4A functions.
Aim:
This proposal asks if the transcription factor HNF4A regulates the production of fasting glucose from fatty acids by the proximal tubule cells of the kidney in diabetes.

Hepatocyte Nuclear Factor 4 alpha (HNF4A, NR2A1) is an orphan nuclear receptor known to have a metabolic role in the liver and pancreas. Pancreatic HNF4A helps regulate glucose metabolism by modulating insulin secretion. Liver HNF4A regulates fasting glucose production from fats by controlling the gluconeogenic enzymes PEPCK and glucose-6-phosphatase, and fatty acid metabolism by regulating Very Long Chain Acyl-CoA Dehydrogenase (VLCAD). Loss-of-function mutations of HNF4A or its response element in target promoters result in Maturity-Onset Diabetes of the Young Type I (MODY I) in humans.

In the kidney, HNF4A is expressed solely in the proximal tubule. This strikingly restricted expression strongly suggests that HNF4A regulates proximal tubule-specific functions such as reabsorption of filtered glucose, PEPCK-mediated gluconeogenesis, lipid metabolism and insulin degradation. Indeed, the proximal tubule produces up to 20% of circulating glucose in humans and accounts for up to two-thirds of renal insulin clearance.

Recent work in our laboratory funded by the NKF has shown that renal HNF4A expression is increased in diabetic nephropathy in humans, and the HNF4A target VLCAD is upregulated in DM humans and fasted ob/ob mice. Our data suggests that HNF4A controls fasting renal glucose production from very long chain fatty acids in diabetic patients. This putative role is consistent with the established metabolic roles of HNF4A in liver gluconeogenesis, fat metabolism, and pancreatic insulin secretion. Our US collaborator Dr Frances Sladek has found that HNF4A is itself regulated by a fatty acid, linoleic acid, consistent with a role for HNF4A in controlling fatty acid breakdown. Therefore, we will ask if renal HNF4A regulates fasting glucose production from fatty acids in animal models of the proximal tubule.

Aim 1 seeks to identify HNF4A renal targets in the gluconeogenic and fatty acid metabolic pathway by expression analysis of kidneys of the existing HNF4A7 isoform knockout mouse. We will verify the co-expression of potential targets with HNF4A in human diabetic nephropathy biopsies.

Aim 2 will determine if HNF4A is required for maintenance of fasting glucose in a Tetracycline-inducible kidney-specific HNF4A knockout mouse model to be created by Dr Zakir Hossain, director of the mouse facility in CeLS.

Aim 3 will determine if administration of the HNF4A ligand linoleic acid increases fasting glucose levels in these Tet-inducible HNF4A knockout mice.

These studies will increase our understanding of the kidney’s role in fasting blood glucose regulation in diabetes, and will set the stage for the development of fatty acid derivatives as drugs to control HNF4A so as to modulate blood glucose levels in diabetic patients.
Increased GATA3 Expression and Th2 Cytokine Profile in DEC1-Transfected Jurkat Cells: A Potential Mechanism of Action of DEC1 in the Pathogenesis of Minimal Change Nephrotic Syndrome

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Introduction:
We have previously demonstrated that interleukin-13 (IL-13) gene expression was upregulated in CD4+ and CD8+ T-cells of children with MCNS in relapse. Using DDRT-PCR, we also identified that the Deleted in Esophageal Cancer 1 (DEC1) gene was significantly increased in CD4+ T-cells of pediatric MCNS patients in relapse. Further studies showed that DEC1, when transfected into Jurkat cells, could inhibit cell proliferation by up to 30%. Cell cycle analysis indicated that DEC1 arrested Jurkat cell cycle progression by blocking its entry into the G2/M phase. Immunofluorescent staining with anti-DEC1 antibody suggested that DEC1 was a cytoplasmic protein, in agreement with PSORT prediction. Therefore, we hypothesized that upregulation of DEC1 in CD4+ T-cells in MCNS relapse, and inhibition of Jurkat cell proliferation by DEC1 may have important implications on the regulation of cytokine synthesis pathways and signaling function in T-cells. This study thus aims to delineate the role of DEC1 in the pathogenesis of MCNS, utilizing DEC1-transfected Jurkat cells, via i) elucidation of pathways regulated by DEC1 gene using microarray, and ii) cytokine expression profiling of DEC1-transfected Jurkat cells.

Method:
Jurkat cells were transfected with DEC1 gene using SuperFect Transfection Reagent (Qiagen). RNA was isolated from the DEC1-transfected cells, reverse transcribed and hybridized into microarray. After normalization and statistical analysis of the data, the differentially expressed genes were further analyzed and classified using Ingenuity Pathways Analysis into functional categories and pathways. For cytokine expression profiling of DEC1-transfected Jurkat cells, Jurkat cells transfected with DEC1 and plasmid alone (plasmid control) were cultured at 5 x 10^6 cells/ml for 72 hours. Cytokine levels in the supernatants were then assayed utilizing the multiplex suspension bead array system.

Results:
Of 3326 genes that were differentially expressed, 546 genes showed regulation above 2-fold. DEC1-transfected Jurkat cells differentially expressed molecules that regulate biological functions like cell signalling (37 molecules, p=0.004), molecular transport (67 molecules, p=0.004), cellular assembly and organization (34 molecules, p=0.005). The analysis also showed greater than 1.5-fold increase in the molecules involved in the NF-κB (transcription factor involved in immune response) signaling pathway. In addition, there was also a greater than 2-fold increase in GATA3 expression, an inducing factor for Th2 polarization and transcription factor of IL-13 gene expression. Results of cytokine profiling of DEC1-transfected Jurkat cells demonstrated a 110% increase in IL-5 level as compared to plasmid controls. There was also a 95% increase in IL-4 levels in DEC1-transfected Jurkat cells, as compared to plasmid controls.

Conclusion:
The above findings suggest that the mechanism of action of DEC1 in the pathogenesis of MCNS may be mediated via the NFκB and GATA-3 pathways. IL-4, IL-5 and IL-13 are found in a gene cluster, regulated coordinately by GATA-3. The findings of increased IL-4 and IL-5 levels in DEC1-transfected Jurkat cells are in accordance with an increase in GATA3 gene expression, further supporting the role of Th2 polarization in this disease.
ERK-Inhibitor AZD6244 Enhances the Anti-Tumour Activity of Sorafenib in a Xenograft Model of Human Renal Cell Carcinoma (RCC)

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Introduction:
Sorafenib, an anti-VEGFR small molecule inhibitor, induces RCC tumour response rate of approximately 40% as monotherapy. However, sorafenib therapy is associated with significant adverse events. In this study, we aim to evaluate the synergistic effect of AZD6244 in combination with sorafenib in a murine RCC xenografts model.

Method:
We successfully generated three patient RCC-derived xenografts in SCID mice. These mice were divided into four groups, each treated with vehicle-only, sorafenib, AZD6244, and combination AZD6244/sorafenib. Molecular changes in the tyrosine kinase receptor signaling pathways, cell cycle, apoptosis and mTOR pathways in response to the above treatment regimes were analysed by western immunoblotting. Apoptosis, microvessel density, MEK signaling and cell proliferation were analysed by immunohistochemistry.

Results:
Sorafenib as monotherapy induced partial tumor growth inhibition and partial inhibition of the PI3K/AKT and MEK/ERK signaling pathways. AZD6244 alone has no significant anti-tumor activity. Combination AZD6244/sorafenib enhances anti-tumor activity of sorafenib and allows dose reduction of sorafenib without compromising its anti-tumor activity. AZD6244/sorafenib treatment caused profound inhibition of PI3K/AKT and MEK/ERK signaling pathways, cell cycle and mTOR proteins expression and apoptosis induction compared to sorafenib monotherapy. Combination AZD6244/sorafenib treatment resulted in profound inhibition of cell proliferation and angiogenesis, and markedly increase apoptosis as evidenced by immunohistochemistry.

Conclusion:
Our findings showed that AZD6244 significantly enhances the anti-tumour effect of sorafenib in RCC, such that combination AZD6244/sorafenib allows halving of sorafenib dosage, thus minimizing the occurrence of adverse effects, without compromising its clinical activity in RCC.
Pathogenesis of Hypercholesterolemia in Minimal Change Nephrotic Syndrome (MCNS)

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Introduction:
Minimal change nephrotic syndrome (MCNS) is the most common cause of childhood idiopathic NS, and is proposed to be a primary Th2 cytokine disorder. A hallmark of MCNS is hypercholesterolemia, a major risk factor for atherosclerosis in children with long-standing treatment-resistant disease. Previous studies in drug-induced rat models of NS have suggested that hypercholesterolemia seen in these models of NS may be secondary to proteinuria. Our laboratory has developed an IL-13 gene overexpression rat model of MCNS, which at Week 10 displayed edema, proteinuria, and hypoalbuminemia. These rats had associated hypercholesterolemia which correlated significantly with serum IL-13. This was associated with significant changes in hepatic expression of cholesterol metabolic genes HMGCR and CYP7A1. Moreover, hepatic protein expression of IL-13 receptors, IL-4Ra and IL-13Ra1, in IL-13 transfected rats indicated possible IL-13 signaling on the hepatocytes. Therefore, we hypothesized that in MCNS relapses, hypercholesterolemia is due to dysregulation of cholesterol homeostasis, mediated by the direct action of Th2 cytokines like IL-13, on the liver, and not secondary to proteinuria alone.

Method:
A transformed mammalian vector containing rat IL-13 gene was electroporated into quadriceps of rat every ten days. Plasma IL-13, cholesterol, albumin and urine albumin levels were assayed weekly to define onset of hypercholesterolemia before proteinuria. Liver tissue RNA and protein were harvested for microarray (Week 10), RT-PCR and Western blot at the onset of hypercholesterolemia and at Week 10. RT-PCR was carried out to validate microarray data.

Results:
A timeline experiment charting the biochemistry of IL-13 transfected rats weekly, showed significant elevation of plasma cholesterol, starting at Week 2, before onset of proteinuria at Week 6. At Week 2, there was also a significant correlation between plasma IL-13 and plasma cholesterol (p<0.001), but not with urine albumin excretion. IL-4Ra and IL-13Ra1 protein expression were demonstrated in the livers of IL-13 transfected rats sacrificed at Week 2 and 10.

Microarray of hepatic gene expression in three IL-13 transfected rats at Week 10 with hypercholesterolemia >3.10 mmol/L showed upregulation of IL-13Ra1 and IL-13Ra2, downregulation of Jak2, STAT5 and downstream molecules LIFR, SHP-2, MAPKs and LDLR, as well as dysregulation of cholesterol metabolic genes such as ABCG5, HMGCR and CYP7A1. MetaCore™ pathway analysis suggested OSM signaling in hypercholesterolemia. RT-PCR validated downregulation of molecules involved in the OSM pathway regulating LDLR transcription, with 5.4-fold downregulation of LIFR, the OSM receptor.

RT-PCR on the liver of rats sacrificed at Week 2, which were hypercholesterolemic and not yet proteinuric showed that the key molecules LIFR and ABCG5 were already downregulated by 4.8 and 10.0 fold respectively.

Conclusion:
Hypercholesterolemia in IL-13 transfected rats preceded proteinuria, suggesting a phenomenon independent of proteinuria, and may be due to IL-13 acting directly on the IL-13 receptors present on hepatocytes. Our results have shown downregulation of LIFR might lead to a decrease in hepatocyte LDLR expression, resulting in decreased clearance of plasma cholesterol. Downregulation of ABCG5, a transporter in cholesterol efflux, might also result in cholesterol and bile acids accumulating within hepatocytes, inhibiting further plasma cholesterol uptake.
Indians May Have Lower Glomerular Filtration Rates which is Unrelated to Protein Intake

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Introduction:
Measured glomerular filtration rates (GFR) in Asians without chronic kidney disease (CKD) are reportedly lower than in Europeans (1, 2). We assess the normal distribution of GFR in a multi-ethnic Asian population without CKD and GFR estimated with the CKD-EPI equation.

Methods:
We prospectively recruited 103 healthy participants (49.5% male, Chinese 34%, Malay 24.3%, Indian 23.2%, and others 19.4%). We measured standardized serum creatinine (Scr) enzymatically, and GFR (all GFR; in mL/min/1.73m²) using 3-sample plasma clearance of 99mTc-DTPA, normalized to body surface area (du Bois). We used 24-hour urine collections to estimate total protein intake (g/day) (3): 6.25 × urine urea nitrogen + 30 × weight. We use linear regression to assess the association of demography with mGFR, and to develop GFR prediction equations. We use the mean age from previously published studies to predict the GFR of those populations. We estimate GFR (eGFR) using the CKD-EPI equation, and assess its performance using the bias (median difference of eGFR - mGFR), precision (IQR), root mean square error (RMSE), and the percentage accuracy of eGFR to within 15%, 30% and 50% of mGFR.

Results:
Population means: age 42.5 ± 14.3 years, body mass index 24.9 ± 4 kg/m², body surface area 1.7 ± 0.2 m², serum creatinine 0.8 ± 0.2 mg/dL, mGFR 101 ± 15.8, and eGFR 104 ± 15.2. Protein intake was similar by ethnicity. By linear regression, predicted GFR = 124.6 – 0.565 × (Age). In the full multivariate model (age, gender, race), GFR was lower in males (-2.58, p = 0.04) and in Indians (-9.3, p <0.001). Predicting mean GFR using data from previously published studies, the GFR for Chinese was similar: GFR 99 (male, mean age 41.6 years); GFR 98 (female, mean age 46.4 years). But is higher in our Indians: predicted GFR 97 (mean age 31.2 years). For eGFR, the bias is 4, precision 17.7, RMSE 14.8. Percentage accuracy of eGFR to within 15%, 30% and 50% of mGFR are 70.9, 93.2, and 99, respectively.

Figure 1 Distribution of measured GFR of healthy participants with age.

Dotted lines = 95% CI for the regression function line
Dashed lines = Prediction interval of the regression function

Conclusions:
Healthy Indians have significantly lower GFR compared to other ethnic groups. This appears to be unrelated to amount of dietary protein intake. After adjusting for age, the mean measured GFR in Chinese participants was similar to Chinese in China, but our Indians had higher GFR than those in India. The CKD-EPI equation estimates GFR accurately in a multi-ethnic Asian population without CKD.

References:
Nutritional Assessments are Valid in Asians but Total Protein Intake per Ideal Body Weight is Valid Only in Chronic Kidney Disease Patients

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Introduction:
Clinical practice guidelines recommend objective nutritional assessments by body mass index (BMI), mid-arm circumference (MAC), corrected mid-arm muscle area (cAMA), and mid-arm muscle circumference (MAMC) (1). It is unclear how protein intake associates with these assessments in an Asian population. Moreover, the standards proposed are inappropriate when risks of clinical outcomes or associated metabolic abnormalities are considered (2, 3). We assess protein intake and its association with nutritional assessments in a multi-ethnic Asian population.

Methods:
We analyzed the 24-hour urine collections of the Asian Kidney Disease Study (4) and the Singapore Kidney Function Study to estimate total protein intake (TPI; g/day) using (5), TPI = 6.25 × urine urea nitrogen + 30 × weight (in kg). We calculated ideal body weight (IDW; kg) = 22.99 × height² (m) (2). We calculated muscle assessments (1):

MAMC = MAC – (π×TSF)
cAMA (men) = [(MAC – π×TSF)²/4π] – 10
cAMA (women) = [(MAC – π×TSF)²/4π] – 6.5

We used t-test, ANOVA, and Chi-square tests where appropriate, taking significance at the 5% level and using JMP 7.0.1.

Results:
There were 232 chronic kidney disease (CKD) patients (4) and 103 healthy participants with mean age 53.5 ±15.1 year; comprising of 51% male, 38.5% Chinese, 29.6% Malay, 23.6% Indian, and 8.4% Others, 20.4% smokers, 35.5% diabetics, and 57.3% hypertensives.

Nutritional assessments
Overall, TPI is associated with MAC (p <0.001), cAMA (p<0.001, and MAMC (p <0.001). TPI divided by IDW (TPI-IDW; g/kg/day) also associates with MAC (26.131472 + 4.1530665 × TPI-IDW; p <0.001), cAMA (23.025138 + 8.1261286 × TPI-IDW; p<0.001, and MAMC (19.487248 + 2.480638 × TPI-IDW; p <0.001) but not when TPI is divided by actual body weight. When examined separately, TPI is associated with MAC, cAMA, and MAMC in both CKD and healthy participants, but is associated with TPI-IDW only in CKD patients.

Conclusions:
Total protein intake is associated with muscle assessments in all participants. TPI divided by IDW should only be used in CKD patients.

References:
Sodium Restriction Should Be Emphasized in Chronic Kidney Disease Stages 1 To 3

Boon Wee Teo, Pek Yee chow, Qi Chun Toh, Evan JC Lee, On behalf of Singapore Kidney Function Study research team
Department of Medicine, Division of Nephrology, Yong Loo Lin School of Medicine, National University of Singapore

Introduction:
Clinical practice guidelines recommend dietary sodium restriction to <100 mmol/L in chronic kidney disease (CKD) patients with hypertension. It is unclear what proportion of CKD patients achieve this target compared to healthy, normal kidney function participants in a multi-ethnic Asian population. Moreover, the effectiveness of dietary counseling in a diverse population with CKD is also unknown. We assess the profile of urine sodium excretion to determine the appropriateness and effectiveness of current clinical practice in a multi-ethnic Asian population.

Method:
We analyzed the 24-hour urine collections of the Asian Kidney Disease Study and the Singapore Kidney Function Study for sodium excretion. We used t-test, chi-square, and non-parametric tests where appropriate. We used linear regression to assess the association of continuous variables of age, height, weight, body mass index (BMI; kg/m²), glomerular filtration rates (GFR; mL/min/1.73m²) with sodium excretion. Significance is taken at the 5% level and analyses were performed using JMP 7.0.1.

Results:
There were 232 CKD patients and 103 healthy participants with mean age 53.5 ±15.1 years and mean sodium excretion 124.9 ± 68.3 mmol; comprising of 51% male, 38.5% Chinese, 29.6% Malay, 23.6% Indian, and 8.4% Others, 20.4% smokers, 35.5% diabetic, and 57.3% hypertensive. The mean systolic and diastolic blood pressures (mmHg) in healthy participants were lower at 113.8 ±14.5 and 67.5 ±9.9, respectively; compared to CKD patients at 133.6 ±21.5 and 71.8 ±10.2, respectively, (p <0.001). Indians excreted more sodium than Chinese (p = 0.0164) and Malay (p = 0.0018). Mean sodium excretion by diuretic use, or diabetic status, and between normal and CKD participants was similar. On average, patients with CKD stages 1 to 3 excreted >100 mmol but overall 40.1% (93/232) excreted <100 mmol, and 10.3% (24/232) excreted <50 mmol/day. The distribution of excretion is similar in normal participants; 37.9% (39/103) excreted <100 mmol, and 11.7% (12/91) excreted <50 mmol. Only 3 patients were on sodium bicarbonate, but the average sodium excretion was not different.

Table 1 Urine sodium excretion and significant associations

<table>
<thead>
<tr>
<th>Sodium excretion (mmol/L)</th>
<th>Univariate P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>142.3 ± 72.6</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>106.8 ± 58.4</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Chinese</strong></td>
<td>120.9 ± 71.9</td>
</tr>
<tr>
<td><strong>Malay</strong></td>
<td>112.3 ± 61.6</td>
</tr>
<tr>
<td><strong>Indian</strong></td>
<td>144.2 ± 67.4</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>133.1 ± 66.3</td>
</tr>
<tr>
<td><strong>CKD stage (n)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal (103)</td>
<td>128.5 ± 66.5</td>
</tr>
<tr>
<td>1 (27)</td>
<td>141.5 ± 68.7</td>
</tr>
<tr>
<td>2 (45)</td>
<td>145.7 ± 80.8</td>
</tr>
<tr>
<td>3 (99)</td>
<td>127.7 ± 67.4</td>
</tr>
<tr>
<td>4 (53)</td>
<td>93.4 ± 51.6</td>
</tr>
<tr>
<td>5 (8)</td>
<td>80.7 ± 43.6</td>
</tr>
<tr>
<td><strong>Intercept</strong></td>
<td>171.923</td>
</tr>
<tr>
<td><strong>Slope</strong></td>
<td>-0.878</td>
</tr>
<tr>
<td><strong>BMI (per kg/m²)</strong></td>
<td>9.923</td>
</tr>
<tr>
<td><strong>GFR (per mL/min/1.73m²)</strong></td>
<td>102.025</td>
</tr>
</tbody>
</table>

Conclusion:
Patients with CKD stages 4 and 5 achieve sodium restriction but more effort should be made to reduce sodium intake in earlier stages of CKD and in healthy individuals. Dietary counseling should be intensified in Indian patients and the dietary sodium intake should be reduced by approximately 20% in the general population.
Prevalence of Prehypertension/Hypertension in an Asian Pediatric Population

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1Department of Pediatrics, Yong Loo Lin School of Medicine, N University Health System, Singapore Clinical Research Institute, Singapore

Introduction:
Pediatric blood pressure (BP) norms vary in different populations and are yet to be established in multi-ethnic Singapore, comprising 74.7% Chinese, 13.6% Malay and 8.9% Indian.

Method:
A cross-sectional study was conducted to establish office BP norms in healthy Singapore school children. BP readings were measured following a standard protocol in 9936 children aged 6-19 years using the mercury sphygmomanometer, and the second and third readings for each child were analyzed. Blood pressure norms were constructed from a subset of children (n=7824, 3725 boys and 4099 girls) with normal weight, using restricted cubic spline quantile regression. Children with medical conditions or on drugs that could affect BP were excluded (n=84). BP normograms were established according to sex, age and height percentiles, based on international definitions for weight and height categories.

Results:
The BP normograms for systolic and diastolic BP in boys and girls are shown below:

<table>
<thead>
<tr>
<th>Weight Category</th>
<th>Normal Weight (n=7824)</th>
<th>Overweight/Obese (n=2028)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prehypertensive</td>
<td>752 (10.0)</td>
<td>283 (14.0)</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>699 (8.9)</td>
<td>316 (15.6)</td>
</tr>
</tbody>
</table>

Based on our normograms, overweight or obese children were 1.5 times more likely to be pre-hypertensive (relative risk 95% CI: 1.28, 1.65) and 1.7 times more likely to be hypertensive (relative risk 95% CI: 1.54, 1.97) than children with normal weight.

Conclusion:
There is a fairly high proportion of prehypertensive and hypertensive children of normal weight in our multi-ethnic population, and this risk increases significantly if the child is overweight.
Knowledge of Chronic Kidney Disease Among Primary Care Patients in Singapore

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¹SingHealth Centre for Health Services Research, Singapore; ²SingHealth Polyclinics, Singapore; ³Department of Medical Social Services, Singapore General Hospital, Singapore; ⁴Department of Medical Social Services, Singapore General Hospital, Singapore; ⁵Department of Renal Medicine, Singapore General Hospital, Singapore

Introduction:
Kidney disease is the 9th leading cause of death in Singapore. In 2006, diabetes mellitus and hypertension accounted for 59.6% and 11.3% of new kidney failure respectively. However, little is known about the knowledge level of chronic kidney disease (CKD) locally.

This study sought to evaluate the knowledge level of CKD among primary care patients in Singapore.

Method:
This was a cross-sectional survey of a convenient sample of 1520 patients from 3 polyclinics in Singapore. Patients with existing CKD or on dialysis were excluded. The self-administered questionnaire included 7 questions on CKD and demographics. One point was given to each correct question with a maximum of 7 points. Respondents were considered knowledgeable if they scored ≥ 4. T-test, chi square and regression tests were used to examine the differences and associations.

Results:
Of 1520 respondents, 1434 completed all 7 questions on CKD and were further analyzed.

Mean age of the respondents was 48.9 ±15 years. 50.4% were male. 39% had above secondary level education. About 62% had a monthly household income of ≤$2000. 618 (43.5%) respondents reported having chronic diseases such as diabetes (234) and hypertension (462).

Mean score for knowledge was 3.59 ± 1.66 and median score was 4.

In multivariate regression, respondents aged between 40 – 60 years (-0.079) and above 60 years (-0.133), Malay (-0.572) and Indians (-0.687), those who had up to primary education (-0.145) and a monthly income of below $2000 (-0.168) (all p<0.05) were less likely to be knowledgeable. (Numbers in parentheses indicate beta coefficients).

Respondents with no chronic illness (0.206) and no children (0.099) were more likely to be knowledgeable. However, this association was observed only in univariate regression analysis.

Conclusion:
Targeted efforts to improve the knowledge of older, lower socioeconomic status patients and those with chronic diseases would be essential in preventing CKD.
Introduction:
Kidney transplantation is the best treatment option for kidney failure, both in terms of quality of life and cost effectiveness. However, while the demand for kidneys has increased, the supply of donor kidneys remains small. This study thus seeks to understand general public’s willingness to donate one of their kidneys while alive and the demographic factors associated with it.

Method:
This was a cross-sectional study of a convenience sample of 1,520 general public seeking attendances at local medical centres. A self-administered questionnaire was completed that included questions on demographics and willingness to donate a kidney. Respondents were aged 18 years and above, without underlying chronic kidney disease (CKD), end stage renal disease (ESRD) on dialysis, or history of kidney transplant.

Results:
Of the 1,520 respondents, the mean age was 49±15 years and 50% were male. Response rate to "willingness to donate kidney while alive", age, gender and race was 93% (or 1,420 respondents). Of the 1,420 respondents, 707 (or 48%) were willing to donate one of their kidneys while alive. Most of the respondents who were willing to donate kidneys were younger (<40 years; p<0.0001), had above secondary level of education (p<0.0001), had household income above S$2,000 (p<0.0001), were not married/single/divorced (p<0.0001), and were working as professionals (p=0.0001). Fear of risks of surgery (M=1.86, SD=0.81) and poorer health consequent to donation (M=1.84, SD= 0.79) were the main reasons for not considering donating one of their kidneys while alive.

Conclusion:
Our findings suggest that demographic factors and concerns of surgical risks and over ill-health post transplant influence willingness to donate a kidney while alive. Addressing these concerns may alleviate anxiety with regard to living kidney donation.
Health-related Quality Of Life (HRQOL) of Patients on Kidney Transplant Waiting List and Factors Impact on Them

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1Department of Renal Medicine, Singapore General Hospital, Singapore; 2Centre for Health Services Research, Singapore Health Services, Singapore; 3Department of Medical Social Services, Singapore General Hospital, Singapore

Introduction:
Waiting times for kidney transplant are long in Singapore with an average waiting time of 7 years. Health-related quality of life (HRQoL) of patients might be affected as a result of the stress of the long wait, the uncertainty of being called to undergo a surgical operation and fear of transplant rejection. The aims of the study were therefore to measure the HRQoL of patients on kidney transplant waiting list and to compare the HRQoL of the study cohort with that of a matched general population, as well as to identify factors which could impact on the HRQoL scores in this group of patients.

Method:
This was a cross-sectional study of kidney transplant waiting list patients managed at a tertiary renal unit in Singapore from May to September 2009 using the Short Form 36-Item Health Survey (SF-36). The SF-36 consists of 8 multi-item subscales that evaluate various aspects of physical and psychological functioning and well-being, which ranges from 0-100 with higher scores indicating better health. These subscales are: 1) Physical Functioning; 2) Role Physical; 3) Bodily Pain; 4) General Health; 5) Vitality; 6) Social Functioning; 7) Role Emotional; and 8) Mental Health. In addition, the eight subscales are aggregated into two summary measures: Physical Component Summary and Mental Component Summary (MCS) scales. A SF-36 normative calculator was used to generate HRQoL scores for the Singapore general population matched with the study cohort’s age, gender and ethnicity. Statistical significance for all tests was set at 5%. Minimal clinically important difference for the SF-36 is defined as mean differences greater than five points for scales score and greater than three points for summary scores.

Results:
There were 265 respondents (response rate= 81%). Our study showed that HRQoL scores for patients on kidney transplant waiting list were lower than the population norms across all subscales. The worst subscale score for patients on the kidney transplant waiting list when compared to the general population, in terms of the highest mean difference, was the General Health (mean difference= 12.7), followed by Role Physical (mean difference= 10.5), Social Functioning (mean difference= 7.1), Bodily Pain (mean difference= 6.1) and MCS (mean difference= 3.9). These subscales scores were also shown to be clinically significantly lower from those of general population. In the univariate analysis, HRQoL of the patients on kidney transplant waiting list was not influenced by age, educational level, a history of kidney transplant, duration on transplant waiting list and years on dialysis. In multivariate analysis, predictors for better HRQoL were those Chinese, married, employed and undergoing haemodialysis. From the analysis, age, gender and household income did not significantly influence the SF-36 across all subscales scores.

Conclusion:
Patients on kidney transplant waiting list had impaired HRQoL than the general population, particularly in the aspects of General Health, Role Physical, Bodily Pain, Social Functioning and MCS scales. More research could be done into reasons for poorer HRQoL among the at-risk patients (those who are non-Chinese, single, unemployed, retirees or housewives) and explore ways of improving their HRQoL.
Health-Related Quality Of Life of Patients on Kidney Transplant Waiting List versus Post-Transplant Patients

Ong SC², Van Der Erf S², Chow WL², Joshi VD², Lim J³, Lim C³, Tee PS¹, Lu YM¹, Kee T¹
¹Department of Renal Medicine, Singapore General Hospital, Singapore; ²Centre for Health Services Research, Singapore Health Services, Singapore; ³Department of Medical Social Services, Singapore General Hospital, Singapore

Introduction:
To date, kidney transplantation is the best treatment for kidney failure patients as it provides survival advantages and is cost-effective. However, there is a mix of evidence found in the international literatures on health-related quality of life (HRQoL) improvement after kidney transplantation. The aim of the current study was therefore to evaluate the difference in HRQoL of patients on kidney transplant waiting list and post-transplant patients in Singapore.

Method:
Patients on the transplant waiting list and post-transplant patients were recruited from Singapore General Hospital between May to September 2009 and administered the Short Form 36-Item Health Survey version 2 questionnaire (SF-36). The SF-36 consists of 8 multi-item subscales that evaluate various aspects of physical and psychological functioning and well-being, which ranges from 0-100 with higher scores indicating better health. These subscales are: 1) Physical Functioning; 2) Role-Physical Functioning; 3) Bodily Pain; 4) General Health; 5) Vitality; 6) Social Functioning; 7) Role-Emotional Functioning; and 8) Mental Health. Statistical significance for all tests was set at 5%. Minimal clinically significant difference for SF-36 subscales scores is defined as mean difference greater than five points for subscales score. Univariate and multivariate analyses were undertaken to evaluate the HRQoL of patients.

Results:
There were 192 post-transplant (response rate=93%) and 265 waiting list (response rate=81%) respondents. The two groups of patients were similar in most of the demographic profile. However, the post-transplants group had more patients with higher level of education level and household income of SGD2000 and above. Apart from Role Emotional and Bodily Pain, all SF-36 subscales scores for post-transplant patients were statistically and clinically significantly better than patients on transplant waiting list. The greatest mean difference observed in the SF-36 subscale scores was in Vitality (mean difference= 8.84; 95% CI= 5.49-12.19). This was followed by General Health (mean difference= 8.26; 95% CI= 5.10-11.42), Social Functioning (mean difference= 7.90; 95% CI= 3.94-11.86), Role Physical (mean difference= 7.75; 95% CI= 3.11-12.38), Mental Health (mean difference= 6.77; 95% CI= 3.59-9.95) and Physical Functioning (mean difference= 5.10; 95% CI= 1.77-8.43). After adjusting for possible confounding factors of age, gender, ethnicity, marital status, employment status, educational level and household income in multivariate analysis, post-transplant status remained a significant factor in determining better HRQoL in our study.

Conclusion:
In Singapore, post-transplant patients have better HRQoL compared with patients on transplant waiting list. Efforts to improve kidney transplant rates as well as reducing transplant waiting times could consequently improve HRQoL of renal failure patients.
Role of Blood Pressure Monitoring in Predicting Left Ventricular Hypertrophy and Arteriopathy

Kar-Hui Ng, Lourdes Paula Resontoc, Yew-Weng Lau, Alfred CL Yip, Hla Yee, Mya Than, Yiong-Huak Chan, Wee-Song Yeo, Cindy Hia, Terence CW Lim, Lieng-Hsi Ling, Swee Chye Quek, Hui-Kim Yap

1Shaw-NKF-NUH Children’s Kidney Centre, University Children’s Medical Institute, National University Health System and Department of Paediatrics, Yong Loo Lin School of Medicine, NUS; 2Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 3Biostatistics Unit, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 4Cardiac, National University Heart Centre, Singapore; 5Paediatrics, University Children’s Medical Institute, National University Health System, Singapore

Introduction:
Children with end-stage renal disease (ESRD) have an increased risk of death from cardiovascular and cerebrovascular disease in young adulthood. This study aimed at determining the role of blood pressure (BP) monitoring and uraemia-related risk factors that may prognosticate intermediate cardiovascular outcomes, determined by 2D-echocardiography, including fractional shortening, ejection fraction and left ventricular mass index (LVMI), and arteriopathy scans, including parameters of arterial wall thickening, arterial stiffness and endothelial dysfunction.

Method:
As part of an ongoing prospective study, 41 paediatric patients with mean age 17.2±4.9 years and mean ESRD duration 4.2±3.7 years were recruited. A subgroup of 29 patients with mean age 17.2±3.8 years and mean ESRD duration 3.3±2.7 years underwent arteriopathy investigations. Serum biochemistry (calcium, phosphate, urea, uric acid, haemoglobin, haematocrit, intact parathyroid hormone) was monitored retrospectively at 3-monthly intervals, dated back to the diagnosis of chronic kidney disease (CKD) stage 2. Serum biochemistry, clinic BP and ambulatory BP monitoring was done prospectively at 3-monthly intervals from the time of recruitment. Serum homocysteine and lipid profile (low density lipoprotein, high density lipoprotein, triglycerides, cholesterol) measured at 6-monthly intervals from the time of ESRD was also collected. End-points taken for analysis include the patients' latest 2D-echocardiography parameters and arteriopathy scan including systolic flow-mediated dilatation, pulse wave velocity, augmentation index (AIx), β, carotid and femoral intima-media thickness). Systolic BP (SBP) and diastolic BP (DBP) scores were calculated by summing the number of abnormal ambulatory BP parameters (abnormal=1, normal=0). An Arteriopathy score was calculated by summing the number of abnormal arteriopathy parameters. Principal component analysis was used to obtain individual predictors for subsequent linear and logistic regression analyses.

Results:
Multivariate linear regression showed time-averaged clinic SBP index (B=6.967, p=0.026) and SBP score (B=7.384, p=0.014) predicted LVMI, DBP score (B=9.133, p=0.007) predicted AIx, and ESRD duration (B=0.613, p=0.013) predicted the Arteriopathy score. Stepwise logistic regression further showed SBP score (OR=3.195, p=0.029) and homocysteine (OR=3.071, p=0.038) as significant predictors of LVMI.

Conclusion:
These results show that hypertension play a key role in affecting both cardiac and vascular abnormalities, and are therefore important cardiovascular risk factors in children with ESRD. Moreover, ambulatory BP parameters were superior to clinic BP in predicting cardiovascular and arteriopathy outcomes.
Is 24-Hr Urinary Metabolic Evaluation in Urolithiasis Useful in the Singapore Context?

Teck Wei TAN, Keng Siang PNG, Yee Mun LEE, Sing Joo CHIA, Yew Lam CHONG
Department of Urology, Tan Tock Seng Hospital

Introduction:
The role of 24-hr urinary metabolic evaluation in urolithiasis in Singapore has not been well-studied. We investigated the prevalence of five metabolic disorders diagnosed through a single 24-hour urine sample in a selected group of patients at risk of recurrent stone formation.

Methods:
A total of 103 patients completed 24-hour urine collection while on a random diet. The collection was made before any medical therapy which could change the urinary milieu. Urinary concentrations of calcium, oxalate, citrate, uric acid, creatinine, sodium, magnesium, phosphate and potassium were measured. A spot urine pH was measured at recruitment. The prevalence of low urine volume, hypocitraturia, hyperuricosuria, hyperoxaluria and hypercalciuria were calculated.

Results:
The commonest disorder, other than low urine volume, was hypocitraturia, which was found in 56% of patients. The prevalence of low urine volume, hyperuricosuria, hyperoxaluria and hypercalciuria were 72%, 17%, 9% and 8% respectively. At least one of the five disorders could be detected in 96% of patients. Even if low urine volume was excluded, 71% of patients still had at least one of other four metabolic disorders detected.

Conclusion:
This study clearly established the usefulness of 24-hour urinary metabolic evaluation in patients at risk of recurrent stone formation. This practice should be advocated and may lead to decreased stone episodes in the long term for these high-risk patients.

Urine Biomarkers in Paediatric Kidney Disease
Mali VP, Lee KS, Yap HK, K Prabhakaran
National University Hospital

Aims:
To analyse selected biomarkers in the voided urine samples of children and compare their differences between dysplasia and surgical obstruction with reference to normal controls.

Methods:
Patients aged 0-18 years were prospectively recruited and grouped into 3 cohorts; normal kidneys (N), dysplastic kidneys (D) and kidneys with obstruction at pelvi-ureteric junction (PUJO). Voided urine was collected. The urinary concentration of epidermal growth factor (EGF) and monocyte chemoattractant peptide-1(MCP-1) concentrations were quantitated by ELISA (R&D Systems). The reproducibility was confirmed by determining minimal intra-assay and inter-assay variations of each sample. The EGF and MCP-1 concentrations of each sample were calculated in duplicate and their respective mean values were taken as the final values. The final EGF and MCP-1 concentrations of each sample were corrected against their respective creatinine concentrations quantitated by Jaffe colorimetric method (Assay Designs). The study was funded by National Kidney Foundation and institutional review board approval was obtained.

Results:
Thirty patients were recruited under the following groups: N=14, D=9 and PUJO=7. There was a trend towards decreased EGF and MCP-1 concentrations in dysplastic kidneys as compared to normal controls. The EGF concentrations were much higher in the operative urine samples taken from the renal pelvis of PUJO signifying that the increased levels detected from voided urine was originating from the PUJO kidney.
Conclusions:
Decreased levels of EGF and MCP-1 in voided urine may be useful to screen for the presence of dysplasia. In the PUJO population, postoperative trends of EGF levels in voided urine may be useful as a screening test to confirm relief of or recurrence of obstruction.
PROJECTS FUNDED BY THE VENERABLE YEN PEI-NKF RESEARCH FUND

BASIC SCIENCE

Effects Of S-Propargyl-Cysteine (SPRC) on Animal Models of Acute Kidney Injury and Chronic Renal Failure and its Underlying Mechanisms
Zhu Yi Zhun
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore

The Origin of Anti-C1q Autoantibodies – A Key Nephritogenic Factor for Proliferative Lupus Nephritis
Lu Jinhua
Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore

Functional Analysis of Mir-196a in Human Renal Cell Carcinoma
Lina Lim
Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore

CLINICAL RESEARCH

Effect of Using an Audiovisual CPR Feedback Device on Chest Compression Rate and Depth
Marcus Ong Eng Hock
Department of Emergency Medicine, Singapore General Hospital

Urinary Biomarkers in Systemic Lupus Erythematosus Patients with Active and Inactive Nephritis
Fong Kok Yong
Department of Rheumatology and Immunology, Singapore General Hospital

MR Micturating Cystourethrography: Feasibility Study using Dynamic T1-Weighted Gradient Echo Gadolinium Detection Pulse Sequence for Diagnosis of Vesicoureteric Reflux
Teh Hui Seong
Department of Diagnostic Radiology, Khoo Teck Puat Hospital, Alexandra Health

Prospective Monitoring Of Volume and Nutritional Status Using Bioimpedance Spectroscopy in Incident Peritoneal Dialysis Patients and Prospective Monitoring of Fluid Status During Episodes of Volume Overload in Prevalent Patients
Marjorie Foo
Department of Renal Medicine, Singapore General Hospital

Prevalence of Vitamin D Deficiency in Pre-Dialysis Chronic Kidney Disease Patients in Singapore
Priscilla How
Department of Pharmacy, National University of Singapore; Department of Medicine (Division of Nephrology), National University Hospital

Urinary Proteomics of Progressive Diabetic Nephropathy
Lim Su Chi
Department of Medicine and Clinical Research Unit, Khoo Teck Puat Hospital

Assessment Of Renal Disease Activity and Response in Lupus Nephritis – Comparison of Agreement in Rating by Rheumatologists and Renal Physicians
Faith Chia Li-Ann
Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital

Association of ACE D Allele with Acute Kidney Injury in Non-Chinese Patients after Cardiac Surgery
Sophia Chew
Department of Anaesthesia, Singapore General hospital

Real-Time Extracorporeal Circuit Blood Flow Measurements: Assessing Correlation with Circuit Longevity During Continuous Renal Replacement Therapy (CRRT)
Tan Han Khim
Department of Renal Medicine, Singapore General Hospital

Minimising Renal Dysfunction in Paediatric Liver Transplant Recipients with Closer Monitoring of Renal Function and Optimising Immunosuppression Using the Cylex®Immuknow™ Assay
Marion Aw
Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore

Prospective One Year Follow Up of Acute Kidney Injury in Septic Patients Admitted to Medical ICU
Mukhopadhyay Amartya
Department of Medicine, National University Hospital
### Biomarkers to Predict Tenofovir Related Renal Toxicity
Lawrence Lee Soon-U
Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore

### Clinical Prevalence and Associated Factors of Erectile Dysfunction in Uremia
P Ganesan Adaikan
Department of Obstetrics & Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore

### Mood, Cognitive, Physical Functioning and Quality Of Life in Older Adults with Chronic Kidney Disease (CKD)
Prof Ng Tze Pin
National University Health System; Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore

### Improving End-Of-Life Care in Renal Patients with a Clinical Coordinated Pathway
Alethea Yee
Department of Palliative Medicine, National Cancer Centre Singapore

### Usefulness Of Ngal as a Biochemical Marker for Acute Kidney Injury in Patients with Sepsis and Cardiac Failure
Wee Choon Peng Jeremy
Department of Emergency Medicine, Singapore General Hospital

### Augmentation of Glutathione Levels Through Oral N-Acetylcysteine Supplementation in Type 2 Diabetic Patients to Increase Resistance to Bacterial Infection
Gan Yunn Hwen
Department of Biochemistry, National University of Singapore

### Reducing Nephrotoxicity of Vancomycin: A Prospective, Randomized Study of Continuous versus Intermittent Infusion of Vancomycin
Jolene Oon Ee Ling
Department of Medicine, National University Health System

### A Multi Protocol Investigation to Study the Effects of Intra-Dialytic Exercise on Solute Removal in Hemodialysis Patients, and Compare That With Hemodiafiltration
Gade Pandu Rangaiah
Department of Chemical and Biomolecular Engineering, National University of Singapore

### BIM Genotypes in Patients with Lupus Nephritis: Associations with Lupus Pathogenesis and Treatment Response
Fong Kok Yong
Department of Rheumatology and Immunology, Singapore General Hospital

### The Clinical and Psychosocial Impact of Living Kidney Donation
Terence Kee Yi Shern
Department of Renal Medicine, Singapore General Hospital

### TRANSLATIONAL RESEARCH

#### Transcriptomal and Molecular Characterization of Tumor Associated Monocytes/Macrophages in Human Cancers
Alvin Wong
Department of Haematology Oncology, National University Health System

#### Phenotypic and Functional Analysis of CD39+ Regulatory T Cells (Tregs) in Kidney Transplant Patients, and their Correlation with Clinical Outcomes
Francisco Salcido-Ochoa
Department of Renal Medicine, Singapore General Hospital

#### The Effectiveness of Self-Management Interventions to Improve Outcomes in Established and Incident Hemodialysis Patients
Konstadina Griva
Department of Psychology, National University of Singapore; National Kidney Foundation

#### High Prevalence of Mupirocin-Resistant Staphylococci in a Dialysis Unit Where Mupirocin and Chlorhexidine are Routinely Used for Prevention of Catheter-Related Infections
Hsu Li Yang
Department of Medicine, National University Health System

#### Hydrogen Sulfide: A Novel Agent to Protect Kidney Against Hypertensive Renal Injury
Bian Jinsong
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore

#### Segmental Renal Gene Expression and Functional Characterization of Renal Drug Transporters in a Rat Model of Type II Diabetes with Progressive Nephropathy
Edmund Jon Deon Lee
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore
Surface Modification of Catheters to Inhibit Infection and Omental Wrapping in Peritoneal Dialysis
Neoh Koon Gee
Department of Chemical and Biomolecular Engineering, National University of Singapore

Portable, Non-Invasive Dry Weight Assessment and Vascular Access Monitoring Using Modern Bioimpedance Analysis For Optimal Management of Hemodialysis Patients
Victor Lee Tswen Wen
Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore

Targeted Inhibition of Signal Transducer and Activator of Transcription-3 Pathway for the Treatment of Metastatic Renal Cell Carcinoma
Gautam Sethi
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore

Activation Of Na+/K+-Atpase with a Rat Derived DR Region Specific -A New Approach to Treat Renal Ischemic Diseases
Prof Bian Jinsong
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore

Thermo-Responsive Magnetic Nanoparticles for Combined Modalities of Kidney Cancer
Lee Chee Wee
School of Applied Science, Temasek Polytechnic

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**BASIC SCIENCE**

**Effects Of S-Propargyl-Cysteine (SPRC) on Animal Models of Acute Kidney Injury and Chronic Renal Failure and Its Underlying Mechanisms**

PI: A/Prof Zhu Yi Zhun
Affiliation: Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore

**Aims/Objectives:**
The aim of this study is to investigate the protective effects of a novel compound, S-propargyl-cysteine (SPRC) on the animal models of acute kidney injury and chronic renal failure and explore its underlying mechanisms. SPRC, which was synthesized in PI's lab by reacting L-cysteine with propargyl bromide, is a structural analog of S-allylcysteine (SAC), one of the major compounds in aged garlic extract. Our lab, for the first time, demonstrated that SAC medicates cardioprotection in acute myocardial infarction via a hydrogen sulfide ($H_2S$)-mediated pathway (Chuah et al., 2007, Am J Physiol.). Compared to SAC, SPRC has showed the properties of increased affinity for cystathionine-$\gamma$-lyase (CSE), the major $H_2S$ producing enzyme. SPRC was demonstrated in PI’s lab to protect both adult rat hearts and neonatal cardiomyocytes from ischemia/hypoxia injury through anti-oxidative and anti-apoptotic mechanisms. Thus, we reasonable hypothesize that SPRC might be a protective agent in kidney injury, as several studies have indicated that SAC has renoprotective and antihypertensive effects in nephrectomized rats.

**Method:**
We set up the animal models for acute and chronic kidney failures and treated the animals with 50, 100 and 200mg/kg/day SPRC and 200mg/kg/day SAC. After collecting the blood and kidney samples, we measured the renal function and did histological and antioxidant enzyme assay.

**Results/Expected results:**
It was found that compared to SAC, SPRC treatment reduced the injury significantly in the acute and chronic kidney failure animal models, indicated by the decreased necrosis grades. SPRC could also increase the...
SOD activity and decrease the ROS production. The function of H2S pathway will be analyzed in the following studies.

**Comments:**
SPRC was found to reduce hypertension and renal damage in the acute and chronic kidney failure animal models. Our data suggested that the renoprotective effects of SPRC may be associated with the antioxidant properties. We will further address the protective mechanism of SPRC via the H2S-mediated pathway, including the measurement of H2S concentration and CSE activity in plasma and kidney as well as the CSE mRNA and protein expression.

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**The Origin Of Anti-C1q Autoantibodies – A Key Nephritogenic Factor For Proliferative Lupus Nephritis**

**PI:** A/Prof Lu Jinhua  
**Affiliation:** Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore.

**Objectives:**
Systemic lupus erythematosus (SLE) is a prototype systemic autoimmune disease characterized by the production of antinuclear autoantibodies. These antibodies preferentially deposit in the kidney glomeruli where they cause inflammatory tissue injuries through complement and leukocyte activation, a pathological condition known as lupus nephritis (LN). Recent studies showed that, besides antinuclear antibodies, anti-C1q autoantibodies are strongly associated with LN induction and flares. The main objective of this study is to determine how these pathogenic anti-C1q autoantibodies are induced in SLE patients. The hypothesis is that C1q binding to apoptotic cell-associated ligands renders these otherwise tolerised molecules immunogenic.

**Method:**
Known apoptotic cell-associated C1q ligands are purified and coated on mouse red blood cells (RBC). If a C1q ligand on RBC induced the autoantibody-reactive epitope, this C1q-ligand complex will be used to immunize mice. The induction of anti-C1q autoantibodies is determined and the autoantibody-producing B cells will be cloned to produce anti-C1q autoantibodies.

**Expected results:**
It is anticipated that calrecitin and gC1qR might induce the autoreactive epitopes on C1q as these proteins are better exposed on the cell surface upon apoptosis. Whether C1q binding to these ligands are sufficient to induce autoantibodies remain uncertain, but we expect that co-administering of danger-associated molecular patterns (e.g. HMGB1) might be sufficient to cause autoreactive B and T cell activation and therefore the production of autoantibodies. If anti-C1q autoantibodies are induced, these B cell clones will be immortalized by hybridoma generation. The produced natural anti-C1 autoantibodies will be valuable reagents for the dissection as to how these antibodies contribute to LN flare.
**Aims/Objectives:**
Renal cell carcinoma (RCC) is the most common form of kidney cancer, with 208,000 people diagnosed globally each year, with 100,000 deaths. Advanced RCC is deadly with a poor prognosis, limited treatment options and low survival rates. A new class of post-transcriptional regulators, known as microRNAs (miRs), is aberrantly expressed in kidney cancer. miRs can either suppress translation or cause mRNA degradation by cleaving mRNA. In spite of rapid progress in miR research, the molecular events which result as consequences of expression of miRs are not characterized. Many groups have investigated the functions of particular miRs in relation to the regulation of specific proteins, where the miRs are either oncogenic or suppressive by targeting their gene of interest. In particular reference to renal cancer, genetic screening of renal carcinoma has revealed a number of miR which are dysregulated. Specifically, miR196a has been reported to be dysregulated in clear cell and papillary renal cell carcinoma. A predicted target of miR196a is annexin-a1, a possible tumor suppressor, which we have worked on for a number of years.

**Method:**
In this study, we will investigate the functional roles of miR196a in renal cell carcinoma in vitro and in vivo. We will clone miR196a and will (i) test its functionality in renal cancer cell lines RCC4 and 786-O, on cell growth, proliferation, migration and chemosensitivity.

**Results/Expected results:**
We predict that miR196a will be growth enhancing, and thus may be an oncogenic miR.

**Comments:**
This project will ultimately define the functional roles of previously uncharacterized miRs, and can allow us to understand the regulation of cellular processes in the promotion of cancer.

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**Aims/Objectives:**
Effect of Using an Audiovisual CPR Feedback Device on Chest Compression Rate and Depth

**PI:** A/Prof Marcus Ong Eng Hock
**Affiliation:** Department of Emergency Medicine, Singapore General Hospital

**Method:**
209 dialysis center nurses and health care personnel were involved in either an AED course or a CPR practice session between January 2010 and December 2010. 5 cycles of 30 chest compressions were performed on a manikin without CPR prompts. After a break of at least 5 minutes the participants performed another 5 cycles with the use of real time audiovisual feedback via the ZOLL E Series defibrillator. Performance data was obtained and analyzed.

**Results:**
With feedback, there was a statistically significant improvement from 39.57% to 46.94% (p=0.009) of the participants being within the target compression depth of 4.5cm and a reduction in those below target from 16.45% to 11.05% (p 0.004). The use of feedback also produced a statistically significant improvement in achieving the target for the rate of chest compression (90-110 compressions per minute) from 41.27% to 53.49; (p<0.001). The mean depth of chest compressions was 4.85cm (SD=0.79) without audiovisual feedback and 4.91 (SD = 0.69) with feedback. For rate of chest compressions, it was 104.89 (SD = 13.74) vs 101.65 (SD = 10.21) respectively. Interestingly, the mean depth of chest compression was less in males than in females (4.61 vs 4.93cm, p = 0.011); and this trend was reversed with the use of feedback.

**Conclusion:**
We conclude that the use of feedback devices helps to improve the quality of CPR during training. However more studies involving cardiac arrest patients requiring CPR are required to determine if these devices improve survival.
Urinary Biomarkers in Systemic Lupus Erythematosus Patients with Active and Inactive Nephritis

PI: Prof Fong Kok Yong
Affiliation: Department of Rheumatology and Immunology, Singapore General Hospital

Aims/Objectives:
It is hypothesized that there are significant differences in the urinary concentrations of specific chemokines and cytokines between active and inactive lupus nephritis disease activity. This proposal aims to determine the urinary concentrations of relevant chemokines and cytokines (MCP-1, VCAM-1, CXCL16, TNFR-1, p-selectin, TWEAK, Lipocalin, Adiponectin) in active and inactive lupus nephritis patients to determine the urinary cytokines profile in active lupus nephritis patients.

Method:
The study assayed 8 chemokines and cytokines concentrations in the urine of patients with or without lupus nephritis using ELISAs. Clinical data were obtained through chart review. The unpaired T test were used for comparison of cross sectional data (e.g. active vs inactive groups) with a p value of < 0.05 considered significant. The correlation of urinary biomarkers with clinical features and MEX-SLEDAI in a prospective manner will determine their clinical utility.

Results/Expected results:
One hundred and one patient-samples were included in the study, comprising 12 with active lupus nephritis (LN), 28 with inactive disease but have history of LN and 61 with inactive disease and have no history of LN. The urinary cytokines results are as shown below.

Comments:
There was no statistical significance when results are compared between groups though there is a trend of elevated MCP-1, TNFR-1, VCAM-1 and Lipocalin concentrations.

Project No : 2008-07-21
Start Date : Jan 09
Expected End Date : Jun 12
Introduction:
To evaluate the feasibility of using MR as an imaging technique for detection of vesico-ureteric reflux (VUR).

Method:
Study received institutional review board approval, and written informed consent was obtained from all patients. A total of 6 patients (age range, 24–35 years, 5 female and 1 male) had VUR detected on fluoroscopic voiding cystourethrography (VCUG) and were recruited into the study. One patient was excluded when MR imaging revealed an intra-uterine pregnancy. These yielded 10 kidney-ureter units (Five were normal, and 5 had VUR detected on VCUG. MR cystography was performed using a 1.5-T MR scanner. Patients were catheterized and gadolinium-enhanced saline was infused into the bladder. A gadolinium detection pulse sequence technique was used. This allowed real-time visualization of images in fluoroscopy window on the console as they were being obtained, was used to detect VUR. When the contrast was seen refluxing into the urinary tract, a three-dimensional (3D) MR gradient echoes sequence was triggered. The result was compared to VCUG.

Results:
Findings at MR cystography and VCUG were concordant in 8 (Four normal; 4 with VUR) of the 10 kidney-ureter units (80%). There was discordance between the two techniques in the remaining 2 kidney ureter units; one had VUR detected on MR cystography only, and the other on VCUG alone. There was also good concordance for the extent of hydronephrosis.

Conclusion:
MR cystography does not involve ionizing radiation and give excellent anatomical depiction of the urinary system. This novel technique holds potential as a screening examination for diagnosing VUR.

Aims/Objectives:
Fluid overload and malnutrition are independent predictors of mortality and morbidity in peritoneal dialysis. The aim is to prospectively monitor fluid and nutritional status in peritoneal dialysis patients using bioimpedance spectroscopy (BIS) monitoring to compare with clinical assessment and correlating it to clinical outcome.

Method:
Fluid overload:
This will be investigated in incident and prevalent PD patients.
Incident patients:
BCM will be performed at designated time points of 0, 1.5, 3, 6, 9 and 12 months during routine visits and will be compared to standard method of assessment by physician i.e. physical examination, blood pressure.
Prevalent patients:
Fluid overload patients will be recruited when they are admitted. They will have daily BCM monitoring, physician will treat patient according to the standard way of assessment and management. The reading of daily BCM will not be made known to the physician and will not impact on patient management and outcome. The patient will have a total of 10-14 days reading till fluid balance is achieved.

Nutritional monitoring:
Incident patients:
At the start of dialysis, patient’s body composition will be measured using BCM. Follow up assessment and advice will be given by diettian. Patient will be assessed over a 12 month period with BCM measurements taken at months 0,1,5,6,8,9 and 12 coinciding with dietary assessment. Dietary assessment will be in the form of subjective global assessment (SGA) at each visit. Serum albumin, potassium and haemoglobin will also be analysed.
Results/Expected results:
Study to start in August 2011

Comments:
Assessment of fluid balance in a dialysis patient is subjective and accuracy variable based on experience of the assessor. BIS readings of fluid and nutritional status if correlates closely with clinical outcome may help to enable more objective, accurate and reproducible assessment of such parameters.

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Aims/Objectives:
Vitamin D deficiency is common in chronic kidney disease (CKD) patients and is associated with elevated parathyroid hormone (PTH) concentration and mineral and bone disorder (MBD). There is also increasing evidence to show that vitamin D deficiency and MBD increase cardiovascular morbidity and mortality in CKD patients. There is a need for early identification and correction of vitamin D insufficiency or deficiency in CKD patients to prevent its long-term complications. However, the vitamin D status of CKD patients in Singapore has not been well described. As such, the purpose of this study is to assess the vitamin D status and determine the prevalence of vitamin D deficiency in predialysis stage 4 and 5 CKD patients in a tertiary academic teaching hospital in Singapore, and its association with parameters of MBD.

Methods:
Patients with stage 4 or 5 CKD who are not yet on dialysis from the outpatient renal clinic at the National University Hospital (NUH) will be recruited into this study. A one-time blood sample will be collected from these patients to determine their serum 25(OH)D, creatinine, phosphorus, calcium, albumin and i-PTH concentrations. These parameters will be compared between the stage 4 and 5 predialysis CKD patients. The patients will also be divided into 3 groups based on their 25(OH)D levels: <12 nmol/L (severe vitamin D deficiency), 12-40 nmol/L (mild-moderate vitamin D deficiency) and 40-75 nmol/L (vitamin D insufficiency), and any association with the laboratory parameters will be determined and compared among the three groups.

Comments:
This research project is currently ongoing and results are not yet available.

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Urinary Proteomics of Progressive Diabetic Nephropathy

PI: Dr Lim Su Chi
Affiliation: Department of Medicine and Clinical Research Unit, Khoo Teck Puat Hospital

Introduction:
Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in Singapore. The rate of progression of DN is highly variable. The risk factors predictive of DN progression are incompletely understood. Understanding these risk factors may help to shed light on mechanisms underlying DN progression. These risk factors may also serve as clinical tools to risk stratify subjects susceptible to renal progression. Hence, there is a need to search for biomarker(s) associated with progression of DN. Urinary proteomes pattern is an intuitively attractive biomarker for DN. We hypothesize that the urinary proteomes in progressive DN differs systematically from stable DN.

Methods:
In this preliminary study, we studied 2 groups (n=5) of males with type 2 diabetes: Group 1: Progressive DN (Δ serum creatinine≥50%) vs Group 2: Stable DN (Δ serum creatinine≤10%) in a two year prospective observation cohort study. Subjects were carefully matched for potential confounders like gender, age, ethnicity and smoking status. We extracted protein from urine using ethanol precipitation. The samples were subjected to de-convolution by 2D Clean Up and quantification by 2D Quant kit before pooled analysis. Using 2 dimensional fluorescence differential gel electrophoresis (2D DIGE), which employs the use of CyDye and differential labeling to allow 2 samples to be simultaneously run on the same gel. The sample will be separated firstly by electrical charges, then by molecular weight. Different wavelengths specific to the dye will be used to scan the gel, giving different sample images for the same gel, which allows the algorithm to compare and contrast global proteome profile and individual protein concentrations. Proteins differentially up-regulated in either groups will be identified by Matrix Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry. The results obtained will be validated by western blot after identification.

Results:
7 differentially up-regulated spots were sent for mass spectrometry and identified. Our preliminary results suggested that the highest up-regulated protein in Stable DN was Basement Membrane Specific Heparan Sulfate Proteoglycan (HSPG) Core Protein with a fold change of 5.78 (p=0.073). The highest up-regulated protein in Progressive DN was identified as Fibrinogen Beta Chain with a fold change of 2.71 (p=0.098).

Conclusions:
Up-regulation of Basement Membrane HSPG Core Protein, which controls the selective permeability of the glomerular basement membrane, could play a protective role in DN by controlling entry and exit of certain molecules. High fibrinogen has been associated with elevated levels of albuminuria, and could be an indication of additional cardiovascular risk with altered clotting mechanisms in diabetics. We plan to validate our preliminary results using western blot experiments.

Project No : 2010-01-01
Start Date   : Jul 10
Expected End Date   : Jun 13
Assessment of Renal Disease Activity and Response in Lupus Nephritis – Comparison of Agreement in Rating by Rheumatologists and Renal Physicians

PI: Dr Faith Chia Li-Ann
Affiliation: Department of Rheumatology, Allergy and Immunology. Tan Tock Seng Hospital

Aims/Objectives:
Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that occurs predominantly in women in the reproductive age group and damages tissues and organs as a result of inflammation from immune complex deposition.

Lupus nephritis (LN) is common amongst our patients with SLE and is a major contributor to the increased morbidity and mortality of these patients. Assessment of activity and response of LN is currently subjective, but there have been newly formulated measures that may help to improve management in LN. This project aims to

1. Compare the correlation of SLICC clinician consensus disease activity indices with SLAM and SLEDAI using case scenarios presented to rheumatologists and renal physicians
2. Retrospectively assess past renal disease activity using the above measures in LN patients to determine effect on current disease activity and renal function
3. Prospectively assess activity and response using these measures in LN patients when therapy is initiated or changed

Method:
Aim 1: Selected case scenarios will be presented to rheumatologists and renal physicians to score with a rating of 0 (no activity) to 3 (severe activity). These scenarios will include clinical data, medications, renal function tests, urinalysis and kidney biopsy results (if available). 160 scenarios have been created through retrospective review of patients case sheets and are being uploaded to a web-based survey platform at present.

Aim 2: LN patients with a 10 year follow up will have their disease activity scored yearly using SLICC renal index, SLAM renal and SLEDAI renal indices. These will be correlated with the last measured renal disease activity and function.

Aim 3: Paired case scenarios with patient’s data 6 months apart after change in therapy will be presented to rheumatologists and renal physicians to be scored and comparison of agreement between the rating and the calculated scores obtained using established indices will be carried out using the chance-adjusted measure of agreement.

Results/Expected results:
We hope that the information obtained will improve the management of LN by allowing greater uniformity of assessment of activity and response of the disease. It may also allow us to better understand the effect of renal disease activity on the renal outcome in LN, and evaluate the response of therapy more accurately.

Comments:
This study is currently in progress and no results are available at this time.

Project No : 2010-01-02
Start Date : Jul 10
Expected End Date : Jun 13
Aims/Objectives: Postoperative acute kidney injury (AKI) after cardiac surgery is a frequent, serious, multifactorial complication with interpatient variability predicted poorly by preoperative clinical and procedural markers. In our preliminary study, we noted that 56% of patients presenting for cardiac surgery developed AKI and apart from common known risk factors, ethnicity was independently associated with the risk of AKI, with Indians and Malays having a higher risk of developing AKI after cardiac surgery. The ACE D allele has been implicated in kidney injury in African Americans and we postulate that the D allele is associated with the increased incidence of AKI in the non-Chinese after cardiac surgery.

Method: 991 consenting patients who underwent cardiac surgery were studied. Clinical covariates were recorded. The primary outcome was AKI, defined as a 25% or greater increase in preoperative to maximum postoperative serum creatinine level within 3 days after surgery. DNA was isolated from preoperative blood and PCR was used to detect the deletion (D) allele and insertion (I) allele of the ACE gene.

Results/Expected results: 49.5% patients have a creatinine rise of 25% post cardiac surgery. Out of 491 patients who develop AKI, 60.9% carry the D allele. A race effect was seen with Indians and Malays having a higher risk of developing AKI compared to Chinese (p≤0.002). In addition, non-Chinese with the D allele have a marginally higher risk compared to Chinese of developing AKI (OR 1.037, CI 0.949-1.134)

Comments: Indians and Malays who have the D allele have a higher risk of developing AKI compared to Chinese. The ACE D allele is linked to increased renal vasoconstriction and this susceptibility in the non-Chinese may be unmasked during cardiac surgery which is associated with problems of atheroembolism and ischaemia-reperfusion injury. This is the first local report linking the D allele in the non-Chinese with development of AKI after cardiac surgery.

Real-Time Extracorporeal Circuit Blood Flow Measurements: Assessing Correlation with Circuit Longevity During Continuous Renal Replacement Therapy (CRRT)

**PI:** A/Prof Tan Han Khim  
**Affiliation:** Department of Renal Medicine, Singapore General Hospital

**Aims/Objectives:**
Real-time pressure measurements is an industry-widely practiced technique adopted on many if not all haemodialysis (HD) and continuous renal replacement therapy (CRRT) machines. However, a study published by this author suggested that pressures do not correlate with circuit longevity much less have any circuit prognostic value. The hypothesis for this proposed NKF-funded research is that flow and velocity measurements of blood and fluids within the CRRT extracorporeal circuit (EC) yields better correlation with circuit patency and longevity, providing a tool for timely circuit intervention and/or change and avert unplanned CRRT down-time. This has been shown to reduce overall delivered dialytic dose and efficacy.

**Method:**
A total of 100 CRRT circuits will be studied in a prospective observational study. Transonic flow probes will be used to measure blood, effluent and replacement flow characteristics, data-logged and analysed for correlation with ultimate spontaneous circuit clotting. Simultaneous pressure measurements will also be obtained.

**Results/Expected results:**
Study is currently pending IRB approval

**Comments:**
If the hypothesis were true, this study could encourage further studies into flow-patency correlation with the potential for a paradigm shift in industrial design of such devices.

**References:**

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Minimising Renal Dysfunction in Paediatric Liver Transplant Recipients with Closer Monitoring of Renal Function and Optimising Immunosuppression Using The Cylex®Immuknow™ Assay

**PI:** A/Prof Marion Aw  
**Affiliation:** Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore

**Introduction:**
Whilst liver transplantation is life-saving for children with end-stage chronic liver disease or acute liver failure, a number of complications are seen in survivors secondary to the long-term use of immunosuppression. These include nephrotoxicity, viral infections and lymphoproliferative disease. The ability to measure adequacy of immunosuppression in individual patients would allow physicians to tailor immunosuppression appropriately, thus avoiding over immunosuppression and the increased risk of infection, or under-immunosuppression and the risk of allograft rejection. At the same time, being able to monitor renal function in an accurate and timely manner, would contribute to preserving renal function in the long term.

**The aims of the study are to:**
1. To determine if the Cylex®Immuknow™ assay would be useful in helping assess adequacy of immunosuppression, thereby allowing appropriate decrease in immunosuppressive drug therapy.
2. To correlate the degree of immunosuppression with the incidence of graft dysfunction, as well as the incidence of post-transplant infections and other complications of over-immunosuppression.
3. To determine if serum Cystatin C would be useful as a regular screen for renal dysfunction in our paediatric liver transplant recipients.

**Method:**
All paediatric liver transplant recipients on follow-up at the University Children's Medical Institute would be invited to participate. Blood will be obtained during out-patient or in-patient clinical visits. This will be sent for the serum Cystatin C measurement as well as the Cylex®Immuknow™ assay. The immunosuppressive regimen the recipients are receiving will be recorded, as well as relevant clinical history at the time of review. The clinical course of the patient, including incidence of acute allograft rejection, and incidence of infections would be recorded, and these events correlated with results obtained from the Cylex®Immuknow™ assay. Serum cystatin C results will be correlated with serum creatinine results, the calculated GFR based on the Schwartz's formula, as well as the
creatinine clearance calculated from the annual 24-hour urine collection. Ethics approval has been obtained from the Institutional Review Board and informed consent will be obtained prior to inclusion in the study.

**Results:**
Nil yet.
The study was approved in March 2011. Patient recruitment has just started.

**Aims/Objectives:**
Acute kidney injury (AKI) is common in Intensive Care Unit (ICU) with reported incidences varying from 1.5% to 24%. AKI is independently associated with high mortality especially in patients requiring renal replacement treatment (RRT). Among survivors up to 1/3 patients may require long term dialysis.

Given the wide variability in the definition of AKI in clinical studies, to foster uniformity in both research and clinical practice, an expert group (Acute Dialysis Quality Initiative [ADQI]) developed a new classification of AKI (Risk, Injury, Failure, Loss, and End-stage renal disease [RIFLE]). This was later modified by the Acute Kidney Injury Network (AKIN).

Long term outcome studies in patients with AKI in critically ill patients show increased mortality in this subgroup even in the post hospitalization period. We are recruiting patients in a prospective observational study of critically ill patients admitted to the Medical ICU with sepsis and AKI. Data is collected up to a period of 12 months following discharge or death, whichever is earlier.

**Method:**
- Centre: Medical ICU, Medical High Dependency unit, National University Hospital, Singapore.
- Study type: Prospective, Observational
- Patient Population: Adult (>18 years) patients with sepsis who are admitted to MICU/MHD during study period and who develop AKI by RIFLE and AKIN criteria.
- Duration: All enrolled patients are to be followed up till 12 months post enrollment or death, whichever is earlier.
- Evaluations: Various demographic, clinical and physiological parameters are noted on admission and at various regular intervals as per protocol. APACHE II will be recorded to assess severity of illness.
- Follow-up: Patients will be followed up by renal physicians. No additional blood tests or follow up will be performed apart from standard of care for the purpose of the study.
Results:
50 patients (mean age 63 years (± SD 16.5, male 58%) have been recruited in this ongoing study between Nov 2010 - Aug 2011. Mean Apache II score is 26.7 (± SD 7.64). 28%, 34% and 38% of patients respectively were in class R (risk), I (injury), F (failure) by RIFLE Criteria and as per AKIN staging, 38%, 20% and 42% patients were in stage 1, 2 and 3 respectively on admission. Out of 50 patients recruited, 21 (42%) patients received some form of RRT. Mortality in ICU and hospital was 16% and 30% respectively. 2 patients died after discharge from hospital before the 3 month follow up. Of the patients discharged from hospital 23 patients are on follow up with renal physicians and none required RRT after discharge from ICU while 4 patients refused to follow up after discharge from hospital. At one month follow up all discharged patients were free from RRT and none had required readmissions. The average creatinine at 1 month is 104 µmol/l (± SD 41.35).

Comments:
Acute kidney injury in critically ill septic patients portends a grim prognosis as evidenced in our ongoing study by the high mortality even in post ICU period.

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Biomarkers to Predict Tenofovir Related Renal Toxicity

PI: Dr Lawrence Lee Soon-U
Affiliation: Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore

Aims/Objectives:
Tenofovir (TFV) is increasingly being prescribed as first-line therapy for HIV and hepatitis B infection. TFV may cause significant renal toxicity through damage to renal tubular cells, possibly by oxidative damage to renal tubular cell mitochondria. This can lead to Fanconi’s syndrome and renal failure. The widespread use of lifelong TFV will increase the burden of renal toxicity in Singapore and globally.

The aim of this research program is to prevent TFV-related renal toxicity by (i) exploring toxicity mechanisms, (ii) discovering early tubular damage biomarkers and (iii) finding predictors of TFV toxicity.

Methods:
The clinical study will recruit HIV patients from existing cohorts. Blood and urine will be assayed for established and novel biomarkers of renal toxicity and compared between the TFV-toxic group and controls without TFV toxicity. We will also correlate these markers with potential predictors of renal toxicity such as genetic polymorphisms and transporter function.

In vitro investigations: Primary human renal proximal tubular epithelial cells (RPET) (Lonza) were maintained in growth medium containing 10% FCS, 5% CO₂ at 37°C. Growth medium was supplemented with three concentrations of tenofovir (T₁ 1.2 x 10⁵ nM; T₂ 1.2 x 10⁴ nM; T₃ 1.2 x 10³ nM), cidofovir (C₁ 3 x 10⁶ nM; C₂ 3 x 10⁵ nM; C₃ 3 x 10⁴ nM) and adefovir (A₁ 3.2 x 10⁷ nM; A₂ 3.2 x 10⁶ nM; A₃ 3.2 x 10⁵ nM). T₃, C₃ and A₃ concentrations represent physiological Cmax (corrected for protein binding). RPET cultured in growth medium without FCS served as negative control. The RPET were treated for 24 and 144 hours.

After fixation, RPETs were stained with ponceau red, washed and destained. The plate was then read at 540 nm cell number per well determined using a standard curve of cell number vs. OD₅₆₀. The CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega) was used for determining cell viability.
Results/Expected results: Clinical program: We expect to discover biomarkers of renal toxicity from patient samples and from the products of the kidney cells exposed to TFV \textit{in vitro}. These biomarkers can then be validated and then applied prospectively.

Preliminary \textit{in vitro} results:

![Renal Proximal Tubular Cell Replication](image)

Preliminary analysis showed that the various concentrations of TFV significantly impaired cellular replicative capacity in the short term; there was cell proliferative recovery after 144 hours exposure. The cell viability experiment results showed similar recovery.

Physiological concentrations of cidofovir resulted in a similar reduction in cell proliferation in the short term with recovery over 144 hours. There was significant, irrecoverable impairment of cell replication and viability at higher cidofovir concentrations.

Physiological concentrations of adefovir resulted in no change to cell replication or viability. Higher concentrations of adefovir led to significant but reversible reduction in cellular replication.

Comments:
These results suggest that in our 2D \textit{in vitro} model, physiological concentrations of tenofovir, cidofovir and adefovir do not impair RPET cellular replication and viability in the medium term (144 hours). Further work is underway to confirm these results in a polarised 3D-cell-culture model and to further understand mechanisms of mitochondrial damage after exposure to these drugs.

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<td>Expected End Date</td>
<td>Dec 13</td>
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Aims/Objectives: Erectile dysfunction (ED) is one of the most prevalent medical conditions among uremia patients and it has a strong negative effect on a couple’s quality of life. In Singapore, where there is considerable number of patients undergoing chronic dialysis therapy, limited epidemiologic and community data exists on the prevalence, severity and clinical correlates of ED in these people.

Method: This clinical study is aimed at assessing the prevalence of sexual dysfunction and the associated risk factors among male uremia patients. Specifically, the project is proposed to evaluate using a validated questionnaire, the parameters of sexual function of the uremia patients and identify the clinical correlates predisposing to derangement. The study subjects will comprise 400-600 men aged 21 - 65 years undergoing dialysis therapy and on management at the National University Hospital, Singapore.

Results/Expected results: This project is expected to provide important population data for extrapolating the correlation of sexual dysfunction and will help to delineate the risk factors of sexual dysfunction among uremia patients in Singapore. It is also hoped that this study will contribute to definite prophylactic and/or management options for improvement of male reproductive health among these patients.

Comments: Life expectancy of patients with uremia has been extended as a result of improvements in dialysis therapy. This accomplishment has led to a new appreciation of problems, previously ignored or not adequately addressed, that affect the well-being of patients with uremia. As a noteworthy example, sexual dysfunction is a widely prevalent and important medical concern with strong negative impact on the couple’s quality of life. Findings from this study will provide the scientific basis for the prophylactic and/or management considerations to moderate the problem of ED in uremia.
Mood, Cognitive, Physical Functioning and Quality Of Life in Older Adults with Chronic Kidney Disease (CKD)

**PI:** A/Prof Ng Tze Pin  
**Affiliation:** National University Health System; Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore

**Aims/Objectives:**  
Poor mood, cognitive and physical functionings are highly prevalent in patients with end-stage renal disease and strongly impact health care and social services. Few longitudinal studies have investigated the impact of mood, cognitive and physical functional impairments on quality of life, hospitalization and mortality outcomes, and the risk factors associated with their presence in persons with dialysis-free and early stage chronic kidney disease (CKD)  
The objectives in this prospective 1-year prospective follow up study of community-living older persons with stage 3 to 5 CKD are:

1. To assess mood, cognitive and physical functioning in older adults with CKD.  
2. To investigate the effect of mood, cognitive and physical functioning on quality of life, mortality and hospitalization.  
3. To investigate risk factors of depression and cognitive impairment, particularly the role of chronic inflammation in influencing mood, cognitive and physical functional impairment and CKD outcomes.

**Methods:**  
This is a prospective cohort study with 1 year follow up of older persons identified with stage 3 to 5 chronic kidney disease (stage 3 to 5; eGFR <60 ml/min/1.73 m²) from the community. Individuals with chronic kidney disease (CKD) are identified from among 3,000 participants in the ongoing Singapore Longitudinal Aging Study (SLAS) cohort who are currently being recruited from South Central Region.  

Data on measures of renal, mood, cognitive and physical functions are collected at baseline and 12 months, together with other measures at baseline including demographic, clinical, nutritional and inflammatory biomarkers (high-sensitivity C-reactive protein, inyterleukin-6, TNF-alpha). Depression is assessed by Geriatric Depression scale (GDS), cognitive performance a comprehensive neuropsychological battery of 9 tests evaluating global cognition (Mini Mental State Examination) and specific cognitive domains including language, memory and executive function.

**Preliminary and Expected results:**  
The baseline recruitment is ongoing. So far we have screened a total of 1487 subjects and identified 136 individuals with CKD (9.1%), comprising 124 (8.3%) with Stage 3 CKD, and 9 (1%) with 4 and 3 (0.2%) with Stage 5 CKD. Among the subjects with CKD, there were 5 (3.7%) with depressive symptoms (GDS≥5) and 28 (21%) with cognitive impairment.  

From analysis of prospective data, we expect that depressive symptoms, cognitive impairment and functional disability each will be prospectively associated with poorer quality of life, greater risk of mortality and hospitalization. Moreover, we also anticipate that inflammatory biomarkers will contribute to depressive symptoms, cognitive impairment and poor CKD outcomes among CKD patients prior to dialysis therapy.

**Comments:**  
Studying older adults with early stage CKD should help to elucidate potential biomedical and psychosocial interventions to reduce adverse outcomes such as kidney failure and ESRD requiring dialysis, decreased quality of life, hospitalizations and mortality. If supported by the findings from this research, possible early interventions may include screening, assessment and treatment of depression, cognitive and physical functional impairment in individuals with CKD.
Improving End-Of-Life Care in Renal Patients with a Clinical Coordinated Pathway

PI: Dr Alethea Yee
Affiliation: Department of Palliative Medicine, National Cancer Centre Singapore

Aims/Objectives:
In 2009, there were 17,054 recorded deaths in Singapore, of which 9,434 (55.3%) occurred in public sector hospitals. Singapore General Hospital (SGH) is the largest tertiary public sector hospital in Singapore with almost 1,500 beds. About 11.3% of all deaths in SGH were accounted for by renal patients, constituting the third highest mortality group in the hospital in the year 2009. To date, there is scant data regarding the quality of end-of-life care received by patients in Singapore. The Liverpool Care Pathway for the Dying Patient (LCP) has been recognized as the gold standard for end-of-life care for dying patients in UK. In 2008, the National Liverpool Care Pathway Renal Steering Group published guidelines recommending appropriate drug prescribing to control symptoms at end-of-life. These recommendations were subsequently incorporated in the renal-LCP. In this study, we aim to determine if adoption of a locally-adapted renal LCP in a renal ward in SGH translated to better end-of-life care for renal patients.

Method:
The renal-LCP was adapted for local use and implemented as a pilot project on a renal ward. A baseline review of 20 consecutive death records was performed followed by implementation of the modified renal-LCP. Post-implementation audit of 20 consecutive patients on the adapted LCP was done, comparing common end-of-life symptoms and other outcome measures.

Results/Expected results:
(Preliminary results, recruitment ongoing) Of the 5 types of common end-of-life symptoms studied in this pilot project (pain, nausea/vomiting, restlessness, respiratory secretions and dyspnoea), there were only 2 uncontrolled symptom at death in the post-implementation group compared to 11 uncontrolled symptoms in the pre-implementation group. There was a 10 to 32% increase in prescription of breakthrough medications for symptom control in the post-implementation group, translating to better symptom care. Inappropriate monitoring of clinical parameters was discontinued in 50% patients in the post-implementation group compared to 25% in the pre-implementation group. The documentation of resuscitation status of the patient was improved, achieving full documentation in the post-implementation group.

Comments:
The preliminary results show an improvement in symptom control and end-of-life care in renal patients on a clinical coordinated pathway.

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Usefulness of NGAL as a Biochemical Marker for Acute Kidney Injury in Patients with Sepsis and Cardiac Failure

**PI:** Dr Wee Choon Peng Jeremy  
**Affiliation:** Department of Emergency Medicine, Singapore General Hospital

**Aims/Objectives:**  
Determine whether serum NGAL is useful in predicting acute kidney injury in renal impaired patients with sepsis or cardiac failure presenting to Emergency Department (ED)

**Method:**  
Patients presenting to the Emergency department meeting the inclusion criteria will be recruited in the trial. Remaining serum samples from routine blood investigations obtained will be used for NGAL levels. Subsequently outpatient follow up records or notes will be traced and any need for renal replacement therapy at 3 months post admission will be noted. The renal replacement registry will be screened for any study patients requiring renal replacement therapy within 3 months post discharge.

**Results/Expected results:**  
The study is pending IRB approval and has not commenced.

Augmentation of Glutathione Levels Through Oral N-Acetylcysteine Supplementation in Type 2 Diabetic Patients to Increase Resistance to Bacterial Infection

**PI:** A/Prof Gan Yunn Hwen  
**Affiliation:** Department of Biochemistry, National University of Singapore

**Aims/Objectives:**  
Type 2 diabetes mellitus has reached epidemic proportion globally and 10% of adults in Singapore are diabetic. Diabetes is frequently accompanied by many devastating complications including the development of diabetic nephropathy that greatly reduce life expectancy and adversely affect the quality of life. Asians are also at higher risk of developing chronic kidney disease (CKD) as a result of diabetes. These patients are at increased risk of developing infections compared to diabetics without CKD. However, diabetic patients with poor glycemic control are generally more susceptible to certain infections. Melioidosis is an infectious disease where Type 2 diabetes presents as a very strong risk factor. We have previously found that immune cells from diabetic individuals with poor glycemic control were defective in controlling intracellular bacterial growth due to defective IL-12 and IFNg production. Furthermore, an altered redox balance resulting in low intracellular glutathione (GSH) to glutathione disulfide couple (GSSG) in diabetic cells is the cause for the low IL-12 production. The GSH to GSSG ratio is directly correlated with the concentration of glycated haemoglobin (HbA1c), an indication of glycemic control in diabetics. Exogenous addition of GSH or N-acetylcysteine (NAC), a precursor to the synthesis of GSH, could restore IL-12 and the microbicidal activity of monocytes and macrophages to diabetic cells. However, we have yet to determine the signaling pathways that are modulated by GSH involved in triggering IL-12 production. We believe that GSH is modulating a signaling step that is defective in the diabetic cells due to severe oxidative stress. Thus, our aim in this proposal is to determine whether oral supplementation with NAC in diabetic patients, an anti-oxidant widely used as a supplement and documented to be safe and well-tolerated, could help improve resistance to infection and improve disease outcome when their cells are examined ex vivo and to determine the mechanism involved. The long term goal would be to develop oral NAC supplementation as an adjunctive and preventive therapy for diabetic patients, particularly for those who are at increased risk of infection such as the ones with CKD.
Method:
1. Determine whether 6-week oral supplementation with N-acetylcysteine in Type 2 diabetic patients with poor glycemic control will improve resistance to bacterial infection by isolating blood from patients before and after supplementation and testing their white blood cells in vitro.
2. Determine the mechanism of how increased concentrations of glutathione modulate the signaling pathway leading to enhanced IL-12 production and whether this mechanism is defective in cells from diabetic patients.

Expected results:
We hope to see that after 6 weeks of daily oral NAC supplementation, the resistance profile of white blood cells from poorly controlled diabetic patients show increased IL-12 production and improve resistance to infection. We will also identify the mechanism of how GSH regulate IL-12 and improve resistance to infection.

Comments:
We believe that the data generated from this proposal will form the basis for the development of the long term goal and inform the study design involving patient trials in the future.

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Reducing Nephrotoxicity of Vancomycin: A Prospective, Randomized Study of Continuous Versus Intermittent Infusion of Vancomycin

**PI:** Dr Jolene Oon Ee Ling  
**Affiliation:** Department of Medicine, National University Health System

**Introduction:**
*Staphylococcus* spp. is the leading cause of surgical site, skin / skin structure and nosocomial bloodstream infections worldwide. In view of its spectrum of activity and cost, vancomycin is the most common agent used for methicillin-resistant *S. aureus* (MRSA) infections in Singapore. Unfortunately, approximately 1 in 5 patients on long-term vancomycin therapy develops nephrotoxicity. In Singapore, there is an increasing trend of treating patients who respond favourably to initial antimicrobial therapy in the outpatient setting. Based on our preliminary data, administering vancomycin via continuous infusion in the outpatient setting is associated with later onset of nephrotoxicity.

**Method:**
This study is a prospective, multi-centre, randomized, controlled study. Two hundred and twenty patients requiring a prolonged course of vancomycin will be enrolled from two hospitals in Singapore over a period of 2-3 years. Eligible patients are those with stable and normal baseline renal function requiring at least 10 days vancomycin for documented infections. The duration of treatment including hospital and OPAT will be determined by the clinician and this typically is 4-6 weeks, or as clinically indicated. Patients will be involved in this study as per duration of treatment prescribed to them by the Infectious Disease physician. Patients will be monitored and assessed daily by study coordinators for signs and symptoms of potential adverse events and complications of IV therapy. Serum creatinine, electrolytes, white blood cell count, liver function test, serum vancomycin concentrations and markers of renal toxicity (urine and serum NGAL, serum and urine cystatin C and urine albumin to creatinine ratio) will be performed weekly or more frequently if clinically indicated. Cultures will be repeated according to clinical need.

**Results:**
The primary outcome will be nephrotoxicity, as defined by the Acute Kidney Injury Network (AKIN) criteria. The secondary outcome will be the...
sensitivity and specificity of other markers (serum and urine cystatin C, serum and urine NGAL) in detecting early nephrotoxicity. Clinical and microbiological outcomes will be gathered to allow us to link these to the toxicity information.

**Conclusion:**
We will test the hypothesis that vancomycin administered by continuous infusion is associated with a later onset and reduced incidence of nephrotoxicity without reduction in clinical efficacy. It is possible that this study will cause clinicians to reconsider the mode by which this commonly used antibiotic is administered.

**A Multi protocol Investigation to Study the Effects of Intra-Dialytic Exercise on Solute Removal in Hemodialysis Patients, and Compare that with Hemodiafiltration**

**PI:** Prof Gade Pandu Rangaiah  
**Affiliation:** Department of Chemical and Biomolecular Engineering, National University of Singapore

**Background:**
Decades of dialysis treatment have saved the lives of patients with end stage renal failure. However, patients’ mortality and morbidity are still high (in some instances, worse than patients with stage 4 cancer patients) even with better designs of dialysis apparatus and treatment protocols. Hemodiafiltration (HDF) is one such protocol which integrates convective clearance along with diffusive clearance, and has been suggested as a better dialysis modality because of its ability to clear higher molecular weight toxins (randomized clinical trials in progress). However, be it HD or HDF, the toxin removal is primarily restricted by inter-compartmental resistance due to cellular membrane or capillary endothelium (Ward et al., 2006). In another scenario, it has been postulated – intra-dialytic exercise reduces this resistance by opening up membrane pores and also by dilating the inter-compartmental membrane (Maheshwari et al., 2011).

**Aims/Objectives:**
The specific aims of proposed study are,
1. Study the improvement in toxin removal due to intra-dialytic exercise.
2. Investigate the mechanism of increased toxin removal during intra-dialytic exercise using toxin kinetic modeling for urea and β2-microglobulin.
3. Compare the quantum of toxin removal during conventional hemodialysis (CHD) with exercise regimen and stand-alone hemodiafiltration (HDF).

**Method:**
A comprehensive representation of patient physiology, the diffusion-adjusted regional blood flow model has been developed to explain toxin kinetics (Maheshwari et al., 2011). In the proposed clinical research, the developed solute clearance model will be employed. A total of 15 patients will undergo three different dialysis protocols (CHD, CHD + exercise regimen, and HDF). The proposed study is open label, self-controlled (within-subject design), single center trial, randomized dialysis sessions, efficacy study. During each dialysis protocol, 10 blood samples, 4 mL each,
will be collected to study the urea and β₂-microglobulin kinetics. To study the efficacy of each dialysis protocol, total spent dialysate will also be collected, and analyzed for total removed toxin mass. Additionally, the toxin rebound will be calculated after 2 hours of dialysis. The conventional dialysis session will be treated as control for each patient. Mode of exercise will be via active cycling movement. Two bouts of exercise (each lasting 20 mins), first during 90-120 min and second during 150-180 min of dialysis session will be given. HDF will be performed in post-dilution in mode with 18L of effluent fluid volume.

**Expected results:**
Exercise to lower extremities will not only increase the cardiac output, but also decrease the inter-compartmental resistance. Decrease in major mass transfer barrier will result in increased toxin movement from remote inaccessible compartments to vascular compartments, and thus toxins will be swept away by increased blood flow to lower extremities. This will result in increased toxin removal. Additionally, toxin removal by intra-dialytic exercise regimen is anticipated to be on par with stand-alone HDF.

**Comments:**
The validity of developed solute clearance model can significantly improve the dialysis care, first by including β₂-microglobulin as a surrogate marker toxin, and second by incorporating intra-dialytic exercise into routine dialysis care. The developed model can also suggest the optimal time of implementation of exercise such that it will result in maximum toxin removal with minimum fatigue to patient.

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**BIM Genotypes in Patients with Lupus Nephritis: Associations with Lupus Pathogenesis and Treatment Response**

**PI:** Prof Fong Kok Yong  
**Affiliation:** Department of Rheumatology & Immunology, Singapore General Hospital

**Aims/Objectives:**
Lupus nephritis is common among local lupus patients and some have treatment resistant diseases. The current study aims to firstly compare the prevalence of the BIM genotypes among a large cohort of lupus patients and normal individuals and to determine whether specific BIM genotype is associated with corticosteroid treatment resistance.

**Method:**
300 patients and 100 normal controls will be studied. The BIM genotype will be assayed by PCR methodology. The clinical data and autoantibodies profiles will be abstracted by chart review with the cumulative corticosteroids dosage from onset of nephritis to remission calculated, and documentation of steroid sparer and/or cytotoxics use. Specific genotypes will be correlated with cumulative corticosteroid dosage. Disease activity and permanent organ damage will be scored using SLEDAI and SLICC Damage Index. Prevalence of the BIM (wild) and BIM (poly) will be calculated and the genetic data will be assessed using the Hardy-Weinberg equilibrium.

**Results/Expected results:**
The main study has just commenced and a pilot study of 5 lupus patients done previously showed the following:

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<th>Treatment</th>
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<th>BIM (Poly)</th>
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<tr>
<td>Oral Prednisolone</td>
<td>7,336 mg</td>
<td>6,563 mg</td>
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<tr>
<td>Oral Azathioprine</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>IV CYC or Oral MMF*</td>
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* IV CYC or MMF refers to IV cyclophosphamide and mycophenolate mofetil

The mean cumulative prednisolone dosages per patient within the 1 year post biopsy period was 12% higher in the BIM (Wild) group compared to BIM (Poly) group.

Comments:
Hence it would be interesting to study a large cohort of lupus patients to determine the relationship between treatment response and specific BIM genotypes.

Project No : 2011-01-08
Start Date                : Jul 11
Expected End Date  : Jun 14

Aims/Objectives:
Kidney transplantation is the preferred treatment for suitable patients with end-stage renal failure (ESRF) because of the survival benefits it offer over dialysis treatments. Furthermore, receiving a kidney transplant from a living kidney donor (LKD) is also preferred due to the superior outcomes compared to kidney transplantation from a deceased donor. Unfortunately, LKD transplant rates in Singapore are very low due to the lack of individuals willing to become donors.

Most of the barriers to LKD transplantation in Singapore appears to be psychosocial and concerns over donor safety. Unfortunately, there has been no research performed locally to address these barriers and concerns. As a result, this proposed study seeks to fill in the knowledge gaps and guide transplant programs in developing initiatives that may help increase LKD transplantation in Singapore.

The aims of this proposed study are as follows:

1. Compared psychosocial outcomes of individuals who have been LKDs to the general public who will act as the control group.
2. Examine various factors that influence psychosocial outcomes of LKDs.
3. Perform a retrospective analysis of clinical parameters of LKDs before and after donation at various time points.
4. Perform a separate and prospective analysis of psychosocial and clinical outcomes of LKDs.

Method:
We propose that the research be conducted in two arms:

(1) Cross-sectional and retrospective arm that run in four phases as follows:

**The Clinical and Psychosocial Impact of Living Kidney Donation**

PI: Dr Terence Kee Yi Shern
Affiliation: Department of Renal Medicine, Singapore General Hospital

Aims/Objectives:
Kidney transplantation is the preferred treatment for suitable patients with end-stage renal failure (ESRF) because of the survival benefits it offer over dialysis treatments. Furthermore, receiving a kidney transplant from a living kidney donor (LKD) is also preferred due to the superior outcomes compared to kidney transplantation from a deceased donor. Unfortunately, LKD transplant rates in Singapore are very low due to the lack of individuals willing to become donors.

Most of the barriers to LKD transplantation in Singapore appears to be psychosocial and concerns over donor safety. Unfortunately, there has been no research performed locally to address these barriers and concerns. As a result, this proposed study seeks to fill in the knowledge gaps and guide transplant programs in developing initiatives that may help increase LKD transplantation in Singapore.

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1. Compared psychosocial outcomes of individuals who have been LKDs to the general public who will act as the control group.
2. Examine various factors that influence psychosocial outcomes of LKDs.
3. Perform a retrospective analysis of clinical parameters of LKDs before and after donation at various time points.
4. Perform a separate and prospective analysis of psychosocial and clinical outcomes of LKDs.

Method:
We propose that the research be conducted in two arms:

(1) Cross-sectional and retrospective arm that run in four phases as follows:
• The first phase would be the survey instruments preparation and development for LKDs and general population controls. Pre-survey focus group discussions and structured interviews would be conducted to sharpen the survey instruments.
• The second phase would be conducting the survey itself.
• The third phase would be providing health assessment for past LKDs to obtain their latest clinical outcomes.
• The fourth phase would be obtaining LKDs’ and the recipients’ past clinical outcomes from medical record office.

(2) Conduct prospective psychosocial evaluation and clinical outcomes extraction for 12 months post-donation for new LKDs and their recipients.

Results/Expected results:
The study is pending IRB approval to commence.

Hypotheses/ Expected results of the study:
1. LKD's psychosocial outcomes are at least comparable to that of the controls;
2. There would be an association of donor's psychosocial outcomes with their current clinical outcomes, quality of relationship with others and the recipient's outcomes;
3. Clinical outcomes of living kidney donors in Singapore should be comparable to outcomes of LKDs reported in other studies.

**PROJECTS - TRANSLATIONAL RESEARCH**

**Transcriptomal and Molecular Characterization of Tumor Associated Monocytes/Macrophages in Human Cancers**

PI: Dr Alvin Wong  
Affiliation: Department of Haematology Oncology, National University Health System

Monocytes/macrophages constitute a major proportion of leukocyte infiltrates in solid tumors and mediate a variety of protumoral functions like angiogenesis, tumor cell proliferation, metastasis and immunosuppression. While several studies have documented the role of tumor associated monocytes/macrophages (TAM) in murine tumor models, investigations on human TAM remain sparse. Renal cell carcinoma (RCC) is one of the human cancers with well-documented immune mediated phenomena such as spontaneous regression. However its clinical response to immunotherapeutic regimes remain unpredictable. The monocyte/macrophage arm of the immune system in human RCC is poorly investigated, but is an area of growing interest in oncology. The present study addresses this area. Transcriptome analysis of monocytes from human RCC patients (RCC-Mo) revealed these cells to be in a transient inflammatory status, characterized by the upregulation of various cytokines, chemokines, growth factors and cell surface receptors. In conjunction, these cells showed higher expression of several C-type lectin/phagocytosis-related receptors and protumoral genes like MMPs, VEGFA and CXCR4. Surprisingly, these cells showed severely impaired inflammatory responses when activated through the Toll-like receptor 4 (TLR4) pathways. This was demonstrated by the drastic downregulation of the inflammatory transcriptome and reduced expression of genes like TNFA, CCL3, IL-1B as well as IL-10 upon ex vivo stimulation. These results were validated by high throughput qPCR as well as an in vitro RCC tumor cell-human monocyte co-culture system. Signaling studies were performed to understand the molecular basis of the refractory phenotype of RCC-Mo upon activation with inflammatory stimuli (Lipid A). These results were also validated using the RCC tumor cells/normal monocyte co-culture system. Functional assays including cytokine profiling done showed an inflammatory as well as immunosuppressive phenotype of the monocytes. Gene expression of frozen tumor RNA showed a similar in-situ profile.
In conclusion, RCC-Mo under basal conditions show an inflammatory phenotype possibly linked with protumoral functions whereas upon activation, they show a refractory state reminiscent of an immunosuppressive phenotype. This combination of protumoral functions and immunosuppressive properties indicate a potential role of these cells in promoting tumor growth and immune evasion. The profile within the tumor corresponds to that in the periphery. Meanwhile further work on this area of RCC immunology continues.

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**Phenotypic and Functional Analysis of CD39+ Regulatory T Cells (Tregs) in Kidney Transplant Patients, and their Correlation with Clinical Outcomes**

**PI:** Dr Francisco Salcido-Ochoa  
**Affiliation:** Department of Renal Medicine, Singapore General Hospital, Singapore; Singapore Immunology Network, Singapore

**Aims/Objectives:**  
Transplantation confers the best treatment opportunity for patients with end-stage organ failure. However, the immune system deploys several effector mechanisms aiming for the destruction of allografts. To avoid transplant rejection, patients receive immunosuppressive drugs to silence their immune system. But these medications come with severe side effects like infections. Tregs, a natural occurring T cell subset, are involved in the maintenance of immune cell homeostasis by downregulating immune responses against pathogens and silencing autoimmune responses. Foxp3 is a crucial transcription factor involved in the development and function of Tregs. In mice, the removal of Foxp3+CD4+CD25+ Tregs accelerates allograft rejection or even precludes the spontaneous acceptance of allografts. In this study, we aim to detect and characterise subsets of Tregs in kidney transplant (KTx) patients to identify potential tolerogenic Treg profiles in blood and kidney tissue biopsies.

**Methods:**  
T cell subsets were characterised in peripheral blood mononuclear cells (PBMC) of 11 kidney transplant patients followed up in our transplant clinic for at least a year after transplantation. At present, we are analysing phenotypic markers on effector T cells and Tregs, as well as cytokine secretion by effector T cells. The analysis was performed using 2 panels utilising 13 colour flow cytometry to identify surface markers (CD3, CD4, CD14, CD19, CD25, CD39, CD49d, CD127, CD45RO, CD45RA, CCR6 and CCR7), and cytokines (IFNγ, TNFα, IL-2, IL-4 and IL-17α), as well as Foxp3. To compare the best readout for cytokine production, PBMC were stimulated with either anti-CD3/anti-CD28 monoclonal antibodies (mAb) or with phorbol 12-myristate 13-acetate plus ionomycin (PMA/IONO) for 5 hours or 18 hours. To prevent secretion of cytokine and retain them intracellularly, cells were treated with Brefeldin A. The cells were then stained with the antibodies, and were acquired on LSR II flow cytometer and analysed using FlowJo software.
Results:
By flow cytometry, the percentage of CD4+CD25+ Foxp3 Tregs varied considerably among these patients (0-7%). Similar variability was found for the CD45RO+CCR6+ T_{REM} subset, and the recently identified CD49d-CD127- (double-negative) Foxp3+ Tregs. The patients also exhibited low levels of CD19 (1-4%). However, significance of these results is not clear at present. Overall, viability of cryopreserved cells decreased profoundly to 50% during stimulation time. Anti-CD3/-CD28 mAb stimulation induced only marginal cytokine secretion whereas with PMA/IONO substantial cytokine production was detected.

Comments:
The differences in the Treg subsets are probably mainly an effect of the particular immunosuppressive drugs the patients receive. In addition, the cells were fragile and their viability was low after cryopreservation. This and the effect of the immunosuppression might explain the reduced response after stimulation. Nevertheless, the preliminary results provide useful data for a detailed characterisation of immune cell subsets in KTx patients. The low yield of cells obtained and the poor viability preclude the design of functional studies on cryopreserved cells. We will conduct extensive phenotypic analysis in KTx patients before and after immunosuppression in a prospective study. In addition, we will analyse immune cells subsets in old KTx biopsy blocks.

| Project No  : 2008-07-22 |
| Start Date   : Jan 09 |
| Expected End Date  : Jun 13 |

The Effectiveness of Self Management Intervention to Improve Outcomes in Established and Incident Hemodialysis Patients

PI: Dr Konstadina Griva
Affiliation: Department of Psychology, National University of Singapore; National Kidney Foundation Singapore

Background:
Hemodialysis a complex and demanding behavioral regimen. The key components comprising treatment are the triweekly process of dialysis and continual nutritional management, medication, and fluid control. A growing appreciation of difficulties associated with following such a regimen has emerged. Poor adherence to treatment is common in hemodialysis patients which may increase risk for poor clinical outcomes. The aim of this randomized controlled trial is to evaluate the effectiveness of a self-management intervention to improve behavioural and biochemical markers of adherence in this patient population.

Methods:
One hundred and sixty-six hemodialysis patients were randomly assigned to the self-management intervention (n = 81) or standard care (n = 86). Participants were assessed at baseline, post-treatment (1 week following completion of program), and at 3 and 9 months follow-ups using self report measures of adherence, beliefs, mood and quality-of-life. The self management intervention is led by renal health care professionals and comprises 4 group sessions using educational, cognitive, and behavioral strategies to enhance effective self-management of fluid, diet and medication.

Results:
Significant group differences were found during the acute-phase analysis (pre to post intervention) in fluid control (p=.001), and diet (p=.006) reflecting improved adherence over time for the intervention group. Concomitant changes were also found in self-efficacy (p=.002) and medication beliefs (p=.015) indicating that intervention reduced patients’ concerns about prescribed medications and increased their confidence in dietary and fluid management. Interview data showed that intervention participants perceived great value in the program and expressed wish for more sessions and regular interaction with other patients. Analysis of long term follow up and biochemical data is currently in progress to ascertain sustainability of effects over time.
Discussion:
The current study provides evidence for the feasibility and effectiveness of self management intervention to promote behavioural change in patients on hemodialysis.

Trial registration: ICRTN 3143403

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Aims/Objectives:
To determine the prevalence of mupirocin- and chlorhexidine-resistant staphylococci in hemodialysis patients in a university hospital following implementation of an antisepsis protocol comprising 2% chlorhexidine bodywash and 2% mupirocin application to anterior nares as well as dialysis catheter exit sites.

Method:
Prospective longitudinal surveillance of all inpatients with end-stage renal disease (ESRD) undergoing hemodialysis via tunneled catheters was conducted between May to August 2009. All suitable subjects underwent nasal and catheter exit-site swabbing, followed by selective culture of staphylococci from the calcium alginate swabs. All staphylococci were typed using pulsed-field gel electrophoresis. Mupirocin minimum inhibitory concentrations (MICs) were tested using Etest strips. Additionally, all isolates were tested for the presence of the high-level mupirocin resistance gene ileS-2, methicillin resistance mec gene, as well as antiseptic resistance genes qacA/qacB and smr.

Results/Expected results:
Thirty-two patients were recruited, of whom 9 had their first hospitalization and hemodialysis. There were altogether 66 hospitalizations, with 140 distinct Staphylococcus spp. isolates cultured, comprising 86 methicillin-resistant coagulase-negative staphylococci (MR-CoNS), 36 methicillin-susceptible CoNS (MS-CoNS), 11 methicillin-resistant S. aureus (MRSA) and 7 MSSA. Staphylococci positive for ileS-2 and high-level mupirocin resistance were isolated from 23 (71.9%) of previously hospitalized patients but none of the 9 patients during their first hospitalization. Three (33.3%) had acquired ileS-2-positive MR-CoNS by their second hospitalization, however. Overall, 60% of all staphylococci cultured were positive for ileS-2, while 93.8% were positive for the antiseptic resistance genes qacA/qacB.

Comments:
In conclusion, the practice of daily chlorhexidine bodywash and mupirocin prophylaxis increases the prevalence of chlorhexidine- and mupirocin-resistant staphylococci in a dialysis unit where mupirocin and chlorhexidine are routinely used for prevention of catheter-related infections.

PI: Dr Hsu Li Yang
Affiliation: Department of Medicine, National University Health System

High prevalence of mupirocin-resistant staphylococci in a dialysis unit where mupirocin and chlorhexidine are routinely used for prevention of catheter-related infections
resistance genes in staphylococci amongst hemodialysis patients using catheters. This limited study suggests that the benefit, if any, of prophylactic antiseptics in patients with long-term hemodialysis catheters may only be transient and continued surveillance for risk-benefit assessments is necessary.

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**Hydrogen Sulfide: A Novel Agent to Protect Kidney Against Hypertensive Renal Injury**

**PI:** A/Prof Bian Jinsong  
**Affiliation:** Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore

**Aims/Objectives:**  
This project was designed to study the protective effects of H₂S on 2K1C renovascular hypertension and then examine the underlying mechanisms.

**Method:**  
Two kidneys-one clip renal vascular hypertension model was created by clipping one of the two kidneys of rats to restrict blood flow to the clipped kidney. Renin-rich juxtaglomerular cells were isolated from rat kidney. cAMP level was examined using a commercial available kit.

**Results/Expected results:**  
We found that both plasma renin and angiotensin II level was significantly higher in this two-kidneys-one-clip renal hypertension model. Treatment with NaHS (an H₂S donor) significantly reversed these effects, but had no significant effect on angiotensin-converting enzyme (ACE). To further study the inhibitory effect of H₂S on renin, we isolated renin-rich juxtaglomerular cells from rat kidneys. These cells are primarily responsible for synthesis, storage and secretion of renin. By measuring cAMP level, we found that H₂S significantly inhibited isoproterenol-stimulated renin release and cAMP elevation.

**Comments:**  
H₂S suppresses renin release from juxtaglomerular cells via suppression of adenylate cyclase AMP pathway.

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Segmental Renal Gene Expression and Functional Characterization of Renal Drug Transporters in a Rat Model of Type II Diabetes with Progressive Nephropathy

**PI:** Prof Edmund Jon Deoon Lee  
**Affiliation:** Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore

**Aims/Objectives:**
To profile the proximal tubule membrane transporter gene and protein expression during the various stages of disease progression in high-fat diet and single low dose streptozotocin-induced type II diabetic rats.

**Method:**
Six-week-old male Sprague-Dawley rats are randomly assigned to three groups as follows: Group 1 (n=6) serves as baseline, Group 2 (n=16) is fed commercial high fat diet, and Group 3 (n=12) is fed commercial regular diet. Group 1 rats (baseline) will be euthanized right after acclimatization. Group 2 and 3 rats will receive a single low dose of streptozotocin at 12 weeks of age. Half of diabetic rats (non-fasting plasma glucose > 250 mg/dl) in Group 2 and 6 age-matched control rats in Group 3 will be euthanized at 15 weeks of age. The remaining one half of diabetic rats in Group 2 and 6 age-matched control rats in Group 3 will be euthanized at 20 weeks of age. Blood and urine samples are collected for biochemical assays. Kidneys are harvested for laser capture microdissection (LCM) followed by real-time PCR, kidney pathology evaluation, and immunohistochemistry.

**Results/Expected results:**
Optimizations of tissue staining, LCM sampling and RNA extraction have been done. The optimum time from the time of cryosection, hematoxylin & eosin staining and LCM sampling until the start of RNA extraction was 20 minutes; the isolated proximal tubules had RNA integrity numbers (RINs) of approximately 6.6. Approximately 4000 proximal tubule cells per cap could be isolated by LCM within 20 minutes. At least 2 LCM caps were required to obtain optimal and reproducible RNA quality and quantity for downstream analysis.

**Comments:**
The actual study is going to be initiated soon, and therefore no actual study results are available at the time of writing.

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Surface Modification of Catheters to Inhibit Infection and Omental Wrapping in Peritoneal Dialysis

**PI:** Prof Neoh Koon Gee  
**Affiliation:** Department of Chemical and Biomolecular Engineering, National University of Singapore

**Aims/Objectives:**
Our goal is to formulate new methodologies for modifying catheter surfaces to inhibit bacterial infection and omental wrapping in peritoneal dialysis. We hypothesize that this can be achieved by modifying the catheter surfaces with a highly hydrophilic polymer in conjunction with immobilized (non-leachable) antimicrobial agent(s) and heparin.

**Method:**
We will undertake following 5 tasks:

**Task 1:** Creating a biocompatible highly hydrophilic catheter surface
Since peritoneal dialysis (PD) catheters are commonly made of silicone or polyurethane, we will investigate how these 2 types of surfaces can be engineered to be highly hydrophilic by surface grafting of polymers such as polyethylene glycol (PEG), hyperbranched polyglycerol (HPG). These graft polymers have a large exclusion volume which inhibits proteins, bacteria and cells from approaching the surface. Flat surfaces will be tested first.

**Task 2:** Immobilization of antimicrobial agent
Bactericidal polymers such as carboxymethyl chitosan (CMCS) will be grafted on the substrate surface, either by itself or in combination with other hydrophilic polymers.

**Task 3:** Immobilization of heparin
The mechanism for omental adhesion involves the formation of a fibrin exudate at the site. The fibrin encourages the migration of leucocytes and fibroblasts to the site leading to collagen depositions and dense adhesions. To enhance the catheter’s anti-thrombin and anti-inflammatory properties, heparin will be conjugated to the hydrophilic polymer coating.

**Task 4:** Co-immobilization of heparin and antimicrobial agent
Optimization of antimicrobial and cell adhesion inhibition property will be carried out by varying (i) ratio of hydrophilic, anti-microbial and heparin groups, and (ii) the manner in which the groups are distributed (ie sequential or random distribution of groups). The optimized conditions obtained with flat substrates will then be applied to actual catheters.

**Task 5:** In vitro evaluation of functionalized catheters
Antibacterial assays of the functionalized catheters will be carried out with Staphylococcus, coagulase-negative Staphylococcus, and Pseudomonas.
species since they are the commonly encountered causative organisms in infections related to PD. In vitro cell adhesion assays will be carried out with fibroblasts and endothelial cells since these two cell types are chosen for their relevance to the omental wrapping phenomenon and the omentum structure.

Results/Expected results:
Preliminary work with silicone surface grafted with CMCS and HPG are encouraging, with 80-90% reduction in the number of adherent bacteria. We are in the process of optimizing the graft density and polymer chain length of HPG as well as evaluating other types of polymer grafts like those based polyethylene glycol (PEG).

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Aims/Objectives:
The majority of patients in Singapore requiring renal replacement therapy are on hemodialysis with a total number of 2800 patients in 2005 based on the latest Singapore Renal Registry report. This figure is not anticipated to decrease any time soon as less than 100 renal transplants are performed annually while 600 new patients are added to the renal registry per year. The median wait time for kidney transplant is 9 years. Hence, it is important that patients who are on hemodialysis receive optimal management.

Method:
Presently, there is no reliable method to assess the dry weight of dialysis patients and clinical assessment is often used to assess the ultrafiltration target. This can result in overhydration or underdialysis in dialysis patients and can lead to increased morbidity and mortality. In addition, vascular access function and patency are essential for optimal management of hemodialysis (HD) patients. Low blood flow rate and loss of patency limit HD delivery extend treatment and may often result in underdialysis. Bioimpedance analysis (BIA), which is well developed in cardiovascular research, may potentially address both clinical issues related to dry weight assessment and vascular access monitoring. BIA has been used to determine extracellular volume (ECV) and/or intracellular volume (ICV) and has been validated by applying dilution methods as the gold standard. Similarly, impedance plethysmography may provide the accurate assessment of blood flow rates across two points to assess vascular access function and patency.

Expected results:
We seek to develop a portable non-invasive vascular access monitoring system based on modern bioimpedance analysis that is able to measure, store, and transmit continuous blood flow data for dry weight assessment and monitoring of vascular access. This will be a useful adjunct for caregivers and clinicians for the surveillance of vascular access and determination of dry weight in patients for optimal hemodialysis.

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**Targeted Inhibition of Signal Transducer and Activator of Transcription-3 Pathway for the Treatment of Metastatic Renal Cell Carcinoma**

**PI:** Dr Gautam Sethi  
**Affiliation:** Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore.

**Aims/Objectives:**  
Renal cell carcinoma (RCC) is an adult-onset epithelial malignancy, accounting for approximately 85% of all kidney tumors with an estimated incidence of > 40,000 new cases diagnosed per year worldwide. The current treatment for advanced metastatic RCC is ineffective because of the poor prognosis and the development of resistance against molecular targeted drugs. Hence, novel agents that are safe and effective are urgently needed. Signal transducer and activator of transcription 3 (STAT3) is constitutively activated in RCC and regulates the expression of various genes that play a major role in promotion of cellular proliferation, survival, invasion and angiogenesis. Hence, STAT3 is a promising therapeutic target and blocking STAT3 signaling could lead to a potential novel therapy for metastatic RCC. This prompted us to investigate the effect of anti-cancer agent zerumbone on STAT3 signaling cascade using RCC cell lines and xenograft mice model.

**Method:**  
The effect of zerumbone on STAT3, associated protein kinases and phosphatase, STAT3-regulated gene products, cellular proliferation, migration, invasion, and apoptosis was analyzed in RCC cells.

**Results/Expected results:**  
We found that zerumbone inhibited STAT3 activation concomitant with suppression of upstream kinases c-Src, JAK1 and JAK2 in RCC cells. Pervanadate reversed the zerumbone-induced downregulation of STAT3, suggesting the involvement of a protein tyrosine phosphatase. Zerumbone downregulated the expression of various STAT3 regulated gene products, such as cyclin D1, Bcl-2, Bcl-xL, survivin, Mcl-1 and vascular endothelial growth factor, and also inhibited migration and invasion of RCC. Finally, zerumbone inhibited proliferation, induced apoptosis and significantly potentiated the apoptotic effects of chemotherapeutic drugs (paclitaxel and capecitabine) used for the treatment of RCC.

**Comments:**  
Overall, the results so far indicate that zerumbone exerts its anti-cancer and pro-apoptotic effects through the modulation of STAT3 activation cascade in RCC cells.

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### Aims/Objectives:
The aim of the proposed study is to optimize the synthesis of a thermo-responsive core-shell magnetic nanoparticle system for controlled release of chemotherapeutic drugs at elevated temperatures.

Renal cell carcinoma (RCC) is the most common malignant tumor arising in the kidney and surgery is currently the only effective way of treating it. It has been reported that chemotherapeutic drugs have limited treatment efficacy. However, studies have shown that heat can improve the efficacy of chemotherapeutic drugs. Therefore, in this study, we propose to utilize the synergistic effects of a combined hyperthermia and chemotherapy treatment for treating RCC. This is achieved through a thermo-responsive core-shell magnetic nanoparticle system which can generate heat under an alternating magnetic field; as temperature rises, the system will release a heightened amount of the loaded drugs.

### Method:
Superparamagnetic Fe$_3$O$_4$ nanoparticles will be coated with thermo-responsive polymer and various process parameters will be varied. The aim of varying the parameters is to create an optimal system that has the highest doxorubicin loading capacity and the lowest doxorubicin leakage in normal physiological conditions.

Drug release studies will be carried out on the drug loaded particles in the absence and presence of a magnetic field at 37°C, 40°C, 42°C and 45°C.

### Results/Expected results:
The combined hyperthermia and chemotherapy treatment could be a powerful treatment alternative for RCC. The proposed system allows the use of chemotherapeutic drugs with fewer occurrences of side effects as the drugs are expected to be released only near the cancerous sites where magnetic field is being applied. Moreover, the system developed in this project may be applied towards the treatment of other cancer types.

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**Thermo-Responsive Magnetic Nanoparticles for Combined Modalities of Kidney Cancer**

**PI:** Dr Lee Chee Wee  
**Affiliation:** School of Applied Science, Temasek Polytechnic

**Aims/Objectives:** The aim of the proposed study is to optimize the synthesis of a thermo-responsive core-shell magnetic nanoparticle system for controlled release of chemotherapeutic drugs at elevated temperatures.

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ACKNOWLEDGEMENTS

The National Kidney Foundation, Singapore Society of Nephrology and Chapter of Renal Physicians would like to thank the following for their support and contributions:

The late Venerable Yen Pei, the leader of Singapore Buddhist Welfare Services and its devotees for their generous contribution to research in renal-related diseases,

Prof Tan Chorh Chuan, President, National University of Singapore, for his kind presence as the Guest-of-Honour and Judge for the Abstract (Oral and Poster) Presentations,

Prof David Lee, Professor Emeritus of Medicine, David Geffen School of Medicine, UCLA, for his kind presence as a Plenary Speaker and Judge for the Abstract (Oral and Poster) Presentations,

A/Prof Tan Say Beng, Executive Director, National Medical Research Council, for his kind presence as a Judge for the Abstract (Oral and Poster) Presentations,

A/Prof Evan Lee, Senior Director, Clinical Division, National Kidney Foundation, for delivering a Plenary Lecture,

Dr Marjorie Foo, Medical Director, Peritoneal Dialysis Programme, Singapore General Hospital, for delivering a Plenary Lecture,

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And all those who have contributed in one way or another to the success of the NKF 1st Scientific Meeting
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