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## ABSTRACT LIST

**CVS—Cardiovascular Science**
**EM—Endocrinology and Metabolism**
**DMM—Dermatology/Molecular Medicine/Medical Physics/Special Technique**
**GIH—Gastroenterology and Hepatology**
**HO—Haematology and Oncology**
**NRI—Nephrology/Rheumatology/Immunology**
**NUS—Neurosciences**
**RM—Respiratory Medicine**

## CARDIOVASCULAR SCIENCE

## ENDOCRINOLOGY AND METABOLISM

## DERMATOLOGY/MOLECULAR MEDICINE/MEDICAL PHYSICS/SPECIAL TECHNIQUE

## GASTROENTEROLOGY AND HEPATOLOGY

## RESPIRATORY MEDICINE

The Organising Committee of the 8th Medical Research Conference and the Medical Science Group, Faculty of Medicine would like to extend their gratitude to

# GlaxoSmithKline Limited

for their continuing support and generous donation.

Last but not least, we would like to thank all speakers, chairmen, presenters and participants for their participation and contribution.

# FOREWORD

The Medical Research Conference (MRC) has been a major annual research event of Department of Medicine for the past seven years. It aims to enable doctors, post-graduate students, and scientists from within and outside the Department as well as the Faculty of Medicine to gather in an informal forum, and critically discuss their on-going research activities, thereby stimulating and sharpening their research insight, and promoting inter-disciplinary collaborations. This yearly event has established itself as an important forum for the promotion of research culture and collaboration. It provides researchers and post-graduate students an important venue for the exchange and stimulation of ideas, and an opportunity especially for junior staff to present their work and develop a lifetime inclination to conducting good research, in response to the needs of our society. With the newly formed Medical Science Group within the Faculty of Medicine we will for the first time hold the MRC under the Group banner. We hope to be able to bridge the gap between basic and clinical research and further nuture the research culture.

We hope that the MRC will stimulate further research collaborations within the University and with other local, mainland, and overseas institutions. Striving for excellence in research has always been an important mission of the University of Hong Kong. Indeed it is important for us to gather our expertise and aim to further the status of Hong Kong in the international research arena. We believe we are well positioned to the challenges ahead, and through our innovation, collaboration, and hard work, will continue to excel.

Prof Karen SL Lam
Group Chairman
Medical Science Group

## PL1 Recent advances in chronic hepatitis B: precore mutants, ccc HBV DNA and treatment

Prof CL Lai. Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong

The natural history of chronic hepatitis B infection is still not well understood. In a recent study of Chinese patients with chronic hepatitis B virus (332 with and 44 without cirrhosis-related complications), 50% of patients <30 years old had precore mutations. The prevalence of precore mutations among hepatitis B e antigen (HBeAg)-positive patients, although lower than that among anti-HBe-positive patients (P=0.031), was already high (44.2%). Median HBV DNA level in anti-HBe-positive patients was $1.5 \times 10^6$-$1.55 \times 10^6$ copies/mL, irrespective of the presence or absence of precore mutations. There was no difference in the prevalence of precore mutations between patients with and without complications (P, not significant). Precore mutations occur in a large proportion of Chinese patients with chronic hepatitis B before HBeAg seroconversion. The development of complications is not related to precore mutations.

There is recent resurgence of interest in the covalently closed circular (ccc) HBV DNA because it is the source for viral replication but is itself resistant to therapy. We adopted a non-PCR Invader assay to measure the cccDNA levels. Total HBV DNA, cccDNA, and human genomic DNA in liver biopsy samples from 15 patients positive for hepatitis B e antigen (HBeAg), 26 patients positive for antibody against HBeAg (anti-HBe) and 16 patients with HBsAg seroclearance were measured.

Anti-HBe-positive patients had lower median total intrahepatic HBV DNA ($p<0.001$) and intrahepatic cccDNA levels ($p<0.001$) than HBeAg-positive patients. cccDNA levels were directly proportional to the total intrahepatic HBV DNA ($r=0.948$, $p<0.001$), while the proportion of intrahepatic cccDNA was *inversely proportional* to the amount of total intrahepatic HBV DNA ($r=-0.850$, $p<0.001$). Low percentage of cccDNA was detected in 29/41 (71%) of the serum samples. The serum HBV DNA levels correlated positively to the intrahepatic total HBV DNA ($r=0.831$, $p<0.001$) and intrahepatic cccDNA ($r=0.524$, $p=0.004$). Six out of 16 patients (37.5%) with HBsAg seroclearance had detectable intrahepatic total HBV DNA levels. Of these, 100% of the intrahepatic HBV DNA was in the form of cccDNA in 4 patients while 71.3% and 91.1% of the intrahepatic total HBV DNA were in the form of cccDNA in the remaining 2 patients.

In the treatment of chronic hepatitis B, many novel nucleoside analogues are under trial. Two of the most promising are entecavir and L-deoxythymidine (LdT). A phase IIb trial is being conducted in 104 patients from 5 countries to assess the safety and antiviral efficacy of LdT, lamivudine, and the combination of LdT+ lamivudine over one year. The trial compares treatment with LdT (600 mg/d), LdT (400 mg/d), LdT (600 mg/d) plus lamivudine (100 mg/d), LdT (400 mg/d) plus lamivudine (100 mg/d), and lamivudine alone (100 mg/d), with equal randomization to the 5 treatments. Preliminary wk 24 data indicate significantly better antiviral effects for LdT-containing regimens (LdT alone and LdT+lamivudine) compared to lamivudine monotherapy.

The future of hepatitis B therapy is combination therapy. This should be available in the near future.

## PL2 Update in the management of atrial fibrillation

Prof CP Lau, Cardiology Division, Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong

Atrial fibrillation (AF) is the commonest sustained arrhythmia, affecting >5% Western population over the age of 65 years. In Hong Kong, our survey suggests that 1.3% of active elderly subjects had AF, and risk factors for AF included hypertension, diabetes mellitus, ischaemic heart disease and valvular heart disease.
Prolonged AF lead to electromechanical remodeling of the atrium. We found that in patients with persistent AF undergoing internal defibrillation, AF resulted in shortening of the atrial effective refractory period (AERP) in the left atrium, but less shortening in the low right atrium. Interestingly the degree of AERP shortening depends on the preceeding duration of AF, suggesting that AF begets AF.

We hypothesized that if AF can be restored promptly, electromechanical remodelling could be prevented or potentially reversed. An implantable atrial defibrillator (IAD) was used in 16 pts with AF. Repeated AF termination lead to a progressive reduction in LA size and AF burden measured by the device. Furthermore, prompt (<4h) termination of AF was associated with less atrial stunning than delayed AF termination, suggestive of the adverse effects of AF on atrial mechanical function. High LA pressure from valve disease or prolonged AF lead to electromechanical feedback which can be an important cause of electromechanical remodeling.

AF can be prevented by either pacing or ablation. Single site atrial pacing with automatic atrial overdrive has been shown to suppress AF burden in pts who required pacemakers. We examined the use of multisite right atrial pacing in pts with bradycardia. In the presence of a betablocker sotalol, dual site pacing prolonged the time to first AF recurrence and AF burden. Pacing both the LA and RA during cardiac surgery also reduce the incidence of post operative AF.

Recent evidence suggests that some types of AF originate in the atrial myocardial sleeves in the pulmonary vein, and elemination of such foci can significantly reduce AF incidence. We introduced a technique of using restoration of AF and mapping of early reinitiation to locate and ablate the focus. Furthermore, as radiofrequency energy that is used for ablation has a higher propensity for thromboembolism and pulmonary vein stenosis, we have introduced the use of transcatheter cryoablation of these pulmonary vein foci. Early results are encouraging.
In summary, while medical treatment remains the mainstay of treatment of AF, non-pharmacological approaches such as implantable devices, pacing and ablation can be useful in a significant proportion of patients. As AF is a heterogeneous disorder, it is likely that these therapies used alone or combination, will be individually tailored patients.

## PL3 Cellular and molecular interactions in synaptogenesis

Professor Benjamin Peng, Ph.D. Professor & Head, Department of Biology, Hong Kong University of Science and Technology, Hong Kong

At the vertebrate neuromuscular junction (NMJ), signal transmission from the nerve to the muscle in causing muscle contraction is mediated by highly efficient release of acetylcholine from the nerve terminal and the sensing of this molecule by acetylcholine receptors (AChRs) in the muscle. This is accomplished by the clustering of AChRs in the postsynaptic membrane opposite to the nerve terminal and the focal accumulation of ACh-containing synaptic vesicles in the nerve terminal. In this presentation, the cellular and molecular mechanisms that govern the development of the postsynaptic membrane will be discussed. Recent studies have established the central role of motoneuron-derived heparan-sulfate proteoglycan (HSPG) agrin as an inducer for NMJ development. Agrin activates the receptor tyrosine kinase MuSK to effect AChR clustering, but this reaction requires a yet unidentified cofactor since agrin does not by itself bind to MuSK. Our study has implicated the role of HSPG-bound growth factors as partners in agrin's action. Once activated, the kinase cascade leads to local actin polymerization which serves as a scaffold for organizing the postsynaptic apparatus. In addition to tyrosine kinase, postsynaptic induction also leads to tyrosine phosphatase activation which helps define the boundary of synaptic AChR clustering and effects dispersal of extrajunctional clusters. Besides receptors, postsynaptic development also involves the assembly of synaptic basal lamina which provides anchorage to extracellular molecules essential for synaptic function such as acetylcholinesterase (AChE). The transmembrane protein complex dystroglycan, via its extracellular interaction with HSPG and its cytoplasmic interaction with the cytoskeleton, appears to play an pivotal role in AChE clustering at the NMJ. These results suggest cellular and molecular mechanisms whereby postsynaptic signaling leads to the assembly of a transmembrane protein complex, consisting of extracellular, membrane-associated and cytoskeletal proteins, that serves to localize structural and enzymatic proteins essential for synaptic transmission.

## PL4 NF-kappaB, an evolutionarily conserved mediator of immune and inflammatory responses

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Vertebrates respond to infection through a combination of adaptive or acquired immunity and innate or natural immunity. The principal feature of acquired immunity is the generation of receptors on B and T-cells that can distinguish between self and non-self, and hence protect the organism from infectious agents such as bacteria or viruses. By contrast, the hallmarks of innate immunity consist of physical barriers and the ability to generate a battery of cytokines upon non-specific recognition of conserved structures on infectious agents such as bacterial lipopolysaccharides (LPS). The cytokines help to mount an inflammatory response and to recruit specialized cells, such as natural killer cells, to the site of infection. The rapid induction in the synthesis of these cytokines is coordinated by the activation of the inducible transcription factor NF-kappaB. NF-kappaB is critical for the inducible expression of many genes involved in the immune and inflammatory responses such as IL-1, IL-2, IL-2Ralpha, IL-6, IL-8, TNF-alpha, TNF-beta, beta-IFN, GM-CSF and serum amyloid A protein. Our recent studies in this area has focused on further understanding the signal transduction pathways that lead to the activation of NF-kappaB in innate immune responses, and the mechanism by which the activity of NF-kappaB is regulated. Recent advances in both of these areas will be discussed.

## PL5 Functional study of expression of the Epstein Barr virus encoded LMP1 protein in nasopharyngeal epithelial cells

A/Prof GSW Tsao, HM Li, XH Wang, YC Wong. Dept. of Anatomy, University of Hong Kong, Hong Kong, Hong Kong.

Epstein Barr virus (EBV) infection has long been postulated to play a crucial role in the pathogenesis of nasopharyngeal carcinoma. EBV infection is an early event in nasopharyngeal carcinoma. The EBV encoded LMP1 protein is expressed during the latent infection of the virus and is commonly detected in nasopharyngeal carcinoma cells. The pathological role of LMP1 in nasopharyngeal epithelial cells remains elusive due to the lack of cell model representative of nasopharyngeal epitheliulm. We have recently established an immortalized cell model from non-malignant nasopharyngeal epithelium. The immortalized nasopharyngeal epithelial cell line is non-tumorigenic and retains the differention properties of normal nasopharyngeal epithelial cells. This newly established nasopharyngeal epithelial cell system has been used to examine the functional property of LMP1 expression. Expression of LMP1 induced agar independent growth (hallmark of malignant transformation in vitro), stimulated growth and downregulated expression of the p16 gene in the immortalized nasopharyngeal epithelial cells. Other biological properties of LMP1 expression are: increased saturation density, downregulated E-cadherin expression, increased cell mobility and invasive property in nasopharyngeal epithelial cells. Gene expression profiling of the LMP1 expressing cells reveals upregulation of genes for cell survival, angiogenesis and metastasis. Our results suggest that LMP1 expression play an important role in promoting the conversion of premalignant nasopharyngeal epithelial cells to invasive cancer cells.

## PL6 Hyperhomocysteinemia and atherosclerosis: role of chemokine and adhesion molecules

A/Prof Karmin O, Department of Pharmacology, Faculty of Medicine, University of Hong Kong, Hong Kong

**Introduction:** Hyperhomocysteinemia is regarded as an independent risk factor for cardiovascular and cerebral vascular disorders. The stimulatory effect of homocysteine (Hcy) on monocyte chemoattractant protein-1 (MCP-1) expression *in vitro* has been suggested to play an important role in Hcy-mediated atherosclerosis. We previously reported that Hcy stimulated MCP-1 expression in endothelial cells, in vascular smooth muscle cells and in monocyte-derived macrophages. The objective of the present study was to investigate whether such stimulatory effect occurred *in vivo* leading to monocyte adhesion to the endothelium.
**Methods:** Hyperhomocystenemia was induced in Sprague-Dawley rats after four weeks of high-methionine diet (serum Hcy levels 4-5 fold higher than the control).
**Results:** The number of monocytes present on the surface of aortic endothelium was significantly elevated in hyperhomocysteinemic rats. There was a significant increase in the expression of MCP-1, vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in the endothelium. Antibodies recognizing MCP-1, VCAM-1 or E-selectin could abolish the enhanced monocyte binding to the aortic endothelium of hyperhomocysteinemic rats.
**Conclusions:** These results suggest that hyperhomocysteinemia alone stimulates the expression of chemokine and adhesion molecules *in vivo* leading to increased monocyte adhesion to the aortic endothelium. Such effect may contribute significantly to the development of atherosclerosis by facilitating monocyte infiltration into the arterial wall.

This study was supported by grants (HKU7346/00M and HKU7249/02M) from the Research Grant Council of Hong Kong.

## CVS-01 Use of a filter-type distal protection device in percutaneous revascularisation of native coronary arteries

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**Introduction** Distal embolization of atherosclerotic and/or thrombotic debris occurs routinely during percutaneous coronary intervention (PCI). Prevention of this phenomenon during saphenous vein graft intervention by a distal protection device (DPD) is associated with a reduction in major adverse cardiac events. There are few data on the use of DPDs in native coronary arteries.
**Methods** The FilterWire EX is a temporary filtration DPD that utilizes a 0.014" guidewire on which is mounted an expandable loop structure attached to a thin porous filter. The filter is made of polyurethane and rotates freely on the end of the guidewire. The loop captures particles which are retained by trapdoor action during retrieval using the delivery sheath. The recommended vessel size is 3.5 to 5.5 mm where the filter loop is placed. The FilterWire EX was used in 35 patients undergoing PCI of native coronary arteries. Clinical and angiographic variables were analyzed.
**Results** The FilterWire EX was used in 20 left anterior descending arteries, 8 right coronary arteries, and 2 left circumflex arteries. The indications for PCI were: stable angina (17; 48.6%), recent myocardial infarction (MI) (8; 22.9%), stable angina (6; 35%), recent unstable angina (5; 14.3%), acute MI primary PCI (4; 11.4%), and acute MI rescue PCI (1; 2.9%). The vessel diameter at FilterWire EX deployment was 2.89 ± 0.54 mm (range 1.71 mm to 4.42 mm; 88.6% <3.5 mm). The wire was used to cross the lesion directly in 23 patients (65.7%), using a conventional guidewire as "buddy wire" in 5 patients (14.3%), requiring lesion predilation in 5 patients (14.3%), and following thrombectomy in 2 patients (5.7%). The device was successfully deployed and retrieved in all cases. Flow impairment was noted in 8 patients (22.9%) after deployment of the device but was restored to normal after retrieval. Reversible vasospasm occurred in 11 (31.4%) patients. There was no death, myocardial infarction, and dissection in each of the patients.
**Conclusion** The use of the FilterWire EX appears to be safe in native coronary arteries even with a diameter <3.5 mm at the site of deployment. There are no complications related to its use. Large randomized controlled trials are needed to prove the efficacy and safety of this device.

## CVS-02 Prevalence of diabetes mellitus (DM) in the Hong Kong Cardiovascular Risk Factor Prevalence Study cohort

BMY Cheung, YB Man, KSL Lam, NMS Wat, JLF Lo, DFY Chau, CY Law, TH Lam,[1] GM Leung,[1] SCF Tam,[2] CH Cheng, CR Kumana, CP Lau. Department of Medicine, Department of Community Medicine,[1] The University of Hong Kong; Clinical Biochemistry Unit,[2] Queen Mary Hospital, Hong Kong

**Introduction:** 2881 randomly chosen Hong Kong men and women participated in the Hong Kong Cardiovascular Risk Factor Prevalence Study in 1995-6. After 6 years the subjects are recalled for follow up. Here we report the prevalence of DM in the restudied subjects.
**Method:** 813 subjects (393 men, 420 women; age 51±12 yrs) were randomly chosen from the cohort and were studied in the morning after overnight fasting. A medical history was obtained from each patient. A 2-hour oral glucose tolerance test was performed.
**Results:** The prevalence of DM in 1995-6 and 2001-2 is 9.6% and 15.5% respectively ($p<0.001$). After adjusting for age, the prevalence of DM has increased by 35.1% ($p<0.001$).
**Conclusion:** The prevalence of DM rises sharply with age, especially after the age of 55. The prevalence of DM in the elderly is alarmingly high and shows a rising trend.

***Prevalence of DM in percentage.*** The number of subjects is given in brackets.

| Age | | <35 | 35-44 | 45-54 | 55-64 | 65-74 | >74 |
|---|---|---|---|---|---|---|---|
| 1995-6 | Male | 2.0 (305) | 5.8 (378) | 7.5 (267) | 18.6 (242) | 21.7 (161) | — |
| | Female | 1.4 (286) | 3.2 (474) | 10.9 (294) | 21.2 (203) | 29.3 (150) | — |
| 2001-2 | Male | 3.3 (30) | 10.3 (97) | 9.6 (114) | 23.9 (67) | 32.9 (70) | 33.3 (15) |
| | Female | 4.3 (23) | 5.2 (115) | 6.0 (150) | 28.4 (67) | 36.2 (47) | 44.4 (18) |

In 2001-2, the prevalence of DM in >64 years is 32.9±5.1% in men and 38.5±6.0% in women.

## CVS-03 Inhibition of the unique repolarisation $K^+$ channel current $I_{Kur}$ by verapamil in human atrial myocytes

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**Introduction:** Verapamil is widely used as an antiarrhythmic drug in patients with atrial arrhythmias, and its $Ca^{2+}$ antagonistc action is usually believed to be the mechanism. The present study was to determine if anti-atrial arrhythmia was related to the blockade of the unique repolarization $K^+$ current ($I_{Kur}$, ultra-rapid delayed rectifier $K^+$ current).
**Method:** Whole-cell patch clamp technique was used to determine $I_{Kur}$ and another voltage-gated current, transient outward $K^+$ current ($I_{to1}$) in human atrial myocytes.
**Results:** It was found that verapamil inhibited $I_{Kur}$ in a dose-dependent manner ($EC_{50}$ = 3.74 mM). The effect was fully reversed upon washout of the drug. At test potential of +50 mV, Verapamil at 5 mM decreased $I_{Kur}$ by 40.3 ± 5.1% (2.68 ± 0.21 pA/pF in control and 1.84± 0.17 pA/pF after verapamil, n=8, $p<0.01$). The inhibition of $I_{Kur}$ by verapamil is voltage-independent. In contrast, verapamil (0.1~50 mM) exhibited a slight increase in $I_{to1}$, but did not show a dose-response fashion. However, verapamil accelerated inactivation of $I_{to1}$. At 1 mM, the time constant of $I_{to1}$ inactivation was reduced to 51.16± 5.29 from 71.74± 3.3 ms of control (+50 mV, n=8, $p<0.01$). Other kinetics of $I_{to1}$ were not affected by verapamil.
**Conclusion:** The present study has demonstrated for the first time that verapamil, a well-known calcium blocker, significantly blocks the unique repolarization $K^+$ current $I_{Kur}$, and revealed a novel antiarrhythmic mechanism of verapamil.

## CVS-04 Effects of genistein on $K^+$ currents in rat ventricular myocytes

Z Gao,[1] CP Lau,[1] TM Wong,[2] GR Li.[1,2] ICSM/Department of Medicine, and [2]Department of Physiology, Faculty of Medicine, The University of Hong Kong

**Introduction:** Previous studies showed that genistein modulated ionic channels in a protein tyrosine kinase- (PTK) dependent or independent way upon species and/or channel types. The present study was designed to determine whether transient outward $K^+$ current ($I_{to}$), sustained $K^+$ current ($I_{sus}$), inward rectifier $K^+$ current ($I_{K1}$) were regulated by genistein in rat ventricular myocytes.
**Methods:** Whole-cell patch technique was applied to record $I_{to}$, $I_{sus}$, and $I_{K1}$ in enzymatically dissociated ventricular myocytes from rat hearts. All experiments were conducted at 22~23°C.
**Results:** Genistein reversibly inhibited $I_{to}$ in a concentration-dependent manner ($IC_{50}$ = 27.8 μM. The compound (50 mM) shifted midpoint of voltage ($V_{0.5}$) for inactivation of $I_{to}$ to -42.5 ±1.0 from –37.6±0.6 mV ($P<0.01$), while the $V_{0.5}$ for $I_{to}$ activation was not significantly altered. In addition, genistein reversibly suppressed $I_{sus}$ with $IC_{50}$ of 17.1 μM. Moreover, the compound at 50 mM reduced $I_{K1}$ at –100 and –50 mV by 40.6±6.2% and 51.4±0.7%, respectively. However, the effects of genistein on $I_{to}$, $I_{Ksus}$, and $I_{K1}$ were not affected by the application of phosphotyrosine phophatase inhibitor (sodium orthovanadate, 1 mM). On the other hand, daidzein (100 mM), an inactive analogue of genistein, did not show significant effect on the three $K^+$ currents. Another type of PTK inhibitor, tyrphostin A23, had no effect on $I_{to}$, $I_{Ksus}$, and $I_{K1}$.
**Conclusion:** 1) The PTK inhibitor genistein, not tyrphostin A23, reversibly inhibited $I_{to}$, $I_{Ksus}$, and $I_{K1}$ in rat ventricular myocytes, and 2) the effects were not affected by the protein tyrosine phosphatase inhibitor orthovanadate. The present study has provided the first information that genistein-induced suppression of $I_{to}$, $I_{Ksus}$, and $I_{K1}$ is independent of PTK inhibition.

## CVS-05 Effects of 293B, a selective $I_{Ks}$ blocker, on transient outward and ultra-rapid delayed rectifier $K^+$ currents in human atrial myocytes

XL Du, CP Lau, SW Chiu, HF Tse, GR Li. ICSM/Department of Medicine, Faculty of Medicine, The University of Hong Kong

**Introduction:** Previous reports demonstrated that chromanol 293B, a so-called selective blocker of slow component of delayed rectifier $K^+$ current ($I_{Ks}$), inhibited other channel currents. It is unclear whether 293B may affect other $K^+$ currents in human atrium.
**Methods:** With whole-cell patch clamp technique, we studied effects of 293B on transient outward $K^+$ current ($I_{to1}$) and ultra-rapid delayed rectifier $K^+$ current ($I_{Kur}$) in isolated human atrial myocytes. Experiments were conducted at room temperature.
**Results:** It was found that 293B reversibly inhibited $I_{to1}$ and $I_{Kur}$ in a concentration-dependent manner, and the effect was not dependent on depolarizing voltage. Concentration for 50% inhibition ($IC_{50}$) was 31.2 μM for $I_{to1}$, and 30.9 μM for $I_{Kur}$. 293B blocked $I_{to1}$ and $I_{Kur}$ with same concentration range, and significant effect was observed from a concentration of 1 μM, and reached maximum effect at 250 μM. Voltage-dependent kinetics of activation and inactivation, and time-dependent recovery from inactivation of $I_{to1}$ were not altered by 293B; however, time to peak and time-dependent inactivation of $I_{to1}$ was significantly accelerated.
**Conclusion:** The results indicate that the so-called $I_{Ks}$ blocker 293B significantly inhibits major repolarization $K^+$ currents, $I_{to1}$ and $I_{Kur}$, in human atrial myocytes.

## CVS-06 Effect of Qigong on hypertension: a randomised controlled study

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**Introduction:** The cause of essential hypertension remains unknown in most patients. Exercise and relaxation therapy are useful in decreasing blood pressure. We conducted a randomised controlled trial to examine the effects of Guolin qigong on blood pressure, other cardiovascular risk factors and psychosocial well-being in patients with mild essential hypertension.
**Method:** After informed consent, each patient was randomised to practice either qigong or conventional exercise daily for 16 weeks. In addition to clinical and laboratory assessments, SF-36, Beck Anxiety Inventory and Beck Depression Inventory version II questionnaires were also completed by the patients.
**Results:** Ninety-one patients were recruited but 3 patients withdrew before the intervention period. Qigong skills were harder to master than conventional exercise. Sodium level increased by 1.18±0.46 mmol/l more in the qigong group at week 16 (p=0.012). Heart rate was higher by 3.2±1.3 bpm in the qigong group at week 4 (p=0.016) but not subsequently. Ambulatory daytime systolic BP as well as night-time systolic and diastolic BP were higher by 4.89±2.11 mmHg (p=0.024), 6.19±2.45 mmHg (p=0.014) and 5.54±1.53 mmHg (p=0.001) respectively in the qigong group at week 16. Systolic and diastolic blood pressure, calcium, cholesterol, renin, weight, BMI, waist, hip, and urine protein significantly decreased in both groups at week 16. General health, mental health, social functioning, anxiety, and depression also improved in both groups. One female in the qigong group developed vestibular neuronitis.
**Conclusions:** Both Guolin qigong and conventional exercise lower blood pressure in patients with mild essential hypertension. It may therefore be an alternative to conventional exercise as part of the non-pharmacological management of hypertension.

Support from the Li Ka Shing Foundation is gratefully acknowledged.

## CVS-07 Earthworm extract as a fibrinolytic agent in healthy men: a randomised, double-blind, placebo-controlled study

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**Introduction:** "Plasmin" (Everpride Pharmaceutical) is a commercially available health food supplement that contains an earthworm extract. Preliminary studies suggested that it might have fibrinolytic properties. We therefore tested its efficacy and safety in a randomised, double-blind, placebo-controlled trial.
**Method:** 30 normal healthy men participated with informed consent and were randomised to either Plasmin 750 mg three times daily or matching placebo capsules for 28 days. Blood samples were taken at 1, 2, 7, 14 and 28 days for haematological and biochemical tests.
**Results:** There were no significance changes in blood count, renal function, liver function, blood glucose, and lipid profile. There was a small difference (1.04s ± 0.31, p=0.002) in the activated partial thromboplastin time (APTT) between Plasmin and placebo. There was no incidence of abnormal bleeding. The number of adverse events (AEs) in the 2 treatment groups (9 AEs in 4 placebo-treated subjects and 7 AEs in 3 Plasmin-treated subjects) was comparable. None of the adverse events were related to trial medication. Plasmin was well tolerated by the subjects.
**Conclusions:** Plasmin is a safe and well-tolerated Chinese medicine. In this short-term study, we have not found any adverse haematological effects. A large clinical trial of long duration is needed to evaluate its efficacy in the prevention of thromboembolic diseases.

## CVS-08 Lipid profile of the Hong Kong Cardiovascular Risk Factor Prevalence Survey cohort

BMY Cheung, YB Man, JLF Lo, DFY Chau, CY Law, KSL Lam, TH Lam,[1] NMS Wat, SCF Tam,[2] CH Cheng, CR Kumana, CP Lau. University Department of Medicine, Department of Community Medicine,[1] The University of Hong Kong; Clinical Biochemistry Unit,[2] Queen Mary Hospital, Hong Kong

**Introduction:** In 1995-6, 2881 randomly chosen Hong Kong men and women participated in the Hong Kong Cardiovascular Risk Factor Prevalence Survey. The subjects are recalled for follow up after 6 years. Here, we report the current lipid profile of the subjects who have been restudied.
**Method:** 813 subjects (393 men, 420 women; age 51±12 yrs) were randomly chosen from the cohort and were studied in the morning after overnight fasting. Body fat was assessed using bioelectrical impedance. Height, weight, waist and hip circumferences were measured. Blood pressure was measured carefully after resting. An oral glucose tolerance test was performed. Venous blood was taken for lipid and glucose measurement.
**Results:** Compared to six years ago, there were no significant changes in body weight and body mass index (BMI), but the waist circumference (WC) increased from 81.5±1.1 to 83.9±1.1 cm (p<0.001). Plasma total cholesterol increased from 5.00±0.10 mmol/L to 5.27±0.10 mmol/L (p<0.001). This was due to a rise in both HDL-C (1.2±0.03 to 1.3±0.04 mmol/L, p<0.001) and LDL-C (3.2±0.09 to 3.4±0.08, p=0.12). Plasma triglycerides were 1.6±0.06 mmol/L in men and 1.3±0.05 mmol/L in women (p<0.001). 126 (16%) and 214 (26%) subjects had diabetes and hypertension respectively. Multiple regression analysis showed that HDL-C was related (R=0.53, p<0.001) to WC (_=-0.34), sex (_=0.24), age (_=0.13), alcohol (_=-0.15), fasting glucose (_=-0.11) and diastolic pressure (_=0.09). LDL-C was related (R=0.21, p<0.001) to age (_=0.1), fasting glucose (_=0.11) and diastolic pressure (_=0.08).
**Conclusions:** Dyslipidaemia is associated with central obesity, high blood glucose and high blood pressure in these subjects that have been randomly selected from the general population. Our data highlight metabolic syndrome as a major problem in Hong Kong.

## CVS-09 Randomised controlled trial of low salt diet in the treatment of hypertension

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**Background:** Non-pharmacological treatment is the preferred initial step in the management of mild hypertension. We compared its efficacy with drug treatment.
**Methods:** 93 patients (M:F, 45:48; age 44 ± 11 yrs) with untreated mild essential hypertension were recruited. After a placebo run-in phase, 73 eligible patients were randomised to drug treatment (with hydrochlorothiazide 25 mg daily [n=19] or metoprolol 100 mg daily [n=14]) or non-pharmacological treatment (lifestyle modification including a low-fat, low-salt, high fibre diet, weight control, smoking cessation, moderating alcohol intake and regular exercise) for 6 months. Additional drugs were allowed after 12 weeks if the blood pressure was not controlled. Left ventricular mass index (LVMI) was determined by echocardiography.
**Results:** In the non-pharmacological group, there was a significant decrease in sodium intake (56 ± 14 mmol/day) and body fat (1.7 ± 0.5%), but the decrease in body mass (1.4 ± 0.4Kg) was small.
There was a significant decrease in ambulatory systolic and diastolic blood pressure in the drug treatment (16±2 mmHg and 10±2 mmHg) and diet group (10±2 mmHg and 6±1 mmHg). Change in sodium excretion correlated with diastolic blood pressure (r=0.44, p=0.02) rather than systolic blood pressure (r=0.35, p=0.08).
**Conclusion:** Non-pharmacological treatment reduces blood pressure slightly, but to a lesser extent than antihypertensive drugs. It can therefore be used in patients with very mild hypertension. In patients with more severe hypertension, non-pharmacological treatment should be implemented in conjunction with antihypertensive medications.

| | N | Diastolic pressure | | Systolic pressure | | LVMI | |
|---|---|---|---|---|---|---|---|
| | | baseline | final | baseline | final | baseline | final |
| non-pharmacological | 38 | 95 ± 1 | 89 ± 1 | 142 ± 2 | 135 ± 3 | 128 ± 5 | 119 ± 4 |
| pharmacological | 35 | 96 ± 1 | 83 ± 2* | 142 ± 2 | 122 ± 3* | 130 ± 6 | 123 ± 5 |

* $P < 0.05$

## CVS-10 Prevalence of obesity in the Hong Kong Cardiovascular Risk Factor Prevalence Survey-2 (CRISPS2)

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**Introduction:** In 1995-6, 2881 randomly chosen Hong Kong men and women were studied in the Hong Kong Cardiovascular Risk Factor Prevalence Survey. In CRISPS2, those subjects are randomly recalled to assess the prevalence of cardiovascular risk factors. Here, we report the interim findings on obesity.
**Method:** 813 subjects (393 men, 420 women; age 51±12 yrs) were studied. The medical history was obtained and the subjects were examined with special attention to blood pressure and indices of obesity. Body mass index (BMI) was derived from the weight (kg) divided by height squared ($m^2$). Each subject had an oral glucose tolerance test. Lipid measurements were performed on fasting blood samples.
**Results:** 4.6% of men and 4.5% of women were obese (BMI ≥30). 41% men and 30% women were overweight (BMI≥25). Compared to six years ago, there were no significant changes in body weight and BMI, but the waist circumference increased from 81.8±1.1 to 84.3±1.2 cm (p<0.001). In men, BMI was related to triglycerides (r=0.37, p<0.001), HDL-cholesterol (r=-0.42, p<0.001), diastolic blood pressure (r=0.31, p<0.001), fasting glucose (r=0.27, p<0.001) and fibrinogen (r=0.11, p=0.04). In women, the BMI also correlated with triglycerides (r=0.35, p<0.001), HDL-cholesterol (r=-0.38, p<0.001), diastolic blood pressure (r=0.34, p<0.001), fasting glucose (r=0.26, p<0.001) and fibrinogen (r=0.16, p=0.002). 126 (16%) and 214 (26%) subjects had diabetes and hypertension respectively. In the overweight or obese, the prevalence of diabetes and hypertension is increased to 28% and 40% in men and 25% and 41% in women respectively. BMI≥25 is associated with diabetes (OR 3.5 [2.3-5.1]) and hypertension (OR 3.0 [2.2-4.2]).
**Conclusions:** The prevalence of obesity in the general population is low compared to western countries, but overweight is common. Overweight is associated with cardiovascular risk factors, so appropriate weight control in the general population seems warranted.

## CVS-11 Randomised trial of candesartan and enalapril (RACE) in the treatment of hypertension – final results

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**Introduction:** The renin-angiotensin system plays an important part in the pathophysiology of hypertension. We compared two ways of blocking the system, using an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker.

**Method:** 22 hypertensive patients (16 men, 6 women; age 47±10 yrs) were randomised to either candesartan 8 mg or enalapril 10 mg daily for 3 months. The dose could be doubled after 6 weeks for blood pressure control. Ambulatory blood pressure monitoring was performed before randomisation and before the final visit. Echocardiography was performed at randomization, 2 and 12 weeks. Flow-mediated dilatation was measured at the brachial artery to assess endothelial function. Exhaled nitric oxide was measured using an integrated chemiluminescent system. The coefficient of variation of left ventricular mass index (LVMI), flow-mediated dilatation (FMD) and nitric oxide measurement were 7%, 5% and 3% respectively.

**Results:** Candesartan and enalapril both significantly lowered systolic and diastolic blood pressure. This was confirmed by ambulatory blood pressure monitoring.

There were no significant differences between the treatments in their effect on LVMI and exhaled nitric oxide. Changes in LVMI correlated strongly with changes in the ambulatory systolic blood pressure ($r=0.62$, $p=0.02$) and diastolic blood pressure ($r=0.61$, $p=0.02$). FMD increased with enalapril but not candesartan treatment ($p=0.04$). Neither treatment significantly altered plasma potassium, creatinine, renin and aldosterone. Fasting blood glucose decreased significantly from 5.6±0.4 to 5.1±0.3 mmol/L in the enalapril group ($p=0.01$) only.

**Conclusions:** Both drugs are efficacious in lowering blood pressure. Candesartan tended to lower blood pressure more than enalapril, which might be the result of the dosages used. Enalapril appears to have a favourable effect on blood glucose and endothelial function. We conclude that candesartan may be used as an alternative to enalapril in the treatment of hypertension, particularly in those who are intolerant of the latter.

| | Baseline SBP | Final SBP | Baseline DBP | Final DBP | Baseline LVMI | Final LVMI |
|---|---|---|---|---|---|---|
| candesartan | 158.2±5.7 | 134.5±4.3* | 100.3±3.9 | 85.3±3.2* | 139.8±12.5 | 134.9±11.1 |
| enalapril | 159.8±4.0 | 145.3±6.1** | 97.4±3.0 | 87.0±3.4* | 127.3±6.4 | 134.5±8.0 |

*$p<0.05$ vs. baseline; ** $p<0.05$ vs. baseline and $p=0.05$ vs. candesartan

## CVS-12 Urotensin II in hypertension

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**Introduction:** Urotensin II (UII) is an 11 amino acid cyclic peptide and is the most potent vasoconstrictor known. UII receptors are found in the heart and arterial vessels, including atherosclerotic plaques, suggesting that urotensin II has a role in cardiovascular diseases. UII circulates in human plasma and its plasma concentration is raised in cardiac and renal failure. The role of UII in hypertension has not been investigated.

**Method:** We studied the plasma level of UII levels in 22 hypertensive subjects (68% male, age 51±10 yrs) and 37 normal controls (49% male, age 51±12 yrs ) with consent from the subjects and approval from the Ethics Committee. Plasma UII was measured using a radioimmunoassay.

**Results:** Plasma UII was 10.6±1.4 pg/ml in normotensive controls and 21.0±3.6 pg/ml in hypertensive subjects ($p=0.003$). UII in men and women were not significantly different. UII did not vary significantly with age in normal healthy subjects ($r=0.09$, NS) but was inversely related to age in hypertensive subjects ($r=-0.35$, $p=0.025$). In hypertensive subjects but not in control subjects, UII was related to diastolic blood pressure ($r=0.31$, $p<0.05$). UII is not related to plasma creatinine or creatinine clearance ($p>0.05$).

**Conclusions:** Our results raise the possibility that this novel and potent vasoconstrictor may have an aetiological role in hypertension or may be involved in the complications of hypertension. Antagonists to urotensin II might be antihypertensive agents with a novel mechanism of action.

## CVS-13 Genetic linkage of beta and gamma subunits of epithelial sodium channel to systolic blood pressure in southern Chinese

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**Background:** Mutations of the genes encoding beta and gamma subunits of the epithelial sodium channel (SCNNIB and SCNNIG) has been found to cause increased or decreased channel function, which leads to development of rare, salt-dependent hypertension or hypotension respectively. Both of these two genes are located on chromosome 16p12. Other DNA variants in or around these genes may contribute to the variation in blood pressure. The objective of the study is to evaluate the genetic linkage between blood pressure and the epithelial sodium channel genes (SCNNIB and SCNNIG) in Southern Chinese.
**Methods:** Sixty families were recruited from the general population of southern Chinese. Each family comprised of various numbers of siblings. All participants had their blood pressure measured and were genotyped at chromosome 16p12 by use of three highly polymorphic microsatellite markers. Sibling pairs with different allele-sharing assessed by identity-by-state (IBS) were grouped to compare their blood pressure difference.
**Results:** The mean systolic and diastolic blood pressure difference between all sibling pairs was 16.5 and 11.0 mmHg respectively. At the D16S420 locus, the difference in systolic blood pressure between siblings that shared two alleles according to IBS (n=38, 11.9 ± 10.6mmHg) was significantly lower than siblings that shared one allele according to IBS (n=70, 19.3 ± 18.0mmHg, p=0.02). Difference in systolic blood pressure in other two loci D16S403 and D16S417 were not significant. In all three loci, difference in diastolic blood pressure was not significant between the sibling pairs.
**Conclusion:** The SCNNIB and SCNNIG genes and chromosome region 16p12 are implicated in the physiological variation of systolic blood pressure among southern Chinese. The findings may contribute to the understanding of salt-sensitive hypertension in Southern Chinese.

## CVS-14 Public knowledge in Hong Kong towards cardiopulmonary resuscitation

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**Introduction:** Prompt delivery of cardiopulmonary resuscitation (CPR) substantially improves the prognosis of out-of-hospital cardiac arrest (OHCA) victims. In spite of this, the rate of bystander-initiated resuscitation is appallingly low. Therefore, we conducted a study to evaluate the general knowledge of basic life support in Hong Kong and to identify areas of improvement.
**Method:** This was a cross-sectional descriptive study (n=357) conducted in a standardized interview format via telephone using a structured and fixed-alternative (multiple-choice) questionnaire. The survey was designed to assess general knowledge under a proposed scenario, and experiences regarding CPR training in Hong Kong.
**Results:** We found that (1) CPR knowledge in Hong Kong was poor with approximately 12% of the population having received any form of training; (2) CPR knowledge even among the previously trained is far from satisfactory; (3) knowledge with regard to circulatory maintenance in basic life support was weakest; (4) the commonest reason for not taking CPR training was due to a lack of spare time.
**Conclusions:** The degree of citizen preparedness in initiating CPR is very poor. There is an urgent need to raise public awareness towards the importance of basic life support. In addition, intensified educational efforts and investigations of new approaches to improve this first stage in the chain of survival are warranted.

## CVS-15 The Cytokine response of rat macrophages to lipopolysaccharide is modulated by adrenomedullin

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**Introduction:** Adrenomedullin (AM) is now recognised to be involved in inflammation. We investigated whether AM is an inflammatory or anti-inflammatory cytokine.
**Method:** Rat macrophages (NR8383) were incubated at $10^6$ cells/well in 3.5-cm tissue culture plates in RPMI 1640 medium containing 1% fetal bovine serum in the absence or presence of 10ng/mL to 1μg/mL AM. Conditioned media of parallel cultures were removed at 0, 3, 6, and 24-hours after AM stimulation. Secretion of cytokines including TNF-α and IL-6 were measured using ELISA. To test whether AM is anti-inflammatory, macrophages were activated by 1ng/mL to 1μg/mL lipopolysaccharide (LPS) in the absence or presence of 1_g/ml AM. The cytokine response was measured in the conditioned media at 0, 3, 6, and 24-hours.
**Results:** Basal secretion of TNF-α and IL-6 were 29.2±2.0 pg/mL and 58.7±2.8 pg/mL respectively. AM increased TNF-α concentration by 115.4±41.4% but reduced IL-6 concentration by 56.3±4.9 % at 24 hours. LPS at 1 ng/mL enhanced TNF-α by 45-fold and IL-6 production by 11-fold. The presence of AM reduced the TNF-α and IL-6 response to 66% and 49% respectively.
**Conclusion:** Our results suggest that AM modulates cytokine secretion from rat macrophages and may thus have a regulatory role in inflammation.

## CVS-16 Increased adrenomedullin expression in the heart, the lung and the mesenteric artery by endotoxin

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Previous studies have shown that the circulating levels of adrenomedullin (AM) are elevated during inflammation. The levels of AM and its messenger RNA (mRNA) in various tissues during the time course of inflammation remain to be determined. To study this, inflammation was induced in rats by intraperitoneal injection of lipopolysaccharide (LPS, 10mg/kg). The tissues were harvested at 0, 1, 3 and 6 hours after LPS administration. Tissue levels of AM were determined by radioimmunoassay. The gene expression levels of AM were determined by solution hybridization-RNase protection assay of preproAM mRNA levels. The preproAM mRNA levels were increased in mesenteric artery and right atrium at 1 hour after LPS injection, in the left ventricle and the lung at 3 and 6 hours after LPS injection and in the right ventricle at 6 hours after LPS injection. These results suggest that AM synthesis increases in these tissues by LPS at the time intervals specified. In addition, AM contents increased in the lung at 3 and 6 hours after LPS injection. From these data and the mRNA result, it is concluded that there is an increase in AM release in the lung. As the plasma AM levels were elevated at 3 and 6 hours, the results show that the lung may be an important organ for AM secretion in the septic state. However, the AM levels in the mesenteric artery were increased at 1, 3 and 6 hours whereas the preproAM mRNA were only elevated at 1 hour after LPS injection. The results would indicate an increase in AM release only at 1 hour after LPS injection and a decrease in AM release at 3 and 6 hours. The responses of the lung and the mesenteric artery in terms of AM secretion are different.
Acknowledgement: RGC grant.

## CVS-17 Follow up of the Hong Kong Cardiovascular Risk Factor Prevalence Survey cohort

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**Introduction:** In 1995-6, 2881 randomly chosen Hong Kong men and women participated in the Hong Kong Cardiovascular Risk Factor Prevalence Survey. The subjects are recalled for follow up after 6 years. Here, we report changes in risk factors in the subjects who have been restudied.
**Method:** Subjects were randomly chosen from the cohort and were studied in the morning after overnight fasting. Height, weight, waist and hip circumferences were measured. Blood pressure was measured carefully after resting. Venous blood was taken for analysis of lipids and glucose.
**Results:** 179 subjects (89 M, 90 F; age 51±13 yrs) were studied. The blood pressure was 117.2±1.9/74.0±1.1 mmHg in 1995-6 and 120.3±1.3/75.0±0.8 mmHg in 2001-2. The systolic and diastolic blood pressure both increased by 3.1±2.3 and 1.0±1.3 mmHg respectively ($p>0.05$). In men, the increase in blood pressure was 0.1±2.1/1.4±1.5 mm Hg whereas in women, the increase was 4.6±1.7/2.6±1.3 mmHg. There was a significant increase in systolic blood pressure in women ($p=0.01$). There were no significant changes in body weight and body mass index (BMI), but the waist circumference increased from 81.8±1.1 to 84.3±1.2 cm ($p<0.001$). Plasma cholesterol increased from 5.04±0.11 mmol/L to 5.28±0.10 mmol/L ($p=0.002$).
**Conclusions:** There was an increase in waist circumference, which was accompanied by an increase in plasma cholesterol. There was an increase in systolic blood pressure in women with age. These changes may reflect the development of the metabolic syndrome in some of the individuals.

## CVS-18 Cellular electrophysiology in swine atrial myocytes

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**Introduction**: Pig is a species commonly used for the studies on cardiac diseases including arrhythmias, but the cellular electrophysiology is not fully understood in pig atrium. The present study was to examine ionic contribution to action potential repolarization in pig atrial myocytes.
**Method**: Single atrial myocytes were enzymatically dissociated from pig left atrium. Transmembrane currents were recorded with whole-cell patch clamp technique at 22~23°C.
**Results**: Classical 4-aminopyrine- (4-AP) sensitive transient outward $K^+$ current ($I_{to}$) was not detected with voltage-steps to between –40 and –60 mV from –50 mV in pig atrial myocytes. Instead, $Ca^{2+}$-activated transient outward $Cl^-$ current ($I_{Cl.Ca}$ or $I_{to2}$) was observed in all cells tested (n = 25). $I_{to2}$ showed a bell-shaped *I-V* relationship, and sensitive to inhibition by the $Cl^-$ channel blocker DIDS (100 μM), and replacement of external $Cl^-$ ion. In addition, a sustained delayed rectifier $K^+$ current ($I_{Ksus}$) was observed in pig atrial myocytes. Current-voltage (*I-V*) relationship of $I_{Ksus}$ showed a property of weak inward rectification. The current density was 4.8 ± 0.7 pA/pF at +50 mV (n = 9), and was highly sensitive to inhibition by 4-AP with $IC_{50}$ of 17.5 μM (n = 6). $I_{Ksus}$ was not affected by 10 mM tetraethylammonium or 0.5 mM $Ba^{2+}$. However, the current showed a strong use-dependence at 1 (-60%) and 2 (-80%) Hz.
**Conclusion**: 1) The classical $I_{to}$ observed in hearts of several species including man is not expressed in pig atrial myocytes; 2) $I_{to2}$ is significant in pig atrial cells; 3) 4-AP-sensitive $I_{Ksus}$, similar to ultra-rapid delayed rectifier $K^+$ in human atrium, is present in pig atrial myocytes, but the current is highly suppressed at normal heart rates; therefore, 4) $I_{to2}$ may play an important role in the repolarization of pig atrium, which is different from human.

## CVS-19 Incidence of postural orthostatic tachycardia syndrome (POTS) in Chinese patients

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**Introduction:** Head upright tilt table testing (TTT) has become an accepted modality to evaluate an individual's susceptibility to autonomically mediated hypotension and bradycardia severe enough to cause syncope. Recent studies have identified a milder form of autonomic dysfunction which is known as Postural Orthostatic Tachycardia Syndrome ( POTS). There is no prior report on the incidence of POTS in Chinese patients(pts).
**Methods:** We reviewed the hospital records of 104 patients (43 male, 61 female; mean age 43±21 years) with history of recurrent syncope who were admitted for TTT in our hospital from 1998 to 2002. A positive TTT is defined as the occurrence of symptomatic hypotension and / or bradycardia during upright tilt. POTS is defined as increase in heart rate of > 30 beats/min or maximum heart rate > 120 beats/min in the first 10 minutes of tilting associated with symptoms of dizziness or syncope.
**Results:** 41(39%) patients had positive TTT. Out of 41 pts, there were 23 female pts and 18 male patients with positive TTT ( mean age 33 ± 20 yrs vs 57 ± 23 yrs, p=0.2). Among those patients with positive TTT, only 2 (5%) had POTS. Both patients diagnosed to have POTS were young female (age: 15 and 16 yrs old, respectively). There are no significant differences in the clinical characteristics between patients with POTS and those with positive TTT.
**Conclusion:** The results of the present study demonstrated that POTS is uncommon (5%) in Chinese patients with syncope. Although there is no clinical predictor for the occurrence of POTS in patients with syncope, TTT is useful for diagnosis of this rare and potentially treatable disorder.

## CVS-20 Clinical characteristics and outcome in Chinese patients with hypertrophic cardiomyopathy

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**Introduction** : Hypertrophic cardiomyopathy ( HCM) is a heterogeneous disease with different prevalence of phenotypic characteristics in different countries.Data on the natural history of HCM in Chinese is sparse. The aim of this study was to describe the clinical characteristics and long term outcome in Chinese patients(pts) with HCM.
**Methods** : We studied the clinical characteristics and long term outcome in 112 pts ( 62 male ,40 female)diagnosed in our hospital ( a tertiary referral center)from 1973 to 2001.
**Results***:* The mean age at presentation was 55 ± 15 yrs. The incidences of different types of HCM are: apical hypertrophy -42% ; ventricular septal hypertrophy- 30% ; concentric-13% and others- 15%. During a mean follow-up of 5.4 years( 1-29 years) from presentation, cardiovascular (CVS) mortality was 7.1% (8/112) and annual cardiovascular mortality was 1.3%. Fifty three( 48%) patients had 1 or more major CVS morbidity : atrial fibrillation (36%), congestive heart failure ( 16%), malignant ventricular arrhythmia (12%), cerebrovascular accident (7%), and infective endocarditis ( 3%). Female sex and the occurrence of atrial fibrillation were identified to be significant clinical predictors of CVS mortality ( $p<0.05$).
**Conclusion***:* HCM in Chinese pts are characterized by a late onset of presentation and high incidence of apical variant of HCM ( 42%), suggesting a different genotypical pattern of HCM as compared to Western population. Although the annual CVS mortality in Chinese HCM pts is low( 1.3%), approximately 50% of pts experience serious CVS complications, especially atrial fibrillation on follow-up.

## CVS-21 Do you know your mice? A lesson from SM22alpha knockout mice

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**Introduction**: SM22alpha is a 22-kD smooth muscle cell (SMC) lineage restricted protein that physically associates with cytoskeletal actin filament bundles in contractile SMCs. To examine the function of SM22alpha, gene targeting as used to generate SM22alpha -deficient ($SM22^{-/-LacZ}$) mice.
**Method**: The gene targeting strategy employed resulted in insertion of the bacterial LacZ reporter gene at the SM22alpha initiation codon permitting precise analysis of the temporal and spatial pattern of SM22alpha transcriptional activation in the developing mouse. Multidisciplinary approach was taken to characterize the SM22alpha knockout mice from the ultrastructual organization to whole animal cardiovascular physiology.
**Results**: Northern and Western blot analyses confirmed that the gene targeting strategy resulted in a null mutation. Histological analysis of $SM22^{+/-LacZ}$ embryos revealed detectable beta-galactosidase activity in the unturned E8.0 embryo in the layer of cells surrounding the paired dorsal aortae concomitant with its expression in the primitive heart tube, cephalic mesenchyme and yolk sac vasculature. Subsequently, during postnatal development, beta-galactosidase activity was observed exclusively in arterial, venous and visceral SMCs. SM22alpha -deficient mice are viable and fertile. Their blood pressure and heart rate do not differ significantly from their control SM22alpha $^{+/-}$ and SM22alpha $^{+/+}$ littermates. Cardiac echocardiography revealed preserved systolic function and no significant differences in chamber sizes. The vasculature and SMC-containing tissues of SM22alpha-deficient mice develop normally and appear histologically similar to those of their control littermates. Deconvolution microscopy confirms the colocalization of SM22alpha to actin filaments. However, electron microscopy revealed a subtle ultra structural defect in the actin filament organization.
**Conclusion**: Taken together, these data demonstrate that SM22alpha is not required for basal homeostatic functions mediated by vascular and visceral SMCs in the developing mouse. These data also suggest that signalling pathways that regulate SMC specification and differentiation from local mesenchyme are activated earlier in the angiogenic program than previously recognized.

## CVS-22 Adrenomedullin suppresses migration inhibitory factor production and cytokine response of rat macrophages to lipopolysaccharide

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**Introduction:** Adrenomedullin (AM) is a vasorelaxant peptide that is also involved in inflammation. Macrophage migration inhibitory factor (MIF) is released from pituitary and macrophages and is an important regulator of inflammation. We investigated the interaction between AM and MIF in cultured rat macrophages.
**Method:** Rat macrophages (NR8383) were activated by LPS in the absence and presence of AM at 1ng/mL to 1μg/mL. MIF, TNF-alpha, IL-6 and IL-1beta were measured by ELISA at 0, 1, 3, 6, and 24-hours. The effect of AM on cytokine response from resting macrophages was determined from cell cultures containing RPMI medium and AM alone.
**Results:** AM increased release of MIF from resting macrophage dose-dependently in the first hour by 36.3 % to 75.7 % (compared to control with no AM) while further production in subsequent 24 hours was not significantly affected by the presence of AM. For LPS-stimulated macrophages, AM also increased MIF secretion dose-dependently in the first hour by 13.5% to 35.4% (compared to control containing LPS but no AM), but reduced further production of MIF by 22.6±6.8% at 24 hours. The suppressive effect was observed even at 1 ng/mL of AM. The presence of AM also reduced the production of TNF-alpha, IL-6 and IL-1beta from LPS-stimulated cells by 66%, 49 % and 30 % respectively.
**Conclusion:** Our results suggest that AM modulates cytokine secretion from rat macrophages and may have a regulatory role in inflammation.

## CVS-23 The Heart Protection Study findings with simvastatin reanalysed by number needed to treat

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**Introduction:** *Number Needed toTreat* (NNT) is superior to *Relative Risk Reduction* (RRR) as a means of assessing clinical trial results. We therefore opted to compare relevant RRRs and NNTs in the *MRC/BHF Heart Protection Study(HPS) with Simvastatin* the largest randomized trial of coronary heart disease (CHD) prevention to date, which recruited patients with prior CHD, diabetes or hypertension [HPS Collaborative Group 2002 Lancet 360:7-22].
**Methods:** Using an Excel spreadsheet to enter event rates, NNTs and respective 95% CIs were calculated and compared with corresponding published (or derived) RRRs.
**Results:** Respective event rates, 5 year NNTs & CIs are shown in the table.
**Conclusions:** NNTs are more discriminating than RRRs. They confirm that the benefits of statins: are clinically significant in terms of all-cause mortality and stroke, and that for major vascular events they are similar in persons with CHD only or diabetes only and in all cholesterol level and age categories.

| Outcome of Interest | | Event Rate (%) | | %RRR | NNT |
|---|---|---|---|---|---|
| | | Simvastatin | Placebo | (95% CIs) | (95% CIs) |
| **All Cause Mortality** | | 12.9 | 14.7 | 12 (5 to 18.) | 57 (37 to 128) |
| **Vascular Mortality** | | 7.6 | 9.1 | 17 (8 to 24) | 66 (44 to 134) |
| **Non-vascular Mortality** | | 5.3 | 5.6 | 4 (* to 15) | 444 (* to 117) |
| **1st Major CHD Event** | | 8.7 | 11.8 | 26 (19 to 32) | 32.7 (26 to 45) |
| **1st Stroke** | | 4.3 | 5.7 | 24 (14 to 33) | 73 (50 to 131) |
| **Revascularisaton** | | 9.1 | 11.7 | 22 (15 to 29) | 39 (29 to 58) |
| **1st Major Vascular Event** | | | | | |
| Total Cholesterol (mmol/L) | <5.0 | 17.7 | 23.1 | 23 (12 to 33) | 19 (13 to 35) |
| | ≥5.0 <6.0 | 16.9 | 24.5 | 23 (15 to 30) | 18 (13 to 27) |
| | ≥6.0 | 21.6 | 26.8 | 19 (12 to 26) | 19 (14 to 30) |
| Age (years) | <65 | 16.9 | 22.1 | 23 (16 to 30) | 19 (15 to 28) |
| | ≥65 <70 | 20.9 | 27.2 | 23 (14 to 32) | 16 (11 to 26) |
| | ≥70 | 23.6 | 28.7 | 18 (9 to 26) | 20 (14 to 36) |
| Prior CHD only | | 16.8 | 22.5 | 25 (17 to 33) | 18 (13 to 26) |
| Prior Diabetes only | | 13.8 | 18.6 | 26 (13 to 37) | 21 (14 to 40) |
| Prior CHD + Diabetes | | 33.4 | 37.8 | 11 (* to 24) | 23 (12 to *) |

* Denotes a negative value indicating an increased event rate in the treated group, renderng further analysis inappropriate; 1st major coronary event = non-fatal MI or CHD death; Revascularisation = coronary and non-coronary bypass and angioplasty; 1st major vascular event = 1st major coronary event, stroke or revascularisation

## CVS-24 Endotoxin increases adrenomedullin expression in heart, lung and mesenteric artery

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**Introduction:** Previous studies have shown that the circulating levels of adrenomedullin (AM) are elevated during inflammation. The levels of AM and its messenger RNA (mRNA) in various tissues during the time course of inflammation remain to be determined.
**Method:** Inflammation was induced in rats by intraperitoneal injection of lipopolysaccharide (LPS, 10mg/kg). The tissues were harvested at 0, 1, 3 and 6 hours after LPS administration. Tissue levels of AM were determined by radioimmunoassay. The gene expression levels of AM were determined by solution hybridization-RNase protection assay of preproAM mRNA levels.
**Results:** PreproAM mRNA levels were increased in mesenteric artery and right atrium at 1 hour, in the left ventricle and the lung at 3 and 6 hours and in the right ventricle at 6 hours, after LPS injection. AM levels in the mesenteric artery were increased at 1, 3 and 6 hours and at 3 and 6 hours in the lung after LPS injection.
**Conclusions:** There is an increase in AM release in the lung, so it may be an important organ for AM secretion in the septic state. However, the response of the lung and the mesenteric artery to LPS in terms of AM secretion appears to be different.

## EM-01 Characterisation of novel fat-derived hormones using proteomics-based approaches

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**Introduction:** Fat tissue (adipose) has recently been recognised as an important endocrine organ that secretes a wide range of hormones involved in the regulation of energy metabolism and cardiovascluar tone. The major objective of this study is to use modern proteomics based approach for systematic identification and characterisation of fat-derived hormones with therapeutic potential.
**Methods:** Secreted proteins from 3T3 L1 preadipocytes and mature adipocytes were separated by high-resolution two-dimensional gel electrophoresis (2-DE). Differentially-secreted proteins from these cells were detected by PDQUEST software, and identified by N-terminal protein sequencing and MALDI-TOF mass spectrometry.
**Results and conclusion:** 1. We have found several posttranslationally modified isoforms of adiponectin, a novel hormone with anti-diabetic, anti-inflammatory and anti-atherogenic functions. Further mass spectromic analysis detected several lysine residues within the collagenous domain that are hydroxylated and glycosylated. Mutational studies indicated that these modifications are critically important for the anti-diabetic and anti-inflammatory functions of adiponectin.
2. We have identified a novel fat-derived hormone, which we named as adipocyspin. 2-DE analysis found that expression of adipocyspin was induced during adipose conversion. Computer modelling suggested that adipocyspin shares structural homology with family members of cysteine protease inhibitors. Functional analysis using recombinant adipocyspin demonstrated that this secretory protein could inhibit adipose conversion of 3T3 L1 preadipocytes. This result suggests that adipocyspin might act as a feedback regulator of fat formation (adipogenesis), and that adipocyspin or its agonists could be used as anti-obesity agents.

## EM-02 Predictors of low bone mass in young Chinese women

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**Introduction:** Low peak bone mass is a major risk factor for postmenopausal osteoporosis. Identifying premenopausal women with low bone mass is a cost-effective approach towards prevention of osteoporosis and related fractures in later life.
**Method:** Demographic information and clinical data were obtained from 544 healthy pre-menopausal Chinese women, aged 18-39 years, who were recruited from the community. Predictors of bone mass were assessed using a standardized questionnaire. Bone mineral density (BMD) was assessed using dual-energy X-ray absorptiometry (DXA) at the femoral neck and lumbar spine. Bone mass was considered low if the T-score was <-1.00, i.e., 1 standard deviation below the peak young mean for the local population.
**Results:** The mean age of the cohort was 31.9 +/- 5.7 years. 19% and 26% of our cohort were classified to be low bone mass at lumbar spine and femoral neck respectively. Multivariate logistic regression model revealed that low body weight was the only independent predictor for low bone mass at both the spine (Odds ratio 5.3, confidence intervals 3.3-8.7, $p<0.0001$) and femoral neck (Odds ratio 4.4, confidence intervals 2.9-6.8, $p<0.0001$) while daily weight bearing time of less than 1 hour was an additional risk factor for low bone mass at lumbar spine (Odds ratio 4.0, confidence intervals 1.1-14.2, $p=0.03$). All other factors including height, menarche age, calcium intake, family history of fracture, use of calcium supplement, contraceptive pill use and smoking or drinking habit were not predictive of peak bone mass in these young women.
**Conclusion:** Low body weight and lack of weight bearing exercise are the two most important risk factors for low peak bone mass in Chinese women. Early intervention in this group of women may reduce the risk for osteoporosis in later life.

## EM-03 Transforming growth factor-beta1 gene polymorphisms and bone turnover, bone mineral density and fracture risk in southern Chinese women

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Genetic contributions play an important role in determining bone mineral density (BMD) and bone turnover. Transforming growth factor-beta (TGF-beta) is abundant in bone and has been implicated as an important regulator of both bone formation and resorption. A T→C polymorphism in exon 1 of the TGF-beta1 gene, which results in the substitution of proline for leucine, has recently been suggested to be associated with higher BMD. In the present study, we analyzed the relationship between TGF-beta1 polymorphism and BMD in southern Chinese women. The TC polymorphic site of TGF-beta1 gene at nucleotide position 29 was analyzed in 366 healthy southern Chinese women by direct sequencing. BMD at lumbar spine and hip region, biochemical markers of bone turnover and PTH level were measured. Among the postmenopausal women (n=239), the prevalence of fragility fractures was significantly higher in individuals with TC genotype ($p<0.05$). Serum alkaline phosphatase and osteocalcin as well as urine N-telopeptide excretion were significantly higher in women with TC than TT or CC genotypes (all $p<0.05$). Women with TC genotype had lower BMD at total hip, femoral neck and trochanteric region. However, after adjusting for age, height, weight and years since menopause, the BMD at both the hip and spine did not differ among the three genotypes. With respect to the premenopausal women (n=127), no difference was seen in the BMD at the hip or spine among the three groups. In conclusion, we observed an association between TGF-beta1 gene polymorphism and bone turnover as well as fragility fractures in postmenopausal southern Chinese women.

## EM-04 Development of a murine model of autoimmune thyroiditis induced with homologous mouse thyroid perioxidase

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Autoimmune Thyroid Disease (AITD) is a common condition affecting mostly female subjects. Thyroid peroxidase (TPO) is a well-characterized autoantigen in AITD. Autoantibodies and autoreactive T lymphocytes to TPO are believed to play a major role in the pathogenesis of lymphocytic thyroiditis. To understand the pathogenic mechanisms of AITD and the role of TPO, we have established a mouse model of lymphocytic thyroiditis by immunizing C57Bl/6(H-$2^b$), CBA (H-$2^k$) and C57Bl/6 × CBA F1 mice with recombinant mTPO (rmTPO) ectodomain comprising amino acid residue 1-837 produced in E.coli. Mice were immunized with 30mg purified ectodomain in complete Freund's adjuvant. Antibodies against rmTPO were detected in the serum of all mice from week 3 onwards. Draining lymph node cells from rmTPO-immunized animals showed dose dependent proliferation to TPO stimulation. Mice sacrificed at day 50 revealed variable degree of thyroiditis with infiltration of mononuclear cells and destruction of thyroid follicles. C57Bl/6 and the F1 mice, in comparison to CBA mice, showed greater degree of thyroiditis and subnormal serum T4 levels. The degree of histological features of thyroiditis corresponded to the biochemical abnormalities of these animals but not to the anti-TPO antibody response. Immunotyping of the thyroid cellular infiltrates showed predominantly CD4+ T cells and B220+ B cells but scanty CD8+ T cells. None of the control mice injected with the purified fusion partner developed anti-TPO antibodies and thyroiditis. Significant weight gain was exhibited in the mTPO-immunized animals, with the degree of hypothyroidism correlated strongly with the amount of weight gain, giving additional supportive evidence of thyroid dysfunction. In conclusion, a genuine mouse model of AITD induced with rmTPO was established which possessed all features of lymphocytic thyroiditis. This model will enable the study and understanding of the autoimmune process of AITD and may assist in the development of new strategies for modulating the pathogenic immune response involved in this disease.

## EM-05 The effect of dietary caloric density on neuropeptide response in mice

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**Introduction** : Hypothalamic neuropeptides, such as pro-opiomelanocortin (POMC), an anorectic peptide and neuropeptide Y (NPY), an orexigenic peptide, play key roles in the regulation of energy balance. The regulation of their expressions may be important in determining susceptibility to diet-induced obesity.

**Methods** : Three strains of mice (C57BL/6J, 129/Sv and A/J) known to have different susceptibilities to diet-induced obesity were used. They were fed a 23.6% fat, w/w, high fat diet (HF) or a 4.3% low fat control diet for two days. In the second experiment, four groups of each strain of mice were placed on diets with different fat contents and caloric densities for a period of six weeks. In both experiments, neuropeptide mRNA expression was measured using real-time quantitative reverse transcription polymerase chain reaction (RT-PCR).

**Results** : The weight gain of the mice over 6 weeks correlated with the caloric density rather than the fat content of the diets. A differential weight gain was observed between strains with C57BL/6J having the lowest threshold in developing obesity, suggesting an influence of susceptibility genes. On acute exposure to a high caloric density diet, C57BL/6J had a paradoxical rise in hypothalamic NPY expression ($p<0.01$) despite a trend to higher caloric intake. After six weeks on diets with increasing caloric density, both C57BL/6J and 129/Sv developed significant suppression in POMC expression compared to those on control diets ($p<0.05$) despite the presence of hyperleptinaemia. In contrast, the least obesity-prone A/J mice mounted a rise in POMC expression on acute exposure to a high caloric density diet ($p<0.05$) and maintained POMC expression even after prolonged exposure.

**Conclusions** : It is proposed that an acute rise in POMC expression in response to a high fat challenge may be a predictor of low susceptibility to adiposity. The dysregulation of POMC neurons in response to high caloric intake may be important in mediating diet-induced obesity.

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## EM-06 The clinical genetics of multiple endocrine neoplasia type 1 in Chinese

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**Introduction** : Multiple endocrine neoplasia type 1 (MEN 1) is characterized by a triad of neoplasia affecting the parathyroid glands, enteropancreatic endocrine tissue and the anterior pituitary gland.

**Methods** : In order to define the prevalence of MEN 1 germ-line mutations in Southern Chinese patients with MEN 1 syndrome, we performed direct sequencing of the entire open reading frame of the MEN1 gene for twelve index patients and their first degree relatives.

**Results** : Six patients had familial MEN 1 syndrome and six had sporadic disease. Nine different germ-line mutations at the MEN1 gene were identified, including three novel mutations (248-249delTT in exon 2, K559X (AAG→TAG) in exon 10 and IVS 2nt+2(G→T) in intron 2). All patients with familial MEN 1 syndrome were heterozygous carriers of a germ-line mutation and MEN 1-related disorders were only evident in their first-degree relatives who also carried the mutation. All patients with enteropancreatic lesion were mutation carriers and the absence of mutation in three apparently sporadic MEN 1 patients with only hyperparathyroidism and pituitary microadenoma might represent the presence of MEN1 phenocopy.

**Conclusions** : The finding of MEN1 germ-line mutation in all patients with familial MEN 1 syndrome suggests that genetic screening should be useful in our population to identify affected individuals within a kindred and allow early detection of MEN1-related tumours.

**Acknowledgement** : This work is supported by CRGC Grant, University of Hong Kong

## EM-07 The beneficial effects of Qigong and conventional exercise on blood pressure and anthropometric indices in type 2 Chinese diabetic patients

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**Introduction:** Exercise is generally accepted as a cornerstone of diabetes management, because it promotes caloric expenditure and improves insulin sensitivity. Qigong is an alternative exercise program, widely practiced in China, which is believed to have the effect of self-regulating various functional activities of the body, leading to alterations in metabolic rate and potential benefits on glycaemic control. In this study we investigated the effects of qigong versus conventional exercise in 100 Chinese type 2 diabetic patients with suboptimal glycaemic control.
**Method:** Patients were randomised to either of 2 groups, to learn and practice the scheduled conventional exercise or qigong program with matched exercise intensity for 18 weeks, under the supervision of a physiotherapist and qigong therapist, respectively. They were also instructed to practice it at home. All medications remained unchanged throughout the study.
**Results:** During the study period neither group showed any significant improvement in HbA1c or insulin sensitivity as measured by HOMA or QUICKI. Qigong had a superior effect on HDL-C, with a significantly larger proportion of subjects having a HDL-C>0.9mmol/l at the end of the study (OR=1.84; p=0.023 versus conventional exercise), but a smaller proportion of subjects maintaining a BMI<23 (OR=0.7; p=0.038). When data from the two groups were combined, an improvement in both systolic and diastolic blood pressure was observed, increasing in magnitude as the study progressed (sBP: -3.42±1.37, p=0.012 at week 10 and –4.52±1.57, p=0.004 at week18; dBP: -3.23±0.98, p=0.001 at week 10 and –5.07±1.15, p<0.001 at week 18). Similar reductions in waist and hip circumferences were also noted (waist: -0.32±0.12cm, p=0.006 at week 10 and –0.49±0.16cm, p=0.002 at week 18; hip: -0.30±0.11cm, p=0.006 at week 10 and –0.38±0.16cm, p=0.015 at week 18).
**Conclusion:** We concluded that long-term exercise, either qigong or conventional exercise, was associated with beneficial effects on various aspects of the metabolic syndrome in patients with type 2 diabetes.
**Acknowledgement:** This study was supported by funding from the Li Ka Shing Foundation.

## EM-08 The effect of glycoxidized LDL on monocytes/ macrophages cell line (THP-1) gene expression

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**Introduction**: Cardiovascular complications are the leading cause of morbidity and morality in patients with diabetes mellitus. Epidemiological studies have shown that hyperglycemia and dylipidemia are two risk factors for diabetic atherosclerosis. Lipoprotein is subjected to both glycation and oxidation in diabetes. The biological effects of glycoxidized LDL have not been well characterized. The objective of this study is to investigate whether glycoxidized LDL induce the gene expression of human monocyte/macrophages, using the THP-1 cell line, by modified lipoprotein.
**Methods**: Glycoxidized LDL was obtaining by incubating LDL with glucose and copper ion for 7 days. The extent of oxidation was measured by TBARS assay. Monocytes/ macrophages cell (THP-1) was incubated with glycoxidized LDL for one day. The gene expressions were quantified using quantative PCR.
**Results**: The oxidation of glycoxidized LDL was significantly higher than native LDL (7.12±2.7 nmol MDA/mg protein versus 1.615±0.459 nmol MDA/mg protein). The gene expression of CD36 has been upregulated by 4.6±1.09 folds when incubated with glycoxidized LDL at 100ug/ml. The gene expression of SR-BI was suppressed by glycoxidized LDL by 4.8±1.64 folds.
**Conclusion:** Glycoxidized LDL regulate the gene expression of CD36 which may increase the binding and internalization of modified LDLs by macrophages. Down regulation of SR-BI may lead to a decrease in cholesterol efflux. These gene expression profiles have suggested some potential mechanisms for the contribution of modified LDL to the development of diabetic atherosclerosis.

## EM-09 High blood pressure is related to obesity in Hong Kong

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**Introduction:** Obesity predisposes to the development of hypertension. We have previously found that blood pressure correlated with indices of obesity in Hong Kong Chinese. We sought to confirm this association in subjects randomly chosen from the general population.
**Method:** 813 subjects (393 men, 420 women; age 51±12 yrs) recruited for the Hong Kong Cardiovascular Risk Factor Prevalence Survey-2 (CRISPS2) were studied. 214 of them were hypertensive (systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90 mmHg or taking antihypertensive medication) and the other 599 were normotensive. The medical history was obtained and the subjects were examined with special attention to blood pressure and indices of obesity. Body fat was assessed using bioelectrical impedence.
**Results:** Diastolic blood pressure (DBP) was related to age ($r=0.16$, $p<0.001$) and indices of obesity including body mass index ($r=0.35$, $p<0.001$), body fat ($r=0.12$, $p=0.004$), waist circumference (WC) ($r=0.40$, $p<0.001$) and waist-to-hip ratio ($r=0.35$, $p<0.001$). Systolic blood pressure (SBP) was also related to age ($r=0.50$, $p<0.001$), WC ($r=0.37$, $p<0.001$) and the other indices of obesity. Multiple regression analysis suggested that WC ($ß=0.40$, $p<0.001$) was the only independent predictor of DBP, whereas age ($ß=0.45$, $p<0.001$) and WC ($ß=0.23$, $p<0.001$) were independent predictors of SBP ($R^2=0.30$, $p<0.001$). DBP correlated with WC in both men ($r=0.32$, $p<0.001$) and women ($r=0.37$, $p<0.001$). Similarly, SBP correlated with WC in both men ($r=0.20$, $p<0.001$) and women ($r=0.40$, $p<0.001$).
**Conclusions:** Our results confirm that blood pressure is related to obesity. WC is a simple measurement and a good predictor of blood pressure, especially in women. As the relationship between blood pressure and obesity is continuous, tackling obesity in the community as well as in hypertensive individuals may reduce the incidence and prevalence of hypertension and other cardiovascular risk factors.

## EM-10 Correlation between plasma phospholipid transfer protein activity and low density lipoprotein subfractions pattern in subjects with type 2 diabetes mellitus

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**Introduction:** Phospholipid transfer protein (PLTP) plays an important role in the remodelling of high density lipoprotein (HDL) by facilitating the transfer of phospholipids between lipoproteins. It may also enhance cholesteryl ester transfer mediated by cholesteryl ester transfer protein (CETP). Since the formation of small dense low density lipoprotein (LDL) also involves lipid exchange between LDL particles and triglyceride-rich lipoproteins, we have investigated the relationship between plasma PLTP activity and LDL subfractions pattern in patients with type 2 diabetes mellitus (DM).
**Methods**: 240 subjects with type 2 DM and 136 controls were recruited. All subjects were non-smokers and non-drinkers. Plasma PLTP activity was evaluated by measuring the transfer of radiolabelled phosphatidyl-choline from liposomes containing radiolabelled phosphatidylcholine to plasma HDL. LDL subfractions were determined by density gradient ultracentrifugation.
**Results:** The diabetic subjects had higher plasma triglyceride ($p<0.001$) and lower HDL ($p<0.001$) than the non-diabetic controls. They also had significantly higher concentration of small dense LDL-III (118.7 mg/dl ± 52.8 vs 88.0 ± 45.3, $p<0.001$). Plasma PLTP activity was increased in the diabetic patients (2.09 umol/ml/h ± 0.54 vs 1.60 ± 0.41, $p<0.001$) and the difference remained significant after adjusting for age, sex, body mass index. There was an association between plasma PLTP activity and HbA1c ($r = 0.17$, $p<0.01$) but no correlations were found between plasma PLTP activity, triglyceride or HDL. However, plasma PLTP activity correlated with small dense LDL-III concentration ($r = 0.29$, $p<0.001$).
**Conclusions:** The diabetic subjects have higher concentration of small dense LDL-III and plasma PLTP activity. The positive correlation between these two parameters in subjects with type 2 DM suggested that PLTP might be involved in the formation of small dense LDL particles by facilitating lipid exchange.

## EM-11 Effect of the microsomal triglyceride transfer protein -493 G/T polymorphism and type 2 diabetes mellitus on LDL subfractions

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**Introduction.** Genetic variation in the microsomal triglyceride transfer protein (MTP) affects the secretion pattern and plasma concentration of apolipoprotein (aopB)-containing lipoproteins and the common functional –493 G/T polymorphism has been reported to influence plasma lipids levels. Recent data suggest that carriers of the T allele might be more sensitive to detrimental factor such as features of the insulin resistance syndrome. Since type 2 diabetes is associated with obesity and insulin resistance, the present study investigated the effect of this polymorphism on plasma lipids, apoB and LDL subfractions in 281 Chinese type 2 diabetic patients and 364 non-diabetic controls.
**Methods.** The polymorphism for each subject was determined by polymerase chain reaction, restriction enzymatic action and subsequent electrophoresis on 3% metaphor agarose gel.
**Results.** The frequency of the rare T allele was 0.162 and 0.126 in subjects with and without diabetes respectively. Diabetic subjects had significant higher body mass index and waist hip ratio than the controls ($p<0.01$). There were no differences in the effect of the polymorphism on plasma lipids and apoB in the 2 groups. However, the TT genotype was associated with a higher concentration of small dense LDL-III than the GT or GG variants in the diabetic subjects ($p = 0.01$) whereas no such effect was observed in the controls. In the diabetic patients, age, plasma triglyceride and the MTP genotype were independent determinants of LDL-III concentrations in linear regression analysis ($R^2 = 10\%$, $p <0.05$) whereas in the controls, only plasma triglyceride and age were important determinants ($R^2 = 15\%$, $p <0.01$).
**Conclusion.** The -493 G/T polymorphism has a minor effect on LDL subfraction pattern in Chinese and the effect is only apparent in the presence of type 2 diabetes.

## EM-12 Up-regulation of aldose reductase activity in cultured mesangial cells overexpressing human aldose reductase gene is associated with increased TGF-beta1 and collagen IV expression

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**Introduction**: Increased flux of glucose through the polyol pathway involving the enzyme aldose reductase (AR) has been implicated in the pathogenesis of diabetic nephropathy. However, the proposed mechanisms to explain the role of AR and its interaction with other pathogenetic pathways, such as enhanced production of advanced glycation endproducts (AGEs),TGF-beta1and extracellular matrix including collagen IV in diabetic nephropathy remain controversial. In the present study, we examined the effect of increased AR activity on TGF-beta1 and collagen IV expression in mesangial cells derived from transgenic mice expressing human AR (hAR) with AGEs-BSA application.
**Methods**: We established a transgenic mouse model expressing hAR in kidney mesangial cells. Primary cultures of the mesangial cells from hAR transgenic (TG) and wildtype (WT) mice were treated with 0.1mg/ml of either AGEs-BSA or non-glycated BSA for 24 hours with and without the addition of an AR inhibitor, zopolrestat (0.01mM). hAR mRNA and protein expression were measured by RT-PCR and Western blotting respectively. AR activity, and mRNA levels of TGF-beta1 and collagen IV in mesangial cells were examined by spectrophotometry and RT-PCR.
**Results**: hAR mRNA and protein expression were demonstrated in TG, but not in WT mesangial cells. AR activity was also increased in TG mesangial cells ($P<0.05$ versus WT mesangial cells). Enhanced AR activity was seen in both WT and TG mesangial cells ($P<0.05$), when treated with AGEs-BSA. The increase in AR activity in the presence of AGEs-BSA was accompanied by increases in TGF-beta1 and collage IV transcripts in mesangial cells, which reached statistical significance only in the cells from TG animals ($p<0.01$). Preliminary data show that this increased TGF-beta1 mRNA expression and AR activity can be partily abolished by the addition of an AR inhibitor.
**Conclusion**: These results suggest that AR may be involved in the increased expression of TGF-beta1 which contributes to the mesangial cell proliferation and matrix protein production observed in diabetic nephropathy, in part through an interaction with AGEs.
**Acknowledgement**: This is an on-going project sponsored by the **UDM Research Grant** 02/04.

## DMM-01 Etiology in 16 cases of toxic epidermal necrolysis and Stevens-Johnson syndrome admitted within 8 months in a teaching hospital

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**Introduction**: 8 patients with toxic epidermal necrolysis and 8 patients with Stevens-Johnson syndrome were admitted consecutively to Queen Mary Hospital between August 2001 and March 2002. The objective of this study was to determine the local aetiology, including the possible role of viral infection as a co-factor.
**Method**: A retrospective analysis of all cases of TEN and SJS treated in Queen Mary Hospital within this 8-month period was carried out. Etiological study including drug, viral serology and PCR was performed in view of the clustering of admissions related to these two conditions.
**Results**: Majority is drug-induced. The most common drug was allopurinol (TEN, 50%; SJS, 13%), followed by anticonvulsants (25%), antibiotics and NSAID. The mean duration of drug exposure prior to the onset of symptoms was 14 days, with a range of 1-40 days. The drugs potentially responsible for TEN and SJS were largely the same. Two cases were probably attributed to drug abuse for recreational purpose, including inhalation of organic solvent and use of oral phenobarbital. One case of SJS was believed to be caused by "traditional Chinese herbs". Only one case of SJS had no likely responsible drug identified. Possible etiological co-factors were cancers (19%), radiotherapy and renal failure.
All the patients were negative for CMV and parvovirus B19 IgM. None of the 12 tested patients had HHV-6 or HHV-7 DNA detected in the plasma. There was no difference in seroprevalence between patients and controls for EBV VCA IgG, parvovirus IgG, HHV-6 IgG or HHV-7 IgG.
**Conclusion**: We concluded that drugs remain the most important etiology. No association with viral infection, including human herpesvirus-6 and parvovirus B19, was detected in the present series. Early diagnosis and prompt withdrawal of suspected drugs remain the most important measures in managing this condition.

## DMM-02 Efficacy and safety of tacrolimus ointment monotherapy in Chinese patients with moderate to severe atopic dermatitis

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**Introduction**: Tacrolimus is an effective immunosuppressant in inhibiting T-cell activation. The objective was to evaluate safety and efficacy of topical tacrolimus ointment in treating signs and symptoms of moderate and severe atopic dermatitis in Chinese patients.
**Method**: This was an open noncomparative multicenter study conducted during the spring and summer seasons in Hong Kong. Treatment of tacrolimus ointment was given to all affected areas twice daily for up to 4 weeks. Topical or systemic corticosteroids were disallowed. Patients were assessed at weekly interval for adverse events and efficacy using the Eczema Area Severity Index (EASI), Physician's Global Evaluation of Clinical Response (PGECR) and Patient's assessment of itch using visual analogue scale.
**Results**: 43 patients aged 3-23 years of age with moderate to severe atopic dermatitis involving 15-90% body surface area at baseline were recruited and 39 patients completed the study. Compared to baseline, the mean EASI decreased by 49% after treatment for 4 weeks ($p<0.001$) and scale for patients' assessment of itch decreased by 43% at Week 4 ($p<0.001$). 25% of patients showed marked or excellent improvement (>75%) in PGECR at week 1 and the percentage was maintained at the end of treatment. Burning sensation at the site of application was the most common adverse event reported. No patients complained of discomfort related to excessive greasiness at the sites of ointment application.
**Conclusion**: We concluded that the short-term monotherapy of tacrolimus in an ointment base was well tolerated during the hot and humid seasons in Hong Kong. It was effective in treating the signs and symptoms of moderate to severe atopic dermatitis in Chinese patients. Tacrolimus ointment provides a new therapeutic modality for the topical treatment of atopic dermatitis, especially in patients suffering from steroid-induced skin atrophy. The main limitation for its use as first line treatment is its cost.

## DMM-03 Q-switched alexandrite laser for the treatment of nevus of Ota increase viscoelasticity of the treated area

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**Background and Objective:**
The mechanism of action by laser or intense pulsed light source to produce the effect of non-ablative skin rejuvenation involves injury to the vessels of the papillary dermis, heating up of the dermis leading to collagen shrinkage and increase in fibroblastic activities. The use of Q-switched alexandrite laser (QS Alex) for the treatment of nevus of Ota based upon the principle of selective photothermolysis and as a result, it should not lead to non-ablative skin rejuvenation. Our objective is to assess the viscoelasticity of nevus of Ota patients after treatment with QS Alex to test the hypothesis that QS Alex can be a tool for non-ablative skin rejuvenation.
**Study design/material and method:**
34 patients with nevus of Ota were treated with QS Alex (mean fluence: 9.5J/cm2, 4-8 treatment sessions per patient). The viscoelasticity of the nevus was assessed by a cutometer at baseline and before each treatment. To ensure consistency, the site of assessment was marked on the translucent paper at the first appointment.
**Result:**
There was significant increase in elasticity and firmness in area treated with QS Alex (p=0.0001 and 0.001 respectively).
**Conclusion:**
When used for the treatment of nevus of Ota, QS Alex can lead to non-ablative skin rejuvenation. Given the theory of selective photothermolysis, such effect is unlikely to be due to laser damage to the surrounding tissue. There may be mechanism other than previously propose that can lead to non-ablative skin rejuvenation in nevus of Ota patients treated with QS Alex.

## DMM-04 The use of 1320 nm Nd:YAG laser for the treatment of acne scar in Asians

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**Background:**
Much work have been done looking at the use of 1320 nm Nd:YAG laser for non-ablative treatment skin rejuvenation of photoageing but its role for the treatment of acne scar has not been well documented.
**Objective:**
To assess subjectively and objectively the role of 1320 nm Nd:YAG laser in the treatment of acne scar.
**Method:**
8 acne scar patients were treated with 1320 nm Nd:YAG laser. All have received treatment at monthly interval for at least 4 months before assessment. All patients were assessed using a structure questionnaire for the degree of scar depth improvement and overall satisfaction. A cutometer was used for the objective measurement of skin elasticity and firmness.
**Result:**
All patients noticed at least mild degree of improvement in term of scar depth with 3 out of 8 patients noticed moderate to significant degree of improvement. Adverse effects including post-inflammatory hyperpigmentation were transient only. Cutometer indicated improvement in elasticity in some but not all parameters. Such observation may be related to the fibrosis nature of some of the acne scars.
**Conclusion:**
1320 nm Nd:YAG laser can be used for the treatment of atrophic acne scar.

## DMM-05 Random control study looking at the use of 1320 nm Nd:YAG laser and intense pulsed light source for non-ablative skin rejuvenation in Chinese

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**Background:**
Non-ablative rejuvenation with a 1320 nm Nd:YAG laser or intense pulse light source has generated much interest. Our objective is to study and compare the use of 1320 nm Nd:YAG laser/intense pulsed light source in non-ablative skin rejuvenation in Chinese
**Method:**
47 patients with Glogau classification type 2 photoageing were recruited into the study and were randomized to receive treatment with either 1320nm laser, or intense pulsed light source. All have received treatment at monthly interval for at least 4 months before assessment. All patients were assessed using a structure questionnaire for the degree of improvement in term of skin texture, pigmentation and wrinkle improvement. A cutometer was used for the objective measurement of skin elasticity and firmness
**Result:**
For subjective degree of improvement, 37% patients treated with 1320nm Nd-YAG recorded moderate to significant improvement as compare to 20% of those treated with IPL. However, in term of pigmentation, all patients treated with IPL recorded at least mild degree of improvement as compared to 33% in the cooltouch group. Cutometer assessment indicated significant improvement in firmness and elasticity of most of the important parameters for 1320nm Nd-YAG but only some of the parameters for IPL.
**Conclusion:**
1320nm Nd:YAG appears to be more effective in the improvement of wrinkle than IPL. However, in term of pigment reduction, IPL appears to be more superior. A combination approach using both 1320nm Nd:YAG and IPL can be particularly applicable in the use of non-ablative skin rejuvenation for Asian.

## DMM-06 Bioinformatics data mining and statistical modeling on the molecular interactions between S-adenosylmethionine decarboxylase and its substrate

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**Introduction & Method:** S-adenosylmethionine Decarboxylase (AdoMetDC) is a rate-limiting enzyme in polyamine synthesis pathways. Significance of its conserved amino acids in relationship to function has been studied with respect to the molecular evolution and phylogenetic division of organisms. This paper attempts to use computational technologies to align and model for the homology of 28 species of AdoMetDCs using 1JEN, the human AdoMetDC, as the template; and to study the sites of molecular interactions, atom-atom interactions and bond-lengths, and docking energies between the 28 AdoMetDCs and 2 models of AdoMet. We have applied data from studies on conserved amino acid mutagenesis,and performed Gramm docking program (3D-models, bond-lengths and docking energies) and Afterdock statistics program (statistical analysis of group-distance-energy) to determine the most probable models for AdoMet and AdoMetDC interactions.

**Result & Conclusion:** Results indicated that the Pro225 and Pro246 of AdoMetDCs are involved in binding the adenine group of AdoMet and the Cys226 serves as an electron donor to facilitate the decarboxylation reaction, and the Ser68 undergoes serinolysis to form Pyruvol68, which in turn serves as an electron sink to break the C—$COO^-$ bond from AdoMet. The sequential binding, activation and catalysis of the reaction are shown to be similar in all the 28 species of pyruvoyl-dependent AdoMetDC reactions, regardless of their highly variation of AdoMetDC amino acid sequences.

**Acknowledgement:** Thanks to Professor A. Danchin of HKU Pasteur Research Centre for the amino acid sequences of 28 species of the enzyme S-Adenosylmethionine Decarboxylase

## DMM-07 Computer generated superstructures of scrapie fibril

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**Introduction**: The conformational change of normal prion $PrP^{C}$ to scrapie prion $PrP^{Sc}$ and its subsequent polymerisation is thought to underlie prion disease. Scrapie fibril deposits are diagnostic of prion disease. In the conversion of $PrP^{C}$ to $PrP^{Sc}$ there is an increase in beta-sheet content from 3% to between ~40%. All the results from various prion structural studies and computer predictions of the $PrP^{Sc}$ points to the involvement of the residues 90-140, which are required for prion infectivity, in the conformational change due to it's relative flexibility and lower thermodynamic barrier to template-assisted conformational change to beta-rich $PrP^{Sc}$. We have attempted to design and engineering the scrapie $PrP^{Sc}$ and its fibril supramolecules by computer technology, in order to understand its disease mechanism.
**Method**: Four methods have been developed and used in our study, namely, protein engineering of $PrP^{Sc}$ 3D structure, GRAMM (Global Range Molecular Matching) computer docking, superstructure models generation, and protein interaction screening.
**Results**: Based on a proposed 3D structure of $PrP^{Sc}$ chosen from six penultimate models of $PrP^{Sc}$ from previous findings and predictions, we constructed a model of $PrP^{Sc}$ using the computer pdb model of the Syrian hamster prion protein which corresponds to the infectious fragment of the scrapie isoform (PDB accession 1B10). We applied the above computer technology to generate a model of the scrapie molecule, and its fibrillar superstructure. Our work described here has provided a pdb model of what has previously been suggested for the structure of $PrP^{Sc}$. This provides structural biologists with a 3D model to manipulate and visualize on computer. Using this computer pdb file we have then used computerized docking methods to find a best fit for two monomers of $PrP^{Sc}$. Our results have demonstrated that there is a low energy interaction for two $PrP^{Sc}$ molecules, which when repeated for other $PrP^{Sc}$ monomers, produces a helical linear fibril of $PrP^{Sc}$ subunits. The structure of the oligomer we have put forward suggests a method of polymerisation of these individual $PrP^{Sc}$ oligomers which is consistent with E.M. data. We have analysed the atom-atom interactions between scrapie units as well as the amino acid to amino acid interactions between fibrils.
**Conclusion**: This study serves as a structural basis for future analysis of disease mechanism. We have also provided a suggestion for the molecular basis of scrapie polymerisation. Computer docking provides a fully replicable and exact method for searching for potential interactions between two molecules, and combined with our models of $PrP^{Sc}$ monomer and oligomer potential drug discovery *in silico* has been made possible.

## DMM-08 Electron paramagnetic resonance (EPR) oximetry: a novelopportunity for monitoring tissue and cellular oxygen concentration in biological systems *in vivo* and *in vitro**

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**Introduction:** One of the important aspects of Electron Paramagnetic Resonance (EPR) technology is to monitor cell and tissue oxygen concentrations in different biological systems. Oxygen broadens the EPR spectral line width of the oxygen sensitive probe, i.e. nitroxides, via Heisenberg exchanges, and this property is used to determine oxygen concentration. To enhance the utilization of EPR spectroscopy, we synthesized several isoindoline nitroxides and evaluated their oxygen sensitivity, metabolic kinetics and cytotoxicity. In addition, we developed a novel EPR technique to detect simultaneously extracellular and intracellular oxygen concentration *in vitro* and, localized and mapped tissue oxygen concentration in different rats and mouse models *in vivo*.
**Method:** Oxygen sensitivity was evaluated by the line width of EPR spectra. Metabolic kinetics was measured by the conversion of nitroxides into hydroxylamines. Cytotoxicity was measured by oxygen consumption rates, trypan blue exclusion and clonogenicity assay. Extra- and intracellular oxygen were measured by using lithium phthalocyanine (LiPc) and 4-oxo-2,2,6,6-tetramethylpiperidine-$d^{16}$-1-$^{15}$N-oxyl ($^{15}$N-PDT) in presence of gadolinium complex. The line widths of EPR spectra were converted to oxygen concentration with calibration curve. With X-band EPR spectroscopy, we observed the oxygen concentration between extra- and intracellular compartments in Chinese Hamster ovary (CHO) cells. With L-band EPR spectroscopy and imaging systems, we measured and mapped the distribution of oxygen in different animal models *in vivo*.
**Results:** (1) Isoindoline nitroxides had the properties of high oxygen sensitivity and low cytotoxicity. (2) A significant gradient of oxygen concentration between the extracellular and intracellular compartments existed in CHO cells. (3) The *in vivo* EPR spectroscopy had high sensitivity for monitoring real-timely tissue oxygen concentration in whole animal, especially in hypoxia and ischemia conditions.
**Conclusion:** The techniques of *in vitro* and *in vivo* EPR spectroscopy can provide useful and even unique information pertinent to the studies of oxygen concentration in different modelling systems.
* This work was supported by Seed Funding for Basic Research, HKU and UDM Department Research Grant.

## DMM-09 Plasma membrane cholesterol homeostasis is essential for preventing oxidative damage in wild-type Chinese hamster ovary cells

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**Introduction:** The interrelation between plasma membrane cholesterol and cellular oxidative damage is unclear yet. Our recent study suggests that plasma membrane cholesterol appears to be a regular barrier to intracellular oxygen concentration. The generation of reactive oxygen species (ROS) is greatly depended on tissue oxygen concentration. In this study, we hypothesize that the abnormal of plasma membrane cholesterol would augment ROS production and increase the vulnerability of the cells to oxidative stress.
**Method:** To test this hypothesis, we investigated intracellular oxygen concentration, ROS production and apoptotic cell death in normal and mutant Chinese hamster ovary (CHO) cells defective in cholesterol synthesis and metabolism. Those cells were exposed to different oxidants such as xanthine/xanthine oxidase, hydrogen peroxide and menadione, etc. Plasma membrane cholesterol was detected with microenzymatic assay and filipin-staining confocal microscopy. Intracellular oxygen concentration was measured with 4-oxo-2,2,6,6-tetramethylpiperidine-$d^{16}$-1-$^{15}$N-oxyl ($^{15}$N-PDT) in presence of gadolinium complex. The spectrum of $^{15}$N-PDT was recorded with X-band electron paramagnetic resonance (EPR) spectroscopy. Oxygen concentration was simulated by the conversion of the EPR line width of $^{15}$N-PDT. ROS was trapped with 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) and detected with X-band EPR spectroscopy. DNA fragmentation was detected by agarose gel electrophoresis and ELISA cell death kit.
**Results:** After treated with the oxidants, the high cholesterol cells had lower intracellular oxygen concentration and higher superoxide level and apoptotic cell death rates than normal cholesterol cells. It is interesting that the low cholesterol cells had higher intracellular oxygen concentration and apoptotic cell death rates than the normal cholesterol cells. The low cholesterol cells showed different levels of superoxide under the treatments of xanthine/xanthine oxidase, hydrogen peroxide and menadione respectively.
**Conclusion:** Plasma membrane cholesterol homeostasis might play an important role in cellular oxidative damage.

* This work was supported by Seed Funding for Basic Research, HKU and UDM Department Research Grant.

## DMM-10 Expression studies of 24 genes on mouse chromosome 17, towards the identification of the gene(s) responsible for adolescent idiopathic scoliosis

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**Introduction:** Adolescent idiopathic scoliosis (AIS) is a spine deformity of unknown etiology which occurs commonly in adolescents. Our laboratory has recently identified a locus associated with adolescent idiopathic scoliosis on chromosome19 by linkage analysis of seven families. This region of chromosome 19 originally encompasses approximately 2 megabases of DNA, further fine mapping has now narrowed this stretch to about 800 kilobases that contain 23 genes or putative genes. This study aims to identify the genes that are expressed in the tissues involved in the phenotypic manifestation of the disease i.e., bone, cartilage, muscle and tendons. This knowledge will help reduce the number of candidate genes we need to screen for causative mutations in our patients. As the region of human chromosome 19 of interest is in synteny on mouse chromosome 17, comparative genomics will be used to that effort.
**Methods:** 1) Bioinformatics is used to identify the human genes on 19p13.3 that have mouse homologues. Primers are designed to amplify cDNAs from the murine sequences. RNAs are extracted from mouse bone, trachea, and muscle. 2) RT-PCR is performed to assay for gene expression in these tissues. Beta-actin is used as positive control for RT-PCR and reference for gene expression while liver and kidney RNAs are used as neutral control. 3) The cDNAs amplified from the RT-PCR are also printed on microarrays and are hybridized to fluorescent cDNA synthesized from RNA of the various tissues. The resulting signals are quantified using a laser scanner and the Quantarray software.
**Results:** 17 of the 23 genes on the locus identified have homologues on mouse chromosome 17. All of these 17 genes are expressed in the mouse tissues examined i.e. bone, muscle, trachea, kidney and liver when assayed by RT-PCR. Microarray experiments revealed that three of the genes studied have high expression in muscle, one in bone and one in trachea.
**Conclusion:** These experiments help prioritise the genes to focus on, in our search for the AIS candidate gene.
**Acknowledgement:** This project is funded by a UDM Seed Grant to K. Chan.

## DMM-11 Patch clamp—a powerful technique for ion channel studies in biomedical science

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The patch clamp is a very powerful technique invented by Nobel Prize Laureates, Drs. Sakmann and Neher. The technique has many applications in Physiology, Pharmacology, Neurology, Heaptology, Nephrology, Gastrointestology, Oncology, etc. I have been working in ion channel studies for many years with patch clamp technique, and I am delighted to present the related information regarding application of patch clamp techniques. The patch clamp has been applying for ionic channel studies in different types of cells, including cardiac and smooth muscle cells, endothelial and epithelial cells, and cancer cells, etc., because the ion channel is everywhere in organisms. As we know, ionic channels play important roles in the cellular physiological activities of many types of cells. In excitable cells, activation of ion channels is related to cell depolarization and then repolarization to complete a physiological activity, such as excitation-contraction in myocardium and skeletal muscle, conduction of excitation impulses in neurons, and secretion in glands. A lot of diseases are actually related to channelopathy closely linked with genes, such as the inherited long QT syndrome involved in disorders of cell membrane $K^+$ channels or $Na^+$ channels. In addition, ionic channels are involved in cell cycling in proliferative cells. For example, in cancer cells blockade of $K^+$ channels significantly inhibits cancer growth by reducing $G_0$ cells to $G_1$ phase. Understanding of physiological roles of ion channels in various types of cells may be helpful in the management and/or study of different diseases

## DMM-12 Isolation and characterisation of proteoglycans from tissues, organs and cell culture—applications in clinically oriented basic research

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**Introduction:** Proteoglycans (PGs) are complex macromolecules consisting of a protein backbone to which one or more glycosaminoglycan (GAG) chain(s) is/are attached. Each GAG chain contains numerous sulphate ester groups, and thus confer to the macromolecule a high net negative charge. PGs have distinct chemical properties, for example, large hydrodynamic size, high buoyant density, and are hydrophobic. The 'class' of a PG is determined by the nature of the GAG chain, whilst the core protein sequence determines the identity and hence the 'name' of the PG. In this respect, *decorin*, a small leucine-rich dermatan sulphate PG is so named since it 'decorates' collagen fibrils. PGs are ubiquitous in all mammalian cells, and they play active roles in diverse functions such as cell migration and proliferation, cell adhesion and signalling, selective permeability of basement membranes, growth factor sequestration, protease inhibition and entry of viruses into cells.

**Methods:** The source of the PG often determines their purification methods. The quantity of tissue and organ samples is usually sufficient to enable classical chemical purification using chaotropic buffers containing protease inhibitors. Membrane intercalated PGs can be isolated by detergent or specific enzymes known to act on the membrane-bound components. For samples in which the PG content is minute, such as tissue culture or explant organ culture, steady state metabolic labelling with [$^{35}$S]-sulphate is the preferred method. Characterization of PGs is according to their anionic charge, density, and hydrodynamic size, by a combination of enzymatic treatment, anion exchange chromatography, cesium chloride centrifugation, gel filtration chromatography, hydrophobic interaction chromatography and electrophoresis. PG synthesis at the level of transcription can be assessed by standard molecular biology techniques.

**Applications and Relevance:** Modulation of PG synthesis has been demonstrated in renal diseases with altered basement membrane understanding of their functions in health and disease, especially their interaction with cytokines and growth factors.permeability, diabetic nephropathy, abnormal angiogenesis, bacterial colonization of the urinary tract or bronchial tree, neural regeneration, and skin maturation. Recent advances in chromatographic and biochemical laboratory analytical techniques have allowed much improved characterization and purification of PGs, thereby resulting in increased.

## DMM-13 Carrier detection in spinal muscular atrophy

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**Introduction:** Spinal muscular atrophy (SMA) is a common autosomnal recessive disorder due to loss of a motor neurons in the spinal cord resulting in failure of innervation of the muscles. The incidence is estimated as 1 in 10,000 with a carrier frequency of 1 in 50. In the most severe form, Type I, disease manifest at <6 months of age and death occurs at <2 yrs. Type II SMA displays an intermediate severity, with onset at <18 mos and patients are never able to walk. In the mildest phenotype, Type III, patients can walk independently and the onset is at >18 months. The disease locus has been mapped to chromosome 5q13 and one of the possible candidate genes, the telomeric survival motor neuron (SMNt) has found to be homozygously deleted in 98.6% of SMA patients. Carriers however, are asymptomatic. Since couples who are both carriers run 25% risk of having an affected child with each pregnancy, the need to establish a method for carrier detection is genuine.
**Method:** Fluorescent allele-specific quantitative PCR (AS-QPCR) method was developed to assess the copy number of SMNt gene in normal, obligate carriers and SMA patients. With the use of Q-PCR TaqMan technology an 89 bp Exon 7 fragment specific for the SMNt gene is amplified. In addition results are expressed with reference to Q-PCR amplification of a β-globin gene fragment (102 bp) as an internal control gene.
**Results:** This AS-PCR method allows specific amplification of SMNt as opposed to the centromeric SMN gene (SMNc), which is present in both carriers and patients. Our method has been validated against another Q-PCR procedure that requires comparison of band intensity between that of the target gene and the normalization gene fragment (i.e. β-globin gene). Results of 20 normals, 27 carriers and 29 SMNA patients are compared using both methodologies.
**Conclusion:** AS-QPCR allows rapid detection of SMA carriers in families where deletion of SMNt gene is the pathogenic mechanism of disease. This method is more specific, robust and less labour intensive than existing methods. It should find widespread use to identify carriers amongst sibs of SMA patients so that they can be counselled for the need of prenatal testing.

## GIH-01 One-year dose of lamivudine reduces serum hepatitis B virus covalently closed circular DNA level

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**Background and aim:** In an on-going study, hepatitis B virus covalently closed circular (HBV ccc) DNA is detectable in the serum of patients with chronic hepatitis B. Serum ccc HBV DNA positively correlates with intrahepatic ccc HBV DNA in liver biopsy specimens (Wong et al., in preparation). The effect of lamivudine on HBV serum cccDNA levels is not known. This study aims to assess the effect of a one-year dose of lamivudine on serum HBV cccDNA levels.
**Methods:** Serum samples from 92 chronic hepatitis B patients were retrospectively studied. Of these, 75 patients received a daily dose of 100mg lamivudine and 17 patients received placebo for one year. cccDNA in serum samples taken at weeks 0, 24, and 52 from the start of the first dose were quantified using an Invader Assay, which specifically detects a single stranded region on the HBV plus strand DNA, thereby distinguishing the ccc form of HBV DNA from the relaxed circular form. Drug resistance mutation (YMDD) was determined by the INNO-LiPA Assay.
**Results:** The baseline median cccDNA levels for the placebo and the lamivudine groups were $4.52 \times 10^6$ and $3.84 \times 10^6$ copies/mL respectively. Compared to baseline values, the median reduction in serum cccDNA levels for the placebo and lamivudine groups at week 24 were 0.02 and 2.03 logs respectively ($P < 0.001$), while those at week 52 were 0.09 and 1.87 logs respectively ($P = 0.001$). Fourteen lamivudine-treated patients developed YMDD mutants by week 52. At week 24, there was no significant difference between the median cccDNA decrease for the groups with YMDD mutants and wild type ($P = 0.659$). However, at week 52, the median cccDNA decrease became significantly less for the patients with the YMDD mutants (mutant vs wild type : 0.81 vs 1.92 logs; $P = 0.005$).
**Conclusion:** One year treatment of lamivudine significantly decreases not only serum total HBV DNA, but also serum ccc HBV DNA. With the development of YMDD mutants, the decrease in serum cccDNA became significantly less.

## GIH-02 HBsAg seroclearance in chronic hepatitis B infection: virological, histologic and clinical aspects

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**Background:** Comprehensive studies of Chinese chronic hepatitis B patients with HBsAg seroclearance are lacking.
**Aims:** To explore the biochemical, virological, histologic and clinical aspects of patients with HBsAg seroclearance.
**Patients and Methods**: Ninety-two patients with HBsAg seroclearance (median follow-up 126 months) and 92 HBsAg-positive controls matched for age, sex and duration of follow-up were studied. Intrahepatic total and covalently closed circular (ccc) HBV DNA were measured quantitatively. The HBV genotype was determined. Liver biochemistry, serum HBV DNA levels and development of clinical complications were monitored.
**Results**: The mean age of HBsAg seroclearance was 48.8 (± 13.81) years. There was a significant improvement in serum alanine aminotransferase levels after HBsAg seroclearance ($p<0.0001$). Patients with genotype B had a higher chance of HBsAg seroclearance compared to those with genotype C ($p=0.014$). Ninety-eight percent of patients had undetectable serum HBV DNA. Thirty-seven percent of patients had low titer of intrahepatic HBV DNA, mainly in the form of cccDNA (71 – 100%). All 14 patients with liver biopsies had near normal liver histology. There was no difference in the risk of development of hepatocellular carcinoma (HCC) between patients with and without HBsAg seroclearance. However, the mean age of HBsAg seroclearance was significantly older in patients with HCC compared to patients without HCC ($p=0.016$).
**Conclusions**: Patients with HBsAg seroclearance had favorable biochemical, virological and histologic parameters. The intrahepatic HBV DNA level was low and predominantly in the form of cccDNA. However, HCC could still develop particularly in patients who had HBsAg seroclearance at older age.

## GIH-03 Role of liver biopsy in the management of liver dysfunction after hematopoietic stem cell transplantation in a hepatitis B virus prevalent patient population

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**Introduction:** Derangement of liver-function-tests (LFT) is common after hematopoietic stem-cell-transplantation (SCT). The role of liver biopsy in such cases has not been defined in hepatitis B virus (HBV) prevalent patients. The impact of liver biopsy in the management of LFT derangement post-SCT in a HBV-prevalent population was examined.
**Materials and methods.** Seventy-five liver biopsies, performed for 323 patients with LFT derangement post-SCT (263 allogeneic, 60 autologous), were analysed. The HBV carrier rate was 13.6%.
**Results.** Significantly more LFT derangements and therefore liver biopsies occurred in allogeneic versus autologous SCT. Prior to biopsy, graft-versus-host disease (GVHD) and HBV reactivation were clinically diagnosed in 70.6% and 25.3% of cases. A definite histopathologic diagnosis was obtained after biopsy in 53 cases, with GVHD, HBV-hepatitis and concomitant GVHD/HBV-hepatitis found in 33%, 21% and 8% of cases respectively. The clinical and histopathologic diagnoses were concordant in 43 cases and discordant in 10 cases. Clinical management was altered in 6/10 discordant cases, 5 of which were due to HBV or hepatitis C virus (HCV) reactivation. Twenty-two biopsies showed non-diagnostic histopathologic features. Twenty of these cases were successfully managed based on clinical diagnoses. The clinical/biochemical features of patients clinically diagnosed to have GVHD did not differ significantly whether or not they were HBV/HCV carriers. However, liver biopsies in HBV/HCV carriers resulted in significantly more treatment alterations as compared with non-carriers.
**Conclusions.** Clinical diagnoses of LFT derangements post-SCT might be adequate for initiation of treatment, but liver biopsy in HBV/HCV carriers were needed as this might impact on management.

## GIH-04 Gastric epithelial expression of trefoil family factor 2 and mucin 6 in normal and *Helicobacter pylori*-infected subjects

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**Introduction**: The role of trefoil family factors (TFFs), a group of small secretory peptides, and mucins, the major components of the mucous viscous gel covering the surface of epithelium, in mucosal defence and healing has been postulated. *H. pylori* infection is associated with gastric mucosal inflammation and injury. The aim of this study was to determine whether *H. pylori* infection is associated with altered expression of TFF2 and MUC6 at the different gastric sites.
**Method**: Gastric biopsy specimens taken from gastric antrum, incisura and body were used for the detection of *H. pylori* infection and histological assessment, and the determination of TFF2 and MUC6 expression by immunohistochemistry.
**Results**: Of the 76 patients recruited, 27 (35.5%) were positive for *H. pylori* infection. Chronic gastritis was present in 26 (96.3%) *H. pylori* positive patients and 7 (14.3%) *H. pylori* negative patients ($P<0.001$). In all 42 (100%) patients with normal mucosa (i.e. without *H. pylori* and chronic gastritis), TFF2 and MUC6 were coordinately expressed in regenerative zone and deep portion of glands of antral mucosa, and only in the regenerative zone of gastric incisura and body mucosa. However, in patients with *H. pylori* infection, TFF2 and MUC6 expression was detected within the foveola of antral mucosa, incisura and body in 59.3%, 44.4% and 11.1%, respectively (all $P<0.05$, compared with normal mucosa). Moreover, TFF2 and MUC6 expression was also detected in the glands of incisura and body mucosa in a proportion (96.3% and 14.8%, respectively) of *H. pylori* infected patients.
**Conclusion:** *H. pylori* infection is associated with extended TFF2 and MUC6 expression in the gastric antral, incisura and body epithelium, which indicates a protective role of these factors in *H. pylori* infection.

## GIH-05 Randomised controlled study of rabeprazole, levofloxacin and rifabutin triple therapy versus quadruple therapy as second-line treatment of *Helicobacter pylori* infection

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**Background and aims:** To test the efficacy of rabeprazole, levofloxacin and rifabutin triple therapy versus rabeprazole-based quadruple therapy for the treatment of *Helicobacter pylori* (*H. pylori*) infection after failure of one or more courses of *H. pylori* eradication therapy.
**Methods:** One hundred and nine patients who failed previous *H. pylori* eradication were randomised to receive 1) rabeprazole 20mg twice daily, rifabutin 300mg and levofloxacin 500mg once daily for 7 days (RRL) or 2) rabeprazole 20mg twice daily, metronidazole 400mg thrice daily, bismuth subcitrate 120mg and tetracycline 500mg four times daily for 7 days (quadruple). Endoscopy with biopsy for CLO test, histology and culture was performed before treatment. Post-treatment *H. pylori* status was determined by $^{13}$C-urea breath test. Metronidazole, clarithromycin and amoxycillin resistance was defined as MIC of >8 ug/ml, >2 ug/ml and >2 ug/ml respectively.
**Results:** The clarithromycin resistance rate (79% versus 21%, P<0.001) and metronidazole resistance rate (89% versus 40%, P<0.001) was significantly higher in patients who have taken these antibiotics in their previous *H. pylori* treatment compared to those who have not. Intention-to-treat and per protocol *H. pylori* eradication rate were 91% / 91% for the RRL group and 91% / 92% for the quadruple group. For patients with double resistance to metronidazole and clarithromycin, the eradication rates were 85% (17/20) in the RRL group and 87% (13/15) in the quadruple group. Compliance was greater than 95% for both regimens.
**Conclusion:** Rabeprazole, levofloxacin and rifabutin based triple therapy and rabeprazole-based quadruple therapy were equally effective as a second-line treatment of *H. pylori* infection.

## GIH-06 The role of cigarette smoking and its interaction with cyclooxygenase-2 in acute ulcerative colitis in mice

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**Introduction:** Epidemiological studies show that smokers are more likely to have Crohn's disease but are less susceptible to ulcerative colitis. Our previous study indicated that passive cigarette smoking exacerbated the Crohn's disease induced by 2,4,6-trinitrobenzene sulfonic acid in rats. However, no direct evidence had been found regarding the protective effect of cigarette smoking on ulcerative colitis. Besides, cyclooxygenase-2 (COX-2) is strongly associated with inflammatory bowel disease for its inhibition could either potentiate or attenuate colitis. The aim of the present study is to evaluate the role of cigarette smoking on ulcerative colitis induced by dextran sulfate sodium (DSS) in mice and to determine whether COX-2 takes part in the inflammatory process in this model.
**Methods:** Acute ulcerative colitis was induced in balb/c mice by giving 5% DSS in drinking water for 7 days. The mice were simultaneously exposed to passive 0% or 2% cigarette smoke (CS) once daily for the same period of time. Colonic tissues were assessed for the inflammatory parameters and COX-2 expression in these animals.
**Results:** DSS in drinking water given for 7 days increased colon weight when compared with the control. Histology findings revealed that there were infiltration of inflammatory cells and loss of glands in the colonic mucosa. An increase in myeloperoxidase (MPO) activity, a marker for neutrophils infiltration, was also observed. Both immmunohistochemistry and Western immunoblotting indicated that COX-2 was induced in the colonic tissues with ulcerative colitis. It was accompanied with an induction of the cellular proliferation. On the other hand, passive CS exposure neither affected the degree of inflammation nor the MPO activity in these animals. However, both COX-2 protein expression and cellular proliferation was increased in colonic tissue in mice with CS exposure alone and synergistically induced in mice when combined with DSS.
**Conclusion:** Passive cigarette smoking did not influence the severity of acute colonic inflammation induced by DSS though it further provoked COX-2 expression and cellular proliferation in colitis animals. Furthermore, COX-2 was induced by CS exposure alone but did not accompany with colitis formation. All these findings indicated that COX-2 was not directly involved in the initiation of inflammation in this acute ulcerative colitis model. However, whether the induction of cigarette smoke on proliferation exerts beneficial effect on ulcerative colitis is undefined and requires further study.

## GIH-07 A comparative study of the acceptance for percutaneous endoscopic gastrostomy and nasogastric tube feeding in patients with swallowing dysfunction

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**Introduction**: Nasogastric Tube (NGT) feeding is the most common form of non-oral feeding for patients with a variety of swallowing dysfunction. Percutaneous Endoscopic Gastrostomy (PEG) feeding is increasingly popular as an alternative to NGT feeding. This study aims to investigate the acceptance for this relatively new form of non-oral feeding by patients and their immediate caregivers.
**Method**: A retrospective cohort of patients who had PEG insertion for swallowing dysfunction between January 1999 and December 2001 at Tung Wah Hospital was identified. They all had a period of NGT feeding before PEG insertion. Opinion survey on patients and their immediate caregivers were done in person or by phone using a semi-structured interview. Data of preference for PEG or NGT feeding were systematically collected.
**Results**: 48 potential patients were identified with a mean age of 72.7. Eighteen were females and 30 were males. Thirty-six had strokes, 7 had other neurological diseases and 4 had head & neck cancers. 10 of 48 patients could be interviewed as 20 were dead and the remaining 18 were not communicable because of dementia or dysphasia. Seven of 10 patients (70%) preferred PEG, 2 of 10 (20%) preferred NGT and 1 of 10 (10%) was neutral. Patients' main reasons for choosing PEG were comfort, good comesis and less likely to slip out. And patients' main reason for choosing NGT was ease of handling. 48 caregivers were interviewed. 34 of 48 caregivers (70.8%) preferred PEG, 7 of 48 (14.6%) preferred NGT and 7 of 48 (14.6%) were neutral. Caregivers viewed that patient's comfort, good comesis and less likely to slip out were also main factors for favouring PEG. Ease of tube replacement and handling and less local complications were main factors favouring NGT.
**Conclusion**: Although PEG is a more invasive method of non-oral feeding, this study revealed a higher acceptance amongst patients and their caregivers for PEG over NGT feeding.

## GIH-08 A study of the profile of percutaneous endoscopic gastrostomy feeding at Tung Wah Hospital

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**Introduction:** Percutaneous Endoscopic Gastrostomy (PEG) is increasingly popular as a form of non-oral feeding. This study aims to investigate the demographic features, diagnoses and commodities, complications, outcome and duration of feeding in patients who required PEG for nutritional support at Tung Wah Hospital.
**Method:** Retrospective case note review of a cohort of patients from January 1999 to 2001 at Tung Wah Hospitial who had received PEG feeding for nutritional support due to a variety of swallowing dysfunction.
**Results:** Fourty-eight patients were identified with a mean age of 72.7. Eighteen were females and 30 were males. Thirty-six had strokes, 7 had other neurological diseases and 4 had head & neck cancers. Of the 36 stroke cases, 26 (72%) had one episode of stroke and 8 (28%) had two or more episodes of stroke; 29 (80.5%) were ischaemic and 7 (19.5%) were haemorhagic in nature. Twenty-nine (60.4%) patients experienced early complications, in which most are minor, and 19 (39.6%) had no early complications. The most frequent early complications were: skin infection (34.5%), tube dislodgement due to deliberately pull out by patients (27.6%) and leakage (13.7%). Sixteen (33.3%) patients experienced late complications (not including PEG tube dysfunction) and 32 (66.7%) had no late complications. The most frequent late complications were: tube dislodgement (68.8%), stoma infection (50%), excessive stoma granulation tissue (31.3%), leakage (13.7%) and gastrointestinal upset (12.5%). Twelve (25%) patients experienced tube dysfunction in the form of fracture button, porosity, clogging or deformity. Seventeen (35.4%) patients required a replacement procedure because of tube dislodgement/dysfunction or severe stoma infection. Ten of 36 (27.8%) stroke patients could wean off their PEG tubes eventually. Successful weaning was significantly associated with fewer episodes of stroke but not with side/type of stroke and number of co-morbidities. As on 30th September 2002, twenty of 48 (41.6%) patients had died. Patients who passed away were older (mean age 76.3 vs 70.1; not statistically significant) and not associated with side/type/number of strokes and number of co-morbidities. There was no procedure-related mortality. The mean duration of feeding ranged from 24 to 708 days with a mean of 252 days.
**Conclusions:** Patients requiring PEG feeding at Tung Wah Hosptial have a range of diagnoses and the main indication for intervention is neurological swallowing dysfunction. Mortality is high in this group of patients. PEG is compatible with long-duration and uncomplicated feeding in over 60% of patients.

## GIH-09 A validated symptoms questionnaire (Chinese-GORDQ) for the diagnosis of gastro-oesophageal reflux disease in Chinese population

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**Background and Aims:** There is no gold standard for the diagnosis of gastro-oesophageal disease (GORD). The aim of this study is to develop a validated GORD symptom questionnaire for Chinese population.
**Methods:** Ninety-five Chinese patients with GORD and 101 healthy Chinese controls were presented with a 20-item GORD questionnaire in Chinese language (Chinese-GORDQ) based on a previous published validated western questionnaire. Quality of life in GORD patients was assessed by SF-36. Standard dose of proton pump inhibitors (PPI) for 4 weeks was prescribed to 35 patients with newly diagnosed GORD. The Chinese-GORDQ was performed before, 4 weeks and 8 weeks after treatment. Concept, content, construct, discriminant validity and reliability of the questionnaire were assessed.
**Results:** All items were considered comprehensible by more than 90% of subjects. Relevance of individual symptoms to GORD ranged from 60% to 100%. Seven items were selected by logistic regression to account for most of the differences between control and GORD patients. Test-retest reproducibility and internal consistency were good with the intraclass correlation coefficient of 0.75 and Cronbach's alpha coefficient of 0.9. A cut-off score of equal or greater than 12 was determined to discriminate between controls and GORD patients with an AUC of 0.91, a sensitivity of 80% and a specificity of 83% by ROC analysis. The Chinese-GORDQ correlated negatively with 5 domains of the SF-36 and discriminated between GORD patients who reported a subjective symptomatic improvement during PPI treatment and symptoms deterioration during withdrawal of PPI treatment.
**Conclusions:** Chinese-GORDQ was easy-to-understand, internally consistent and reproducible. It predicted global symptom change, and the symptom severity scores correlated negatively with quality of life. It is suitable for epidemiological studies to assess the frequency and severity of GORD and interventional studies of GORD in Chinese population.

## GIH-10 Long-term prospective follow-up of endoscopic oesophagitis in southern Chinese—prevalence and spectrum of the disease

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**Aims:** To study the prevalence, clinical characteristics and long-term outcome of oesophagitis in Chinese patients.
**Methods:** Clinical and endoscopic data were prospectively collected from consecutive patients who underwent upper endoscopy from 1997 to 2001. Patients with endoscopic oesophagitis were graded according to the Los Angeles system and analysed according to their clinical presentation, endoscopic details, *Helicobacter pylori* status, NSAIDs history, co-morbidity and mortality.
**Results:** A total of 22628 upper endoscopies were performed in 16606 patients. Of these, 631 (3.8%) had endoscopic oesophagitis, 14 had benign oesophageal stricture (0.08%) and 10 had Barrett's oesophagus (0.06%). Most patients (94%) had either LA grade A or grade B oesophagitis. Patients who died during follow-up had a significant higher incidence of co-morbid illness (100% versus 63%, $P<0.001$). By Cox regression analysis, presence of gastrointestinal bleeding ($P=0.008$), advanced age ($P=0.004$) and the use of Ryle's tube ($P=0.043$) were identified to be independent factors associated with mortality.
**Conclusions:** Complicated gastro-oesophageal reflux disease is uncommon in Asian population. Advanced age, use of Ryle's tube and the presence of gastrointestinal bleeding were associated with poor long-term outcome, which was a reflection of the severe underlying co-morbidity.

## GIH-11 Coping strategies, illness perception, anxiety, and depression of patients with idiopathic constipation: a population-based study

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**Introduction:** Functional constipation is a common problem in clinical practice with important psychological elements. We investigated the prevalence of functional constipation in an Asian population, and the interplay between functional constipation, anxiety/depression state, perception and the use of coping strategies.
**Methods:** 3282 patients were interviewed by telephone survey. Constipation was diagnosed by Rome II criteria. The coping ability was assessed by a set of coping categories designed by the authors. Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale.
**Results:** The reliability of the questionnaire was high (kappa values ranged: 0.4-0.9, ps < 0.05). 14% of the general population has constipation. 57.4% of the constipated subjects were aware of having constipation. Patients who knew they suffered from constipation perceived that this problem had a greater impact on their lives ($p < 0.001$); and seeked more medical consultation ($p < 0.001$). The anxiety and depression scores were $6.68 \pm 5.35$ and $6.25 \pm 4.93$ in the constipated subjects, versus $2.89 \pm 3.09$ and $3.72 \pm 2.45$ in the healthy controls (ps < 0.001). Taking prescribed medicine, change eating habit, drink more water, and abdomen massage were effective coping strategies in relieving constipation symptoms. Taking prescribed medicine, exercising, change thinking style, and seek support from others were effective in relieving anxiety.
**Conclusion:** Constipation, associated with anxiety and depression, is prevalent in the general Asian population. Effective physiological or psychological coping strategies, which are affected by illness perception, can alleviate constipation symptoms or anxiety/depression symptoms. We propose a link exists among constipation, anxiety/depression, perception and coping strategies.

## GIH-12 Concordant CpG island methylation in hyperplastic polyposis

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**Introduction**: The CpG Island Methylator Phenotype (CIMP) is a newly described mechanism for carcinogenesis in colorectal carcinomas and adenomas characterized by methylation of multiple CpG islands. The causes of CIMP are unknown. We studied CIMP in hyperplastic polyps (HPs), with emphasis on patients with multiple HPs or large hyperplastic polyposis.
**Method:** Methylation of *p16*, MINT1, MINT2, MINT31 was analyzed by methylation-specific polymerase chain reaction in 102 HPs, 8 serrated adenomas, 19 tubular adenomas, and 9 adenocarcinomas from 17 patients with hyperplastic polyposis (more than 5 HPs) and in 16 sporadic HPs from 14 additional patients.
**Results:** Sporadic HPs were CIMP-negative at any loci, but 43% of HPs from hyperplastic polyposis were CIMP-high (methylation at more than 1 locus, $P = 0.00001$). Methylation among the four loci was correlated within HPs (odds ratio 3.41, $P=0.002$), and the methylation status of HPs within the same patient was also correlated (odds ratio 5.92, $P=0.0001$). Methylation of multiple loci in HPs was present primarily in patients with right-sided hyperplastic polyposis and/or serrated adenomas ($P=0.0009$) and was associated with the absence of K-ras proto-oncogene mutations (odds ratio 5.08; $P = 0.03$).
**Conclusion:** Concordant CpG island methylation of HP in hyperplastic polyposis supports that some patients have a hypermethylator phenotype characterized by methylation of multiple HPs and other colorectal lesions. This represents a novel and distinct morphogenetic pathway for colorectal cancer and is related to patient-specific factors such as carcinogenic exposure or genetic predisposition.

## GIH-13 CpG island methylation in aberrant crypt foci of the colorectum

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**Introduction:** Aberrant crypt foci (ACF) are postulated to be the earliest precursor lesion in colorectal carcinogenesis, and CpG island methylation has been described as an important molecular pathway.
**Methods:** We studied methylation, K-ras mutation, Chromosome 1p loss and microsatellite instability in 27 ACF from 2 patients with familial adenomatous polyposis (FAP) and 34 ACF from 10 patients with sporadic colorectal cancer (CRC). Methylation status of ACF at p16, MINT1, MINT2, MINT31, MGMT, and hMLH1 was assessed by Methylation specific PCR. K-ras mutation was studied by automated sequencing method, loss of heterozygosity at chromosome 1p, and microsatellite instability were assessed by DNA fragment analysis using ABI Prism and GENESCAN.
**Results**: 89%(24/27) of ACF from FAP patients were dysplastic, in contrast, 82% (28/34) of ACF from the patients with sporadic CRC were heteroplastic (P = 0.000001). Methylation was more frequent in sporadic than FAP ACF (53%, 18/34 versus 11%, 3/27; P=0.002). K-ras mutation was present in 7% (2/27) of ACF from FAP patients but in 38% (13/34) of ACF from patients with sporadic carcinomas (P = 0.013) and more common in heteroplastic ACF (12/31) than in dysplastic ACF (3/30) (P = 0.02). Chromosome 1p loss was identified in 3 ACF, all were heteroplastic. Strong associations of ACF methylation with K-ras mutation (P=0.007) and with loss of chromosome 1p (P=0.04) were observed.
**Conclusion**: Our findings suggest epigenetic differences in methylation in ACF are analogous to the heterogeneity of phenotypic and genetic characteristics in FAP and sporadic CRC. We propose a heteroplastic ACF-adenoma-carcinoma sequence in addition to the existing dysplastic adenoma-carcinoma sequence.

## GIH-14 Differing DNA methylation patterns of colorectal cancers in Middle Eastern countries

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**Background:** The epidemiology of colorectal cancer (CRC) differs between Middle Eastern and western countries, but the molecular characteristics have not been studied extensively. The CpG island methylator phenotype (CIMP) pathway is a recently described molecular mechanism in colorectal carcinogenesis characterized by methylation of multiple CpG dinucleotide islands, which results in transcriptional silencing of genes.
**Aim:** To study the methylation patterns of CRC from Middle Eastern countries in comparison to a western population.
**Materials and Methods:** Methylation at p16, MINT1, MINT2, MINT31 and hMLH1 was assessed by methylation-specific polymerase chain reaction in 248 CRC from Egypt, Jordan, and Turkey and compared to data from 97 United States cases, including multivariate analysis.
**Results:** Jordanian CRC had more methylation than the other two Middle Eastern countries and the western population (p = 0.04), but the frequency of methylation of the hMLH1 mismatch repair gene showed no differences. Multivariate analysis showed that factors associated with lower methylation rates were young age (p= 0.004), and left-sided (p = 0.001) and rectal (p = 0.05) location of carcinomas. Medullary carcinoma was strongly associated with hMLH1 methylation (OR=22, 95% CI = 3.6-134.4).
**Conclusion:** CRC from Middle Eastern countries and the United States have differing methylation patterns, which may result from different environmental exposures.

## GIH-15 CpG island methylation in carcinoid and pancreatic endocrine tumours

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**Introduction:** Carcinoid tumours and pancreatic endocrine tumours (PETs) are uncommon neuroendocrine neoplasms and their genetic alterations are not well characterized. CpG island methylation is a mechanism of gene silencing. The aim of this study was to evaluate CIMP in carcinoid tumours and PETs.
**Methods:** We studied 16 carcinoid tumours, 11 PETs, and 22 associated normal mucosa or pancreas. Methylation status of the p14, p16, COX2, MGMT, ER, THBS1, RAR-beta, CACNA1G, and multiple endocrine neoplasia type-1 (MEN1) genes, and of MINT1, MINT2, MINT25, MINT27 and MINT31 loci was evaluated by methylation specific-PCR or combined bisulfite restriction analysis. Chromosome 9p loss was analysed by ABI Prism and GENESCAN.
**Results**: Carcinoid tumours were frequently methylated at *RAR-beta*, *MGMT*, *p16*, *COX2*, *p14*, *THBS1*, and *ER* ranging from 25% to 63% of tumours. Other CpG islands were infrequently methylated or unmethylated. The adjoining normal mucosa was also methylated for *ER*, *COX2*, and *RAR-beta*, but methylation at *p14*, *p16*, *THBS1*, and *MGMT* was tumour-specific. By contrast, PETs and normal pancreas were frequently methylated only at *ER*. Methylation was more frequent in carcinoid tumors than PETs at *MGMT* (25% versus 0%, P = 0.03), *THBS1* (44% versus 9%, P = 0.04), *p14* (44% versus 9%, P = 0.04) and *RAR-beta* (25% versus 0%, P = 0.03), respectively. Chromosome 9p loss was present in 18% (2 of 11) of PETs, and in none of 16 carcinoid tumours. Methylation of the *p16* gene also was present in 45% (5 of 11) patients with carcinoid tumours or pancreatic endocrine tumours metastatic to liver versus 6% (1 of 6) patients without (P = 0.01).
**Conclusion**: Our study indicates that methylation profile of carcinoid tumours differs from PETs, reflecting different molecular pathogenesis.

## GIH-16 Epigenetic and genetic alterations in duodenal carcinomas are distinct from biliary and ampullary carcinomas: frequent G-to-A *K-ras* and *p53* mutations due to *MGMT* methylation and microsatellite instability due to *hMLH1* methylation

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**Introduction:** Carcinomas of the extrahepatic bile ducts, ampulla of Vater, and duodenum are uncommon, and their epigenetic and genetic alterations are not well characterized. We therefore compared the methylation profile and genetic alterations in 18 biliary, 10 ampullary, and 14 duodenal carcinomas.
**Methods:** We evaluated methylation at *p16, p14* and *hMLH1* by methylation specific-PCR, and at *COX 2*, *MGMT*, *ER*, *RAR-beta*, and *CACNA1G* genes, and MINT1, MINT2, MINT25, MINT27 and MINT31 loci by combined bisulfite restriction analysis; mutation of *K-ras*, *p53*, *p16*, and *p14* genes by sequencing; loss of heterozygosity of chromosome 9p; and microsatellite instability (MSI).
**Results:** Duodenal carcinomas were more frequently methylated than biliary carcinomas at *p14* (P=0.04), *MGMT* (P=0.01), MINT1 (P=0.01), MINT25 (P=0.02), MINT27 (P=0.002), *RAR-beta* (P=0.03), and *ER* (P=0.0002), and than ampullary carcinomas at *p14* (P=0.02), *RAR-beta* (P=0.01), and *ER* (P=0.01). In contrast the methylation profiles of biliary and ampullary carcinomas were not different. Site-specific concordant methylation of different sets of genes and loci was present in bile duct and duodenal cancers suggesting the presence of a hypermethylator phenotype. *MGMT* methylation was associated with G-to-A mutation in *K-ras* (P=0.001) and *p53* (P=0.048), and *hMLH1* methylation was associated with MSI-high (P=0.001).
**Conclusion:** Our findings indicate that the methylation profile and genetic alterations of duodenal carcinomas are distinct from biliary and ampullary carcinomas, and that tumor-specific concordant methylation influences gene mutations and MSI-high. Carcinomas with concordant methylation of multiple CpG islands are frequently present in duodenum, and are associated with G-to-A *K-ras* mutations and MSI-high due to methylation of *MGMT* and *hMLH1*, respectively.

## GIH-17 Soluble E-cadherin is an independent pre-therapeutic factor for long term survival in gastric cancer

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**Introduction:** Gastric cancer remains the second leading cause of cancer-related deaths in the world, but a satisfactory tumor marker is currently unavailable for gastric cancer. Soluble E-cadherin has recently been found to have prognostic value in gastric cancer. We aim at evaluating whether pre-therapeutic serum soluble E-cadherin is an independent factor predicting long-term survival in gastric cancer.
**Patients and Methods:** 116 patients with histology proven gastric adenocarcinoma were included. Pre-therapeutic serum was collected and soluble E-cadherin was assayed using a commercially available ELISA kit. The patients were followed up prospectively at the outpatient clinic.
**Results:** There were 75 men and 41 women with a mean age of 66 ± 14 years. Forty eight percent of tumours were located in the gastric antrum. The median survival was 11 months. The mean pre-therapeutic value of soluble E-cadherin was 9159 ng/ml (range, 6002 to 10025 ng/ml), and that of CEA was 11 ng/ml (range, 0.3 to 4895 ng/ml). On multivariate analysis, soluble E-cadherin is an independent factor predicting long-term survival. Ninety percent of patients with serum level of E-cadherin greater than 10000 ng/ml had survival less than 3 years (P = 0.009).
**Conclusions:** Soluble E-cadherin is a potentially valuable pre-therapeutic prognostic factor in patients with gastric cancer.

## GIH-18 PIN1 is over-expressed in hepatocellular carcinoma (HCC) and correlates with an increased beta-catenin and cyclin D1 protein levels

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**Introduction**: In hepatocellular carcinoma, the expression of beta-catenin and cyclin D1 is increased, which may be of pathogenetic significance. As mutations of the *beta-catenin* gene are only found in around 20% of cases, other factors are involved in the accumulation of beta-catenin and cyclin D1. PIN1, a peptidyl-proplyl-isomerase, has been shown to stabilize both beta-catenin and cyclin D1, and to up-regulate *cyclin D1* gene expression. We hypothesize that the beta-catenin and cyclin D1 accumulation in some of the HCC is contributed by PIN over-expression.
**Methods**: The expression of PIN1 in 23 paired-samples of neoplastic and non-neoplastic liver tissues was examined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry and Western blot analysis. Immunohistochemistry was also performed on another 28 paired archival samples of HCC to detect PIN1, beta-catenin and cyclin D1 expression.
**Results**: Compared with paired non-neoplastic liver tissues, 12 of 23 (52%) HCC samples showed an increase in *PIN1* expression by semi-quantitative RT-PCR. These cases also showed beta-catenin accumulation, and sequencing of exon 3 of the *beta-catenin* gene did not show any mutation. Together with the archival materials, PIN1 was found to be over-expressed by immunohistochemistry and Western blot analysis in 26 of 45 tumors (58%), all of which had concomitant accumulation of beta-catenin. Another 5 cases had beta-catenin accumulation without PIN1 over-expression, so that the overall frequency of beta-catenin over-expression was 68% (31/45). In 3 cases with beta-catenin accumulation but no PIN1 over-expression, 2 cases showed mutation in the exon 3 of the *beta-catenin* gene. Finally, 19 of 26 cases with PIN1 over-expression also had increase in cyclin D1 expression.
**Conclusion**: PIN1 expression is increased in a significant proportion of HCC. There is a positive correlation between PIN1, and beta -catenin and cyclin D1 expression, which suggests that PIN1 may be critically involved in hepatocarcinogenesis.

## GIH-19 Evaluation of an automated immunochemical faecal occult blood test for colorectal neoplasia screening in a Chinese population

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**Background and Aim:** Most commercial fecal occult blood tests (FOBT) used for colorectal cancer (CRC) screening in western populations are guaiac based, developed manually, subjective and sensitive to dietary components. Preliminary studies showed their unsuitability for screening in Chinese population. The aim of this study is to evaluate the performance characteristics of a human hemoglobin specific automated immunochemical FOBT, Magstream 1000/Hem Sp, in a Chinese population referred for colonoscopy.
**Methods:** 250 consecutive patients referred for colonoscopy and met the study inclusion criteria took samples for the immunochemical FOBT, without dietary restrictions, from two successive stool specimens. Tests were developed by an automated machine having an adjustable sensitivity threshold. The sensitivity, specificity and positive predictive value for detecting colorectal adenomas and cancers were calculated according to manufacturer's instruction, using a range of sensitivity levels.
**Results:** The sensitivity, specificity and positive predictive value for detecting significant colorectal neoplasia (adenomas $\geq$ 1.0 cm and cancers) at the optimal threshold level were 62%, 93% and 44% respectively. The test is easy to use and not dependent on operator experience.
**Conclusion:** An automated immunochemical FOBT is a robust, convenient and useful tool for colorectal cancer screening in Chinese population.

## GIH-20 Cytogenetic and fluorescence in situ hybridisation characterisation of esophageal carcinomas showed clonal chromosomal aberrations and *CCND1* amplification

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**Introduction:** The genetic mechanisms underlying the development of esophageal carcinomas are not well defined. The aim of the study is to characterize the cytogenetic aberrations that may be important for esophageal squamous cell carcinoma (SCC) tumorigenesis.
**Material and Methods:** Tumour samples and their surrounding tissues of 6 patients with esophageal SCC were studied by cytogenetic and molecular cytogenetic technigues.
**Results:** Cytogenetic analyses of four squamous cell carcinomas (SCC) of the esophagus showed clonal chromosome aberrations involving numerous complex numerical and structural abnormalities. Chromosomal bands or regions preferentially involved were 11q13, 8q10, 21q10, 3p10-p11, 1p11-q11, 5p11-q11 and 14p11-q11. For the first time, recurrent aberrations were identified in esophageal SCC, including homogenous staining region (hsr), isochromosomes i(3q) and i(21q), and ring chromosome. Losses of chromosomal material dominated over gains. Recurrent imbalances included under-representation of 4p13-pter, 5q14-qter, 9p22-pter, 10p, 11p13-pter, 12p13-pter, 17p10-pter, 18p11-pter, 21p, and 22p; and over-representation of 1q25-qter, 3q, 7q, and 8q. Interestingly, hsr at different chromosomal regions occurred in three of four cases. With the application of fluorescence in situ hybridization (FISH) and multicolor COBRA FISH using specific DNA probes, it could be shown that in two cases, the hsr was derived from chromosome 11 material and that the amplicon included *CCND1*.
**Conclusion**: These observed chromosomal aberrations and imbalances provide important information for the further molecular genetic investigation of esophageal SCC. *CCND1* might be an important target in 11q13 amplification, and that amplification of this gene might be crucial in the tumourigenesis of esophageal SCC.
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## GIH-21 Restoration of XAF1 expression inhibits gastric and colonic tumorigenesis *in vivo*

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**Introduction:** XAF1 ( XIAP-Associated Factor 1 ) is identified as a novel XIAP (X-linked inhibitor of apoptosis ) binding partner. XAF1 antagonizes the anti-caspase activity of recombinant XIAP and reverses the protective effect of XIAP overexpression in cell lines. In this present study, we investigated the role of XAF1 as a tumour suppressor.
**Method:** pcDAN3-XAF1 and Adeno-XAF1 plasmids were constructed. Adeno-XAF1 virus was generated. Stable transfectants were established and characterized. Tumourigenesis was evaluated by the formation of solid tumours in athymic nude mice xenograft.
**Results:** Expression of XAF1 was detected only in 50% gastric and colon cancer cell lines, and 54% human colon cancer tissues expressed low level of XAF1 mRNA compared with adjacent normal colon tissues. Restoration of XAF1 expression induced apoptosis in gastric and colon cancer cells. Overexpression of XAF1 suppressed anchorage-dependent and -independent growth, induced cell cycle arrest, and increased the sensitivity to chemotherapeutic drugs. Furthermore, infection of adeno-XAF1 induced apoptosis in gastric and colon cancer cells. In nude mice, overexpression of XAF1 suppressed tumorigenesis in stable gastric and colon cancer transfectants expressing XAF1. Gastric and colon cancer cells infected with adeno-XAF1 either lost their tumourigenic potential or formed tumours of smaller size and markly delayed onset when compared with those infected with adeno-LacZ and uninfected cells.
**Conclusion:**These results demonstrated that XAF1 is a tumour suppressor and has strong proapoptotic activity in gastric and colon cancer. XAF1 may be an ideal candidate for cancer therapy.

## GIH-22 Increased serum levels and epithelial expression of macrophage migration inhibitory factor in gastric cancer

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**Introduction:** Macrophage migration inhibitory factor (MIF) plays a pivotal role in inflammatory and immune diseases, and is also implicated in carcinogenesis. This study aimed to determine if serum levels and gastric epithelial MIF expression are associated with gastric precancerous lesions and cancer.
**Method**: Ninety patients (M/F, 54/36, age, 56±16 years), 15 with normal gastric mucosa, 15 with *H. pylori*-associated chronic antral gastritis, 20 with intestinal metaplasia in the antrum, and 40 with antral adenocarcinoma, were included in this study. Immunohistochemistry was used to determine MIF expression in gastric epithelial cells, and enzyme-linked immunosorbent assay (ELISA) was used to measure serum MIF. Five gastric cancer cell lines (AGS, MKN-45, MKN-28, MGC-803 and SGC-7901) and one non-malignant gastric cell line (GES-1) were cultured for 24 hours. MIF protein in the supernatant was measured by ELISA, and MIF mRNA in cultured cells was determined by reverse transcription-polymerase chain reaction.
**Results***:* MIF expression in epithelial cells was weak in normal mucosa (12%), but increased in gastritis (52%), intestinal metaplasia (66%) and gastric cancer (96%) ($P<0.001$, ANOVA). Serum MIF level was also increased with the pathological changes (576±82 pg/ml in normal mucosa, 2100±349 pg/ml in gastritis, 4498±253 pg/ml in intestinal metaplasia and 9737±1249 pg/ml in gastric cancer, $P<0.001$, ANOVA). There was a significant correlation between epithelial MIF expression and serum MIF level ($R=0.776$, $P<0.001$). Expression of MIF protein and mRNA was increased in all cancer cell lines, compared to the non-malignant cell line.
**Conclusions:** Advanced gastric pathology is associated with a higher MIF expression in epithelial cells. The epithelial MIF expression directly correlated with serum MIF concentrations. Hence MIF plays a role in gastric carcinogenesis and serum MIF levels may be an important biomarker for diagnosis and prognosis.

## GIH-23 Cigarette smoke promoted human xenograft tumors through the upregulation of cyclin $D_1$ and cyclin-dependent kinases

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**Introduction:** Previous studies reported that cigarette smoke could delay gastric ulcer healing, however, its effect on gastric tumor growth is not well established. Cell cycle control is important in carcinogenesis and cancer growth. Cyclin $D_1$ and cyclin-dependent kinase (Cdk) are necessary for the transition from early to mid $G_1$ phase. These cell regulators are actively involved in human gastric cancer. This study aims to delineate the biological pathways of how cigarette smoke promotes gastric cancer in humans.
**Method:** Male athymic BALB/c nude mice were used as a human gastric cancer model to study the biological actions of cigarette smoke. Human gastric carcinoma cells (AGS) were implanted into the gastric walls of the mice. Cells were pretreated in the presence or absence of cigarette smoke extract (CSE, 100 μg/ml) prior to the injection of cells into the stomach.
**Results:** Cigarette smoke promoted the growth of xenograft tumors, which was accompanied with the upregulation of cyclin $D_1$. Overexpression of cyclin $D_1$ were found in all tumor tissues, and cigarette smoke further upregulated this protein by 30%. Tumor tissues had a 5-fold increase in the expression of Cdk6, and cigarette smoke promoted this protein upregulation. Cdk4, another important binding partner of cyclin $D_1$, was also induced by cigarette smoke when compared with the normal tissues. In CSE-treated group, the expressions of tumor suppressor proteins ($p21^{WAF1/Cip1}$ and $p27^{Kip1}$) were decreased by 20% and 30% respectively in tumor tissues.
**Conclusion:** These results suggested that cigarette smoke promoted the growth of gastric tumor through the modulation of cell cycle regulatory proteins. High expression of cyclin $D_1$ and Cdks are important parameters for the progression of gastric tumor growth induced by cigarette smoke. This study provides an insight to develop a new strategy for the treatment of gastric cancer in humans.

## GIH-24 Mechanism of inflammation-associated colonic tumorigenesis promoted by cigarette smoke

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**Introduction:** Our previous study showed that passive cigarette smoking promoted inflammation-associated adenoma formation in colon, but the underlied mechanism remains unknown. 5-lipoxygenase (5-LOX) is an enzyme that converts arachidonic acid to 5-s-HETE, which in turn is converted to $LTA_4$ and then to $LTB_4$, $LTC_4$ and $LTD_4$. Several studies have suggested that 5-LOX and its products can enhance tumorigenesis. The purpose of the present study was to investigate the possible involvement of 5-LOX in the tumorigenesis enhanced by cigarette smoke in the colon.
**Method:** Male balb/c mice were allocated into 4 groups: control, cigarette smoke (CS), dextran sulfate sodium (DSS) and DSS+CS. They were given water or 3% DSS in drinking water for 7 days to induce colitis, with or without 1-hour daily exposure to 2% CS. They were then allowed to drink water for 14 days. The cycle of 7-day DSS ± CS/14-day $H_2O$ treatment was repeated twice. Mice were sacrificed either immediately (when no adenoma was found) or 1 month after the three cycles of treatments (when cigarette smoke promoted inflammation associated adenoma formation). Colon tissues were collected for assessment.
**Results showed that:** (1) After 3 DSS cycles, 5-LOX protein expression was significantly increased in the DSS+CS group compared with the control and the DSS groups. Similar results were found with vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) which are important mediators for tumor progression and angiogenesis; (2) One month after the cycles, 5-LOX was enhanced only in adenomas induced in the DSS+CS group but not in the DSS group, while VEGF and MMP-2 were both increased in the tumors induced in either DSS or DSS+CS group. In addition, we also found that mice treated with MK886 (a specific 5-LOX inhibitor, 20mg/kg P.O. once daily) throughout the whole 3 DSS cycles significantly reduced tumor formation promoted by cigarette smoke. This was associated with the down-regulation of VEGF and MMP-2 expression. Moreover, we found that cigarette smoke extract had a direct effect on colon cancer cells to release these angiogenic mediators.
**Conclusion:** Taken together, these results strongly suggest that 5-LOX is involved in the formation of colonic adenoma which is promoted by cigarette smoke. This action is probably via the VEGF and MMP-2 activation on the angiogenic pathway.

## GIH-25 Prevalence and clinical spectrum of gastroesophageal reflux disease in Chinese population—a population based study

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**Background and aims:** Population-based data of gastroesophageal reflux disease (GERD) in Chinese population are lacking. The aim of this study is to study the prevalence and clinical spectrum of GERD and its complications in Chinese population and the effects of co-existing anxiety and depression on health care utilization.
**Methods:** 3605 ethnic Chinese households, randomly selected by a computer-assisted telephone interviewing system were invited to participate in a telephone survey using a validated GERD questionnaire and the Hospital Anxiety-Depression Scale (HAD). GERD was defined as heartburn and/or acid regurgitation.
**Results:** 2209 subjects (58% female, mean age of 40.3) completed the interview (response rate 61%). The annual, monthly and weekly prevalence of GERD symptoms were 29.8%, 8.9% and 2.5% respectively. Sex, age and socio-economic status were similar between subject with GERD and subjects without. By multiple logistic regression analysis, GERD symptoms were associated with NCCP (OR 2.3, 95% CI 1.7-3.1), dyspepsia (OR 1.9, 95% CI 1.4-2.5), globus (OR 1.8, 95% CI 1.2-2.7), acid feeling in stomach (OR 5.8, 95% CI 4.5-7.5) and NSAID intake (OR 2.3, 95% CI 1.5-3.6), but not with dysphagia, bronchitis, asthma, hoarseness, pneumonia and history of smoking, alcohol, coffee, tea and aspirin intake. GERD patients had a significantly higher mean anxiety and depression score, and required more sick-leave when compared to subjects without. The frequency of heartburn ($P<0.001$), severity of acid regurgitation ($P<0.001$), female sex ($P<0.001$), higher socio-economic status ($P<0.004$) and the degree of anxiety ($P<0.001$) were independent factors associated with health seeking behaviour in GERD patients by multiple logistic regression analysis.
**Conclusion:** The prevalence of GERD was considerably lower than the western population. The frequency of heartburn, severity of acid regurgitation, female sex, higher socio-economic status and the degree of anxiety were independent factors associated with health care utilizations in GERD patients.

## HO-01 Activation of MAPK signaling pathway is essential for Id-1 induced serum independent prostate cancer cell growth

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Id proteins are a group of helix-loop-helix (HLH) transcription factors that lack the DNA binding domain. These proteins act as dominant inhibitors of basic HLH transcription factors by heterodimerization. The HLH protein Id-1 has been suggested to play a positive role in cell proliferation and tumorigenesis of many types of human cancers. However, little is known about the molecular mechanism involved in the function of Id-1. We have shown in an earlier study that Id-1 may stimulate cell proliferation by inactivation the function of $p16^{INK4a}$/pRb pathway (Carcinogenesis 23: 721, 2002). In this study, using four stable Id-1 transfectant clones, we investigated the involvement of MAPK signaling pathway in the Id-1 induced serum independent prostate cancer cell growth. Our results demonstrated that ectopic Id-1 expression in prostate cancer cell line, LNCaP, led to the activation of Raf/MEK1/2 signaling pathway. In addition, inhibition of MEK1/2 phosphorylation by one of its inhibitors, PD98059, resulted in the decreased cell cycle S phase fraction and cell growth rate, suggest that activation of MAPK signaling pathway is essential for Id-1 induced prostate cancer cell proliferation. Furthermore, treatment with antisense oligonucleotide complementary to Id-1 mRNA in PC-3 and DU-145 prostate cancer cells resulted in a decreased Id-1 expression which was accompanied by decreased Egr-1 protein, one of the downstream effectors of the Raf/MEK1/2 pathway. Our results suggest for the first time that the function of Id-1 is associated with MAPK signaling pathway activation and indicate a possible mechanism in which Id-1 regulates prostate cancer cell growth and tumorigenesis. (Supported by RGC grants to YCW [HKU7186/99M and HKU7314/01M] and Area of Excellence Scheme, UGC of HKSAR, China [Project No. AoE/P-10/01]).

## HO-02 Significance of MAD2 expression in mitotic checkpoint control and cellular sensitivity in nasopharyngeal carcinoma cells

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**Introduction:** Nasopharyngeal carcinoma (NPC) occurs with a high incidence in Hong Kong. Chromosomal abnormalities have been commonly found in NPC, but the underlying mechanism is not well understood. MAD2 (mitotic arrest deficient 2) protein is the key factor controlling the mitotic checkpoint and down-regulation of MAD2 has been found in several types of human cancer.
**Methods:** Western blotting, DAPI staining, flow cytometry and BrdU staining techniques were used to study the mitotic checkpoint control on 5 NPC cell lines. Expression of MAD2 was achieved in one of the NPC cell lines using an inducible expression vector, and the effect of MAD2 expression on chemodrug sensitivity were determined by PI staining and colony forming assay.
**Results:** We found that the mitotic checkpoint was defective in two out of five (40%) of the tested NPC cell lines which was associated with reduced expression of MAD2. Ectopic expression of MAD2 in NPC cells conferred cellular sensitivity to one of the mitotic targeting drugs, vincristine.
**Conclusion:** Our findings support a new model of nasopharyngeal carcinogenesis in which a defective mitotic checkpoint characterized by the reduced expression of MAD2 contributes to chromosomal instability. And expression of MAD2 in NPC cells can lead to sensitization of NPC cells to certain anticancer drugs.

## HO-03 Sequential cytogenetic and molecular cytogenetic characterisation of an *SV40T* immortalised nasopharyngeal cell line transformed by Epstein-Barr virus latent membrane protein-1 (LMP1) gene

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**Introduction:** We have successfully established an *in vitro* cell culture system of nasopharyngeal (NP) cells through transfection with the viral oncogene *SV40T*. The aim of the present study was to identify the genetic events that might be critical for cell immortalization, and the cytogenetic aberrations associated with transfection of the Epstein-Barr virus *LMP1* gene.
**Materials and methods:** Cytogenetic and molecular cytogenetic analyses were performed on four sublines derived from a newly established, *SV40T* immortalized NP cell line, NP69. Two sublines were also transfected with and expressed the *LMP1* gene.
**Results:** A total of 7 cytogenetically related subclones were identified, all having highly complex karyotypes with massive numerical as well as structural rearrangements. Centromeric rearrangements, in the form of isochromosomes and whole-arm translocations, were prevalent. Cytogenetic evidence of gene amplification, i.e., homogeneously staining region (HSR), was detected at chromosomal loci 1q21 in all metaphase cells analyzed. The karyoytpic interpretations were further confirmed by multicolor COBRA FISH, which also showed that part of the HSR contained genetic material from chromosome 20. Extensive clonal evolution could be observed by the assessment of karyotypic variation among different subclones and individual metaphase cells. The evaluation of clonal evolution enabled the temporal order of chromosome aberrations during cell immortalization and malignant transformation to be identified. A striking karyotypic similarity was found between sublines expressing *LMP1* and an NPC cell line, with loss of genetic materials from chromosome arm 3p being an important recurrent observation. More interestingly, the karyotypic features of NP69 were also similar to those of many epithelial malignancies.
**Conclusion:** Our observations suggest that serial transformation of NP cell lines might provide a useful *in vitro* model for the study of the multistep neoplastic transformation of NP cells.
**Acknowledgement**. This work was supported by the Kadoorie Charitable Foundation, and research grants from the University of Hong Kong, and the Swedish Cancer Society.

## HO-04 Differential effects of 9-cis, 13-cis and all-trans retinoic acids on the neuronal differentiation of human neuroblastoma cells

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**Introduction:** Neuroblastoma is one of the commonest pediatric tumors and is derived from neural crest precursors. Differentiation therapy is potentially useful in this tumour. Retinoic acid (RA) is an effective differentiating agent for a variety of tumors *in vitro*.
**Materials and methods:** A human neuroblastoma cell line IMR-32 was used as an *in vitro* model to examine and compare the effectiveness of three naturally occurring RA isomers, 9-*cis* (9c), 13-*cis* (13c) and all-*trans* (AT) RA, in mediating growth differentiation and neuronal differentiation in neuroblastoma.
**Results:** At concentrations of 0.1 and 1 μM, all 3 RA isomers significantly inhibited the proliferation of IMR-32 cells when compared with control cells that were treated with solvent alone. At 1 μM, 9c- or 13c-RA led to a significant decrease of the rate of cellular proliferation to ~20% and ~28% respectively ($P<0.001$), whereas AT-RA was less effective, leading only to a decrease of ~14% ($P<0.001$). Furthermore, all 3 RA isomers up-regulated the expression of the neurofilament protein NF200 in IMR-32 cells after 14 days of treatment. The expression of NF160 was also up-regulated by all 3 RA isomers, but AT-RA induced a higher NF160 expression. The protein expression of tyrosine hydroxylase (TH) was also up-regulated by all 3 RA isomers, although the expression level of TH induced by 13c- and AT-RA was higher than that of 9c-RA. AT-RA (1 μM) induced a 4.5-fold increase in the expression of TrkA in IMR-32 cells, as compared with a ~3-fold increase induced by 9c- and 13c-RA. Western blot analysis using antibodies that detected both the full length and truncated TrkB revealed that 13c-, 9c- and AT-RA slightly down-regulated the expression of TrkB. No truncated TrkB was observed. All 3 RA isomers showed similar effectiveness in mediating the down-regulation of TrkB expression. Finally, IMR-32 cells expressed all RARs (α, β, γ) and RXRs (α, β, γ) isoforms. The expression of RARα, RARβ and RARγ was significantly up-regulated by 13c-RA, 9c-RA and AT-RA. The expression of RXRα was increased by treatment with 13c- and AT-RA, but only slightly by 9c-RA. However, the expression of RXRβ1 was down-regulated by 9c-RA and AT-RA, but unaffected by 13c-RA. Treatment with 13c- and AT-RA resulted in more than 2.5-fold increase in the expression of RXRγ. Similar treatment with 9c-RA led to a 2-fold up-regulation of RXRγ.
**Conclusion:** These results imply that distinctive molecular pathways might be involved in the *in vitro* differentiation of neuroblastoma with different RA isomers. Our observations suggest that the differential actions of RA isomers might be exploited in sequential or synergistic treatment strategies in order to maximize their effectiveness in the differentiation therapy of neuroblastomas.

## HO-05 Effect of arsenic trioxide on the cell proliferation of human neuroblastoma cell line IMR-32 cells

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**Introduction:** Neuroblastoma is one of the commonest pediatric tumors and is derived from neural crest precursors cells. Spontaneous regression and maturation of neuroblastoma to ganglioneuroma suggest that differentiation therapy might be potentially useful. Arsenic trioxide ($As_2O_3$) is an effective therapeutic agent for acute promyelocytic leukemia (APL), and induces both differentiation and apoptosis of leukemic cells *in vivo*. However, the underlying molecular mechanisms are not fully understood. In this study, we used a human neuroblastoma cell line, IMR-32, as our *in vitro* model to study the potential application of $As_2O_3$ as a differentiation agent for neuroblastoma.
**Method:** IMR-32 cells were treated with increasing concentrations of $As_2O_3$ (0.1 to 5.0 μM). MTT assays were performed to examine the effect of $As_2O_3$ on cellular proliferation. Western blot analysis was performed to identify the potential signaling molecules involved.
**Results:** At high concentrations of $As_2O_3$, cellular proliferation of IMR-32 was significantly inhibited (~35% at 1.5 μM and ~78% at 5.0 μM). Western blot analysis of differentiation markers suggested that $As_2O_3$ induced differentiation of IMR-32 cells at low concentrations (≤2.5 μM), but led to apoptosis at high concentrations (5.0 μM). $As_2O_3$ induced the phosphorylation of p42/44 MAP kinases (Erk1/2) and protein kinase C (PKC) in a dose and time-dependent manner. Pre-treatment of IMR-32 cells with Ro-31-8220 (1 μM), a specific PKC inhibitor, partially blocked the effect of $As_2O_3$.
**Conclusion:** We conclude that $As_2O_3$ is capable of inhibiting the cell proliferation of a human neuroblastoma cell line IMR-32 via cell differentiation and apoptosis. Signaling molecules such as Erk1/2 and PKC might play crucial roles during the anti-proliferation action of $As_2O_3$. The study was supported by the Kadoorie Charitable Fund.

## HO-06 Oral arsenic trioxide in the treatment of relapsed acute promyelocytic leukaemia

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**Introdution.** Arsenic trioxide ($As_2O_3$) induces a remission in over 90% of patients with relapsed acute promyelocytic leukaemia (APL). To date, only the intravenous (i.v.) preparation of $As_2O_3$ has been used. We have recently developed an oral preparation of $As_2O_3$ that achieves blood levels of elemental arsenic comparable with those of i.v. $As_2O_3$ (Eur J Clin Pharmacol Oct 11, 2002 online). In this study, the efficacy and safety of oral $As_2O_3$ were evaluated.
**Materials and methods.** Twelve consecutive unselected patients with relapsed APL were treated with oral-$As_2O_3$ (10 mg/day) until remission.
**Results.** Eight patients in first relapse achieved a second complete remission (CR2) after a median of 37 days of oral-$As_2O_3$. Subsequent consolidation with idarubicin or oral-$As_2O_3$ plus all-trans retinoic acid (ATRA, 45 mg/m$^2$/day) resulted in continuous CR2 in seven patients (median follow-up: 12 months). Four patients in second relapse (from CR2 induced by intravenous-$As_2O_3$) achieved CR3 after a median of 31 days of treatment with oral-$As_2O_3$/ATRA. After subsequent consolidation with oral-$As_2O_3$/ATRA, all had remained in CR3 at a median follow-up of 14 months. Patients in CR were negative for *PML/RARA* 3-6 months post oral-$As_2O_3$ treatment. Toxicity of oral-$As_2O_3$ was mild, including leucocytosis without the ATRA syndrome, skin rashes, headache and transient liver function derangement. Cardiac arrhythmias were not observed.
**Conclusion.** These results show that oral-$As_2O_3$ is a safe and efficacious treatment for relapsed APL.

## HO-07 Fludarabine, mitoxantrone and dexamethasone (FND) treatment of T-cell large granular lymphocyte leukaemia: clinical and molecular remission

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**Introduction:** T - large granular lymphocyte (LGL) leukaemia is an uncommon indolent T cell malignancy often presenting with pure red cell aplasia (PRCA) in Chinese patients. The optimal treatment is unclear. Responses have been obtained with methotrexate, cyclophosphamide, cyclosporin A (CsA) and splenectomy. However, refractory relapses and dependence on CsA are major problems. Furthermore, molecular eradication of the leukaemia is often not possible.
**Patients and methods:** Nine patients with T-LGL leukaemia were treated with fludarabine, mitoxantrone and dexamethasone (FND). All patients showed increased $CD3^+CD4^-CD8^+$ LGL with clonal T cell receptor (TCR) gamma gene rearrangement by polymerase-chain-reaction (PCR). FND was given at a median of 28 months post-diagnosis, in two cases as the primary therapy, and in seven cases after previous treatment with cyclophosphamide, anti-thymocyte globulin, chlorambucil and CsA.
**Results:** Clinical remission (CR) was obtained in five patients after one course of FND. Three completed six courses of FND, resulting in CR and molecular remission (MR, PCR for TCR gamma gene negative) for a median of 17 months. MR was obtained in another three patients, who still had persistent anemia that responded to CsA. Six episodes of neutropenic fever occurred in 38 treatment courses. The overall CR rate was 56% and MR rate was 67%.
**Conclusions:** FND is an active regimen for patients with T-LGL leukaemia.

## HO-08 Arsenic trioxide in comparison with chemotherapy and bone marrow transplantation for the treatment of relapsed acute promyelocytic leukaemia

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**Intoduction:** The best overall treatment strategy for patients with acute promyelocytic leukaemia (APL) in relapse with chemotherapy, bone marrow transplantation (BMT) or arsenic trioxide ($As_2O_3$) based therapy remains undefined.
**Patients and methods:** We reviewed the clinical course and treatment outcome of 143 APL cases seen in four major hospitals in Hong Kong over a ten-year period.
**Results:** Complete remission (CR) was attained in 113 cases (79%) with all-trans-retinoic acid (ATRA) and chemotherapy. Relapse occurred at a median of 16 months in 54 cases, with a 3-year disease free survival (DFS) of 56%. Post-relapse treatment was successful in 41 cases (76%), giving an actuarial 3-year overall survival (OS) of 81% from CR1. Three different protocols were used : chemotherapy alone (n=19), allogeneic BMT (n=14), and $As_2O_3$ based regimen (n=21). Chemotherapy was associated with the highest therapy-related-mortality (TRM) at 53%, giving a CR2 rate of 47%. TRM was 36% for BMT. The CR2 rate for $As_2O_3$ based regimen was 100% with no TRM. However, 38% of $As_2O_3$ treated patients had subsequent relapses, which were further salvaged in 75% by combined $As_2O_3$ + ATRA. The actuarial OS for the three protocols leveled off by two years at 82% for $As_2O_3$, 43% for BMT and 23% for chemotherapy (p=0.0004).
**Conclusion:** Our results suggest that $As_2O_3$ may be superior to chemotherapy and BMT for the treatment of APL in relapse.

## HO-09 Quantification of circulating Epstein-Barr virus (EBV) DNA in the monitoring of EBV related lymphoid malignancies

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**Background:** Increased levels of circulating Epstein-Barr virus (EBV) DNA are found in patients with EBV related lymphoma. We quantify serial serum EBV-DNA to assess its use in disease monitoring.
**Material and methods:** Quantification of EBV-DNA was done with real time quantitative polymerase chain reaction (Q-PCR) using primers and TaqMan probes for 2 EBV genes (*Bam*HI fragment and EBNA). The genes were cloned into plasmids to serve for Q-PCR standardization. Cell free DNA was extracted from serial serum samples, and the presence of EBV was confirmed by *in-situ* hybridization for EBV encoded RNA (EBER). Patients with EBV-ve lymphomas and stem cell transplantation (SCT) recipients were also studied to assess fluctuations of EBV-DNA attributable to chemotherapy / immunosuppression.
**Results:** A total of 287 samples from 61 patients (43 at diagnosis, 7 disease-free follow-up only,11 SCT control) were analyzed. The quantification of *Bam*H1 and EBNA show similar results. The range of EBV-DNA detected was $<2x10^4$ to $2.83 \times 10^{11}$ genome copies/ml (7-log variation). At diagnosis, EBV-DNA for 27 EBER+ve lymphomas ranged from $3.8 \times 10^4$-$1.3 \times 10^{11}$(median $2 \times 10^8$). EBV-DNA was raised in all 13 NK/T lymphoma, 5 post transplantation lymphoma and 4 angioimmunoblastic T cell lymphoma. For 19 cases of other histology, EBV-DNA was high in 5 EBER+ve cases (2/12 Hodgkin lymphoma, 2/6 B cell lymphoma, 1/3 peripheral T cell lymphoma) but lower in remaining 16 EBER-ve cases ($<2 \times 10^4$-$2.1 \times 10^7$, median $3 \times 10^6$). Disseminated disease give highest levels, and no patient with level>1 x $10^9$ survived. Serial follow-up in 20 EBV+ve cases showed wide and rapid fluctuations in 16 treated cases, and persistent raised levels in 4 untreated cases. 11 SCT patients also showed EBV-DNA fluctuations ($<10^4$-$10^6$) but 13 EBER+ve cases in remission had undetectable ($<2 \times 10^4$) levels,
**Conclusions:** High levels of circulating EBV-DNA were present in patients with EBER+ve lymphomas, but there is overlap with EBER-ve cases, especially with immunosuppression. As levels change rapidly with treatment and recurrence, a single assay is of limited value. Moreover, since 4-5 log rises occurred in days and pre-dated clinical disease by similar time, pre-emptive therapy is difficult. However, persistent absence was associated with remission, and very high level relate to grave prognosis.

## HO-10 Treatment of leukemic relapses after allogeneic hematopoietic stem cell transplantation (HSCT) with intensive chemotherapy followed by infusion of peripheral blood HSC from the initial donor

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**Background:** Leukemic relapses after allogeneic hematopoietic stem cell transplantation (HSCT) are associated with a poor prognosis. We prospectively investigated the utility of intensive chemotherapy followed by infusion of peripheral blood HSC (PBHSC) from the initial donor as salvage treatment of leukemic relapses post-HSCT. The treatment aimed at leukemia cyto-reduction, acceleration of hematopoietic recovery, and enhancement of the graft-versus-leukemia (GVL) effect.
**Material and methods:** Forty consecutive patients with leukemic relapses after allogeneic HSCT were treated. The initial chemotherapy (ICE) comprised idarubicin (6 mg/m$^2$/day $\times$ 5), cytosine arabinoside (Ara-C, 600 mg/m$^2$/day $\times$ 5), and etoposide (150 mg/m$^2$/day $\times$ 3). PBHSC mobilized with granulocyte colony stimulating factor (G-CSF) from the initial marrow donor was infused 48 hours after the completion of ICE. A total of 48 courses of chemotherapy were administered. Donor DNA chimerism (in 37 patients) and minimal residual disease (MRD) in the marrow (in 8 patients) were monitored with polymerase chain reaction (PCR) with fluorochrome labeled primers for microsatellite markers and reverse transcription PCR respectively.
**Results:** The patients consisted of 19 men and 21 women at a median age of 34 (17-52) years. The initial leukemias were chronic myeloid leukemia (CML, n=7), acute myeloid leukemia (AML, n=21), and acute lymphoblastic leukemia (ALL, n=12), with a median time to relapse post-HSCT at 10 months. In 5 patients with isolated extramedullary disease (EMD), full donor chimerism was preserved. In 32 patients with marrow relapse, chimerism was variable at a median of 30% (0-94 %) donor DNA. The median doses of PBHSC were: $2.3 \times 10^6$/kg CD34$^+$ cell; $5.4 \times 10^8$/kg lymphocytes; and $6.3 \times 10^4$/kg colony forming unit – granulocyte macrophage. Neutropenia lasted a median of 12 (9 – 18) days, with amphotericin B used in 13 cases for a median of 7 (2 – 18) days. Neutropenic death occurred in three (15%) CML patients. Of the 37 engrafting patients, 30 (75%) eventually achieved complete remission (CR). Six patients died of refractory disease, and one was alive on palliative chemotherapy. The response rate for CML (14%) was much lower than AML (84%) and ALL (85%). Relapse of leukemia occurred in 15/30 CR cases (EMD in 10 cases), and 10 patients died in remission from graft-versus-host disease (GVHD, n=6) and sepsis (n=4). The 1-year overall survival (OS) was 48% (ALL: 64%, AML: 52%, CML: 21%), which reached a plateau at 20%. MRD monitoring showed disease eradication in 4 disease-free patients, but remained positive in 4 relapsing cases. Significant GVHD occurred in 18 cases, which was unrelated to lymphocyte dose (p=0.51) or donor DNA chimerism (p=0.58), but was strongly correlated with chronic GVHD from the previous HSCT (p=0.003 RR=1.8). Only the type of disease (AML vs ALL vs CML) (p=0.05) and the type of relapse (EMD vs medullary) (p=0.05) were predictive of CR. The donor DNA percentage did not affect CR rate (p=0.21), but significantly influenced the duration of OS (p=0.037). There was significant improvement in OS in acute leukemia cases treated with PBHSC when compared with 42 historical control cases (p=0.0003). However, clinically significant improvement in long-term survival was only seen in cases undergoing a second allogeneic HSCT (p=0.0001).
**Conclusion:** Intensive chemotherapy supported by infusion of PBHSC is a safe and feasible method to achieve CR after post HSCT relapse of acute leukemia. However, a second HSCT may be needed to improve survival. For CML patients relapsing as blastic crisis, intensive chemotherapy may not be of benefit.

## HO-11 Adoptive transfer of cellular immunity against varicella-zoster virus in bone marrow transplantation patients by vaccinating their seropositive donors with live-attenuated vaccine

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**Introduction:** Patients after bone marrow transplantation (BMT) are at risk of varicella-zoster virus (VZV) reactivation, manifests clinically as herpes zoster (HZ). There is no effective prophylaxis and live-attenuated vaccine given to immunocompromized patients poses risk of vaccine strain reactivation. We have demonstrated that BMT from VZV seronegative donors were more often complicated by HZ, suggesting that natural VZV immunity in seropositive donors might confer protection in BMT patients[1]. In this study, we investigate whether live-attenuated vaccine given to seropositive donors can further boost their humoral and/or cellular immunity and whether this immunity can be transferable to patients after BMT.

**Method:** Live-attenuated VZV vaccine was given to seropositive donors 4-8 weeks before BMT. Humoral immunity was measured by VZV specific IgG by complement fixation test and specific cellular immunity was measured by responder cell proliferation ([$^3$H] thymidine incorporation) and cytokine release (interferon-gamma [IFN] levels in the medium), after stimulation with VZV antigen. Both cellular and humoral immunities were measured in donors prior to vaccination and at the time of BM harvest and in patients prior to transplantation and at 3, 6, and 12 months after BMT. Written consents were obtained from patients and donors and approval was obtained from ethics committee.

**Results:** Immunity measurement was completed in one patient-donor pair. VZV vaccination to the seropositive donor led to a 41-fold increase (relative to pre-vaccination) in T-cell immunity (measured by both thymidine incorporation and IFN level) at two months post-vaccination. VZV specific T-cell immunity of the patient, who was transplanted with BM from the donor and was not vaccinated himself, showed a transient increase (relative to pre-BMT) that peaked at six months post-BMT by 16.6-fold (thymidine incorporation) and 11.1-fold (IFN level) and returned to pre-BMT level one year after transplantation. There was no significant increase of humoral immunity in both donor and patient.

**Conclusion:** Data from the first patient-donor pair suggested that VZV vaccination may boost cellular immunity of seropositive donor and the immunity is transferable to patient post-BMT. More donor-patient pairs are being recruited to confirm the results and to see if this can prevent HZ.

**Reference 1.** Leung AYH et al. *Haematologica*. 2002; 87(4): 444-6

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## HO-12 Identification and characterisation of cellular targets for adenovirus E4orf6 protein

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**Introduction:** The adenovirus E4 region open reading frame 6 (E4orf6) is the third oncoprotein of adenovirus, in addition to the E1A and E1B. We had previously demonstrated that the expression of E4orf6 under the transcriptional control of a zinc-inducible promoter in 10-3 cells (a stable subline derived from 293 cells) resulted in cell cycle arrest at S/G2 phase. To characterize the molecular basis for cell cycle arrest in 10-3 cells, we examined whether E4orf6 expression affected the level of cellular proteins that were key regulators of cell cycle progression. Furthermore, we characterized the potential cellular target proteins of E4orf6.

**Method:** Western blotting and immunofluorescence assays with specific antibodies were performed to investigate the levels of cellular proteins and endogenous adenoviral E1 products with induction of E4orf6 in 10-3 cells. A yeast two-hybrid system was used to screen a human kidney cDNA library for isolation of cellular proteins that interacted with E4orf6.

**Results:** In addition to an elevation of the protein level of cyclin D1, the steady-state levels of the three human CDC25 phosphatases (CDC25A, B and C) as well as the E1A and E1B-55kD proteins were altered following the expression of E4orf6. Two members of basic helix-loop-helix (bHLH) protein family were found by the yeast two-hybrid system to interact with E4orf6. Deletional analysis showed that E4orf6 interacted with the C-termini of both bHLH proteins. Further reporter assays confirmed that the interaction of E4orf6 with the bHLH proteins led to relieve the transcriptional repression by these proteins.

**Conclusion:** E4orf6 may modulate cellular factors in permissive cells to induce cell transformation. CDC25 proteins appear to be the major targets for E4orf6 in disrupting cell cycle regulation. The interaction of E4orf6 with the bHLH proteins requires further investigations to define the downstream effector genes.

## HO-13 Non-myeloablative allogeneic peripheral stem cell transplantation in multiple myeloma: 2 years experience in a single center

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Multiple myeloma (MM) is considered non-curable by chemotherapy alone and the role of standard allogeneic stem cell transplantation (allo-SCT) remains controversial because of relatively high treatment related mortality (TRM). Non-myeloablative allo-SCT is associated with satisfactory engraftment but less toxicity which is essential in the heavily pretreated elderly patient with multiple myeloma. We report our 2 years experience on 10 MM patients receiving non-myeloablative allogeneic PBSC transplantation using fludarabin (30mg/$m^2$)/TBI (150 Gy) as conditioning. 8 patients had full HLA-matched siblings donor, 1 had a one major HLA-antigen mismatched siblings and the last one had a full HLA-matched daughter donor. All patients had active disease before SCT with satisfactory engraftment before D21. No TRM was observed during the whole follow up period. Acute GVHD developed in 3 patients (2 at grade III and 1 at grade IV) and 7 patients had chronic GVHD (3 acute GVHD cases included). At a median follow up of 1 year, 2 patients had complete remission (CR), 3 patients had partial response (PR), 3 patients had no response (NR) and 2 patients were too early for assessment. No relapse case was observed. Only one NR patients had donor lymphocyte infusion (DLI) and 2 NR patients had thalidomide. In conclusion, non-myeloablative allo-SCT in multiple myeloma is effective with less toxicity and TRM. However, a longer follow up period and more cases are required before a definite conclusion can be reached.

## HO-14 Traditional Chinese medicine: effect on bone marrow and peripheral blood cell counts and enzyme induction

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Herbal Medicines are important source of pharmacologically active compounds. While studying metabolism of chloramphenicol succinate (CAPS) by bone marrow, we observed that marrow and blood sample obtained from a single marrow donor (A) contained large amounts of an enzyme that metabolized CAPS to 3 metabolites within 15 min of incubation. While other 2 donors (B and C) marrow also metabolized CAPS to 3 metabolites after 3 hours incubation and in remaining 72 marrow samples CAPS was metabolized to one metabolite after 3-24 hrs incubation. Both marrow and blood cell counts (WBC, RBC, Platelets) were also high in this donor (A) as compared to all other marrow donors' marrow and blood cell counts. This donor was pre-treated with TCM (Siu Fung San) for his allergic reaction 10 days prior to marrow donation. Some *in vitro* cell culture studies were also conducted to see its effects on cell proliferation and CAPS metabolising enzyme. We could not see any enzyme induction effect by using *in vitro* method. It is possible that when given orally herbs may undergo metabolism and active components may be distributed to each tissue. These active components may induce cell proliferation, enzymes and protein synthesis. We conclude that "Siu Fung San" a Traditional Chinese Medicine taken prior to marrow and blood donation by our one marrow donor may be responsible for his high bone marrow and peripheral blood cell counts and large amount of succinate dehydrogenase.

## HO-15 Chloramphenicol succinate a competitive substrate and inhibitor of succinate dehydrogenase: relation to its mechanisms of toxicity

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**Objective/Method:** Chloramphenicol succinate (CAPS) causes marrow depression and in some cases severe aplastic anaemia. Molecular mechanism of this toxicity is still unknown. We studied Ex-vivo metabolism of this antibiotic by human bone marrow to investigate how it is metabolized and which enzyme is involved. To study metabolism marrow samples were incubated with CAPS. To investigate involvement of succinate dehydrogenase (SDH) in CAPS metabolism, marrow samples and rat liver mitochondria were incubated with CAPS in the presence and absence of a known SDH activators and inhibitors at $37^0$C. Detection of metabolites was carried out by HPLC.
**Results:** In 72 marrow samples, CAPS was slowly metabolized to chloramphenicol (CAP). In 20 marrow samples, flavin adenine dinucleotide (FAD) enhanced CAP formation but reduction of FAD to $FADH_2$ was inhibited. While in 3 samples it was metabolized to CAP, nitroso-CAP and another metabolite. Marrow incubated with FAD (control) showed $FADH_2$ peak. No CAP formation was observed when marrow and CAPS were incubated with malonate and 3-NPA. Mitochondria metabolized CAPS to CAP. FAD, succinate and malonate enhanced CAP formation but reduction of $FADH_2$ was inhibited. While, oxaloacetate, 3-NPA and nitroso-CAP inhibited CAPS metabolism to CAP.
**Conclusions:** These studies demonstrate that CAPS is a competitive substrate for SDH. In marrow and mitochondria, it is oxidized to CAP by SDH and nitro metabolites formed may be responsible for inhibition of SDH. In some marrows presence of SDH isoenzyme may be responsible for both oxidation and nitroreduction of CAPS to CAP, nitroso-CAP and possibly hydroxylamino-CAP. SDH may be a target for CAPS induced marrow toxicity.

## NRI-01 Anti-beta2 glycoprotein I (beta2GPI) antibodies facilitates phagocytosis of apoptotic neutrophils in patients with systemic lupus erythematosus (SLE)

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**Background** : Increased apoptosis provides a continuous source autoantigens and has been suggested to have a pathogenic role in SLE. We have previously shown patients with SLE have impaired clearance of apoptotic bodies. In this study, we evaluated whether anti-beta2GPI antibodies, a subset of antiphospholipid antibodies, may have an effect on the phagocytosis of apoptotic bodies by macrophages in these patients.
**Methodology**: Patients satisfying the 1982 revised ACR criteria for SLE and normal controls were recruited for study. Peripheral blood mononuclear cells were separated into neutrophils and monocytes by double gradient centrifugation. Neutrophils were left to undergo spontaneous apoptosis while macrophages were derived from monocytes growing in culture medium. Apoptotic neutrophils were fed to macrophages from SLE patients or normal controls with or without beta2GPI (100 _g/ml) in the presence or absence of goat anti-human beta2 GPI antibodies (10 _g/ml) and examined for the number of macrophages with ingested apoptotic neutrophils under light microscopy (phagocytic index).
**Results**: beta2GPI alone was not shown to increase phagocytosis of apoptotic neutrophils by macrophages (33.2 ± 9.5% vs. 31.8 ± 9.4%, $p$=0.67). However, the addition of anti-beta2GPI antibodies enhanced macrophage phagocytosis of apoptotic neutrophils from 31.8 ± 9.4% to 49.7 ± 6.8% in SLE patients ($p$=0.03). Anti-beta2GPI alone had no effects on the macrophage phagocytosis index. Anti-beta2GPI antibodies plus beta2GPI were also found to facilitate phagocytosis in normal controls but the changes did not reach statistical significance (51.1 ± 14.8% vs. 25.7 ± 12.6%, $p$=0.07). Fcgamma receptor blockade by mouse immunoglobulin IgG1 (50 ?g/ml) did not reverse the facilitation in phagocytosis by anti-beta2GPI antibodies (49.7 ± 6.8% vs. 37.1 ± 20.4%) ($p$=0.47).
**Conclusion**: Anti-beta2GPI antibodies significantly enhance phagocytosis of apoptotic neutrophils by macrophages in patients with SLE. This process is dependent on the presence of beta2GPI and the uptake is not mediated by Fcgamma receptor on the surface of macrophages.

## NRI-02 Efficacy of chemoprophylaxis for tuberculosis infection in patients with systemic lupus erythematosus in an endemic area

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**Background:** Chemoprophylaxis has been advocated for protection against tuberculosis (TB) in immunocompromised hosts. Evidence for the efficacy of chemoprophylaxis in patients with systemic lupus erythematosus (SLE) receiving immunosuppressive doses of corticosteroid is lacking. In this study, we examined the efficacy of isoniazid (INAH) prophylaxis in SLE patients in Hong Kong.
**Methodology:** Records of patients from an inception cohort (1989-2001) of Chinese patients with SLE were reviewed. Episodes of TB that were diagnosed before or after the onset of SLE but before the commencement of immunosuppressive drug treatment were identified. Because of the previous lack of consensus on the use of chemoprophylaxis, INAH (300 mg/day) prophylaxis was prescribed to some patients (chemoprophylaxis group) and not others (non-chemoprophylaxis group) at the discretion of the attending physician when the patient had an exacerbation of SLE requiring immunosuppressive doses of corticosteroid equivalent to prednisolone ≥ 15mg daily. The outcome of these 2 groups of patients was followed for recurrence of TB. A chi-square test was used for statistical analysis.
**Results:** There were 652 patients in the cohort. The mean ± SD (range) age of patients at the time of study was 44.6 ± 11.3 (22-78) years and the age at onset of SLE was 32.4 ± 12.1 (13-71) years. The mean duration of disease of these patients was 14.2 ± 7.4 (median 13.0; range 1-31) years. 101 episodes of TB from 84 patients were identified. The female to male ratio was 76:8. 44 episodes were prophylactically treated with INAH while 57 episodes were not. The rates of recurrence of TB in the chemoprophylaxis and non-chemoprophylaxis groups were 1.56 and 1.51 per 100-patient-year after 13.1 ± 7.6 and 10.4 ± 5.8 years of follow up respectively ($p$=0.61). However, extrapulmonary TB has higher preponderance for recurrence than pulmonary TB (0.89 and 2.82 per 100-patient-year respectively). Patients in the chemoprophylaxis group were also found to have a higher rate of relapse of SLE (0.26 ± 0.26/patient-year) and had higher cumulative dose of corticosteroid than those in the non-chemoprophylaxis group (0.15 ± 0.23/patient-year) ($p$=0.01). A further case-control analysis were therefore carried out matching 30 prophylactic INAH treated and 30 non-INAH treated episodes for these 3 factors. No difference in the rates of recurrence of TB could be detected between these 2 groups (1.55 and 2.17 per 100-patient-year for chemoprophylaxis and non-chemoprophylaxis groups respectively) ($p$=0.73).
**Conclusion:** INAH is ineffective as a prophylactic agent in the prevention of recurrence of TB infection in SLE patients requiring the use of immunosuppressive drug treatment.

## NRI-03 Sex hormones and apoptosis and immunoglobulin production in systemic lupus erythematosus

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**Background**: SLE is an autoimmune disease that affects predominantly female of reproductive age. Previous studies have suggested an immunomodulatory role of sex hormones in the pathogenesis of SLE.
**Objectives**: To examine the effects of various sex hormones on apoptosis and immunoglobulin production by peripheral blood mononuclear cells (PBMCs) in SLE patients.
**Methodology**: Patients who satisfied the 1982 ACR criteria for SLE were recruited. PBMCs were obtained from patients and controls and were cultured with sex hormones at various concentrations, namely 17beta-estradiol (3 and 30 ng/ml), testosterone (3 and 30 ng/ml) or prolactin (20 and 200 ng/ml) for 48 hours. Expression of Annexin V (Anx V), a marker for apoptosis, and bcl-2, an intracellular regulator of apoptosis, was measured by flow cytometry. Supernatants from these cell cultures were examined by ELISA for immunoglobulin (Ig) production. Analysis was made according to the sex of the subjects and the menstruation status of female subjects.
**Results**: 17beta-estradiol (30 ng/ml) was found to induce a higher anx V expression on PBMCs in menstruating and postmenopausal SLE (1.06 $\pm$ 0.10 and 0.93 $\pm$ 0.08 respectively) ($p$=0.04). Testosterone, on the other hand, increased anx V expression in postmenopausal SLE and controls (1.00 $\pm$ 0.08 and 0.92 $\pm$ 0.03 respectively) ($p$=0.048) and reduced bcl-2 expression in menstruating SLE patients. Prolactin was found to increase Ig level in male controls and post-menopausal SLE. (291.5 $\pm$ 80.5 ng/ml and 127.3 $\pm$ 54.8 ng/ml respectively ($p$=0.06). Combination of prolactin and 17beta-estradiol reverses the increased Anx V expression found with 17beta-estradiol alone in menstruating SLE.
**Conclusion:** 17beta-estradiol was found to induce apoptosis more readily in menstruating and postmenopausal SLE patients suggesting a role in regulation of apoptosis. Testosterone, on the other hand, tended to induce higher anx V expression on PBMCs in postmenopausal SLE patients and controls. A stronger pro-inflammatory effect was observed on PBMCs from patients than controls on exposure to prolactin.

## NRI-04 Antirheumatic drug prescribing pattern: a survey of Hong Kong physicians

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**Objective:** To describe the current anti-rheumatic drug prescribing patterns for patients with rheumatic disorders by practicing physicians in Hong Kong.
**Methods:** A questionnaire was sent to 1,000 randomly selected physicians in 2001. The first section covered the demographics for the responding physicians and the second section asked about the management of various conditions and prescribing patterns. 3 case-scenarios were provided at the end of the questionnaire and respondents were asked how each case should be managed. The multiple choice format was used for most questions.
**Results:** 342 (34.2%) physicians responded, the majority (222 [64.9%]) of whom were general practitioners while 52 (15.2%) were general internists. The mean ± SD number of years in current practice was 16.4±10.6. The physicians reported that degenerative joint disease (61.9%), inflammatory arthritis (16.0%) and soft tissue rheumatism (14.5%) were the most frequently encountered rheumatic disorders in their practice. They reported that a range of drugs were used: oral nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) – 95.4%; analgesics – 80.4%; selective cyclooxygenase-2 inhibitors (Coxibs) – 80.1%; topical NSAIDs – 61.4%. Concerning the use of nonselective NSAIDs, 62.5% and 31% of the respondents cited efficacy and safety respectively as the most important factors in their choice of drugs. 92.4% reported co-prescribing gastroprotective agents (GPAs), particularly antacids (71.5%) and H2-blockers (58.3%), with nonselective NSAIDs. Either prevention for potential gastrointestinal (GI) side-effects (45.7%) or both treating GI symptoms and prevention for potential GI side-effects (51.1%) were cited as the primary reason for co-prescribing GPAs with nonselective NSAIDs. 82.3% of the physicians reported to be familiar with COX-2 inhibition. History of GI disease (97.8%), old age (50.0%) and renal impairment (34.5%) were reported to be the determining factors for the use of Coxibs over nonselective NSAIDs. 44.0% of physicians reported that they co-prescribed GPAs with Coxibs.
**Conclusion:** Antiinflammatory drugs were commonly used to treat rheumatic conditions in Hong Kong. While most physicians felt efficacy was important in their choice of drugs, there was an overwhelming use of GPA reflecting doctors' concerns over the GI side-effects of anti-inflammatory drugs.

## NRI-05 Leflunomide (LEF) versus low dose methotrexate (MTX) in adult Asian patients with active rheumatoid arthritis (RA)

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**Objective:** To assess the efficacy and safety of LEF 20 mg/day *vs* MTX 7.5-10 mg/week in adult Asian patients with active RA.
**Methods:** Randomised, double-blind, placebo-controlled, multicentre study design. Patients with active RA (modified Disease Activity Score 28 [DAS28] of >3.2) were assigned to receive LEF or MTX for 16 weeks.
**Results:** 301 patients (LEF: 151; MTX: 150) were included in an intention to treat analysis. There were no differences in the baseline characteristics between patients in the 2 groups. Using the DAS28 score criteria, the responder rates on LEF and MTX were 65.3% and 68.5% respectively (mean [95% CI] difference: -3.2 [-14.5% - 8.2%]; p=NS). Additionally, no differences were found between the 2 groups with respect to mean changes in tender and swollen joint counts, ESR, physician's global assessment and duration of morning stiffness. However, compared with MTX treatment, patients on LEF had significantly better adjusted mean reduction in general health assessment (LEF *vs* MTX: 27.6 *vs* 22.5, p=0.012), patient's global assessment (LEF *vs* MTX: 29.7 *vs* 24.0, p=0.007), and pain intensity scores (LEF *vs* MTX: 28.7 *vs* 22.3, p=0.003). The overall incidence of clinical and laboratory adverse reactions between the 2 groups was similar. Alopecia was more commonly reported in the LEF group (LEF *vs* MTX: 26/151 *vs* 8/150, p=0.002) but there was no withdrawal due to this side-effect.
**Conclusion:** LEF 20 mg/day is as efficacious as MTX 7.5-10 mg/week in adult Asian patients with active RA. However, LEF is more effective than MTX with respect to patient's general health status. Additionally, LEF is as safe as MTX in Asian patients.

## NRI-06 Lymphocyte apoptosis, macrophage function and disease activity in systemic lupus erythematosus (SLE)

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**Introduction**: Increased lymphocyte apoptosis and defects in macrophage removal of apoptotic cells have been suggested to contribute to the development of SLE. The aim of this study was to investigate the relationship between peripheral lymphocyte apoptosis, macrophage function as determined by the serum levels of neopterin and γ-interferon (γ-IFN), and SLE disease activity.
**Methods**: Peripheral apoptotic lymphtocytes (AL) were detected by annexin V-FITC staining and flowcytometry. Serum levels of neopterin and γ-IFN were measured by ELISA. SLE disease activity was determined using the systemic lupus activity measure (SLAM) and the serum titer of anti-dsDNA antibodies.
**Results**: (1) The % of AL in the peripheral blood of active SLE patients was significantly higher (13.07±7.39 %, *n*=30) than that of the inactive SLE patients (4.47±3.39 %, *n*=8, *p*<0.001) and normal controls (5.13±3.37 %, *n*=11, *p*<0.001). (2) Serum levels of neopterin in SLE patients were significantly higher (1.39±1.10 μg/dl, *n*=22) than in controls (0.26±0.19 μg/dl, *n*=20, p<0.01). (3) Serum levels of γ-IFN in active SLE patients were elevated (58.97±34.52 ng/l, *n*=15) when compared with controls (28.06±2.35 ng/l, *n*=16, p<0.05). (4) The % of AL correlated significantly with serum levels of neopterin (r=0.446, p<0.05, n=22) and SLAM score (r=0.533, p<0.001, n=38), but not with the serum levels of γ-IFN. The SLAM score was also correlated with the serum levels of neopterin (r=0.485, p<0.05, n=22) but not with those of γ-IFN.
**Conclusions**: Our study supported the hypothesis that increased lymphocyte apoptosis has a pathogenic role in SLE. The increased levels of serum neopterin may suggest an attempt of the patients' macrophage system to remove the apoptotic cell excess. Since serum levels of neopterin correlated with the overall lupus disease activity, they may be regarded as an index of SLE disease activity.

## NRI-07 A new model of health identified through qualitative and quantitative analysis of the Chinese Arthritis Impact Measurement Scales 2. Cultural issues in the translation and validation of health status measures

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**Introduction:** The purpose of this study was to examine the underlying concepts of health identified from factor analysis of a recently validated Chinese Arthritis Impact Measurement Scales 2 (CAIMS2) and qualitative data collected by the Delphi technique.
**Methods:** An expert panel (rheumatologists, physicians, allied health professionals and patients) was invited to derive the constructs of the culturally relevant health model. They were also asked to identify items in the CAIMS2 which were most valued as measurement of impact of arthritis. A principal-component analysis was performed on these responses to identify the major components of health. Item analysis was performed to select items that could most effectively evaluate each factor of health status affected by arthritis from the CAIMS2. Cronbach's alpha was used to evaluate internal consistency of scales of each item of the CAIMS2 using data from the previous validation study (n = 242). Intra-class correlation (ICC) was used to analyze the test-retest reliability. The psychometric properties of the items selected through qualitative research and quantitative analysis were compared so that the items with best face validity as well as empirical validity and reliability were chosen for the short form.
**Results:** The expert panel had identified the following components of health: physical, psychological/mental, spiritual, free from disease, free from pain, well-being, no restriction on activities of one's choice, social capabilities, enjoy work, sleep and positive thinking as health components. 4 components of health were derived from factor analysis accounting for 68.4% of the variance: 1) physical, 2) upper limb function, 3) psychological and 4) self-care. This model was different from the 5 factor model (physical, symptom, effect, social interaction, role) of the original AIMS2. 22 items were selected to make up the CAIMS2 – short form. This achieves a 61% reduction in length of the CAIMS2. Reliability of scales was satisfactory (Chronbach alpha range: 0.6096 to o.9109). Test re-test reliability of the four domains were physical: ICC = 0.9473, upper limb function: ICC = 0.7327, psychological: ICC = 0.7875 and self-care: ICC = 0.6955. Difference in the scores of CAIMS2 and CAIMS2 – short form were less than 10%.
**Conclusion:** Physical, psychological and functional needs are important for arthritis patients. However, variable perceptions of health exist among different ethnic groups. Cautions should be taken to ensure construct and content validity in cross cultural health status study.

## NRI-08 A randomised controlled study on the rehabilitation of rheumatoid arthrits patients with the use of psychological and occupational therapy

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**Purpose:** To evaluate the efficacy of combined psychological treatment and occupational therapy (PSYOT) in improving the psychological well-being, functional status and decrease of pain of patients with rheumatoid arthritis (RA) in comparison with educational information group (ED) and control group (CR).
**Method:** 49 RA patients recruited from the out -patient clinic of Queen Mary Hospital were randomly assigned into three groups: PSYOT (n=19); ED (n=14); CR (n=16). Patients in PSYOT group participated in six intensive practical training sessions in stress management and coping skills, relaxation training, use of protective orthosis, joint protection and energy conservation technique. Patients in ED group received educational information on the understanding of RA, joint protection and energy conservation technique. Patients in the CR group received routine medical treatment as the other groups for the period studied. Measures of psychological well-being, anxiety and tension levels, pain level and functional status were obtained at the baseline and at the post- treatment (at the completion of the training programme).
**Results:** Patients in the PSYOT group showed significant reduction in both anxiety and pain levels after completing the programme ($p = 0.002$; $p = 0.05$ respectively). Measures in psychological well being and hand & finger function of this group also improved. Patients in ED group also showed a significant reduction in anxiety level ($p = 0.05$) and an improvement in hand & finger function as compared to patients in the CR group. Patients reported that they were more able to manage their problems in activities of daily living after receiving combined psychological treatment and occupational therapy.
**Conclusion:** This study supported that a combined PSYOT intervention is more effective than pure education and routine medical treatment for the rehabilitation of people with RA, particularly in managing pain, anxiety and functional daily activities.

## NRI-09 Use of health status measurement scales among arthritis patients with low educational level

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**Purpose:** Many researchers had advocated the use of self-administered health status measures such as Arthritis Impact Measurement Scales 2 for clinical evaluations in rheumatology. Frequently, illiteracy and patients' age are factors limiting the use of this form of assessment. The aim of this study was to evaluate the validity and reliability of using Chinese Arthritis Impact Measurement Scales 2 (CAIMS2) as a health assessment tool for patients with low educational level.
**Methods:** CAIMS2 was administered to 71 subjects [RA=14, OA=57] with arthritis using a standardized interviewing format. Reliability of scales, test-retest reliability and construct validity was evaluated and compared to statistical results of a previous study on validity and reliability of CAIMS2 among literate clients.
**Results:** Inter-item reliability of the CAIMS2 interviewed version [ICC: 0.6512 to 0.9825] was comparable to that of the self-administered version [ICC: 0.8552 to 0.9594]. Test-retest reliability of the CAIMS2 interview version [r = 0.526 to 0.922] was, however, lower than the self-administered version [r = 0.77 to 0.95].
**Conclusion:** Cautions have to be taken in administering CAIMS2 in an interviewed version. Further studies on health status measurement among illiterate patients is necessary to improve the feasibility of using such scales in clinical settings.

## NRI-10 Effects of *Ganoderma lucidium* (Lingzhi) on cell proliferation and cytokine production of synovial fibroblasts from rheumatoid arthritis

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**Introduction:** *Ganoderma lucidium* (Lingzhi), a medicinal mushroom, has been suggested to have immunomodulatory functions. The major chemicals with these effects appear to be polysaccharides and triterpenes. The purpose of this study was to investigate the effects of Lingzhi on RA synovial fibroblast proliferation and cytokine production including interleukin-1beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-alpha) and monocyte chemotactic protein-1 (MCP-1).
**Method:** Tissue samples were collected from patients with RA. The primary culture of synovial fibroblasts was incubated with hot water extract of fresh Lingzhi polysaccharides for 24hr, 48hr 72hr and 96 hr at various concentrations. Proliferation of synovial fibroblasts was measured by thymidine incorporation and cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA).
**Results:** Lingzhi had no effects on synovial fibroblast proliferation. RA synovial fibroblasts spontaneously produced IL-6, IL-8 and MCP-1 but not IL-1 beta, IL-10 and TNF-alpha. Lingzhi had a slight dose and time dependent stimulatory effect on RA synovial fibroblast in IL-6 and MCP-1 production. Linghzhi also inhibited the production of IL-8 in a dose and time dependent manner but it exerted no effects on IL-1 beta, IL-10 and TNF-alpha production.
**Conclusion:** *Ganoderma lucidium* has slight to moderate stimulating effects on un-stimulated RA synovial fibroblast in the production of IL-6 and MCP-1 and inhibitory effects on IL-8 production without stimulating synovial fibroblast proliferation. The effects of Lingzhi on cytokine synthesis by stimulated synovial fibroblasts, which mimic active disease, are currently being studied.

## NRI-11 Impact of advanced glycation end products on human renal tubular epithelial cell function*

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**Background:** The pathophysiology of diabetic nephropathy is not well understood. Much attention has been focused on the role of advanced glycation end products (AGEs). Glycated albumin is known to stimulate extracellular matrix production in glomerular mesangial and endothelial cells. However, the degree and rate of renal function deterioration correlates more with tubulointerstitial damage than glomerular injury. Data on whether AGEs perturb renal tubular epithelial cell (TEC) function are scarce.
**Method:** Human TECs obtained by primary culture were exposed to glycated bovine serum albumin (gBSA, prepared by incubating purified BSA in 0.5 M D-glucose in 0.2 M phosphate buffer at 37°C for 8 weeks, followed by dialysis to remove low molecular weight reactants and glucose) or nonglycated BSA (BSA incubated without glucose) as control, at doses of 0.5 to 4 mg/ml for 6h. The expression of proinflammatory and profibrogenic cytokine, extracellular matrix protein, and adhesion molecule genes were quantified by RT-PCR.
**Results:** From 1 mg/ml and beyond, gBSA, but not BSA, dose-dependently upregulated IL-8, ICAM-1, and TGF-beta gene expression in TEC. Additionally, significant upregulation of collagen, fibronectin, and MCP-1 transcripts were observed at 4 mg/ml, but not below. There was no difference in the expression of C3, PDGF, and RANTES genes after or between gBSA and BSA challenge.
**Conclusion:** We conclude that gBSA modulates the fibrogenic capacity of TEC. These findings offer new insight into the mechanisms contributory to tubulointerstitial injury and fibrosis in diabetic renal disease.
*Funded in part by the Hong Kong Society of Nephrology Research Grant 2001/2.

## NRI-12 *In-vitro* and *in-vivo* induction of interleukin-8 expression by albumin in proximal tubular epithelial cells: an extended study*

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**Introduction**: We have previously demonstrated that albumin superinduces the expression of IL-8, a potent chemokine, in cultured human renal proximal tubular epithelial cells (PTEC). In this study, we explored the intracellular signaling mechanisms leading to heightened IL-8 production in the nephrotic mileu.
**Method**: Human PTEC was obtained by primary culture. Endocytosis of albumin was assayed by flow cytometry. IL-8 gene and protein expression was measured by RT-PCR and ELISA, respectively. The role of NFkappaB was determined by electrophoretic mobility shift assay, and confirmed with the NF-kappaB inhibitor, pyrrolidine dithiocarbamate (PDTC), and a decoy peptide. Intracellular generation of reactive oxygen species (ROS) was detected by a specific fluorescence probe. IL-8 expression in kidney biopsies of nephrotic subjects was studied by immunohistochemistry and *in-situ* hybridization.
**Results**: Receptor-mediated endocytosis of albumin by PTEC significantly induced IL-8 gene expression, which was localized by *in situ* hybridization to the proximal tubules in nephrotic kidney tissues. Apical albumin stimulated IL-8 secretion in both directions, with basolateral secretion predominating. Albumin activated nuclear translocation of both the p65 and p50 subunits of NF-kappaB. There was corresponding upregulation of intracellular ROS. Albumin-induced tubular IL-8 secretion was abrogated by PDTC, or the cell-permeable peptide, SN50, but not the mutant sequence, SN50M. Histologic scores, graded in an observer-blinded fashion, for IL-8 immunostaining were higher in nephrotic than non-nephrotic subjects ($P = 0.039$), and correlated with the magnitude of proteinuria ($r^2 = 0.332$, $b = 0.116$ [95% CI, 0.029-0.203], $P = 0.012$).
**Conclusion**: Albumin is a strong stimulus for tubular IL-8 expression both *in vivo* and *in vitro*, which occurs via NF-κB-dependent pathways. Our findings provide evidence that tubule-secreted IL-8 is important in the pathogenesis of tubulointerstitial inflammation in the proteinuric state.
*Supported by the UDM Research Grant 00/01.

## NRI-13 Effects of a NOS inhibitor in structural and functional of peritoneal membrane in chronic uremia peritoneal dialysis rat model

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**Introduction:** Glucose based dialysis solution (G) and uremia increase peritoneal angiogenesis leading to a decrease in ultrafiltration (UF) in a chronic peritoneal dialysis (PD) exposure model in rats. Increased activity of Nitric oxide synthase in the peritoneal membrane leading to a higher effective peritoneal vascular surface area with loss of UF capacity in long term PD patient was noted. To investigate whether long term therapy of a NOS inhibitor, L-NMMA (N) could improve the alternations both functional and structural in a chronic PD rat model with an improvement of UF volume.
**Method:** 32 Wistar rats were divided into three groups and had catheter implantation. 1 week after catheter insertion, 70% subtotal nephrectomy (N× ) was performed in two groups. All of them received G for 16 weeks and N was given after infusion of 8 weeks of G for two groups, The groups were N × GN (n=12), N × G (n=12) and GN (n=8). At week 16, 4-hour peritoneal membrane permeability tests were performed before sacrificed and tissues were obtained for morphological studies.
**Reuslts:** MTAC Cr: Mass Transfer area coefficients of creatinine, Gabs: Glucose Absorption, TCUF: Total capillary UF rate, NUFR: Net UF rate, Dip Na: minimum D/P Na, *p<0.05 vs GN.
**Conclusion:** The study showed uremia played a major role in the development of significant alternations namely, neoangiogenesis and fibrosis and less transcellular water transport in the N× groups. However, the study did not showed LNMMA have any effect on both functional and structural alternations. With further analysis, the reasons for the mechanisms and the pitfalls may be clearer.

| | Bld Urea (mmol/L) | MTAC Cr (ul/min) | Gabs (%) | TCUF (ul/min) | NUFR (ul/min) | Dip Na (%) | Vessels (n/field) | Fibrosis grade |
|---|---|---|---|---|---|---|---|---|
| GN | 5.7±0.7 | 181±74 | 70±3 | 73±9 | 26±16 | 87±1 | 6±1 | Mild to moderate |
| N×G | 12±4.1 | 123±19 | 69±4 | 74±7 | 40±18 | 90±2* | 16±7* | Severe* |
| N×GN | 11±4.3 | 128±28 | 69±3 | 73±9 | 34±11 | 89±2* | 14±4* | Severe* |

## NRI-14 The effects of spent dextrose- or amino acid-based peritoneal dialysis fluids on peritoneal mesothelial cell function and ultrastructure

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**Introduction:** Dextrose-based peritoneal dialysis fluids (PDFs) have been implicated in progressive functional and structural deterioration of the peritoneal membrane. PDF with amino acids as the osmotic agent have recently been introduced aiming to improve the nutritional status of PD patients. There is little data on the effects of amino acid-based PDF on the function and ultrastructure of human peritoneal mesothelial cells (HPMC), which play a critical role in peritoneal membrane pathophysiology.
**Methods:** We investigated the effects of two commercially available PDFs, with dextrose (1.5% Dianeal) or amino acid (1.1% Nutrineal) as the osmotic agent respectively, obtained after intra-peritoneal equilibration, on HPMC proliferation (MTT assay and cell counting) and viability (LDH release), IL-6 secretion (commercial ELISA), and ultrastructure (scanning and transmission electron microscopy).
**Results:** Exposure of HPMC to 1.5% Dianeal reduced cell proliferation, total cellular protein synthesis, IL-6 secretion, and cell attachment, but prolonged the cell doubling time on recovery, and increased LDH release ($P<0.0005$, $P<0.001$, $P<0.0001$, $P<0.0001$, $P<0.007$, and $P<0.005$ respectively). 1.1% Nutrineal reduced HPMC proliferation ($P<0.005$), increased IL-6 secretion ($P<0.0005$), but did not affect cell attachment, LDH release, protein synthesis, or cell doubling time. Ultrastructural studies of HPMC exposed to Dianeal showed cell flattening, increased cell surface area, reduced microvilli, and intracellular organelle compatible with dysfunctional mitochondria. In contrast, the ultrastructural morphology of HPMC was relatively preserved after incubation with Nutrineal.
**Conclusion:** Our results showed that HPMC ultrastructure, viability, and protein synthesis were better preserved with amino acid-based PDF, compared to conventional dextrose-based PDF. The significance of IL-6 induction by Nutrineal remains to be elucidated.

## NUS-01 Chondroitinase ABC promotes axonal regeneration of Clarke's neurons beyond the spinal cord injury scar

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**Introduction:** We have previously shown that enzymatic digestion of chondroitin sulfate proteoglycan (CSPG) at the injury scar promotes the axonal regeneration of Clarke's nucleus (CN) neurons into an peripheral nerve graft after spinal cord hemisection. The present study examined whether digestion of CSPG using chondroitinase ABC promoted the regeneration of CN neurons across the scar into the rostral spinal cord in neonatal and adult rats.
**Method:** Following hemisection of the spinal cord at T11, either vehicle or chondroitinase ABC was applied onto the lesion site. The postoperative survival periods were 2 and 4 weeks. Regenerating CN neurons were retrogradely labeled by Fluoro-Gold injection at spinal cord level C7.
**Results:** In the sham group, there was no regeneration of injured CN neurons in both neonatal and adult rats. Treatment with chondroitinase ABC in neonates resulted in 11.8% and 8.3% of the injured CN neurons regenerated into the rostral spinal cord, 2 and 4 weeks respectively. In adults, there were 9.4% and 12.3%, 2 and 4 weeks respectively, of the injured CN neurons regenerated their axons to the rostral spinal cord. After chondroitinase ABC treatment, the immunoreactivity for CSPG was dramatically decreased around the lesion site in both neonatal and adult animals.
**Conclusion:** Our results show that degradation of CSPG with chondroitinase ABC can promote the axonal regeneration in the spinal cord. These results further support the hypothesis that CSPG is inhibitory to the regeneration of neurons in the spinal cord after traumatic injury.
**Acknowledgement:** This work was supported by grants from the Hong Kong Research Grants Council and the University of Hong Kong.

## NUS-02 Early release of mitochondrial cytochrome c and the subsequent activation of caspase-3 are involved in the apoptotic death of neonatal motoneurons after injury

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**Introduction:** We examined the mode of spinal motoneuron (MN) cell death after peripheral nerve injury in neonatal rats.
**Method:** Following root avulsion at C7 spinal segment in neonatal day 1 rats, either PBS or caspase inhibitors was applied onto the lesioned area. The postoperative period was 1, 2, 4, 6, 18, 24 and 48 hours. The time course of apoptosis was assessed by using TUNEL, nuclear staining and expression of cytochrome c and active casapse-3.
**Results:** Apoptotic features were first recognized in degenerating MNs by 6 hours after root avulsion. This was confirmed by both TUNEL and nuclear staining. Cytochrome c was released from the mitochondria into the cytosol as early as 1 hour following the lesion. Cytochrome c was localized preferentially in a diffuse pattern 1 hour after root avulsion whereas near the plasma membrane in normal motoneurons. By 6 hours after the injury, the active form of caspase-3 was first visualized by immunohistochemistry. Treatment with a caspase inhibitor Ac-DEVD-CHO could not completely block the activation of caspase-3 and the release of cytochrome c, whereas the administration of a pan caspase inhibitor Boc-D-FMK delayed the release of cytochrome c for more than 48 hours. These results implied that besides caspase-3, other caspase family members should be taken into account.
**Conclusion:** Because the release of cytochrome c and the activation of caspases play crucial roles in the apoptotic pathway, these results suggest that the mechanism of spinal motoneuron death after root avulsion in developing animals is apoptotic. Accordingly, inhibition of caspases may be a potentially important mean for rescuing immature MNs from injury.
**Acknowledgement:** This work was supported by grants from the Hong Kong Research Grants Council and the University of Hong Kong.

## NUS-03 Reducing calcium-mediated endoplasmic reticulum stress could attenuate beta-amyloid peptide neurotoxicity

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**Introduction:** Beta-amyloid peptide (Ab) has been proposed to play an important role in the pathogenesis of Alzheimer's disease. Exposure of Abeta could trigger disturbance of cellular calcium homeostasis in cultured neurons. Calcium depletion in the endoplasmic reticulum (ER) is one of the major causes of calcium toxicity. Since Ab could trigger ER stress in neurons, we hypothesize that modulation of calcium-mediated ER stress could protect neurons from Abeta neurotoxicity.
**Methods:** Primary cultures of cortical neurons from 17-day-old embryos of Sprague-Dawley rats were set up. The neurons were pretreated with three ER calcium release modulators, 2-aminoethoxydiphenyl borate (2APB), Xestospongin C (XeC) or FK506 for 2 hours, followed by the treatment with Abeta. In order to assess their neuroprotective effects against Abeta, the release of lactate dehydrogenase, quantification of apoptotic nuclei stained with 4',6'-diamidino-2-phenylindole (DAPI) and assessment of PARP cleavage were examined. Intracellular free calcium levels, expression of ER stress proteins and caspase-3 activity were also monitored so as to understand the mechanism underlying the neuroprotective effects of the three drugs.
**Results and Conclusion:** Our results showed that 2APB, XeC and FK506 significantly attenuated Abeta neurotoxicity. These drugs could reduce calcium depletion-induced ER stress and subsequent caspase-3 activation. Taken together, these results reveal that the modulation of ER calcium release may be a pharmacological target in future therapeutic approaches of Alzheimer's disease
**Acknowledgement:** This work is supported by Hong Kong Research Grant Council (HKU 7305/00M and HKU 7292/02M) to JH and seed funding research grant to RCCC (2001-2002).

## NUS-04 Delayed application of GDNF can decrease the NOS expression and rescue injured motor neurons in adult rat with C7 spinal root avulsion

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**Introduction**: Our previous studies have shown that spinal motoneurons express neuronal NOS and then die following root avulsion injury. Expression of nNOS and death of spinal motoneurons due to root avulsion can be prevented if GDNF is applied intrathetically on the lesion site immediately after avulsion. It is unknown whether delayed treatment with GDNF could still prevent the death of motoneurons after root avulsion. This hypothesis is examined in the present study.
**Method**: At 2 weeks after avulsion of C7 spinal root, the laminectomy was made at the C-7 segment and a small piece of gelfoam pre-soaked in the solution of 2 microliters normal saline or GDNF at 10 microgram/microliter was placed in contact with C7 segment. At different surviving days, the nNOS expression rate and motor neuron survival rate were detected by NADPH-histochemistry and neutral red counter stain.
**Results**: NOS expression rate of the motor neurons was 36.46%, 56.49%, 32.73% or 15.13% at 2 weeks, 3 weeks, 4 weeks or 6 weeks after avulsion. With a single dose GDNF, started at 2 weeks after avulsion, the NOS expression rate was markedly reduced to 7.30%, 7.99% and 9.24% at 3 weeks, 4 weeks or 6 weeks. In lesion animals, the motor neuron survival rate was 85.95%, 80.5%, 59.66% or 36.32% at 2 weeks, 3 weeks, 4 weeks or 6 weeks after avulsion. But in lesion animals treated with GDNF, the survival rate was increased to 94.33%, 93.75% or 91.83% % at 3 weeks, 4 weeks or 6 weeks.
**Conclusion**: Our data show that during a 6 week process of neurodegeneration a single dose GDNF treatment, though delayed up to 2 weeks, can still inhibit the expression of nNOS and allow substantial rescue of injured motor neurons.

## NUS-05 Diffusion tensor imaging in the evaluation of Wallerian degeneration in paediatric stroke: work-in-progress

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**Introduction**: Wallerian degeneration, the anterograde degeneration of axons and myelin sheaths after proximal axonal or cell body injury, is known to occur after cerebral infarction. In this pilot study, we aim to evaluate if diffusion tensor imaging (DTI), using the indices of fractional anisotropy (FA) and mean diffusivity (MD), can detect and quantify Wallerian degeneration in paediatric middle cerebral artery (MCA) strokes and to compare the findings with conventional MR imaging.
**Methods**: Nine children with unilateral MCA infarctions were studied. Axial T1-weighted, proton density and T2-weighted images, as well as DTI were performed using a Signa 1.5 Tesla imager. Quantitative values of FA and MD were obtained by manually placing regions-of-interest (ROI) in the infarction, and selected areas along the ipsilateral corticospinal tract, i.e. the posterior limb of the internal capsule (PLIC) and cerebral peduncle (CP). Identical ROIs were placed in the matched contralateral regions. The corticospinal tract FA was derived by the mean value of PLIC FA and CP FA. The presence of signal intensity changes in the internal capsule and cerebral peduncles on T2-weighted images were recorded. Statistical comparisons between two sides were performed using the Student's t-test.
**Results:** WD was detected on conventional T2-weighted imaging by hyperintense signal in the PLIC in 4 children and CP in 1 child. The FA of the infarction, PLIC and CP were reduced, and the MD of the infarction and PLIC were increased on the ipsilateral side compared to the contralateral side in all children, whilst the MD of the CP was increased in six children. The mean FA ratio of the ipsilateral to the contralateral side in the infarction, PLIC, CP was 0.45, 0.77 and 0.79 respectively and the differences were statistically significant in all sites ($p=0.029$, $p=0.014$ and 0.008 respectively). The mean MD ratio of the ipsilateral to the contralateral side in the infarction was 2.55 and this difference was statistically significant ($p<0.001$). The differences in MD in the other sites were not statistically significant.
**Conclusion:** DTI is more sensitive than conventional MRI and can be used to detect and quantify WD. Further studies are required to determine if the measurement of FA in Wallerian degeneration can be used as an indicator of neuromotor outcome.

## NUS-06 Diffusion tensor imaging for the evaluation of treatment-induced neurotoxicity in childhood medulloblastoma

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**Introduction:** We propose the use of diffusion tensor MR imaging (DTI) to evaluate treatment-induced white matter (WM) injury in childhood medulloblastoma survivors and also aim to determine if fractional anisotropy(FA) can be used as an index for evaluation of treatment-induced neurotoxicity.
**Methods:** 13 medulloblastoma survivors who were treated with surgery, cranial irradiation and chemotherapy were evaluated. Conventional MR imaging and DTI were performed using a 1.5 Tesla imager. FA maps were generated using the FUNCTOOL software. Voxel-based comparison between the patient and control groups was performed with SPM99. Contrasts (1 –1) and (–1 1) were employed for the detection of positive and negative activations. FA of selected supratentorial WM sites (frontal periventricular WM, parietal periventricular WM and corona radiata) were also measured by placement of regions-of-interest (ROI). ROIs of similar size were placed on identical sites as far possible in the healthy age-matched controls. FA (sum of frontal and parietal WM and corona radiata FA) was compared with age at treatment, time interval after treatment and intellectual outcome (deterioration of school performance). Two-tailed paired t-test was used for detection of statistical significance.
**Results:** Patients were between 3 -17 yrs of age at treatment (mean: 8.2 yrs) and time-interval between treatment and MR imaging ranged between 1–11 yrs (mean: 3.7 yrs). Voxel-based comparison showed areas of activation in the periventricular WM, especially parietal WM, and corona radiata. Using ROIs, mean FA of patients was reduced in all sites compared to controls, with a reduction of between 15.6% and 19.2%. The reduction was statistically significant in the parietal WM and corona radiata ($p=0.011$ and $p=0.040$ respectively) FA reduction of the groups £ 5 years ($n=5$) and >5 years of age ($n=8$) at treatment was 61.1% and 34.8% respectively and FA reduction in the group with < 5 years ($n=8$) and ≥ 5 years interval ($n=5$) since treatment was 35.4% and 60.3% respectively. These differences were however, not statistically significant. Comparing school performance, FA reduction of those with mild deterioration ($n=5$) and those with moderate/severe deterioration ($n=8$) was 19.9% and 60.6 % respectively and this difference was statistically significant ($p=0.041$).
**Conclusion:** Loss of anisotropy occurs in the periventricular white matter of post-treatment medulloblastoma survivors and this loss is significantly greater in those with poor intellectual outcome. DTI is therefore useful in detection and monitoring of treatment-induced neurotoxicity.

## NUS-07 Headache co-morbidity and seizure control in patients with epilepsy

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**Introduction:** Headache (HA) and epilepsy (EP) are common neurological diseases with significant morbidities and mortalities and pose enormous economic burden to our societies. They share clinical characteristic including episodic nature and response to anticonvulsants, and they potentially share similar pathophysiological mechanisms, for example, channelopathies. Here, we hypothesis that the presence of headache comorbidity in patients with epilepsy is associated with poor seizure control.
**Methods:** We studied 904 consecutive patients with seizure disorders in the Epilepsy clinic of Queen Mary Hospital. We successfully interviewed 474 patients with a previously validated, standardized questionnaire to screen for HA comorbidity. Patients with non-epileptic seizure, mental retardation or dementia were excluded. The HA diagnosis was subsequently validated by an independent neurologist who was unknown to individual EP diagnoses and the relevant investigation results. All patients were prospectively followed-up for 12 months to optimize their antiepileptic drugs (AEDs) therapy aiming at achieving a seizure free state. During which, HA diagnosis was also blinded from the attending neurologist for AEDs optimization. No specific treatment, other than simple analgesic, was prescribed for HA during the study period.
**Results:** HA comorbidity was found in 157 patients with EP. Among which, 61 of them could not achieve a seizure free state after a 12 months period. In contrast, only 68 of 315 epileptic patients without HA comorbidity failed to achieve a seizure free state. ($p< 0.000$, Odd ratio = 2.308, 95% Confidence interval 1.519 – 3.507).
**Conclusions:** Headache is a common co-morbidity for patients with epilepsy and associates with a poor seizure control state. This indicates that monitoring headache comorbidity of patients with epilepsy is equally important. Further study is needed to determine if modification of headache comorbidity could improve seizure control.
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## NUS-08 Postictal psychosis related lateral temporal hyperperfusion

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**Introduction:** Postictal psychosis is a rare complication of epileptic seizure characterized by reversible psychotic symptoms after flurries of seizure attack. It was attributed to a phenomenon similar to Todd's paralysis without definite proof. We hypothesis regional hyperperfusion cerebral SPECT defect is associated with postictal psychosis complicating epileptic seizure. Two years ago, we reported our preliminary data of 99mTc-HMPAO cerebral SPECT findings in two patients with PIP. This study is an extension of our previous work.
**Methods:** We prospectively recruited patients with postictal psychosis and performed 99mTc-HMPAO SPECT scan during PIP. Interictal scans were performed at least 4 weeks apart from the PIP and taking off from anti-psychotic medications, if any. The average uptake ratios for each predefined brain region of interest (ROI) were normalized to cerebellum. Semi-quantitative analysis of normalized rCBF was performed and asymmetry index (ASI) was calculated during interictal state and during postictal psychosis.
**Results:** We identified six consecutive patients with PIP including two patients we reported previously. Statistically significant difference could be identified between interictal ASI and PIP ASI over lateral temporal region ($p=0.017$). No statistical significant difference could be observed between interictal ASI and PIP ASI for other ROIs. No statistical difference could be detected between the mean normalized count for each individual ROIs and no association could be detected between lateralization of seizure onset and hyperperfusion abnormalities.
**Conclusions:** This study provides further support to our earlier report of lateral temporal hyperperfusion in patients with postictal psychosis. Although hyperperfusion abnormality in SPECT can be found in Todd's paralysis, such findings are more commonly found in patients with cerebral hyperactivity conditions. Considering the clinical characteristics of postictal psychosis of preceded lucid interval and crescendo–decrescendo clinical course, our findings suggest an alternative pathogenic mechanism for the development of postictal psychosis, for example, activation of subcortical circuit. Furthermore, our observations may be related to the pathogenesis of force normalization.

## NUS-09 A prevalence study of epilepsy in HKSAR, China

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**Introduction**: Epilepsy is a common disorder. Epidemiological data is crucial ital for physicians and health care administrators for taking care patients with epilepsy. In this communication, we report the the epidemiology data of the Hong Kong West (HKW) region of Hong Kong Special Administrative Region (HKSAR).
**Methods***:* With the implementation of clustering system in our clinic since 1996, the epilepsy clinic of Queen Mary Hospital is managing a vast majority of adult patients (> 15 years old) with chronic seizure disorders resided in the HKW region where hosted an adult population of 475,900. Seven hundred and thirty-six patients [female 42.9%, male 57.1%, mean 40.8, SD 13.6] with epilepsy were recruited. All patients underwent EEG examination and each subject was independently assessed by two epileptologists for diagnosis and classified according to ILAE recommendations.
**Results:** The prevalence rate of active epilepsy at or above 15 years of age was 1.54 in 1,000 at the prevalent date (January 1, 2002). 285 (38.8%) had idiopathic epilepsy syndromes, 100 (13.6%) had cryptogenic, and 285 (38.8%) had a remote symptomatic etiology. Seizure type was partial in 408 patients (55.4%) and generalized in 285 (38.8%). Thirty-one patients (4.2%) had positive family history. Interestingly, common idiopathic generalized epilepsy syndromes like juvenile myoclonic epilepsy (0.68%) and childhood absence epilepsy (0.95%) were uncommonly encountered.
**Conclusions:** In summary, this clinic based epidemiological study provides crucial data for epilepsy service development and research in HKSAR. Further hospital based or, preferably, door-to-door population based epidemiological study is indicated to ascertain the population based epidemiologic data for epilepsy with patients resided in HKW of HKSAR.
**Acknowledgment**: This study was supported, in part, by University Department Medicine research grants from University Department Medicine, The University of Hong Kong (to GCYF).

## NUS-10 A large Chinese kindred with familial ALS without SOD1 mutation

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**Introduction:** Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disorder with progressive muscle weakness and wasting. About 10% of familial ALS (FALS) cases have SOD1 mutations.
**Methods:** We ascertained a large Chinese kindred with autosomal dominant FALS. All consented family members underwent detailed clinical, electrophysiological and, if indicated, pathological examination.
**Result:** A total of 16 members (12 living) were classified as affected. Eighteen living (eight affected and ten unaffected) members were available for study. Historical review of the clinical features, and clinical, electrophysiological and pathological assessments showed a phenotypic spectrum in this family, from typical ALS (N=8), who rapidly deteriorated with progressive muscle wasting, weakness and respiratory failure to a group (N=8) with very slowly progressive predominantly lower motor neuron lesion. The clinical features of a member from each group appear to lie between the two ends of this spectrum. Mutation screening for SOD1 mutation were all negative in all 5 exons.
**Conclusion:** The interesting phenotypic spectrum observed in this family is distinctive although similar FALS families with heterogenous phenotypes were reported within the same pedigree. Further studies to identify the causative gene/s in this ALS kindred are indicated.
**Acknowledgment**: This study was supported, in part, by Liu Po Shan/Dr Vincent Liu Endowment Fund for Motor Neurone Disease, Faculty of Medicine, The University of Hong Kong (to GCYF/SLH).

## NUS-11 Gene mapping of familial amyotrophic lateral sclerosis

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**Introduction:** Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disorder characterized by gradual death of motor neurons in cerebral cortex, brain stem, and spinal cord. The pathogenetic mechanism remains unclear for the vast majority of cases. About 10% of ALS cases are familial (FALS). Cu/Zn superoxide dismutase (SOD1) gene accounts for about 10% of autosomal dominant FALS and the gene(s) responsible for the rest of ALS/FALS remain(s) to be found.
**Method:** We recruited a large Chinese kindred without SOD1 mutation for linkage analysis. Peripheral blood samples were collected and DNA were extracted from peripheral lymphocyte. We screened the family with ~ 400 polymorphic microsatellite markers. The genotyping data were subjected to model-based and model-free linkage analysis.
**Result:** Using MLINK of LINKAGE (Ver 5.2) package, we found a maximum LOD score of 4.357, $?_{[m=f]}$=0.0 at a microsatellite marker located at distal long arm of chromosome 8. Multipoint analysis by GENEHUNTER (Ver 1.2) revealed a maximum multipoint LOD score of 3.909 and NPL score 9.209. Haplotype analyses revealed a critical region which spanned 10.18-cM on chromosome 8.
**Conclusion:** We identified a 10.18-cM critical FALS region on chromosome 8. Further analyses using positional cloning and candidate gene approach are indicated to delineate the underlying genetic defect for FALS in this family.
**Acknowledgment**: This study was supported by Liu Po Shan/Dr Vincent Liu Endowment Fund for Motor Neurone Disease, Faculty of Medicine, The University of Hong Kong (to GCYF/SLH), by the University Department Medicine, The University of Hong Kong (to GCYF and TS Cheng), and by the Neurology Donation Fund.

## NUS-12 Magnetic resonance imaging and diffusion tensor imaging in Chinese neonates with hypoxic ischemic encephalopathy

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**Introduction:** Several studies have suggested the potential utility of diffusion tensor imaging (DTI) in the evaluation of brain injury in the asphyxiated neonates.
**Method:** We present our initial experience with DTI in Chinese neonates (gestational age 37 to 41 weeks, age 1 to 9 days of which there were 3 females) who were diagnosed as having hypoxic injury on clinical examination and the severity of the insult was graded using Apgar scores and Sarnat staging. Magnetic resonance imaging (MRI) with DTI was performed in eighteen neonates (eight with hypoxic ischemic encephalopathy [HIE] and ten with no cerebral pathology). The specific areas of interest were chosen in selected white matter (WM) areas: the posterior limb of the internal capsule, frontal WM, occipital WM, central WM, and temporal WM. The apparent/average diffusion coefficient (AADC) and relative anisotropy (RA) were compared between neonates with HIE and those without cerebral pathology using One-Way ANOVA.
**Results:** Abnormality on MRI was noticed in 3 of 8 neonates with HIE of different clinical stages. One neonate in Sarnat stage I and Apgar score of 10 showed periventricular changes as the MRI abnormality. Of the 2 neonates in Sarnat stage II and Apgar score of 3, one had periventricular changes and another showed thalamic abnormality. In contrast, DTI abnormality was noticed in all 8 neonates. A marked decrease of the AADC values was found in the posterior limb of the internal capsule, frontal WM and occipital WM in neonates with HIE. In addition, the RA values were marked reduced in HIE-affected neonates over the frontal WM and occipital WM.
**Conclusion:** The lower AADC in the capsule indicates active myelination and the presence of myelin, while the lower RA in the cerebral WM (e.g. frontal WM and occipital WM) over the site of injury indicates reduced directionality of diffusion in these brain areas and suggests that central fiber tracts have been destroyed or their subsequent development would be impaired. Based on our initial experience, we conclude that the DTI is potentially useful in understanding the basis of the neurologic deficits and DTI has a better correlation with the Sarnat staging and Apgar scores than MR imaging.

## NUS-13 The fractional anisotropy of cerebral gliomas on diffusion tensor magnetic resonance imaging

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**Introduction**: Unlike conventional diffusion-weighted magnetic resonance imaging (MRI), diffusion-tensor imaging (DTI) permits the calculation of an apparent average diffusion constant (AADC) and fractional anisotropy (FA). DTI characterizes diffusive transport of water and provides additional structural information of the brain tissue. Several studies have described the DTI abnormalities of brain tumors, including an increase in AADC within the tumoral tissue and a decrease in FA within and around the tumor.
**Method**: We used DTI to describe deviation or distortion of fibers in normal white matter (WM), tumoral WM, peritumoral WM and WM adjacent to the brain tumor in a cohort of ten patients with confirmed cerebral gliomas. MRI scanning was performed using a 1.5 T GE Signa system with version 9.1 software. The FA from the specific areas on the side of brain tumor was compared to that of the corresponding WMs over the contralateral hemiphere using student's t test and compared among the specific areas using one way ANOVA
**Results**: The median age was 53 years old, and there were 5 females. The FA was reduced in the tumoral and peritumoral brain tissue ($P<0.001$). The abnormal FA in the tumor was much lower than that in peritumoral brain tissue ($p<0.001$). In the apparently distorted WM adjacent to the tumor, there were no difference in the FA between the two sides.
**Conclusion**: DTI maps may provide useful information about WM architecture and its alteration due to the tumors. FA provided details of anatomic information on relationships between tumors and nearby WM tracts, which may assist the interpretation of neurological findings and preoperative planning.

## NUS-14 Visual cortical activations on functional magnetic resonance imaging upon stimulation of vision-implicated acupoints

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**Introduction**: Cho and colleagues previously reported very close correlations between the visual cortical activations on functional magnetic resonance imaging (fMRI) following stimulation with visual light and those following conventional acupuncture.
**Method**: In this study, we compared the brain activations on fMRI obtained during visual stimulation using light-emitting diodes (LED) flashing at 8 Hz with that obtained during conventional or electro- acupuncture (at 2 or 20 Hz) applied to 4 vision-related acupoints (BL60, BL65, BL55, and BL67) over the lateral aspect of the right foot in 18 healthy volunteers. fMRI was performed using a 1.5 T magnetic resonance scanner with standard scanning parameters. Activation periods of 30 s were alternated with rest periods of 30 s. First, fMRI was performed with visual stimulation. Next, fMRI was repeated with conventional and then electro- acupuncture.
**Results**: When compared to positive activations on fMRI over the visual cortex upon LED stimulation, similar activations were seen in 10 subjects during conventional acupuncture and in 8 and 7 subjects, respectively, during electro-acupuncture at 2 and 20 Hz. Negative activations were also seen bilaterally in the occipital lobes and temporal gyri in 13 subjects during conventional acupuncture.
**Conclusion**: Acupuncture may exert its effects via modulating the activity of relevant brain sites. Our results also show that electro-acupuncture is useful for future studies.

## NUS-15 A functional magnetic resonance imaging study comparing brain activations during language task with activations during electrical stimulation of language-implicated acupoints

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**Introduction:** Functional magnetic resonance imaging (fMRI) can visualize brain activations during various task states. Acupuncture may mediate its effects via modulation of brain activities.
**Method:** We compared the brain activations on fMRI during a word generation task with the activations during electrical stimulation of two language-implicated acupoints in 17 healthy Mandarin-speaking male Chinese volunteers aged between 19 and 26 years. All subjects were strongly right handed according to a handedness inventory.
**Results:** Using a standard fMRI protocol and a word generation paradigm, significant activation was seen in the left and right inferior frontal gyri (Brodmann's area [BA] 44, 45) as well as the left superior temporal gyrus (BA 22, 42). Stronger activation with a larger volume was seen in the left hemisphere. Bipolar electrical stimulation of the language-implicated acupoints, SJ8 (11 subjects) or DU15 (6 subjects), without the word generation paradigm in the same cohort produced significant activation in the right inferior frontal gyrus (BA 44, 46) and in the left and right superior temporal gyrus (BA 22, 42), respectively. In contrast, bipolar electrical stimulation of the adjacent non-acupoints failed to produce any significant brain activation.
**Conclusion:** Ability of acupuncture over SJ8 or DU15 in selective activation of the language-implicated cortical sites may be related to the benefit of acupuncture on SJ8 or DU15 in language disorders. Nevertheless, stimulation of SJ8 or DU15 failed to activate the main cortical sites for language (left BAs 44, 45, 46); only subsidiary language areas are activated.

## NUS-16 Implications of hydrocephalus upon presentation of tuberculous meningitis

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**Introduction:** Tuberculous meningitis (TBM) is a serious disorder and hydrocephalus is a known complication of TBM that may occur early or late. The clinical implications of hydrocephalus upon initial presentation of TBM are uncertain.
**Methods:** From January 1997 to September 2001, adults patients diagnosed as TBM in our hospital were studied. Patients referred from other centers for management of hydrocephalus with TBM were excluded. Patients with hydrocephalus on initial or subsequent CT scans were assessed by surgeon and operated if necessary. A standardized regime of anti-TB therapy guided treatment. Patients were followed up regularly for at least 1 year after commencement of anti-TB drugs. A modified Barthel index of 12 or less at 1 year after treatment and mortality were criteria for poor prognosis. Clinical, radiological, microbiological data were analyzed.
**Results:** A total of 31 patients had TBM diagnosed during the study period. Nine of the 31 had hydrocephalus on CT scan upon presentation, and 8 of the 9 required urgent neurosurgical intervention. Of the 22 patients without hydrocephalus on presentation, only 1 developed hydrocephalus subsequently. Age, sex, duration of presenting symptoms and CSF parameters were indifferent between patients with or without hydrocephalus on presentation. Unsteady gait and ataxia ($p=0.001$) were more common in patients with hydrocephalus. Despite having similar Glascow coma scale on presentation ($p=0.838$) , patients with hydrocephalus on presentation were more likely to develop into stage 2 or 3 disease ($p=0.045$) and more likely to develop complicating strokes ($p=0.012$) due to cerebral infarcts ($p=0.007$), with poorer prognosis compared with patients without hydrocephalus on presentation ($p=0.004$).
**Conclusion:** Hydrocephalus upon presentation is common in local TBM patients (29%). This is unrelated to late diagnosis or delayed presentation, suggesting possible abrupt intense inflammatory response to MTB. It seems to be a marker of severe TBM with high risk factor complicating cerebral infarcts associated with poorer prognosis. TBM must be considered for patients who present with hydrocephalus.

## NUS-17 Seronegative myasthenia gravis in Hong Kong Chinese

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**Introduction:** Acquired myasthenia gravis (MG) is an autoimmune disease due to anti-acetylcholine receptor antibodies (anti-AChR) that damage nicotinic acetylcholine receptors on neuromuscular junctions. Variable proportions of generalized MG patients were reported to have undetectable serum anti-AchR level, designated as seronegative MG (SNMG) but without clear definitions of SNMG. One group reported that antibodies against muscle-specific kinase was the pathogenetic basis of SNMG. The exact pathogenesis of SNMG is not yet certain and genuine frequency is unknown.
**Methods:** Patients with MG in neurology clinic were studied with clinical, radiological, serological, electrophysiological and histological data reviewed. Patients with initial negative anti-AChR had the assay repeated at least 12 months apart from the first one, together with anti-striational antibody test. Patients with family history of MG had EMG results repeated in details to exclude congenital MG. Patients with repeatedly negative anti-AChR binding assays had serum tested for anti-AChR modulating antibodies (a bioassay using cultured muscel cells) and P/Q type calcium channel antibodies to look for Lambert-Eaton myasthenic syndrome. Only patients with repeatedly negative anti-AchR binding and anti-striational antibodies, negative for anti-AchR modulating and calcium channel antibodies, and with congenital MG excluded were defined as SNMG.
**Results:** A total 52 MG patients were studied, 21 had pure ocular MG and 31 had generalized MG. Three had turned seropositive upon repeated assay and 1 had positive test for modulating antibodies only. One patient with family history of had congenital MG upon detailed EMG. Only 2 of the 31 generalized MG patients (6.5%) were SNMG. One of the 2 generalized SNMG patient had thymic hyperplasia. No SNMG patients had thymoma. SNMG is more common in pure ocular MG patients.
**Conclusion**: Generalized SNMG is rare. Strigent criteria are required for diagnosis of SNMG. Thymic hyperplasia, but not thymoma, can occur in SNMG. We postulate that a subgroup of SNMG patients may have low titer of high affinity anti-AchR undetectable by current assays.

## NUS-18 An epidemiological study of motor neuron disease in Hong Kong

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**Background:** In a previous epidemiological study conducted in 1990s, the incidence and prevalence of motor neuron disease (MND) in Hong Kong Chinese were found to be low compared to the worldwide figures. Moreover, the incidence of MND had been reported to increase steadily over the last few decades in other parts of the world. In this communication, we reported our epidemiological data of MND in the Hong Kong West region of Hong Kong Special Administrative Region of China and compared the results with that of the previous study.
**Method:** We identified the subjects from the Hospital Authority Database by searching the admission records of Queen Mary Hospital, between 1997 and 2001, using ICD-9 codes of 335. Each retrieved case record was reviewed independently by at least two neurologists. The clinical diagnosis and classification of MND were based on the revised El Escorial criteria.
**Results:** Of 50 identified subjects, 27 subjects were recruited in the present study. Among which, 22 (81%) were definite MND and 5 (19%) were probable MND. Among definite MND, 15 (68%) were limb onset. The male to female ratio was 2.8:1. All subjects were Chinese. The number of new cases from 1997 to 2001 was 22. Thirteen cases were died in this period, and 14 patients were surviving as of December 31, 2001. Therefore, the annual incidence was 4.4 with an incidence rate of 0.77/100,000/year, the point prevalence at December 31, 2001, was 2.4/100,000, the average annual mortality was 2.6, and the mortality rate was 0.46/100,000/year. The mean survival time was 27.1 months (range: 7 to 65 months, SD 17.9). The mean age of onset was 51.5 years (range: 30 to 77 years, SD 11.7), with a peak observed between 55 and 59 years. Eleven cases (40%) had their disease-onset before 50 years of age.
**Conclusion:** In this study, the incidence and prevalence of MND among Hong Kong Chinese remained low compared with the worldwide figures. However, comparing to the previous epidemiological data of MND collected between 1989 and 1992, we noticed a trend of increase in the overall incidence (148%) as well as prevalence (152%) over the last decade. Moreover, the incidence of MND among the younger age group was also increased. Our data warrant a territory wide epidemiological study to document the changes in MND epidemiology in Hong Kong over the last decade.
**Acknowledgment:** This study was supported by Liu Po Shan/Dr Vincent Liu Endowment Fund for Motor Neurone Disease, Faculty of Medicine, The University of Hong Kong (to GCYF/SLH), and by the University Department Medicine, The University of Hong Kong (to GCYF and TS Cheng).

## NUS-19 A study of hemiplegic shoulder pain at Tung Wah Hospital

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**Introduction:** Hemiplegic shoulder pain was reported in 30-40% of stroke patients in Western literature and found to be more common during the spastic phase of motor recovery. It is our impression that the incidence of hemiplegic shoulder pain in Hong Kong is not as high. This study aims to investigate the incidence, diagnosis and association factors of shoulder pain in stroke survivors at Tung Wah Hospital, one of the regional rehabilitation centre of the Hong Kong West cluster.
**Method:** Retrospective case note review of all 114 stroke patients who were admitted into Tung Wah Hospital Stroke Rehabilitation Unit from March to June 2002. Hospital notes were meticulously scrutinised including prescription details for any consumption of analgesics and all records of the doctors, nurses, physiotherapists and occupational therapists.
**Results:** As on September 30, 2002, all patients were followed for at least 3 months. Nine of 114 of patients were found to have hemiplegic shoulder pain giving an incidence of 7.8%. The occurrence of shoulder pain was not significantly related to the motor power or muscle tone of the hemplegic arm. There were also no relations with age/sex of patients; the type/side of strokes and other medical comorbidities. Causes of shoulder pain were mainly adhesive capsulitis, impingement syndrome, biceps tendinitis and glenohumeral subluxation. Most shoulder problems responded to simple analgesics and physical modalities.
**Conclusion:** The incidence of hemiplegic shoulder pain is rather low as compared with that reported in other Western countries but the pattern of shoulder problems is quite similar. We postulated that the low incidence of shoulder pain could be related to our active prevention programme during rehabilitation through staff, patient and caregiver training and education on proper positioning and handling of the hemiplegic arm. Furthermore, the Chinese culture, which usually values a higher pain tolerance, may be another contributing factor.

## NUS-20 Reduced susceptibility to ischaemic brain damage following photochemical stroke in transgenic mice overexpressing the amyloid precursor protein

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**Introduction**: Amyloid precursor protein (APP) is the source of beta-amyloid, the principal component of amyloid plaques in the brain of Alzheimer's disease. Mice overexpressing APP have an increased vulnerability to brain ischaemia induced by endovascular middle cerebral artery occlusion. In this study, we investigate the role of APP in ischaemic brain damage due to photochemically induced thrombosis of cortical microvessels in transgenic mice overexpressing APP. Non-transgenic mice were used as a control group.
**Method**: The brains of transgenic mice overexpressing APP and non-transgenic mice were illuminated with a cold light source through the intact skull for 15 min or 3 min at 1 min following an injection of 0.1 mL or 0.04 mL of Rose Bengal respectively. Infarct volume was assessed 48 hours later from the triphenyltetrazolium chloride-stained brain slices.
**Results**: The relative infarct volume in the transgenic mice and non-transgenic mice following 15 min of photochemically induced thrombosis was 7.87±1.25% (mean±S.E.M.; n=3) and 14.47±4.16% (n=4), respectively. Thus, the infract volume in the transgenic mice was reduced by 45.6% ($P<0.05$). The relative infarct volume in the transgenic mice and non-transgenic mice following 3 min of photochemically induced thrombosis was 1.53±1.36% (n=3) and 3.48±0.64% (n=4), respectively. Thus, the infarct volume in the transgenic mice was reduced by 56.0% ($P=0.05$).
**Conclusion**: We conclude that the mice brain overexpressing APP is less susceptible to ischaemic damage due to photothrombosis of cortical microvessels. Our results are different from the reported findings when focal ischaemia was induced by endovascular middle cerebral artery occlusion.

## NUS-21 Clinical features and risk factors of cognitive impairment after stroke in Hong Kong Chinese

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**Introduction**: Stroke is recognized as an important cause of dementia. The goal of the present study is to examine a series of clinical features and risk factors of cognitive impairment after stroke.
**Method**: A standard protocol was applied to 185 consecutive unselected stroke patients within two weeks after onset of their strokes. Demographic and clinical data were collected. Barthel Index was used to assess the activities of daily living and Mini Mental Status Examination (MMSE) was used to assess the cognitive function. The cutoffs on MMSE score for cognitive impairment were selected according to Crum's criteria and the education level.
**Results**: Seventy-seven stroke patients (41.6%) had cognitive impairment or worse. Cognitive impairment was unrelated to age, gender, marital status, handedness, type of stroke (ischaemic/haemorrhagic), side of the lesion, location of stroke, smoking habit, drinking habit, diabetes mellitus, and hyperlipidaemia. Low education level, low Barthel Index and hypertension were the independent predictors of cognitive impairment in logistic regression analysis ($P<0.05$).
**Conclusion**: The local incidence of cognitive impairment after stroke is high. Education level, functional impairment and hypertension may increase the risk of cognitive impairment after stroke.

## NUS-22 Research strategies in molecular signaling of neuronal apoptosis in Alzheimer's disease

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**Introduction:** Neuronal loss is a key issue in the pathogenesis of Alzheimer's disease (AD). It is evident that neuronal apoptosis is one mode of neurodegeneration. Among all the factors leading to neuronal apoptosis, _-amyloid peptides (A_) are the most important toxin in AD pathogenesis. Therefore, the molecular signaling events of neuronal apoptosis receive much attention in AD research.
**Methods:** Different cell culture models were employed to prove the involvement of a particular protein kinase in A_ neurotoxicity. To verify the involvement of a protein kinase, both the phosphorylation of the kinase and its substrate had to be examined. Having done these experiments, the next step was to elucidate how significance of a kinase in A_ neurotoxicity by using molecular biology technique to transfect neurons over-expressing wild-type or negatively mutated kinase. Furthermore, neurons from knockout and the wild-type mice were used to prove the findings from genetically manipulated neurons. Apart from these experiments, investigation of whether the kinase involved in real clinical case was another important strategy to show the significance of the kinase in the pathogenesis of AD. Afterwards, it is also essential to show how a particular kinase incorporates into other well-known apoptotic pathways.
**Results and Conclusion:** By using these strategies, we have demonstrated that a novel double-stranded RNA-dependent protein kinase (PKR) is significantly involved in A_-induced neuronal apoptosis. It also plays significant roles in the pathogenesis of AD.
**Acknowledgement:** The study was supported by Seed Funding Grant 2001-2002 to RCCC, and RGC grant (HKU 7305/00M) to JH.

## NUS-23 A longitudinal study on functional decline and health services utilisation in fallers and recurrent fallers in community dwelling Chinese older adults in Hong Kong

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**Introduction**: Falls and fall-related injuries commonly led to functional decline, disability and increased hospitalizations or other health care services utilization. There was no local data on the functional impact and health services utilization in fallers for Chinese elderly people in Hong Kong.
This study investigated the functional decline and health services utilization for fallers in community dwelling Chinese elderly in Hong Kong.
**Method:** A population based elderly sample (age 65 years or over) of 1517 subjects were recruited in 1998. Baseline face-to-face interviews and direct clinical and functional assessment were performed for this cohort. Falls were monitored 2-monthly for one year. Re-assessment of their physical function was performed one year later. Data on the utilization of health care services were also documented for one year.
**Results:** 401 falls occurred over 1 year. The prevalence of falls, single fallers and recurrent fallers were 26.4%, 19.4% and 4.75% respectively. Injuries occurred in 76.6%. Fallers experienced a greater decrease in Barthel Index for basic Activities of Daily Living, Instrumental Activities of Daily Living, gait speed and Total Mobility Score. Recurrent fallers experienced the largest degree of decline in all the four functional measures. 216 subjects (14.8%) had been hospitalized. Fallers, recurrent fallers, had greater numbers of visits in clinics, visits at the Accident and Emergency Department and hospitalizations. Based on the current public health care cost data, elderly fallers would consume approximately HK$552 millions more than non-fallers per year for the whole of Hong Kong.
**Conclusion:** Elderly fallers experienced greater declines in their functional ability and utilized much more health care services than the non-fallers in Hong Kong. Interventions which could reduce falls in our elderly would reduce their functional decline as well as saving the health care dollars.
(Acknowledgement: Research Grant of Health Services Research Committee of Hong Kong).

## NUS-24 Screening tests for Alzheimer's disease: a comparison of four short tests in the Hong Kong Chinese

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**Introduction:** The prevalence of dementia was 6.1% and was predicted to increase significantly with population ageing in Hong Kong. With the availability of symptomatic treatments for Alzheimer's disease, the current challenge is to find tools to identify patients who had Alzheimer's disease in the early stage. This study investigated the values of the Delayed Word Recall Test, Mini-Mental Status Examination, Abbreviated Mental Test and Alzheimer's Disease Assessment Scale - cognitive subscale to discriminate between elderly persons with Alzheimer's disease and cognitively normal elderly persons in the Hong Kong Chinese.
**Method:** Chinese elderly subjects with Alzheimer's disease (AD) were recruited from the Queen Mary Hospital Memory Clinic. Cognitively normal elderly persons were recruited from community elderly centres. Every subject underwent a detailed assessment protocol including a detailed history, physical examination and neuropsychological tests as well as the four tests in this study (i.e. Delayed Word Recall Test, the Mini-Mental Status Examination, the Abbreviated Mental Test and the Alzheimer's disease Assessment Scale-cognitive subscale). These four tests all had Chinese Cantonese versions. The Mini-Mental Status Examination (MMSE), Abbreviated Mental Test (AMT) and Alzheimer's disease Assessment Scale-cognitive subscale (ADAS-cog) had all been validated previously in Hong Kong. The diagnosis of AD was based on the NINCDS-ADRDA criteria (i.e. probable Alzheimer's disease). The tests' scores were analysed by the Receiver Operating Characteristic (ROC) curves. The Area under the Curve (AUC) was computed for each test. The sensitivity, specificity, positive and negative predictive values would be compared using the cut-off criteria derived from the ROC curve analysis.
**Results:** 150 subjects were recruited from Feb. 2001 to Oct. 2002. 52 were AD and 98 were cognitively normal subjects. The cut-off scores for AD were $\leq 8$, $\leq 22$, $\leq 2$ and $>13$ for AMT, MMSE, DWRT and ADAS-cog respectively. From the AUC analyses, the Delayed Word Recall Test (DWRT) had the best discriminative power (AUC=0.99) among the four tests with a sensitivity of 94%, specificity of 100%, positive and negative predictive values of 100% and 99.6% respectively.
**Conclusion:** The Delayed Word Recall Test is a good screening test with very high sensitivity, specificity, positive and negative predictive values, which is suitable for use in the early detection of Alzheimer's disease.
**(Acknowledgement: UDM Research Grant, Department of Medicine)**

## NUS-25 The role of nitric oxide on regeneration of paraventricular nucleus and supraoptic nucleus following hypophysectomy

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**Introduction**: The mammalian neurohypophyseal system exhibits a high degree of structural plasticity and recovery of neuroendocrine function following a broad array of physiological and anatomical manipulations. This study investigates the role of nitric oxide (NO) on neuronal regeneration of paraventricular nucleus (PVN) and supraoptic nucleus (SON) in developing and adult rats.
**Methods**: NOS expression and neural regeneration on the floor of the third ventricular were detected by using nitric oxide synthase (NOS) histochemistry and scanning electronic microscope (SEM).
**Results:** Preliminary results have shown that the role of nitric oxide on regeneration of PVN and SON in developing rats seems different from that in the adult. Instead of that the process of regeneration is invariably accompanied by the up-regulation of NOS in the adult, there was no significant increase of NOS activity in SON and PVN neuronal perikarya and neurites in the adjacent median eminence in PN 7, PN 14 and PN 21 rats following hypophysectomy. Despite the fact, large complexes of apparent neurites remained upon the floor of the third cerebral ventricle by SEM in PN 7, PN 14, PN 21 rats by 4 weeks posthypophysectomy as in the adult. It has been also demonstrated that neural regeneration is more robust in the adult than in developing rats by SEM. Most interestingly, we found that the entire process of neurohypophyseal temporary introduction of the antagonist of NOS, L-NAME at 25 or 50 mg/kg per day for ten days immediately after surgery, which is nearly similar in the adult rats those received continuous administration of the NOS inhibitor for 4 weeks after hypophysectomy.
**Conclusions:** This data suggests that NO is a critical initiator for the process of regeneration and regrowth of magnocellular (SON/PVN) axons into the median eminence regeneration in the adult. Our further study aims at investigating the mechanisms.
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## RM-01 Clinical value of ciliary assessment in bronchiectasis—a cross sectional study

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**Introduction:** Although ciliary abnormalities have been studied for several decades, probably owing to technical requirements, a large scale prospective study on the clinical significance of abnormalities of ciliary beat (other than frank immotility and dyskinesia) and ultrastructure has not been conducted.
**Methods:** We have evaluated the prevalence and clinical significance of ciliary beat frequency and ultrastructural defects on the nasal respiratory mucosa obtained from 152 stable patients with idiopathic bronchiectasis (100F, 57.7±15.2 yrs) and 127 control non-smoking subjects (58F, 56.0±24.2 yrs). Ciliary beat assessment was performed by using a photomultiplier method established in our laboratory, and ultrastructure was assessed by using high resolution transmission electron microscopy.
**Results:** Bronchiectasis patients had significantly slower ciliary beat frequency ($p<0.05$), and more percent of patients showing central and peripheral microtubular defects (OR 14.4, 95% CI 5.6-36.8), namely extra peripheral microtubules, "9+1", "8+2", and compound cilia ($p<0.05$), but not microtubular disarrangement, extra matrix or ciliary tail abnormalities ($p>0.05$), than controls. Bronchiectasis patients also had more percent cilia with any ultrastructural, microtubular defects, compound cilia, and ciliary tails than controls ($p<0.05$). Ciliary beat frequency did not correlate with clinically relevant parameters ($p>0.05$). However, the percent of cilia with central, but not peripheral, microtubular defects correlated with 24h sputum volume ($r=0.40$, $p=0.001$, and $r=–0.04$, $p=0.70$ respectively) and $FEV_1$ ($r=-0.24$, $p=0.01$, and $r=0.00$, $p=0.99$ respectively).
**Conclusions:** Our results, therefore, strongly suggest a pathogenic role for central microtubular defects in the development of idiopathic bronchiectasis. It is hoped that future research into ciliary ultrastructural phenotyping could help diagnose and prognosticate, and further the understanding of the pathogenesis of this distressing and untreatable disease.
(Supported by a Hong Kong RGC Grant)

## RM-02 Inhaled steroid therapy and *Pseudomonas aeruginosa* infection and exhaled nitric oxide in bronchiectasis

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**Purpose:** Endogenous NO metabolism is involved in the pathogenesis of many respiratory and other systemic diseases. We have recently shown that exhaled (e)NO production is not significantly different between healthy volunteers and patients with bronchiectasis, except those with chronic *Pseudomonas aeruginosa* (PA) infection had significantly lower eNO than their counterparts and controls. While eNO is reduced by treatment with inhaled corticosteroid in asthma, the effects of such treatment on eNO in patients with bronchiectasis, who also have significant airway inflammation, is unknown.
**Methods:** eNO was measured by using an automatic chemiluminescence analyzer (Sievers NO Analyser280) at steady expiration on 60 stable patients with bronchiectasis, who received either fluticasone 500μg BID (n=30, 9F, 54.1±16.1yr) or matched placebo (n=30, 13F, 52.7±15.8yr). Patients with PA (n=16) at steady state were also assessed.
**Results:** There was no significant difference in eNO levels between fluticasone and placebo patients over the study period. There was no correlation between baseline eNO with age, $FEV_1$, FVC, 24h sputum volume or the number of bronchiectatic segments. Patients with *Pseudomonas aeruginosa* (PA) infection, but not their counterparts, displayed a correlation between 0- and 52-week eNO levels. PA infection was associated with significantly lower eNO levels among the patients.
**Conclusion:** Our data, derived from the first controlled study, showed that inhaled corticosteroid therapy has no significant effect on eNO production in bronchiectasis. The data of this longitudinal study also confirm our previous finding that eNO is reduced on PA-infected patients with bronchiectasis, and this phenomenon is likely to reflect a down-regulatory effect of PA infection on intrinsic airway NO production.

## RM-03 Serum superoxide dismutase levels correlate with disease activity markers in stable bronchiectasis

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**Purpose:** Increased levels of manganese superoxide dismutase (Mn SOD) and decreased catalase expressions at the transcriptional levels as compared with adjacent normal lung tissues have recently been reported by our group in non-small cell lung cancer. While the role of local antioxidant levels are clearly related to apoptosis and accummulation of genetic damages thus perpetuating tissue damage, little is known the levels of SOD among patients with bronchiectasis, a common chronic inflammatory and infective disorder among the Chinese. We have therefore performed this prospective study to evaluate the levels of SOD in the serum and sputum of patients with stable bronchiectasis, and attempted to correlate these with disease activity parameters on these patients.
**Methods:** After a baseline follow up of 3 consecutive weekly visits, fresh sputum and serum were obtained from out-patients at the research clinic. SOD was measured by standard biochemical methods on serum and sputum sol phase. Lung function, 24h sputum volume, exacerbation frequency, sputum microbiology and the number of lung lobes affected by bronchiectasis were determined for each patient. Correlations between these parameters and serum and sputum SOD levels were determined.
**Results:** There were 85 subjects (29M) recruited, and there were no significant difference in serum or sputum SOD levels between the genders, patients with and without *Pseudomonas aeruginsoa* infection, different aetiology groups for bronchiectasis, or smoker and non-smokers ($p>0.05$). There was a negative correlation between serum SOD with FVC % predicted ($r=-0.25$, $p=0.02$) and positive correlation with 24h sputum volume ($r=0.27$, $p=0.01$). There were otherwise no significant correlations between serum SOD with the other aforementioned parameters ($p>0.05$, data not shown). Sputum SOD levels did not correlate with the aforementioned disease activity parameters ($p>0.05$).
**Conclusion:** Serum levels of SOD correlates with disease activity and severity markers in bronchiectasis and this strongly suggests an important role for oxidants in the systematic effects of bronchiectasis.

## RM-04 Computed tomographic evaluation of the role of craniofacial and upper airway morphology in obstructive sleep apnoea subjects in Chinese

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**Introduction**: It has been postulated that craniofacial factors have a relatively bigger contribution to development of obstructive sleep apnea in Chinese than in Caucasians. This study was designed to evaluate the contribution of craniofacial factors using computed tomography in the development and severity of obstructive sleep apnea in Chinese.
**Method**: Subjects were recruited from the Sleep Clinic of the University Department of Medicine, Queen Mary Hospital. Standard in-laboratory polysomnogram (PSG) were performed and manually scored using standard criteria. Cephalometric parameters and pharyngeal cross-sectional areas at the level of velopharynx (VA) and hypopharynx (HA) were measured from computed tomographic scan (CT). The roles of these parameters and other anthropometric and demographic characteristics in the development of OSA (apnea hypopnea index, AHI $\geq 5$) and in the determination of severity of OSA were explored by multiple logistic and multi-nominal regression analysis.
**Results**: Ninety two subjects recruited and 56 had AHI $\geq 5$. Compared with normal subjects, those with OSA were heavier and older and had smaller velopharynx size and VA/HA ratio, lower positioned hyoid bone, longer soft palate, and more relative retrognathism. In a multi-nominal regression model, after controlling for body mass index and age, subjects with severe OSA (AHI > 30) had more relative retrognathism and longer soft palate, while those with mild/moderate OSA had larger velopharynx to hypopharynx ratio.
**Conclusion**: In our Chinese cohort, craniofacial factors and upper airway morphology contributed to development of OSA. Having controlled for obesity, relative mandibular retrognathism was associated with more severe OSA.

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## RM-05 A randomised controlled study of the effectiveness of continuous positive airway pressure, oral appliance and conservative measures in the treatment of mild to moderate obstructive sleep apnea

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**Introduction**: Continuous positive airway pressure (CPAP) is considered the standard treatment for severe obstructive sleep apnea (OSA) but studies on the effectiveness of different treatment options for mild to moderate OSA are limited. The aim of our study was to evaluate the effectiveness of lifestyle modification measures (CM) alone and in combination with CPAP or oral appliance (OA) in improving symptoms and quality of life of subjects with mild to moderate OSA.
**Method**: Subjects were recruited from two sleep laboratories of two hospitals in Hong Kong. Recruitment was aimed at newly diagnosed mild to moderate sleep apnea subjects. These subjects were then randomized into three different treatment groups: CM, CM + CPAP, and CM + OA. Polysomnogram was performed both at baseline and 10 weeks after treatment while still using the assigned treatment. Sleepiness were assessed with Epworth Sleepiness Score, and quality of life were assessed with generic and disease specific questionnaires (health related quality of life score SF-36 and sleep apnea quality of life index SAQLI) at inclusion and after 10 weeks of treatment.
**Results**: 94 subjects finished the study. There was moderate decrease in AHI in CM+OA group and a greater decrease in CM+ CPAP group, while the AHI of subjects managed with CM alone remained the same. The relief of sleepiness was the greatest in CPAP group and smallest in CM group. Quality of life of subjects was also restored to that of normal level in the CPAP and OA groups after treatment while no change was observed in the CM group. The self reported compliance to OA was better than that of objectively measured CPAP compliance.
**Conclusion**: Both CPAP and OA are effective in treating symptomatic mild to moderate OSA while CM alone is of little effect.

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## RM-06 A study on the effect of testosterone replacement on the development of obstructive sleep apnoea (OSA)

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**Introduction**: Obstructive sleep apnoea syndrome (OSA) is a common disease, it is characterised by snoring, repetitive occlusion episodes of upper airway resulting in desaturation and excessive daytime sleepiness. The male preponderance of this disorder, coupled with the reported development of OSA in patients following testosterone administration, suggest that androgens play a role in the pathogenesis of this disorder. One of the reasons for testosterone replacement lead to OSA might be redistribution of fat at neck region lead to decrease in muscle tone, or increase in muscle bulk around neck region result in narrowing of upper airway. The primary goal of the study is to find out the role and mechanism of androgen in the pathogenesis of OSA.
**Method**: Subjects were recruited from Endocrine Clinic of one regional hospital and one University hospital. Recruitment was aimed at newly diagnosed hypogonadal male requiring testosterone administration. Polysomnogram (PSG) was performed at baseline, 24 and 48 weeks after testosterone treatment. Body habitus, body fat and serum testosterone level were monitored regularly. Magnetic resonance imaging of the neck and abdomen were performed at baseline, 6 and 12 months after testosterone replacement for measuring.
**Results**: Four subjects recruited and two had been reassessed with PSG after 24 weeks of testosterone replacement. Their body mass index increased as well as apnea hypopnea index (AHI). One of them who had mild OSA at baseline showed moderate OSA on reassessment PSG. The other one who had no OSA at baseline remained free from OSA.
**Conclusion**: To be drawn after study finished

*This study was supported by the UDM research fund

## RM-07 Inhaled corticosteroid therapy in bronchiectasis—a 12-month study

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**Background:** The clinical efficacy of inhaled corticosteroid (ICS) therapy has not been evaluated on patients with bronchiectasis, despite the presence of chronic airway inflammation in this condition. We have, therefore, performed a double-blind randomized study on patients with stable bronchiectasis.
**Methods:** After a baseline follow up of 3 consecutive weekly visits, 86 patients were randomised to receive either fluticasone 500μg BID (n=43, 23F, 57.7±14.35 yr) or matched placebo (n=43, 34F, 59.2±14.16 yr), and reviewed regularly for 52 weeks.
**Findings:** Altogether 35 and 38 patients from the fluticasone and placebo groups completed the study respectively. Compared with placebo, ICS therapy was associated with significantly more patients showing improvement in 24h sputum volume (OR 2.5, 95%CI 1.1-6.0), but not exacerbation frequency, $FEV_1$, FVC or sputum purulence score. Significantly more fluticasone patients with *Ps. aeruginosa* infection at baseline had improvement in 24h sputum volume (OR 13.3, 95%CI 1.8-100.1) and exacerbation frequency (OR 13.5, 95%CI 1.8-101.1), compared with their placebo counterparts. Other predictors for improvement in post-treatment 24h sputum volume with fluticasone therapy include baseline 24h sputum volume <30ml (OR 2.7, 95%CI 1.1-6.7), exacerbation frequency ≤2/yr (OR 5.1, 95%CI 1.2-22.7), and sputum purulence score >5 (OR 2.7, 95%CI 1.0-6.9). Both groups of patients had improvement in respiratory symptoms after treatment, but ICS therapy was associated with significantly less patients complaining of cough (p=0.03).
**Interpretation:** Our results show that inhaled corticosteroid therapy, over a 52-week duration, is clinically beneficial to patients with bronchiectasis, particularly those with *Ps. aerurginosa* infection

## RM-08 A quantitative high resolution computed tomography assessment of patients with stable bronchiectasis

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**PURPOSE:** To evaluate the clinical relevance of HRCT findings using a quantitative HRCT protocol to assess severity of bronchial wall thickening, extent of bronchiectasis, and presence of small airway abnormalities and mosaic pattern.
**MATERIALS AND METHODS:** Sixty Chinese with steady state bronchiectasis underwent thoracic HRCT scan and full lung function tests. Exacerbation frequency/year and 24h-sputum volume were determined. Extent of bronchiectasis, bronchial wall thickening, presence of small airway abnormalities and mosaic attenuation were evaluated in each lobe, including lingula. Differences between sex and smoking status on HRCT, lung function and clinical parameters were tested using either independent sample t-test or Mann-Whitney rank sum test. Spearman's correlation was used to evaluate associations between clinical, lung function and HRCT scores. Multiple regression analyses were performed to determine HRCT parameters that best predict lung function and clinical parameters adjusted for smoking.
**RESULTS:** Exacerbation frequency was associated with bronchial wall thickening (r=0.32, p=0.03); 24h sputum volume with bronchial wall thickening, small airway abnormalities (r=0.30, 0.39, $p<0.05$), and $FEV_1$, $FEV_1$/FVC and $FEF_{25-75}$ (r=-0.33, -0.29, -0.32; $p<0.05$). Extent of bronchiectasis, bronchial wall thickening and mosaic attenuation was respectively related to $FEV_1$ (r=-0.43 to -0.60 $p<0.001$), $FEF_{25-75}$ (r=-0.38 to -0.57; $p<0.001$), FVC (r=-0.36 to -0.46, $p<0.01$), and $FEV_1$/FVC (r=-0.31 to -0.49, $p<0.01$). After multiple regression bronchial wall thickening remained a significant determinant of airflow obstruction, while small airway abnormalities remained associated with 24h-sputum volume. Women in general had milder disease than men, but showed more HRCT-functional correlations.
**CONCLUSIONS:** This study has established a link between morphologic HCRT parameters and clinical activity, and emphasised the role of BWT in bronchiectasis.

## RM-09 Inhaled fluticasone therapy improves quality of life in bronchiectasis

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**Purpose:** Bronchiectasis is a common chronic infective and inflammatory airway disease among the Chinese. St George's Respiratory questionnaire (SGRQ) is a standardized quality of life instrument consisting of 50 items grouped under three components, namely symptoms, activity and impact. Although conventional structural and disease activity markers are important end points for assessment of treatment efficacy, assessment of quality of life parameters is increasingly being recognized as one of the most important outcome measures. We have, therefore, employed our recently validated Hong Kong version of SGRQ(-HK) to assess the effects of inhaled steroid treatment among patients with stable bronchiectasis.
**Methods:** We have prospectively assessed the SGRQ-HK on 76 stable bronchiectasis patients, who received either fluticasone 500μg BID (FG, n=38, 20F, 57.7±14.6 yr) or matched placebo (PG, n=38, 33F, 58.9±14.2 yr) administered via the Accuhaler device for 24 weeks in a double-blind randomized fashion. SGRQ-HK assessment was performed by the same research assistant who had no knowledge of the treatment protocol. The three domains of SGRQ-HK, namely symptoms, activity and impact scores, as well as the total combined scores, which took account of weighting of each of these components, were calculated for baseline and 24-week.
**Results:** After 4-week treatment with inhaled steroid therapy, the fluticasone patients had improvement (i.e. lower score) in symptom (baseline median 46.0, 4-week 31.3, $p<0.001$), activity (34.8, 33.5, 0.61), impact (28.8, 24.9, 0.13) and total (33.7, 28.5, 0.01) scores when compared with baseline data. After 24-week treatment with fluticasone, symptom (36.2, $p=0.03$), activity (26.8, 0.04), impact (20.7, 0.003) and total (25.2, 0.003) scores all significantly improved when compared with baseline. In contrast, patients who received placebo treatment did not have significant changes in the aforementioned parameters ($p>0.05$) except there was an improvement of symptom score at 4-week (38.5), when compared with baseline (49.1, $p<0.001$). There were no significant difference in the pre- and post- treatment lung function parameters ($p>0.05$).
**Conclusion:** We conclude that quality of life parameters improve with inhaled corticosteroid therapy in patients with bronchiectasis. Results of this study support the use of inhaled steroid therapy on these unfortunate patients

## RM-10 Air-trapping in beta thalassaemia major (TM)

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**Introduction:** We aim to describe and quantify HRCT features of the lung in TM patients, determine the incidence and pattern of lung dysfunction and correlate HRCT findings with lung function parameters.
**Methods:** TM patients above 8 years of age were randomly selected and prospectively evaluated with HRCT (in full inspiration and expiration) and pulmonary function tests (PFT). Presence of focal bronchial and parenchymal lesions and air trapping were recorded. A semi-quantitative air-trapping score (ATS) and lung function parameters were compared. Patients were divided in groups with air trapping (ATS +) and without air trapping (ATS-). Mann-Whitney tests was used to detect any significant differences in PFT indices between ATS + and ATS-. We performed multiple logistic regression to study with relationship with PFT indices and the presence of air trapping. $P<0.05$ was used to indicate statistical significance.
**Results:** There were 42 patients aged 9 to 40 years old (mean: 24 years). None were active smokers or asthmatic. Patients were asymptomatic apart from cough in four patients. The predominant finding on HRCT was air trapping, detected in 11 patients (26.2%). One patient had lower lobe bronchicetasis. No parenchymal lesions were found. PFT was normal in four patients (9.5%). Impaired DLCO as the only abnormality was present in 13 patients (33.3%). Twelve patients (28.5%) had restrictive lung disease, of which eight were mild (TLC 70%-79%), three were moderate (TLC 60%-69%) and one was severe (TLC < 60%). Obstructive lung disease was seen in four patients (9.5%) and three patients (7.1%) had a mixed obstructive-restrictive pattern. PFT was suggestive of small airways disease in four patients (9.5%). Using Mann-Whitney test, there were statistically significant differences in the mean values of FEV1, FEF 25-75%, FEF 50%, FEF 75% in the ATS- and ATS+ groups ($p=0.012$, 0.001, 0.021, 0.019 and 0.004 respectively). Using simple logistic regression analysis, there was a statistically significant association between FEV1, FEF 25-75%, FEF 50%, FEF 75% and air trapping ($p=0.02$, 0.007, 0.038, 0.028 respectively). With multiple logistic regression, only FEF 25-75% was significantly associated with air trapping ($p=0.006$, adjusted odds ratio=0.90, 95% lower CI=0.83, 95% upper CI=0.97, adjusted $R^2=0.49$).
**Conclusion:** TM patients have a varied and mixed pattern of lung dysfunction. Air trapping is the predominant finding on HRCT, suggestive of small airway disease and this is supported by correlation with FEF25-75%. The cause of restrictive lung disease is probably multifactorial and not limited to interstitial lung disease. The relationship of iron overload with small airway disease is yet to be determined.

## RM-11 HRCT evaluation and immunohistochemistry of bronchiectactic airways: is there a relationship?

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**Introduction*:*** This study evaluates the relationships between HRCT parameters and collagen IV density in bronchiectatic airways.
**Method**: 18 bronchiectatic [13 females (65.52 ± 11.45yrs)] subjects underwent thoracic HRCT scan and endobronchial biopsies (EBB). 15 [5 females (57.46 ± 11.99 yrs)] otherwise healthy patients investigated for suspected lung cancer served as controls for EBB. Extent of bronchiectasis, bronchial wall thickening (BWT), global bronchiectasis score and small airway abnormalities on HRCT were qualitatively graded in bronchiectasis subjects. EBBs of all subjects and controls were stained with mouse anti-collagen IV antibodies, amplified using LSAB method. Each section was divided into zone 1 [0-8μm below basement membrane (BM)] and zone 2 [9-16μm below BM]. Positively stained collagen IV density, colour saturation, intensity of staining, and collagen fibril density were obtained for each zone using QWIN image analysis package. Correlation between immunohistochemistry and HRCT findings were evaluated.
**Results:** Bronchiectatic subjects had significantly higher collagen IV density and fibril density in zones 1 ($p < 0.01$) and 2 ($p < 0.01$) compared with controls. There was no difference between saturation and intensity between the 2 groups. Zone 1 collagen IV density was correlated with BWT score ($r=0.51$, $p=0.03$), and mean collagen IV density (zone 1 & 2) was associated with global bronchiectasis score ($r=0.53$, $p=0.02$).
**Conculsion:** Collagen density was higher in bronchiectatic airways than in normal airways. There is an association between immunohistochemistry of EBBs and HRCT parameters in bronchiectasis whereby the BWT and global bronchiectasis scores reflected collagen IV deposition, and probably airway remodelling.

## RM-12 Functional significance of CT quantified bronchial wall thickening in bronchiectasis

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**Introduction:** Qualitative evaluation of bronchial wall thickening (BWT) and extent of bronchiectasis have been proven to have significant functional correlation in bronchiectasis. In asthma studies, BWT has also been noted to be involved in airway remodeling. This study quantifies BWT on HRCT to determine its relationship with lung function and to compare its utility with qualitative evaluation.
**Method:** 18 bronchiectatic [13 females (65.52 ± 11.45yrs)] underwent thoracic HRCT scan and lung function tests. BWT was graded in each lobe (n=6) using previously validated scoring system (0-3) based on thickness of bronchial wall relative to the external diameter (EDB) of dilated bronchi perpendicular to the axial plane. Summation of scores from all 6 lobes provided the overall score. Internal bronchial diameter (IBD), EBD and accompanying arterial diameter (AD) were also quantified on a workstation at pre-selected segmental bronchi. The IBD and EBD of the thickest bronchus in each lobe were also measured. Relationships between HRCT and lung function parameters were evaluated.
**Results:** BWT score correlated with FVC, $FEV_1$, $FEF_{25-75}$ and PEF ($r=-0.65, -0.76, -0.55, -0.75$; $p<0.05$). Mean $IBD_T/EBD_T$ of thickest bronchi, and mean $IBD_{SB}/AD_{SB}$ and $IBD_{SB}/EBD_{SB}$ of segmental bronchi were respectively correlated with $FEV_1/FVC$ ($r=0.51, 0.53$ and $0.54$, $p<0.05$) and $FEF_{25-75}$ ($r=0.52, 0.60$, $p<0.05$ and $r=0.47$, $p=0.05$). Regression analysis revealed that BWT score was independently related to FVC, $FEV_1$ and PFR while $IBD_{SB}/EBD_{SB}$ and $IBD_T/EBD_T$ were significant determinants of $FEV_1/FVC$ and $FEF_{25-75}$ respectively. $IBD_{SB}/EBD_{SB}$ was therefore a measure of BWT and airflow obstruction in larger airways whilst $IBD_T/EBD_T$ reflected smaller airway obstruction.
**Conclusion:**These results have confirmed the clinical utility of both qualitative and quantitative HRCT evaluation of BWT in bronchiectasis, which reflect airway remodeling in large and small airways. These HRCT parameters could represent a non-invasive method of monitoring treatment response and disease progression of the bronchial tree.

## RM-13 Down-expression of inducible nitric oxide synthase (iNOS) and endothelial (Enos) proteins and mRNA iNOS in bronchiectasis in vivo

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**Introduction:** We have recently reported a lowering of exhaled nitric oxide (eNO) production in bronchiectasis among patients with *Pseudomonas aeruginosa* infection. The precise mechanism(s) and occurrence of reduction in eNO production, which potentially correlates ciliary beat frequency and other mechanisms for host defence in the airways, have not been investigated.
**Methods:** We have determined the expression of inducible (iNOS) and constitutive (eNOS) nitric oxide synthase (NOS) proteins including mRNA iNOS in endobronchial biopsies (EBB) obtained from 10 stable patients with idiopathic bronchiectasis (age= 54.1 ±14.4 yrs; $FEV_1$ 63.4 ±18.0%; FVC 65.6 ±18.1%; 24h sputum 12.8±1.2 ml), and 10 control subjects who underwent bronchoscopy for suspected bronchial carcinoma (age=60.6 ±11.2 yrs). Samples were fixed and processed routinely for histology under standardized protocols. Four mm paraffin sections were stained with iNOS and eNOS antibodies using DAKO Envision Kits. Intensity of immunoreactivity (luminance i.e. darker stain had lower grey scale value) was quantified using a Leica QWIN Image Analyser. Riboprobes for human iNOS were used for non-isotopic in-situ hybridization (Roche).
**Results:** Our results showed that iNOS and eNOS protein expressions were significantly down-regulated ($p<0.05$) in bronchiectatic mucosa (n=10; 175.5±2.1; 168.6±2.3 respectively) when compared with controls (145.2±2.5; 145.5±2.2). mRNA iNOS expression was lower in the bronchiectasis than in control non-bronchiectasis.
**Conclusions:** We conclude that iNOS and eNOS expressions are down-expressed in stable bronchiectasis patients. This down-regulation of eNO production is theoretically related to chronic infective state of the bronchiectatic airways, and has major implications in the pathogenesis of bronchiectasis. This interesting phenonmenon should be explored further.
(This abstract is funded by: a CRCG grant of the University of Hong Kong)

## RM-14 Why do family doctors prescribe antibiotics for upper respiratory tract infection?

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**Introduction**: Upper respiratory tract infection (URTI) is the commonest condition for patients to attend their doctors. The prescribing behaviours of family doctors in Hong Kong towards URTIs and the major clinical factors which might affect these behaviours were studied.
**Methods**: Members of the Hong Kong College of Family Physicians were surveyed between August and December 2001 and 801 of them completed the questionnaire with an overall response rate of 65.0%.
**Results:** Purulent nasal discharge, purulent sputum, persistent fever over 3 days, patients looking unwell, exudates on throat, inflamed eardrum and cervical lymphadenopathy made over half of the respondents likely or very likely to prescribe antibiotics for URTIs. Those in private practice and those who graduated from Hong Kong were more likely to prescribe antibiotics while those who had obtained fellowship of the College were less likely to do so. Vocational training and higher qualifications in family medicine/general practice however revealed minimal effect.
**Conclusion:** Our results showed that many doctors are still prescribing antibiotics when they encounter URTI patients presenting with clinical factors which have been proven to have no or little benefits from antibiotics.
**Acknowledgements**: This work was made possible by a grant from the Research and Conference Grants of the University of Hong Kong.

## RM-15 Molecular and conventional epidemiology of tuberculosis in Hong Kong—a population-based prospective study

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**Objective**: The purpose of the study was to determine the pattern of transmission of tuberculosis in Hong Kong by conducting a prospective molecular and conventional epidemiology study
Methods**:** All notified patients residing in the Island of Hong Kong with culture positive tuberculosis were recruited for the study from May 1999 to October 2000. Contact investigation was carried out and demographic and clinical information was obtained. The restriction fragment length polymorphism (RFLP) technique was used to determine DNA patterns of M. tuberculosis isolates.
**Results:** Of the 702 isolates that had RFLP analysis with the IS6110 probe, only 11 (1.6%) had 5 bands or less. 169 (24.5%) of the 691 remaining patients had sputum isolates that belonged to clusters. Significant predictors of belonging to clusters were permanent residency vs new or nonresidents (RR 4.57 95% 1.39-14.9) in Hong Kong and a history of travel to Mainland China in the past 2 years (RR 1.74, 95% CI 1.22-2.47). Risk factors such as alcohol and drug abuse, imprisonment, and HIV infection were not important.
**Conclusion:** While the majority of active tuberculosis in Hong Kong is due to reactivation, there is still a proportion of disease due to recent transmission that is higher than expected. The lack of epidemiological link in the majority of clustered patients suggests transmission of disease could be through casual contact.

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## RM-16 Differential regulation of cytokine-and phorbol ester-induced activation of nuclear factor kappa B by *Pseudomonas aeruginosa* pyocyanin in human airway epithelial cells

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**Introduction:** Non-cystic fibrosis bronchiectasis is a common disease in Chinese, and its pathogenesis is poorly understood. Gradual destruction of the airways occurs from a combination of chronic airway inflammation and infection. Many patients eventually harbour *Pseudomonas aeruginosa* (PA), which produces pyocyanin (PYO), a blue phenazine pigment, in their lower respiratory tract. This study investigates the effects of PYO on cytokine- and phorbol ester-induced activation of NFkappaB in stable transfected A549 cells.
**Method:** The human lung epithelial cell line, A549, was stably transfected with the reporter gene construct (p6kappaBtklucneo) containing six tandem NFkappaB motifs (GGGACTTTCC), and subsequently maintained in medium containing 0.5mg/ml G-418. After treatment for 6h with cytokines and drugs, cells were harvested in reporter lysis buffer by one freeze-thaw cycle. Bioluminescence in the cell lysates was assayed using a commercially available luciferase reporter gene assay kit (Promega).
**Results:** Cytokines (IL-1beta or TNF-alpha) or phorbol 12,13-dibutyrate (PDBu) activated NFkappaB-driven luciferase activity in a dose-dependent manner. In the presence of pyocyanin, the IL-1beta- and TNF-alpha-induced activation was significantly attenuated [$1.0 \pm 0.1$ vs $0.43 \pm 0.04$ and $0.45 \pm 0.05$ for cytokine alone and PYO plus IL-1beta (1ng/ml) or plus TNF-alpha (10ng/ml) respectively; $n = 4 - 5$], while the PDBu-induced activation was significantly potentiated ($1.0 \pm 0.03$ vs $1.46 \pm 0.16$ for PDBu alone and PYO plus PDBu at $10^{-7}$M respectively; $n = 4$).
**Conclusion:** We conclude that potentiation of NFkappaB-dependent transcription by PDBu in PYO-treated cells may imply the involvement of PKC pathway in the chronic airway inflammation in bronchiectasis.
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## RM-17 Oral coriolus versicolor polysaccharide peptide is beneficial by slowing the progression of lung cancer

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**Purpose:** Non-small cell lung cancer (NSCLC) is the commonest cause of cancer death in HK and most patients present in advanced stages thereby missing the opportunity of curative surgical treatment. *Coriolus versicolor cov-1* PSP has been anecdotally assumed to have anti-cancer effects, and is consider as capable of alleviating symptoms in cancer patients. These high desirable claims have not been investigated in a controlled fashion.
**Methods:** We have performed a double-blind placebo-controlled randomized study to evaluate the effects of 3-day administration of PSP (3 capsules TDS) on patients with advanced NSCLC, who have completed standard treatment with chemotherapy and/or radiotherapy.
**Results:** 34 patients were recruited into each of the PSP (n=34; 11F; age 61.3±11.3 yr; and TNM II 1, IIB 1, IIIA 1, IIIB 10, IV 18) and placebo (PG; n=34; 11F; age 55.3±10.7 yr; and IIIA 1, IIIB 12, IV 18)) arms. The two arms did not differ in their previous treatment with chemotherapy, radiotherapy or surgery (0>0.05). No adverse reaction attributable to the trial medications, was reported by either treatment groups. Altogether 10 patients could not complete the study due to clinical deterioration. Of these, 2 cases were from PSP and 8 from PG (p=0.03). There was no significant difference in the body mass index (p=0.78), total calorific intake (0.58), Global Health Status (0.77), quality of life parameters of EORTC-C13 (>0.05), although PSP treatment was associated with improvement in total leucocyte count in blood (0.003) and body fat content (0.02) after treatment.
**Conclusion:** Our preliminary data show that PSP treatment could be associated with slower deterioration in the clinical course of patients with advanced NSCLC, and have no adverse reactions. Our results not only suggest that further clinical trials should be conducted on the effects of PSP on NSCLC, but also show that standard GCP trial criteria could be applicable to research in alternative medicine.
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## RM-18 Clinical outcome of advanced lymphoepithelioma-like carcinoma of the lung after chemoradiotherapy

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**Background:** Lymphoepithelioma-like carcinoma (LELC) of the lung, an Epstein-Barr virus-associated undifferentiated carcinoma, is a rare form of non-small cell lung cancer. It predominantly affects young non-smoking Asians and is believed to be frequently resectable. However, there has only been limited experience with the use of palliative chemotherapy and radiotherapy for the treatment of advanced LELC of the lung.
**Methods:** We prospectively recruited cases of LELC of the lung with a standard clinical protocol in a tertiary respiratory referral center. Patients with confirmed advanced LELC of the lung were given chemoradiotherapy treatment.
**Results:** There were 13 patients (5 males, age 47.9 ± 9.4 years, median follow-up 22 months) with advanced LELC of the lung (1, 7, and 5 patients at TNM stage IIIA, IIIB, and IV) who received systemic chemotherapy and radiotherapy. The primary chemotherapy regimens included 5-fluorouracil/leucovorin/cisplatin (FLP) [n=10], mitomycin C/ifosphamide/cisplatin (MIP) [n=1], paclitaxel/carboplatin (TC) [n=1], and gemcitabine/cisplatin (GC) [n=1]. The response rates to FLP were 60% partial response, 10% stable disease, and 30% progressive disease. Partial responses were also observed in patients given MIP and GC. The one given TC had progressive disease. Ten patients were given local radiotherapy. Six patients received salvage chemotherapy when disease progressed after primary chemotherapy. The overall median survival for patients given FLP was 20.6 ± 6.5 months.
**Conclusion:** The encouraging response to combination chemotherapy (FLP) supports its use, along with radiotherapy, in unresectable LELC of the lung.

## RM-19 Efficacy and safety profiles of a combination of gemcitabine and ifosfamide on Chinese patients with advanced non-small cell lung cancer

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**Purpose:** Chinese patients with non-small cell lung cancers often present differently from their Western counterparts, although they often present in unrestable stages rendering curative surgical treatment not possible. Combination of gemcitabine and ifosfamide has theoretical advantages but the precise safety profile and efficacy are unknown locally.
**Methods:** We have, therefore, performed this open-labelled study to prospectively recruit patients with stage III or IV NSCLC who have not received previous chemotherapy with informed consent. A combination of Gemcitabine (1g/m2, day 1 and 8) and Ifosfamide (3g/m2, day 1) was administered in 4-weekly cycles. Patients with clinical response or static disease received 6 cycles if tolerated. Chemotherapy was withdrawn in the event of clinical or imaging evidence of disease progression.
**Results:** 26 patients (F=12, mean age 55.3±9.9 yrs, stage IIIB/IV=12/14) completed the study with evaluable response. Of these, 5 (19.2%) showed partial response, and 3 (11.5%) showed static disease, and 18 (69.2%) showed progressive disease. Four patients (15.3%) needed dose reduction or omission, usually on day 8 of the respective cycle, for WHO Grade I–III neutropenia. Two patients (7.7%) had WHO Grade I and II thrombocytopenia. All of them recovered spontaneously without developing severe life-threatening complication. Other patients managed to complete each cycle on time with targeted dose administration.
**Conclusion:** The combined administration of Gemcitabine and Ifosfamide in chemo-naïve advanced NSCLC patients was well tolerated in this study and gave rise to a comparable response rate as previously reported. Our results would help respirologists and oncologists in the management of Chinese patients with advanced NSCLC.

## RM-20 Exhaled nitric oxide (eNO) level is not related to quality of life (QoL) parameters in non-small cell lung cancer (NSCLC)

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**Purpose:** Although eNO measurement has become highly popular as a non-invasive monitoring means for many respiratory and systemic diseases, and NO metabolism appears to be involved in the pathogenesis of many neoplastic diseases, little is known on the relationship between eNO and clinical, immunological, and QoL parameters in NSCLC.
**Methods:** Patients with histologically or cytologically confirmed NSCLC were recruited from the University Department of Medicine at Queen Mary Hospital. Clinical parameters, baseline blood tests, serum immunoglobulins (Ig), and QoL parameters (EORTC, Rottadam, HADS) were collected. eNO was measured by using an automatic chemiluminescence analyzer at steady expiration.
**Results:** There were 68 subjects (45M, 58±11yrs) recruited with 39 adenocarcinomas, 15 squamous cell carcinomas, and 14 undifferentiated NSCLC. TNM stages were 3A, 3B, and 4 on 2, 28 and 38 respectively. Four subjects had previous lung resections, 50 had chemotherapy, and 33 had radiotherapy. The serum IgA levels were significantly higher in undifferentiated NSCLC (1888±280mg/dl) than squamous cell carcinomas (1512±129 mg/dl) and adenocarcinomas (1389±54mg/dl) [p=0.029]. Performance status (Karnovsky scale) significantly correlated with haemoglobin (r=0.35, p=0.003), total white cell count (r=-0.40, p=0.001), and neutrophil count (r=-0.43, p<0.001). Multiple regression model identified haemoglobin as the only independent predictor of performance status (p=0.032). Lung function index FEV1/FVC significantly negatively correlated with eNO in the whole group (r=-0.39, p=0.001), in adenocarcinomas (r=-0.38, p=0.02), in males (r=-0.44, p=0.003), in well differentiated carcinomas (r=-0.49, p=0.01), and in stage 4 disease (r=-0.367, p=0.028). eNO only correlated with neuropathy score (p=0.03), but not to the overall QoL score.
**Conclusion:** Increased eNO appears to be related to airflow obstruction, thus underlying airway disease, rather than QoL among patients with NSCLC.

## RM-21 Genomic aberrations in lung cancer: a study with comparative genomic hybridisation and analysis of loss of heterozygosity

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**Introduction:** Lung cancer is the commonest cancer in Hong Kong. A distinguishing feature of theHong Kong lung cancer patients is that there are relatively more female patients. Among these Chinese female patients, 70% is of adenocarcinoma. Most of them are non-smokers. The very high frequency in female and the lack of association with smoking are in contrast to those in the West suggesting that different genetic factors may be involved.
**Method:** To investigate the genomic changes, comparative genomic hybridization (CGH) was performed. Tumor genomic DNA and normal DNA were differentially labeled with biotin-16-dUTP and digoxigenin-11-dUTP using nick translation. After competitive co-hybridization, we analyzed the color ratio profiles to determine genetic aberrations. We further characterize common deleted chromosome regions more precisely by performing loss of heterozygosity. We used multiplex PCR amplification of 9 microsatellite repeat polymorphisms for this study. The amplified PCR products were analyzed using a sequencer model Genetic analyzer.
**Results**: A non-random pattern of loss or gain of chromosomal material can be defined. The pattern is different from those of the Western patients. Deletions of genetic material was repeatedly found at chromosomes 5p, 3q24-29, 3q10-q24, 8q, 1q, 8p, 7p, 10q22, 11q14-21, 12q15; whereas overrepresentation occurs frequently at chromosomes 19p13.1-13.1, 17p, 3p, 8p, 20p, 18q, 6q21-25. LOH at chromosome 5p and chromosome 12q15 were detected in these tumors. Five discrete loci of LOH were found at 5p15.3, 5p15.2, 5p14.3, 5p13.1 and 5p12. In chromosome 12q, LOH were found at both loci of 12q15.
**Conclusion:** Our results may imply the presence of putative proto-oncogenes and tumor suppressor genes of considerable importance for the pathogenesis of lung carcinoma in the Chinese lung cancer patients.

## RM-22 Review of 5 “big gun” antibiotics

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**Background:** There is strong evidence of increasing emergence of bacterial resistance to broad-spectrum antibiotics, probably related to indulgent usage. The aim of this study was to assess use and if possible facilitate more appropriate prescribing of some potent so-called broad-spectrum “big gun” intravenous antibiotics, namely: imipenam, meropenam, cefipime, ceftazidime and pipercillin/tazobactam (Tazocin)

**Methods:** With the endorsement of the Department of Medicine and the Hospital Management Committee, an audit into the prescribing of the above mentioned “big gun” antibiotics coupled with an immediate concurrent feedback (ICF) strategy has been initiated in the Department of Medicine general wards. In corresponding wards, inpatients prescribed any of the aforementioned “big gun” antibiotics are flagged by the pharmacy computer together with their bed number. Specially trained staff visit ach patient on the next working day and the reason for such prescribing is logged (as far as possible). The later indication is reviewed by a panel from the Departments of Medicine and Microbiology. ICF is then instituted (in the form of a memo) addressed to respective the respective prescriber with a cc to the supervising MO, whenever the indication is deemed inappropriate according to the five pre-defined criteria. The latter criteria were: 1) no genuine infection 2) chemoprophylactic use 3) where use of ‘lesser’ gun is deemed appropriate 4) combination of two or more “big gun” antibiotics 4) empirical treatment of community acquired infection in non neutropenic patients (except in patients with severe sepsis, high level immunosupression or failure to respond to more appropriate first line antibiotics). Each memo details the prescribing incident and makes explicit recommendations.

**Results:** The trend in prescribing behaviour for these “big guns” will be presented and compared to the results of our preliminary audit.

## CVS-25 Effects of intramyocardial autologous bone marrow cell implantation on serum concentrations of angiogenic factors and cytokines

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**Background.** Recent experimental studies demonstrated that intramyocardial implantation of bone marrow cells (BMCs) promotes neovascularization and increases local concentrations of angiogenic factors (basic fibroblast growth factor [bFGF] and vascular endothelial growth factor [VEGF]) and angiogenic cytokines (tumor necrosis factor alpha [TNF]) in ischemic myocardium. Whether intramyocardial implanted BMCs release these angiogenic factors and cytokines into the systemic circulation remain unclear. Short or long-term increase in the serum concentration of these angiogenic factors or cytokines may lead to systemic adverse effects.
**Methods**. We performed electromechanical mapping (NOGA, Biosense) guided intramyocardial injection of autologous BMCs in 9 patients (8 males, mean age: 61±9 yrs) with medically refractory angina (mean anti-angina medications: 4±1) and failed previous conventional revascularization procedures. The serum concentrations of VEGF, bFGF and TNF were measured by using ELISA test in triplicate at baseline, Day 1 and Day 90 after the procedure.
**Results**. Successful autologous BMCs injection was performed in all patients at 12 ischemic regions (mean numbers of injection: 15±4 per patient). At 90 days, there was a significant improvement in anginal symptoms, reduction in sublingual nitrate consumption and increase myocardial perfusion as assessed by magnetic resonance imaging (all $p<0.05$). There were no significant increases in serum concentration of VEGF and bFGF after the procedure ($p>0.05$, Table). TNF was undetectable in the serum in any patient before or after the procedure
**Conclusions**. Our initial results demonstrate that intramyocardial implantation of BMCs improved myocardial perfusion and patient's symptoms, but did not affect the circulating concentration of angiogenic growth factors or cytokines. This finding has important implication on the long-term safety of this novel cell-based angiogenic therapy for neovascularization.

| Baseline | Day 1 | Day 90 | |
|---|---|---|---|
| **VEGF (pg/ml)** | 165±156 | 263±168 | 271±100 |
| **BFGF (pg/ml)** | 4.7±8.8 | 3.2±4.8 | 1.9±3.5 |