HONG KONG MEDICAL JOURNAL 香港醫學雜誌
Volume 23 Number 1 **February 2017**
SUPPLEMENT 1

22nd Medical Research Conference, 14 January 2017

Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Small tiny RNAs control plasma cell maturation and antibody production

HM Jäck
Division of Molecular Immunology, Nikolaus-Fiebiger-Center, Department of Internal Medicine III, University of Erlangen-Nürnberg, Germany

Our long-term goal is to understand how one class of interfering non-coding RNAs, the so-called micro-RNAs (miRNAs), regulates and fine-tunes the differentiation of mature B cells into effector cells, that is, memory B cells and antibody-secreting plasma cells. miRNAs control the expression of specific target genes at the post-transcriptional level by binding to target sequences, eg, in the 3′-untranslated region of mRNAs, which, depending on the degree of the binding, results either in a block of translation or an accelerated degradation of the respective target mRNA. Lineage-specific deletion of the miRNA-processing DICER protein as well as of individual miRNAs revealed the importance of miRNA pathway in early steps of central B cell maturation. However, the mechanisms by which miRNA-dependent circuits control the antigen-induced phase of B cell activation of mature naive B cells and their subsequent differentiation into effector cells remain largely elusive.

To change this situation, we established a transgenic knock-in mouse line with a floxed allele of DGCR8, an essential subunit of the nuclear miRNA processing complex. B cell–specific deletion of DGCR8 at various stages of B cell maturations showed that miRNA processing is essential for central B cell maturation as well as the antigen-induced differentiation into effector B cells.

We have also obtained genome-wide miRNA expression profiles of all major mouse B cell subsets, including long-lived plasma cells, and found a profound upregulation of one miRNA in plasma cells. Ectopic expression of this plasma cell signature-miRNA in primary mouse B cells accelerated the differentiation into antibody-secreting plasmablast, as indicated by upregulation of CD138 and enhanced secretion IgM secretion. We also verified several targets of this miRNA, eg, Bach2 and MiTF, both of which are part of the transcriptional circuit that controls germinal centre reactions and plasma cell differentiation. Finally, we will show that this plasma cell signature miRNA delays development in mice carrying a genomic deletion of this plasma cell-specific miRNA.

These studies will provide new molecular insights into regulatory circuits that control the production of antibodies and could potentially lead to new avenues for vaccine development as well as diagnosing or treating diseases associated with aberrant plasma cell development, eg, primary antibody deficiencies, plasma cell malignancies, and autoimmune disorders.

Acknowledgement
Supported by grants TRR130 and GK1660 from the Deutsche Forschungsgemeinschaft to H.-M. J.

Plenary 2

PD-1/PD-L1 targeted immunotherapy: expanding the indication in the management of non-small cell lung cancer

J Kim
Department of Thoracic and Cardiovascular Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Korea

Cancer is definitely a systemic disease and surgery is the typical modality of local control. For the early-stage lung cancer [(stage I, II, and some of IIIa (N2)], surgery has been the mainstay of treatment. Unfortunately, more than 50% of such patients would succumb due to recurrence of the lung cancer, mainly systemic metastasis. Therefore, systemic treatment should be considered as a part of treatment even for the very early-stage stage patients.

However, without the strong evidence of prolongation of recurrence-free survival (and also overall survival), inadvertent application of adjuvant or neoadjuvant application would induce harmful effect rather than benefit. Study design of adjuvant clinical trial is very difficult because of the fact that there is no measurable (identifiable) lesion after curative resection. To define the role of systemic treatment in early-stage lung cancer, the following should be considered; (1) expected survival benefit and risk, (2) potential candidate for the systemic treatment, and (3) the way of response assessment.

There have been several studies of the adjuvant systemic treatment, which have proved marginal-to-moderate benefits. At the current time, most of the guidelines recommend the systemic adjuvant cytotoxic chemotherapy to the patients with stage Ib (>4 cm), stage II, or stage IIIA. In regard to the target-directed therapy, no data have been reported for the strong recommendation yet.

Another important area to be considered would be the role of surgery in advanced cases. It can be cytoreductive surgery or salvage resection before or after systemic treatment, respectively. Nonetheless, there has been almost no information about the indication, timing, approach, extent of resection, and so on.

Recent advances in immune-targeted therapies have led to a major paradigm shift in the treatment of advanced non–small-cell lung cancer (NSCLC). In particular, the programmed death 1 (PD-1) immune checkpoint receptor and its ligands, programmed death ligand 1 (PD-L1) and PD-L2, have become promising targets for immunotherapy, and two humanised anti–PD-1 antibodies, nivolumab and pembrolizumab, are currently approved in several countries for the treatment of NSCLC.

The fairly favourable outcomes in advanced NSCLC naturally lead to consideration of the application in the early-stage cases as adjuvant treatment. Currently several adjuvant trials are underway using several anti-PD-1/PD-L1 antibody drugs. Not yet reported, the outcome seems promising, but is not just optimistic. As proved in the advanced cases, the effect has not been satisfactory in the cases with low mutation burden or non-inflamed cases, and the level of PD-L1 expression is low.

To define the potential candidate for the adjuvant or neoadjuvant treatment of PD-1/PD-L1 targeted immunotherapy, there should be studies about (1) risk assessment (aggressiveness/recurrence prediction), and (2) immune status of tumour and host. And, in regard to the response assessment, we may consider the following as surrogate predictors: (1) changes in CTC, cfDNA, or serological biomarkers and (2) changes of immune profiles of tumour or tumour microenvironment of the newly developed lesions.

And also, there should be more studies of combined local control (surgery) and systemic control (immunotherapy); either as cytoreductive surgery or salvage surgery; either as before, during and after immunotherapy; either as adjuvant, neoadjuvant or palliative purpose; either as monotherapy or combination treatment with chemo/radiotherapy.

Emergence of Zika virus and associated neurological complications

Plenary 3

DH Hamer
Center for Global Health and Development, Department of Global Health, Boston University School of Public Health, United States

Zika virus has gone from relative obscurity to great notoriety during the last decade. Since an outbreak in Yap, Micronesia in 2007, the Asian clade of Zika virus has spread rapidly through the South Pacific to Brazil and then to most of Latin America, the Caribbean, and the United States. Zika is most commonly spread by *Aedes aegypti* but may also be transmitted sexually, through contaminated blood products, and potentially close contact with the tears or saliva of viremic patients. Theories on why Zika has emerged include host factors including immunologically naive populations, antibody-dependent enhancement, viral mutation, and spread via travel.

Although frequently asymptomatic, clinical manifestations include rash (may be severely pruritic), fever, arthralgias, fatigue, and conjunctivitis. Complications are predominantly neurological. These range from paraesthesias to Guillain-Barré syndrome and, less commonly, myelitis and encephalitis. The current outbreak in the Americas has been notable for recognition of the congenital Zika syndrome, which is consistent with a fetal brain disruption sequence characterised by craniofacial disproportion (with microcephaly in severe cases), redundant scalp skin, and occipital bone prominence. This devastating syndrome most commonly results from infection in the first trimester of pregnancy but may occur as late as the third trimester. Clinical manifestations of the congenital Zika syndrome include irritability, hypertonicity, hyperexcitability, convulsions, dysphagia, arthrogryposis, hip dysplasia, macular atrophy, hearing loss, and neurodevelopmental delay. Radiological findings commonly include intracranial calcifications, reduced brain mass, hydrocephalus, and pachygyria.

Definitive diagnosis is based on polymerase chain reaction detection of Zika virus RNA in plasma, urine, blood, or cerebrospinal fluid. Anti-Zika IgM and IgG assays suffer from cross-reactivity with antibodies to dengue and other flaviviruses so these require confirmation with plaque reduction neutralisation tests. Prevention measures include avoidance of travel to areas with ongoing transmission, insect repellents, larvicides, insecticides, and drainage of breeding sites. Several promising vaccine candidates are in phase 2 trials.

Update on hepatitis C virus management

Plenary 4

CL Lai
Department of Medicine, The University of Hong Kong, Hong Kong

There are around 130-150 million chronic hepatitis C virus (HCV) carriers globally, leading to an annual mortality of 500 000. Asia has the largest HCV population in the world, with China alone having more HCV-infected persons than Europe and America taken together.

The incidence of HCV genotype 6 was first identified in a collaborative study between Hong Kong and Scotland, being around 27%. Subsequently it is shown that Vietnam and Myanmar have the highest incidences of HCV genotype 6, 36-49%. The transmission of genotype 6 is predominantly through intravenous drug use.

The first study of the treatment of HCV, with the traditional interferon for 6 months, was published in 1986. In three decades, with the arrival of the direct-acting antivirals (DAAs), the CURE rate for HCV-infected patients (that is sustained response for >12 weeks after treatment) has increased from 6% to 95-100% with 12 weeks of oral treatment.

The goal of treatment is obviously to reduce all-cause mortality. Meta-analysis of interferon treatment shows that sustained responders have a lower rate of cirrhosis progression as well as development of hepatocellular carcinoma. Another encouraging finding is that late relapses after 4-5 years of follow-up occur in only 1-2%. Long-term follow-up data for DAAs are yet to be published.

The licensed agents for the treatment of HCV genotype 1 and 4 include: Harvoni (a single table combining ledispasvier, a NS5A inhibitor, and sofosbuvir, a NS5B inhibitor); Viekira pak (4 tablets combining dasabuvir, a NS5B inhibitor, ombitasvir, aNS5A inhibitor, and paritaprevir, a protease inhibitor which has to be potentiated by ritonavir, a P450 inhibitor); Zepatier (a single tablet combining grazapatier, a protease inhibitor, and elbasivir, a NS5A inhibitor). Harvoni is also licensed for genotypes 5 and 6.

A pan-genotypic drug was licensed in 2016. This drug is Epclusa, a single tablet combination of sofosbuvir and velpatasvir, another NS5A inhibitor. With Epclusa, the cure rate for genotypes 1, 2, 4, 5, and 6 is 99-100%; and for the most difficult-to-treat genotype 3, still 95%.

No change in regimen is required with the occurrence of resistance-associated polymorphisms for most of these drugs, except for Zepatier where a 16-week course is required.

The main problems still existing for the eradication of HCV are: the very high cost of the treatment, the lack of awareness of the infection in the general population, and the possibility of re-infection with continued intravenous drug use.

1

Is multiplex system better? Comparing the diagnostic performance of BioPlex with conventional methods in autoantibody study in systemic lupus erythematosus

YL Au[1], WK Ip[1], YT Chan[1], CS Lau[2]
[1]Department of Pathology, Queen Mary Hospital; [2]Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Methods of autoantibodies detection have evolved markedly in recent years. BioPlex is a new multiplex technology, which has the advantage of high throughput. This study was to compare BioPlex with conventional techniques in anti-nuclear antibody (ANA) screening and specific extractable nuclear antigen (anti-ENA) antibodies tests in a cohort of local Chinese lupus patients.

Methods: A total of 140 consecutive Chinese patients with systemic lupus erythematosus (SLE) from Queen Mary Hospital lupus clinic and 41 healthy controls were included. Clinical data were retrieved from the electronic hospital record. BioPlex 2200 ANA screen was compared with indirect immunofluorescence (IIF). In addition, the detection of ENA antibodies among the BioPlex test panel was compared with traditional in-house countercurrent immunoelectrophoresis (CIEP), enzyme-linked immunosorbent assay (ELISA), and line blot.

Results: The sensitivity of BioPlex ANA screen in the SLE cohort was 91.4%, which was comparable to that of IIF (sensitivity, 90.7%). The specificity among the healthy control by BioPlex was 95.1%, whereas the specificity by IIF was 85.4%. Overall, BioPlex achieved the best agreement with ELISA in ENA profile study. The agreement was over 90% with most of the antibodies tested by ELISA, whereas the agreement was least with CIEP, which ranged from 85.6% for anti-Sm to 93.9% for anti-Ro. Overall, BioPlex and ELISA have a higher sensitivity while CIEP has the best specificity. In terms of the disease association, anti-Sm detected by CIEP has the best positive predictive value and specificity for lupus nephritis compared to other techniques.

Conclusion: BioPlex 2200 ANA screen showed comparable sensitivity to IIF in a local lupus cohort. In terms of ENA profile study, the BioPlex system demonstrated similar performance with ELISA, CIEP, though less sensitive; it remains the best method in terms of disease specificity.

2

Entirely patient-specific primers/probes increases the applicability of allele-specific oligonucleotide real-time quantitative polymerase chain reaction to >90% in detection of minimal residual disease in multiple myeloma

Y Bai, CS Chim
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Allele-specific oligonucleotide real-time quantitative polymerase chain reaction (ASO RQ-PCR) is a standard technique for the detection of minimal residual disease (MRD) in multiple myeloma (MM). However, applicability is low due to the presence of somatic hypermutation of the immunoglobulin (Ig) gene. This study aimed to improve the applicability of ASO RQ-PCR for MRD study in MM.

Methods: Clonality was detected by stepwise PCR amplifying IgH VDJ, IgH DJ, or IgK VJ rearrangements. Complementarity-determining region 3 (CDR3) and mismatch against consensus reverse primers/probes were identified by sequencing. ASO RQ-PCR was performed by ASO primers with consensus and patient-specific primers/probes respectively.

Results: Clonality and CDR3 were identified in all 13 cases. ASO RQ-PCR was successful in three (23.1%) cases by consensus primers/probes only. Among 12 cases with IgH rearrangements, one to eight mismatches were found in consensus probes in six cases and consensus reverse primers in five cases. Using ASO forward or reverse primers with entirely patient-specific primers/probes, RQ-PCR was successful in eight (61.5%) cases with a sensitivity of 5x10-4-10-5. Moreover, using standard curves constructed by plasmid clones of patient-specific CDR3 sequence, RQ-PCR yielded a superior applicability (92.3%, 12 cases) and sensitivity (10-4-10-5) than diagnostic DNA. Finally, in one patient achieving serological complete response before autologous stem cell transplantation (ASCT), ASO RQ-PCR indicated presence of MRD positive after induction, which turned negative after ASCT.

Conclusion: Entirely patient-specific primers/probes increased the applicability of ASO RQ-PCR to >90% in MM. Moreover, ASCT resulted in disappearance of MRD despite serological complete response.

3

Allogeneic haematopoietic stem cell transplantation for non-NK peripheral T-cell lymphoma in Chinese patients: a comparison with contemporary B-cell cohort

TSY Chan, YY Hwang, AKW Lie
Division of Haematology, Medical Oncology and HSCT Unit, Department of Medicine, Queen Mary Hospital, Hong Kong

Objective: Peripheral T-cell lymphoma (PTCL) is a group of heterogeneous non-Hodgkin's lymphoma (NHL) derived from post-thymic mature T lymphocytes. Data in Chinese population with allogeneic haematopoietic stem cell transplantation (HSCT) for PTCL are scarce. In this study, we reviewed the results of allogeneic HSCT for PTCL performed in Hong Kong Chinese patients, and compared it with a contemporary B-cell lymphoma cohort.

Methods: From 2006 to 2015, all Chinese patients who underwent allogeneic HSCT for PTCL and B-cell NHL were recruited. Patients with extranodal NK/T-cell lymphoma were excluded. Indications of transplant included refractory disease to frontline chemotherapy or relapsed disease after chemotherapy.

Results: Overall, 14 patients (male, n=6; female, n=8; median age, 51.5 years) with PTCL underwent allogeneic HSCT with the following diagnoses: (1) PTCL not otherwise specified, n=4 (29%); (2) anaplastic large cell lymphoma, n=6 (43%); (3) angioimmunoblastic T-cell lymphoma (AITL), n=3 (21%) and enteropathy-associated T-cell lymphoma (EATL), n=1 (7%). Six (43%) patients were in partial remission and eight (57%) were in complete remission before transplant. The median follow-up was 57 months. Acute graft versus host disease (GvHD) occurred in six (43%) patients and chronic GVHD occurred in four (29%). The 5-year overall survival (OS) and disease-free survival (DFS) was 78.6% and 71.4%, respectively. Histological subtypes, remission status, and conditioning regimen did not impact the OS and DFS. In a contemporary B-cell NHL cohort, 47 patients received allogeneic transplant (male, n=26; female, n=21; median age, 50 years). The median follow-up time was 20 months. Breakdowns of diagnoses were as follows: (1) diffuse large B-cell lymphoma, n=24 (51%); (2) follicular lymphoma, n=6 (13%); (3) mantle cell lymphoma, n=12 (26%); (4) Burkitt lymphoma, n=4 (8%); and (5) mucosa-associated lymphoid tissue (MALT) lymphoma, n=1 (2%). Acute GVHD occurred in 14 (30%) patients and chronic GVHD occurred in 18 (38%). The 5-year OS and DFS was 67% and 58%, respectively.

Conclusion: Allogeneic HSCT provides a potential cure for Chinese patients with relapsed/refractory PTCL, and is comparable with B-cell NHL. The encouraging results from this study require further evaluation in prospective trials.

4

Lifelong burden of vitamin D deficiency increases cardiac events and death unravelled by an exome chip-derived multi-loci genetic risk score: a Mendelian-randomised study

YH Chan, TH Lam, GN Thomas, CQ Jiang, PC Sham, CM Schooling, BJ Cowling, KK Cheng, KS Lam, HF Tse
Division of Cardiology, Department of Medicine, Queen Mary Hospital, Hong Kong

Background: Prior cardiovascular (CV) studies on vitamin D were short-termed and conflicting. We investigated whether a novel multi-loci genetic risk score for lifelong-deficient vitamin D exposure infer causality to clinical cardiac events and death under Mendelian randomisation approach.

Methods: We studied serum 25-hydroxyvitamin D levels among 5772 subjects in a prospective clinical cohort. Overall, 12 pre-specified candidate single-nucleotide polymorphisms (SNPs) involved along the vitamin D biosynthetic, activation/receptor pathways from prior genome-wide association studies were studied. We constructed a 12-point genetic risk score (GRS) based on six SNPs associated with serum 25-hydroxyvitamin D level (rs2282679, rs4588, rs7041, rs1155563, rs1993116, rs2060793; all $P<0.05$) for CV risk prediction. Study endpoints were acute coronary syndrome (ACS)/myocardial infarction (MI), ischaemic stroke, congestive heart failure (CHF), peripheral vascular disease (PVD), CV death, and combined CV endpoints.

Results: After a mean follow-up duration of 58.4 ± 27.8 months, incident events of ACS, MI, CHF, ischaemic stroke, PVD, and CV death were 99 (1.7%), 191 (3.3%), 431 (7.5%), 98 (1.7%), 24 (0.4%), and 47 (0.7%), respectively. Total combined CV event was 660 (11.4). Vitamin D GRS was associated with combined CV events (P=0.009). Kaplan-Meier analysis showed that lifelong vitamin D exposure as determined by GRS was associated with improved survival from combined CV events (log-rank=12.9, P=0.012). Adjusted for potential confounders, genetic vitamin D status was independently predictive of reduced combined CV events (hazard ratio=0.96; 95% confidence interval [CI], 0.93-0.99; P=0.011). Mendelian randomisation analysis (Wald's estimate method; Fieller's Theorem for 95% CI) showed that lifelong vitamin D exposure has a causal protective effect against the risk of combined CV events (odds ratio=0.885; 95% CI, 0.806-0.970; P=0.009).

Conclusion: Lifelong vitamin D exposure is causally protective against combined incident CV events.

Acknowledgement
This study was funded by the Health and Medical Research Fund, HKSAR (Project no. 10111531)

5

Oral treatment with herbal formula B307 alleviates skeletal muscle atrophy from hind-limb unloading

XK Chen[1], C Zheng[2], CH Wu[3], JSK Kwan[1]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Sports Science and Physical Education, The Chinese University of Hong Kong, Hong Kong
[3]Department of Life Science, National Taiwan Normal University, Taiwan

Introduction: Muscle atrophy can result from physical inactivity, chronic bedrest, and ageing. Traditional Chinese herbal formula B307 is a health supplement with multiple potential protective functions of organs. We investigated the effects of herbal formula B307 on muscle atrophy using the hind-limb unloading (HU) model in mice.

Methods: Eight-week-old ICR mice were randomly divided into four groups: Sham, B307, HU, and HU+B307 groups. HU model was established in the HU and HU+B307 groups for 14 days. Mice in the B307 and HU+B307 groups were given oral B307, while the Sham and HU groups were treated with the vehicle for 14 days. Phenotypes and biomarkers of muscle atrophy were examined using laser Doppler, luminol chemiluminescence, immunohistochemistry, and western blotting.

Results: The muscle mass of mice in the HU and HU+B307 groups was significantly lower than the Sham group ($P<0.05$). Muscle mass was significantly higher in the HU+B307 group versus HU group ($P<0.05$), but no significant difference was found between the Sham and B307 groups. Reactive oxygen species (ROS) of blood, expressions of tumour necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), and caspase-3 in the skeletal muscle was significantly higher in the HU versus Sham and B307 groups ($P<0.01$), while alleviated after B307 treatment in the HU+B307 group ($P<0.05$). Blood flow in the hind-limb and whole body increased after B307 treatments ($P<0.05$). However, no significant differences in muscle strength, B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), caspase-9, and endothelial NOS (eNOS) expression in the skeletal muscle of mice were found between the four groups.

Conclusion: Oral B307 treatment may alleviate skeletal muscle atrophy from HU. Potential underlying mechanisms include improvement in blood flow and downregulation of oxidative stress, inflammation, and apoptosis.

6

Whole-exome sequencing identified novel genetic mutations in a pedigree of familial myeloproliferative neoplasm

CY Cher[1], NKL Ng[1], SSY Lam[1], BL He[1], D Ying[2], CH Au[3], TL Chan[3], ESK Ma[3], R Liang[3], YL Kwong[1], AYH Leung[1]
[1]Division of Haematology, Department of Medicine; [2]Department of Psychiatry, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong
[3]Department of Pathology, Hong Kong Sanatorium & Hospital, Hong Kong

Introduction: Myeloproliferative neoplasm (MPN) encompasses a group of diseases characterised by increased proliferation of erythroid, megakaryocytic, or granulocytic cells in the bone marrow. Clinically, MPN includes chronic myeloid leukaemia (CML) carrying Philadelphia-chromosome (BCR/ABL translocation) as well as polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) that carry gene mutations such as janus kinase 2 (JAK2), calreticulin (CALR), and thrombopoietin receptor (MPL). Approximately 10% of MPNs showed familial occurrence, suggesting inheritance of genes that make them susceptible to MPN.

Methods: A family with three members affected by ET, PV and CML across two generations were identified; another three members were shown to have consistent elevated platelet count. DNA were extracted and subjected to whole-exome sequencing (WES), co-segregated variants were filtered and validated by Sanger sequencing. Intronic JAK2 haplotypes were also tested in these family members.

Results: All family members being tested in this study were shown to carry homozygous JAK2 haplotypes, previously shown to predispose to MPN. WES result has identified several candidate genes co-segregated in affected family members. Truncating mutation in *ZNF467* was of particular interest because of its role in transactivating *STAT3*, a gene shown to induce MPN upon deletion.

Conclusion: We conclude that WES was able to identify candidate gene potentially responsible for the pathogenesis of MPN.

Is endothelial function related to plasma level of lipocalin-2 in man?

7

BMY Cheung[1,2,3,4], Y Wang[2,3,5], KSL Lam[1,2,3], HF Tse[1,2,3,4]
[1]Department of Medicine; [2]Partner State Key Laboratory of Pharmaceutical Biotechnology; [3]Research Centre of Heart, Brain, Hormone and Healthy Aging; [4]Institute of Cardiovascular Science and Medicine; [5]Department of Pharmacology and Pharmacy; The University of Hong Kong, Hong Kong

Background: Recent animal studies suggested that lipocalin-2 plays a key role in the development of hypertension, atherosclerosis, and endothelial dysfunction. Lipocalin-2 knockout mice are resistant to the harmful effects of high fat diet and do not develop hypertension or atherosclerosis. We therefore hypothesised that lipocalin-2 is also related to endothelial function in man.

Methods: We measured plasma lipocalin-2 concentration in 245 subjects (201 men, 44 women; mean [standard deviation] age, 68 [9] years) who had measurements of brachial artery flow-mediated dilatation (FMD). Among these subjects, 151 had hypertension, 80 had diabetes mellitus, and 240 were on lipid0lowering therapy. Brachial artery diameter and flow velocity were measured using a 7.5-MHz ultrasound probe. Scans were taken at baseline, 5 minutes after tourniquet inflation and after sublingual glyceryl trinitrate spray. The percentage change in brachial artery diameter following reactive hyperaemia was calculated. The coefficient of variation of FMD determination was 5%.

Results: Plasma lipocalin-2 correlated with serum creatinine (ρ=0.23, P<0.001) but not FMD (ρ=0.005, P=0.94). FMD correlated inversely with age (ρ= –0.14, P=0.03). Diabetes was associated with a lower FMD (P=0.044).

Conclusion: Plasma level of lipocalin-2 is unrelated to FMD. Endothelial function is influenced by many factors, including ageing and diabetes as found in this study, and also diet and drug therapy. The correlation of lipocalin-2 with creatinine is consistent with it being a marker of renal injury.

Acknowledgement
This work was supported by RGC GRF grant HKU 763312.

Relationship between waist circumference and glycohaemoglobin in the US population

8

KYC Cheung, AJ Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Obesity predisposes to type 2 diabetes, but the relationship between glycohaemoglobin (A1C) and waist circumference has not been well studied. We therefore evaluated the strength of this relationship and the usefulness of waist circumference in predicting the A1C level.

Method: A total of 1009 men and 991 women aged ≥20 years from the United States National Health and Nutrition Examination Survey 2011-2014 were included. A1C level 6.5% (≥48 mmol/mol) was used as the cutoff for diabetes.

Results: There was a linear relationship between A1C and waist circumference in men (P<0.001) and in women (P<0.001). The association between the two variables was significant after adjusting for age, hypertension, lipid levels, race, smoking, and alcohol consumption. Each 10-cm increase in the waist circumference was associated with an increase in A1C of 0.15% in men and 0.10% in women. The area under the receiver operating characteristic curve (AUROC) to predict A1C ≥6.5% (≥48 mmol/mol) was 0.685 for men and 0.714 for women. The negative predictive values corresponding to waist circumference cutoffs of 102 cm and 88 cm were 96.6% for men and 97.4% for women.

Conclusion: There is a significant linear relationship between A1C and waist circumference. Waist circumference alone is already a good criterion for A1C testing in the US general population. Our findings pave the way for a multivariate prediction model with higher predictive value.

HLA-B*38:02:01 predicts carbimazole/methimazole-induced agranulocytosis

9

CL Cheung[1,2,3,4], CW Sing[1], CSM Tang[3,5], VKF Cheng[1], M Pirmohamed[6,7], CH Choi[8], CS Hung[9], EYF Lau[10], KF Lee[11], MWH Mak[11], JYY Leung[12], TW Wong[12], AYY Ho[13], KW Chan[14], VHF Hung[14], V Tam[15], SC Siu[16], PC Sham[3,5], BMY Cheung[2,4], ICK Wong[1,4,17], KCB Tan[2], AWC Kung[2]

[1]Department of Pharmacology and Pharmacy, [2]Department of Medicine, [3]Centre for Genomic Sciences, [4]The State Key Lab of Pharmaceutical Biotechnology, [5]Department of Psychiatry, The University of Hong Kong, Hong Kong
[6]Department of Molecular and Clinical Pharmacology, The University of Liverpool, United Kingdom
[7]Royal Liverpool and Broadgreen University Hospital NHS Trust, Liverpool, United Kingdom
[8]Queen Elizabeth Hospital, Hong Kong
[9]United Christian Hospital, Hong Kong
[10]Pamela Youde Nethersole Eastern Hospital, Hong Kong
[11]Kwong Wah Hospital, Hong Kong
[12]Ruttonjee & Tang Shiu Kin Hospitals, Hong Kong
[13]Tuen Mun Hospital, Hong Kong
[14]Princess Margaret Hospital, Hong Kong
[15]Caritas Medical Centre, Hong Kong
[16]Tung Wah Eastern Hospital, Hong Kong
[17]School of Pharmacy, University College London, United Kingdom

Introduction: Little is known about susceptibility to antithyroid drugs (ATD)–induced agranulocytosis.

Methods: We performed a genome-wide association study (GWAS) involving 20 ATD-induced agranulocytosis patients and 775 healthy controls. The top finding was further replication in additional cases and controls.

Results: A single-nucleotide polymorphism rs185386680 showed the strongest association with ATD-induced agranulocytosis in GWAS (odds ratio [OR]=36.4; 95% confidence interval [CI], 12.8-103.7; $P=1.3x10^{-24}$) and replication (OR=37; 95% CI, 3.7-367.4; $P=9.6x10^{-7}$). HLA-B*38:02:01 was found to be in complete linkage disequilibrium with rs185386680. High-resolution human leukocyte antigen (HLA) typing confirmed that HLA-B*38:02:01 was associated with carbimazole (CMZ)/methimazole (MMI)–induced agranulocytosis (OR=265.5; 95% CI, 27.9-2528.0; $P=2.5x10^{-14}$). The positive and negative predictive value of HLA-B*38:02:01 in predicting CMZ/MMI-induced agranulocytosis was 0.07 and 0.999, respectively. Approximately 211 cases need to be screened to prevent one case of CMZ/MMI-induced agranulocytosis.

Conclusion: HLA-B*38:02:01 predisposes to CMZ/MMI-induced agranulocytosis. Screening for the risk allele will be useful in preventing agranulocytosis in populations where the frequency of the risk allele is high.

Exome-array association analysis reveals an Asian-specific coding variant in *PAX4* associated with type 2 diabetes in Chinese

10

CYY Cheung[1], CS Tang[2], A Xu[1], CH Lee[1], KW Au[1], L Xu[3], CHY Fong[1], KHM Kwok[1], WS Chow[1], YC Woo[1], MMA Yuen[1], JSH Hai[1], BMY Cheung[1], KCB Tan[1], TH Lam[3], HF Tse[1], PC Sham[4], KSL Lam[1]

[1]Department of Medicine, [2]Department of Surgery, [3]School of Public Health, [4]Department of Psychiatry, The University of Hong Kong, Hong Kong

Introduction: Genome-wide association studies (GWASs) identified many common type 2 diabetes (T2DM)–susceptibility variants. We performed an exome-array association analysis to identify additional T2DM susceptibility variants in the Chinese population.

Methods: A total of 5640 Hong Kong Chinese individuals were genotyped using an Asian Exome-chip. We performed single-variant association test on 77 468 single nucleotide polymorphisms (SNPs). Overall, 15 SNPs were followed up in an independent Chinese cohort comprising 12 362 individuals. A combined analysis involving 7189 cases and 10 813 controls was conducted.

Results: An Asian-specific coding variant p.Arg192His (rs2233580) in *PAX4*, and two variants at the known loci, *CDKN2B-AS1* and *KCNQ1*, were significantly associated with T2DM at exome-wide significance ($P_{discovery}<6.45x10^{-7}$) in the discovery stage. *PAX4* rs2233580 was associated with age at diabetes diagnosis. This SNP was replicated and demonstrated a stronger association that reached genome-wide significance ($P_{meta}=3.74x10^{-15}$) in the combined analysis.

Conclusion: We identified the association of a *PAX4* Asian-specific missense variant p.Arg192His with T2DM in an exome-array association analysis, supporting the involvement of *PAX4* in the pathogenesis of T2DM. Our findings are suggestive of *PAX4* being a possible effector gene of the 7q32 locus previously identified from GWAS among Asians.

Acknowledgement
This work was supported by the Hong Kong Research Grant Council: Theme Based Research Scheme (T12-705/11) and Collaborative Research Fund (HKU2/CRF/12R).

Haemorrhagic transformation of ischaemic stroke: risk factors and prognostic implication

11

RHK Cheung[1], KH Chan[1], R Lee[2]
[1]Department of Medicine, [2]Department of Radiology, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: Haemorrhagic transformation (HT) complicating ischaemic stroke is associated with significant morbidities and mortality. The clinical implications of HT have not been explored locally. This study aimed to determine the risk factors and clinical implications of HT complicating cerebral infarction in the Hong Kong Chinese population.

Methods: This was a retrospective case-control study of consecutive patients admitted to Queen Mary Hospital with acute ischaemic stroke (IS) between 1 January 2007 and 31 December 2011. HT was diagnosed with examination of repeated brain neuroimaging (computed tomography or magnetic resonance imaging) performed within 2 weeks of IS onset. Patients with IS without repeated neuroimaging within 2 weeks, and patients with transient ischaemic attack or intracranial haemorrhage were excluded. HT was classified according to the European-Australasian Acute Stroke Study (ECASS) II criteria. Poor clinical outcome was defined as mortality within 90 days or modified Rankin scale score >2 at completion of rehabilitation or around 90 days.

Results: Of 718 patients recruited, 66 (9.2%) received intravenous (IV) thrombolysis and 117 (16.3%) developed HT—HI1, 12 (1.7%); HI2, 3 (0.42%); PH1, 46 (6.4%); PH2, 54 (7.5%); PH at remote site, 2 (0.28%). HT was independently predicted by IV thrombolytic therapy (odds ratio [OR]=2.86; 95% confidence interval [CI], 1.58-5.18), cardioembolic stroke (3.65; 2.23-5.97) and prior warfarin use (2.85; 1.27-6.39). At 90 days, 138 (19.2%) patients died. At completion of rehabilitation or around 90 days, 462 (64.3%) had poor outcome. The 90-day and 5-year mortality rates were significantly increased in patients with PH2 (hazard ratio=1.86; 95% CI, 1.07-3.24 and 1.53, 1.01-2.30, respectively). Multivariate analysis showed PH2 to be an independent predictor of poor outcome (OR=2.14; 95% CI, 1.04-4.40).

Conclusion: IV thrombolytic therapy, cardioembolic stroke, and prior warfarin use were independent predictors of HT. PH2 was associated with increased risk of poor outcome at approximately 90 days and mortality at 5 years.

Magnetic resonance spectroscopy demonstrates neuronal loss and altered glutamatergic neurotransmission in Alzheimer's disease

12

C Chiu, MCQ Yang, A Har, HKF Mak, JSK Kwan
Department of Medicine, The University of Hong Kong, Hong Kong

Background: The role of the anterior cingulate cortex (ACC) is involved in the default mode network during resting state, and is dysfunctional in ageing and Alzheimer's disease (AD). We used magnetic resonance spectroscopy (MRS) to study the biochemical and metabolite profile in patients with AD, and compared with cognitive-normal healthy controls (HC) with no cognitive complaints.

Methods: In a cross-sectional study, 12 age-matched HC and 11 AD patients underwent ^{1}H-MRS using ACC as the region of interest. We measured choline (Cho), creatine (Cr), N-acetyl-aspartate (NAA), myo-inositol (mI), and glutamate/glutamine complex (Glx), and quantified them using internal water as reference.

Results: Compared to HC, AD patients had significantly lower Cho (AD, 2.09 ± 0.52 mM; HC, 3.51 ± 0.78 mM; P<0.001), NAA (AD, 7.80 ± 2.52 mM; HC, 15.27 ± 2.90 mM; P<0.001), and Glx (AD, 6.74 ± 1.90 mM; HC, 17.45 ± 4.17 mM; P<0.001). However, Cr (AD, 18.05 ± 2.52 mM; HC, 16.66 ± 1.84 mM; P=0.185) and mI (AD, 12.00 ± 4.10 mM; HC, 8.82 ± 3.84 mM; P=0.089) showed no significant differences.

Conclusion: Our findings are consistent with current literature supporting the evidence of neuronal loss and altered glutamatergic neurotransmission in AD. MRS may be sensitive for studies of early AD, mild cognitive impairment, and subjective cognitive decline.

13

Event-related potential using task-based electroencephalogram may differentiate between mild cognitive impairment and normal controls

MCQ Yang, JW Li, G Wong, J Kwan
Department of Medicine, The University of Hong Kong, Hong Kong

Background: New non-invasive biomarkers to diagnose early Alzheimer' disease are needed. We investigated the role of event-related potential (ERP) using task-based electroencephalogram (EEG) in differentiating patients with mild cognitive problems from cognitive-normal healthy controls.

Methods: In a pilot cross-sectional study, two patients with subjective cognitive decline (SCD), two with mild cognitive impairment (MCI), and two healthy controls (HC) underwent 128-channel EEG whilst performing two cognitive tasks: go/no-go (GNG using one hand) and prospective memory (PM using two hands) paradigms. ERP components N200 and P300 were selected for GNG task, whilst N300 and parietal positivity were selected for PM task.

Results: For PM task, HC group had significantly higher parietal positivity, higher N300 mean amplitude in posterior location, and higher N300 latency than those of the MCI group. The posterior regions of the brain registered more statistically significant differences than the anterior regions, which may signify the functional neuroanatomy of the PM task. For GNG task, no significant differences in ERP were observed. Results were variable for the SCD group.

Conclusion: Our pilot results indicate that ERP using task-based EEG may have the potential to differentiate between MCI and healthy controls. Sensitivity of the prospective memory task appears to be higher than the go/no-go task, probably because it involves using two hands simultaneously, hence more challenging on the working memory and executive function.

14

The C-terminal domain of hepatitis B core protein regulates hepatitis B virus transcription through recruitment of histone-modifying enzymes

CK Chong, CYS Cheng, SYJ Tsoi, FY Huang, WK Seto, CL Lai, MF Yuen, DKH Wong
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: The hepatitis B core protein (HBc), a component of the hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) mini-chromosome, has been suggested to play a role in HBV transcription. The C-terminal domain (CTD) of HBc possesses four arginine-rich nucleic acid-binding clusters (clusters I to IV). We aimed to identify specific residue(s) or region(s) in HBc CTD essential for its interaction with cccDNA and to investigate the possible mechanism in HBV transcriptional regulation.

Methods: A total of 17 HBc CTD mutants (MT1-MT17) were created by site-directed mutagenesis. Expression plasmids containing wild-type or mutant HBc were transiently co-transfected with plasmid-free full-length HBc-negative HBV genome into HepG2-NTCP cells. HBV RNA, DNA, and HBsAg were measured by qRT-PCR, qPCR, and ELISA, respectively. The association between cccDNA and mutant HBc, as well as various histone modifying enzymes were assessed by chromatin immunoprecipitation (ChIP).

Results: Compared with wild-type HBc, HBc clusters III and IV mutants (MT6-MT17) had significantly reduced level of total HBV RNA (all $P<0.05$). All mutants had significantly lower level of intracellular encapsidated HBV DNA than wild-type HBc (all $P<0.05$). The level of secretory HBsAg of all but one HBc mutant (MT2-MT17) were significantly decreased ($P<0.05$). ChIP experiments demonstrated a relatively smaller degree of association between HBc clusters III and IV mutants and cccDNA (MT3-MT17; $P<0.05$). The relative recruitment level of histone-modifying enzymes CPB, P300, and PCAF were also lower in the HBc clusters III and IV mutants (MT7, MT12) than in the wild-type HBc (all $P<0.05$).

Conclusion: We demonstrated that HBc CTD mutants in cluster III and IV shown impaired viral transcription through their reduced association with cccDNA and the recruitment of histone-modifying enzymes, suggesting that HBc CTD clusters III and IV may be potential therapeutic target to control HBV replication.

15

Network meta-analysis on efficacy of biologic disease-modifying anti-rheumatic drugs (bDMARDs) in psoriatic arthritis patients intolerant of tumour necrosis factor-α inhibitor

AC Chu, MF Tsoi, Y Fei, TT Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Biologic disease-modifying anti-rheumatic drugs (bDMARDs) and target-specific DMARDs (tsDMARDs) have demonstrated their better efficacy over placebo for treating patients with psoriatic arthritis (PsA). However, no trials directly compared different bDMARDs and tsDMARDs. Therefore, we performed a network meta-analysis to obtain indirect comparisons between different bDMARDs and tsDMARDs in tumour necrosis factor-α inhibitor (TNFi)–intolerant PsA patients.

Methods: The literature was searched using ISI Web of Science, Scopus, Medline, Cochrane library, Clinicaltrials.gov, and EMBase. For inclusion, randomised controlled trials must report the proportion of TNFi-intolerant PsA patients achieving ACR20 response as a study outcome. This outcome was analysed with reference to placebo and ustekinumab 45 mg using R statistics version 3.3.1 with netmeta version 0.9.1.

Results: Seven studies with altogether 1114 patients were included in this network meta-analysis. Compared to placebo, all bDMARDs and tsDMARDs were superior to placebo except abatacept (odds ratio [95% confidence interval]: abatacept 10 mg/kg: 2.22 [0.33-15.18]; abatacept 30/10 mg/kg: 2.86 [0.50-16.43]; abatacept 3 mg/kg 2.27 [0.36-14.45]). Using ustekinumab 45 mg as reference, all bDMARDs and tsDMARDs showed similar ACR20 response (odds ratio [95% confidence interval]: ustekinumab 90 mg: 0.91 [0.43-1.93]; abatacept 10 mg/kg: 0.65 [0.08-5.40]; abatacept 30/10 mg/kg: 0.84 [0.12-5.94]; abatacept 3 mg/kg 0.67 [0.09-5.17]; apremilast 20 mg BID: 1.96 [0.41-9.49]; apremilast 30 mg BID: 1.66 [0.34-8.14]; secukinumab 150 mg: 0.87 [0.28-2.67]; secukinumab 300 mg: 1.95 [0.53-7.17]; secukinumab 75 mg: 0.67 [0.22-2.10]; tofacitinib 10 mg BID: 0.84 [0.30-2.34]; tofacitinib 5 mg BID: 0.93 [0.33-2.60]).

Conclusion: Except abatacept, all the biologics and target-specific DMARDs in the meta-analysis showed similar efficacy and are superior to placebo.

16

Selegiline suppressed cigarette smoke medium (CSM)–induced oxidative stress, inflammation and apoptosis via monoamine oxidase–B inhibition in airway epithelial cells

YT Cui[1], KWK Liu[1], MSM Ip[1,3], JCW Mak[1,2,3]
Departments of [1]Medicine and [2]Pharmacology; [3]Research Centre of Heart, Brain, Hormone and Healthy Aging; The University of Hong Kong, Hong Kong

Background: Cigarette smoking is the major risk factor for the development of chronic obstructive pulmonary disease (COPD). Cigarette smoke (CS) is a rich source of oxidants, and is thought to disrupt the oxidant-antioxidant balance in the lung, thus inducing inflammation and apoptosis. Selegiline, an inhibitor of monoamine oxidase (MAO)-B, has long been used as an adjuvant to the levodopa therapy for Parkinson's disease. The aim of this study was to explore the effect of selegiline on cigarette smoke medium (CSM)–induced oxidative stress, inflammation, and apoptosis in human airway epithelial cells (AECs) and the underlying mechanisms involved.

Methods: BEAS-2B cells were treated with 2% CSM following a 30-minute pretreatment of 100 nM selegiline. After treatment, medium was collected for the measurement of interleukin 8 (IL-8) release by ELISA; cells were reserved for flow cytometry as a measurement of apoptosis and reactive oxygen species (ROS); cell lysate was extracted for measurement of antioxidant enzymes activities and Western blot analysis.

Results: CSM (2%) treatment increased MAO-B activity, ROS levels, and pro-inflammatory marker IL-8 release in AECs. Pretreatment with selegiline inhibited the CSM-induced elevation of MAO-B activity, attenuated CSM-induced ROS levels and IL-8 release. In addition, CSM treatment elevated anti-oxidant enzyme superoxide dismutase (SOD) activity, but inhibited catalase (CAT) activity and decreased GSH/GSSG ratio. Pretreatment of selegiline normalised CSM-mediated SOD and CAT activities and GSH/GSSG ratio. Selegiline suppressed CSM-induced nuclear translocation of nuclear factor erythroid 2–related factor 2 (Nrf2) and cytosol translocation of its negative regulator, BTB and CNC homolog 1 (Bach1), thus decreased protein expression of heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1). In addition, selegiline also reversed CSM-induced phosphorylation of IKK, reduction of cytoplasmic I kappa B expression and nuclear translocation of nuclear factor-kappa B p65 subunit, leading to attenuation of IL-8 release. CSM also increased apoptotic cells, cytochrome c, cleaved caspased-3, and pro-apoptotic protein bax expression. Pretreatment of selegiline suppressed CSM-induced apoptosis and apoptosis-associated protein expression.

Conclusion: These findings confirmed that MAO-B inhibitor is capable of reversing CSM-induced oxidative stress, inflammation, and apoptosis via Nrf2/NF-κB pathways for the first time in human bronchial epithelial cells, which may provide a promising therapeutic strategy for COPD.

Acknowledgement
This work was supported by HKU Stanley Ho Matching Grant and YC Chan Scientist Award.

17

Optimal duration of dual antiplatelet therapy after drug-eluting stent implantation: a pairwise and network meta-analysis of randomised controlled trials

Y Fei, MF Tsoi, TT Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The optimal duration of dual antiplatelet therapy (DAPT) after drug-eluting stent (DES) implantation has been debated. Limited evidence was reported by only one clinical trial directly comparing short-term (<12 months) and extended (>12 months) DAPT. Therefore, we performed a network meta-analysis to assess the risks and benefits of different DAPT durations.

Methods: We searched for clinical trials randomising patients to receive different durations of DAPT after DES implantation and included those reporting frequencies of cardiovascular and bleeding events. Both a frequentist approach and a Bayesian framework were used with R statistics.

Results: We included 12 randomised controlled trials with 34 920 patients. Extended DAPT reduced the frequency of myocardial infarction (odds ratio [OR]=0.56; 95% confidence interval [CI], 0.46-0.68 and OR=0.58; 95% CI, 0.44-0.77), and stent thrombosis (OR=0.44; 95% CI, 0.30-0.65 and OR=0.49; 95% CI, 0.29-0.82) when compared to 12-months and short-term DAPT, respectively. However, it increased the risk of major bleeding (OR=1.53; 95% CI, 1.21-1.93 and OR=2.58; 95% CI, 1.62-4.10). Extended DAPT increased all-cause mortality (OR=1.27; 95% CI, 1.03-1.57) when compared to 12-months DAPT.

Conclusion: No superiority of short-term over extended DAPT was identified. DAPT duration should be individualised according to the benefit-risk profile of each patient. Patients at high bleeding risk could have a shorter DAPT duration; those with low bleeding but high ischaemic risk could consider a longer duration.

18

Network meta-analysis of cardiovascular outcomes in randomised controlled trials of new antidiabetic drugs

Y Fei, MF Tsoi, TT Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Evidence from clinical trials that directly compared the effect of new antidiabetic drugs on cardiovascular outcomes in patients with type 2 diabetes mellitus (T2DM) is limited. Therefore, we performed a network meta-analysis to assess the cardiovascular safety of these drugs.

Methods: We searched for randomised controlled trials on new antidiabetic drugs in T2DM patients with established cardiovascular disease or cardiovascular risks. New antidiabetic drugs included glucagon-like peptide-1 (GLP-1) receptor agonists (RAs), sodium-glucose co-transporter 2 (SGLT-2) inhibitors, and dipeptidyl peptidase-4 (DPP-4) inhibitors. Data were analysed using frequentist approach and Bayesian framework in R.

Results: We included seven randomised controlled trials with 62268 T2DM patients. GLP-1 RAs and SGLT-2 inhibitor reduced major adverse cardiovascular event (MACE; odds ratio [OR]=0.89; 95% confidence interval [CI], 0.82-0.97 and OR=0.85; 95% CI, 0.73-0.99, respsecitvely), and cardiovascular mortality (OR=0.85; 95% CI, 0.73-0.99 and OR=0.61; 95% CI, 0.49-0.77) when compared to placebo, respectively. SGLT-2 inhibitor reduced cardiovascular mortality when compared to GLP-1 RAs (OR=0.72; 95% CI, 0.54-0.95) and DPP-4 inhibitors (OR=0.61; 95% CI, 0.47-0.78).

Conclusion: SGLT-2 inhibitor was more beneficial in reducing cardiovascular mortality than GLP-1 RAs and DPP-4 inhibitors. DPP-4 inhibitors did not reduce MACE and cardiovascular mortality when compared to GLP-1 RAs and SGLT-2 inhibitor. None of them increased cardiovascular risks compared to placebo.

19

Uncoupling protein 1–deficient mice exhibit increased mitochondria biogenesis in subcutaneous white adipose tissue

Y Gao, Q Liu, TS Feng, XY Hui, AM Xu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Uncoupling protein-1 (UCP-1) is well known for its dominant function in non-shivering thermogenesis. Interestingly, UCP1-deficient mice are not obese under normal conditions, but how these mice dissipate extra energy in an UCP-1–independent manner is not fully understood.

Methods: UCP1 knockout (KO) mice and the wildtype controls were fed with high-fat high-glucose diet. The oxygen consumption of the mice was monitored by the Comprehensive Lab Animal Monitoring System. Biomarkers for mitochondrial activity and biogenesis were evaluated through western blot and real-time polymerase chain reaction. We also used electron microscopy and histological imaging to observe changes and differences in mitochondria.

Results: Compared to wildtype controls, UCP1 KO mice gained less body weight and adiposity, which was accompanied by increased oxygen consumption. The interscapular brown adipose tissue (BAT) of UCP1 contains larger lipid droplet and abnormal mitochondria. In contrast, the subcutaneous white adipose tissue (scWAT) exhibited a higher oxygen consumption and elevated number of mitochondria. Further analysis showed that the the key components in mitochondria biogenesis, AMPK and peroxisome proliferator activator receptor gamma co-activator (PGC1α) were significantly activated/increased in scWAT of UCP1 KO mice.

Conclusion: The scWAT of UCP1 KO mice have developed a compensatory thermogenic mechanism, which is probably mediated via increased mitochondria biogenesis.

20

Long-term outcome of relapsed acute promyelocytic leukaemia treated with oral arsenic trioxide-based re-induction and maintenance: a 14-year prospective follow-up study

H Gill, YL Kwong
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: For acute promyelocytic leukaemia (APL) in second complete-remission (CR2), optimal post-remission strategy remains undefined. Efficacy of arsenic trioxide (As_2O_3) in relapse with prior As_2O_3 exposure is unknown. Risk factors for central nervous system (CNS) relapse are unclear.

Methods: A total of 71 APL patients in first relapse (R1) were studied. Oral-As_2O_3-based re-induction was administered. Risk factors for a second relapse (R2) and CNS involvement were determined using logistic regression. The leukaemia-free and overall survivals following oral-As_2O_3-based re-induction were determined using Cox-proportional hazard regression.

Results: Oral-As_2O_3-based re-induction resulted uniformly in CR2, irrespective of previous As_2O_3-exposure. Overall, 69 (97%) patients received oral-As_2O_3-based CR2 maintenance. At a follow-up of 66 (range, 15-169) months, 43 (61%) patients were still in CR2, with 10-year leukaemia-free-survival (LFS) of 57%. On the other hand, 28 patients developed R2. Oral As_2O_3-based re-induction led to CR3 in 25 (89%) patients. Post-CR3 strategies included autologous/allogeneic stem cell transplantation and oral-As_2O_3 maintenance. At a post-CR3 follow-up of 33 (range, 10-119) months, 10 patients were still in CR3. The 5-year and 10-year overall survival (OS) of the R1 cohort was 81.3% and 74.1%, respectively. Time to R1 from CR1 of ≤24 months was the only adverse factor for LFS and OS, with 10-year LFS and OS of 68.7% and 90.8%, respectively for R1 >24 months from CR1. CNS involvement occurred in 17 patients, with eight still surviving. Relapse during oral-As_2O_3 therapy was the only significant risk for CNS involvement. As_2O_3 remained effective despite repeated As_2O_3 exposures.

Conclusion: Oral-As_2O_3 maintenance was an effective post-remission strategy for CR2.

21

Developing a transgenic zebrafish model with haematopoietic stem cells–specific expression of human internal tandem duplication of FMS-like tyrosine kinase 3 (*FLT3-ITD*) mutation

BL He, YH Leung
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Recent advent in next-generation sequencing has generated an unprecedented amount of genetic information in myeloid malignancies. However, the functional validation of genetic abnormality is time-consuming by conventional mouse model. Knowledge about zebrafish haematopoiesis accumulated over the past two decades and novel genome-editing technologies in this model organism have made it a unique and timely research tool for the study of human blood diseases.

Methods: The mouse haematopoietic stem cells–specific enhancer *Runx1*, human *FLT3*-ITD, and *eGFP* sequence was cloned into the Tol2 destination vector by multisite gateway cloning strategy. The resultant plasmid DNA pDestTol2-Runx1:FLT3-ITD-eGFP (50pg/embryos) was co-injected with transposase mRNA (50 pg/embryos) into the cytoplasm of one-cell stage zebrafish embryos for transgenesis. F0 embryos shown evident green fluorescent protein (GFP) expression were raised to adult (F0).

Results: F1 stable line Tg(Runx1:FLT3-ITD-eGFP) was identified by outcrossing the individual F0 with wild-type zebrafish. FLT3-ITD+ cells were observed in the blood circulation and the thymus region (definitive haematopoietic site in zebrafish) as shown by in-situ hybridisation of GFP surrogate marker.

Conclusion: This transgenic zebrafish line will not only uncover the underlying mechanism of FLT3-ITD-mediated pathogenesis but also help to identify novel compounds targeting FLT3-ITD+ leukaemia.

Acknowledgement
We acknowledge Zebrafish Core Facility in Li Ka Shing Faculty of Medicine.

22

Risk of hepatitis B virus reactivation in rheumatic patients with positive HBsAg undergoing biologics treatment

C Ho
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: In Hong Kong, the chronic hepatitis B virus (HBV) carrier is around 8%. Use of immunosuppressive therapy may potentially reactivate chronic HBV infection. Prior to use of biologics treatment for rheumatic diseases, it is imperative that all patients are screened for hepatitis status.

Methods: This descriptive analytical study was conducted on 266 patients prior to receiving biologics treatment for their underlying rheumatic diseases. Serum levels of hepatitis B surface antigen (HBsAg), hepatitis B antibodies (anti-HBs and anti-HBc), aspartate aminotransferase, alanine transaminase (ALT) and alkaline phosphatase, venous blood samples were obtained. If patient was positive for either HBsAg or anti-HBc, another sample of the serum was sent to the laboratory to determine viral load by measuring HBV DNA in IU/mL. HBV reactivation is defined as a >1 log10 IU/mL increase in serum HBV-DNA level or the detection of previously undetectable HBV-DNA, and serum ALT elevation >2-3 times the upper limit of normal (ULN) (4).

Results: The mean age of the patients was 52.3 ± 15.3 years, with an age range of 19-86 years; 81 (30.5%) and 185 (69.5%) cases were male and female, respectively, with mean age of 47.9 and 54.6 years. The mean level of liver enzyme, ALT, was 38.9 ± 47.1. Twelve (4.5%) cases were HBsAg positive, 85 (32%) cases were anti-HBs positive, and 54 (20.4%) cases were anti-HBc positive. Overall, 43 (79.7%) cases were both positive for anti-HBs and anti-HBc; 11 (20.3%) cases were anti-HBs negative but anti-HBc positive; and 42 (16%) cases were only positive for anti-HBs. All patients positive for HBsAg anti-HBc were HBV DNA negative before starting biologics treatment. The mean biologics treatment duration was 41 months (range, 1-140 months). The mean peak ALT level was 59.2 ± 73.7 (range, 45-579). Eight cases have ALT elevation >2 x ULN (range, 96-579) without increase in serum HBV-DNA.

Conclusion: No HBV reactivation during 355 treatment course of both anti-TNF and non-TNF therapy, or 904.4 patient-years biologic treatment.

23

Targeted next-generation sequencing of cancer-associated genes in hepatocellular carcinoma identifies somatic deleterious mutations in genes related to genome integrity and cell adhesion

FY Huang, DKH Wong, WK Seto, CL Lai, MF Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Genetic variability of hepatocellular carcinoma (HCC) remains largely unknown, creating a challenge to effective molecular-based target therapies in HCC patients. With the goal of further exploring the molecular complexity of HCC, we investigated the somatic mutations of six cancer-associated genes (*ARID1A, IRF2, HNF4α, FAT4, PIK3CA,* and *TP53*) in HCC pathogenesis-related pathways.

Methods: The spectrums of somatic mutations in the six cancer-associated genes were analysed in eight paired tumour and adjacent non-tumour tissue samples by targeted next-generation sequencing. Mutations predicted to have damaging effects on protein function were validated by Sanger sequencing.

Results: Concurrent mutations of the six genes were detected in all paired tumour and non-tumour samples. Single nucleotide variants (SNVs) were identified in both coding and non-coding regions while insertion/deletions (indels) were only detected in non-coding regions. The most frequent mutated genes detected were *FAT4* (6.7%), *PIK3CA* (2.8%), *IRF2* (2.4%), *HNF4α* (2.3%), *TP53* (1.6%), and *ARID1A* (1.3%). Twelve non-synonymous SNVs in the exonic regions predicted to have deleterious effects on protein functions were only identified in TP53 (important for genome integrity; 3/12) and FAT4 (important for normal cell adhesion; 9/12) genes. Among these 12 SNVs, significant SNVs detected in tumour samples but not in their adjacent non-tumour samples included R117S and Y88S in *TP53* gene (3/8 [37.5%] and 2/8 [25%], respectively) and A4977T and I3602L in *FAT4* gene (both 1/8, 12.5%).

Conclusion: Somatic inactivation of tumour suppressor genes *TP53* and *FAT4* may be key tumourigenic events in hepatocellular carcinogenesis. If validated in a larger cohort, our findings may identify potential pathways for future development of targeted therapies for HCC.

24

The interaction of non-alcoholic fatty liver disease and chronic hepatitis B: results from a matched-case control study of 1202 patients

RW Hui, LY Mak, WK Seto, KS Liu, KS Cheung, J Fung, CL Lai, MF Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The potential interaction between chronic hepatitis B (CHB) and non-alcoholic fatty liver disease (NAFLD), two of the most prevalent liver diseases worldwide, has not been well-defined. Controlled attenuation parameter (CAP) is a non-invasive method to quantify steatosis. This study aimed to use CAP to determine the association between NAFLD, metabolic parameters, and hepatitis B virologic factors in CHB patients.

Methods: From December 2014 onwards, we recruited CHB patients without concomitant liver diseases or secondary causes of steatosis. Liver biochemistry, serum hepatitis B virus (HBV) DNA, and metabolic parameters were measured. Recruited patients underwent liver stiffness (LS) and CAP measurements using transient elastography (Fibroscan, Echosens, Paris). Steatosis was defined as CAP ≥222 dB/m. Metabolic syndrome was defined using the International Diabetes Federation definition. Steatotic and non-steatotic (controls) CHB patients were matched according to age, gender, and antiviral treatment status in a 1:1 ratio.

Results: A total of 1202 CHB patients (mean age, 51.8 years; 51.4% male; 90.7% hepatitis B e-antigen (HBeAg) negative) were included in the analysis, with 601 matched steatotic and non-steatotic patients. Overall, 696 (57.9%) patients were on nucleoside analogue therapy for a median duration of 76 months (6-220 months). Metabolic syndrome (40.6% vs 11.2%) and its individual components were more prevalent in steatotic patients than in controls (all P<0.001). Steatotic patients also had higher LS than controls (5.4 vs 5.0 kPa; P<0.001). In multivariate analysis, central obesity, reduced high-density lipoprotein, increased triglyceride and body-mass index (BMI) were independent predictors of steatosis (odds ratio=2.8, 1.5, 1.7 and 1.4, respectively; all P<0.05), while LS was not a significant factor. Among treatment-naïve patients, HBV DNA levels were significantly lower in steatotic individuals than in controls (3.0 vs 3.4 log IU/mL; P<0.05), while in multivariate analysis, steatosis was independently associated with low HBV DNA levels (odds ratio=0.860; P<0.05).

Conclusion: Metabolic syndrome components and BMI were the main determinants of steatosis in CHB patients, resembling the NAFLD risk factors in non-HBV infected patients. Moreover, LS was not independently associated with steatosis. In treatment-naïve patients, HBV DNA was an independent negative predictor of steatosis. The relationship between steatosis and HBV replication warrants further investigation.

25

The effects of non-alcoholic fatty liver disease on liver fibrosis in chronic hepatitis B patients on nucleoside analogue therapy: a matched-case control study

RW Hui, LY Mak, WK Seto, KS Liu, KS Cheung, J Fung, CL Lai, MF Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The potential synergistic effect of non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis B (CHB) on hepatic fibrosis has not been well-investigated. Liver stiffness (LS) and controlled attenuation parameter (CAP) are non-invasive methods to quantify fibrosis and steatosis, respectively. This study aimed to use LS and CAP to determine the effect of NAFLD on fibrosis in CHB patients on nucleoside analogue (NA) therapy.

Methods: From December 2014 onwards, CHB patients on NA therapy and without concomitant liver diseases or secondary causes of steatosis were recruited. Liver biochemistry, serum hepatitis B virus (HBV) DNA, and metabolic parameters were measured. Recruited patients underwent LS and CAP measurements using transient elastography (Fibroscan, Echosens, Paris). Significant fibrosis was defined using the European Association for the Study of the Liver guidelines (≥9 kPa for patients with normal alanine aminotransferase). Metabolic syndrome was defined using the International Diabetes Federation criteria. Patients with CHB with and without significant fibrosis were matched according to age, sex, NA type, and duration of treatment in a 1:3 ratio.

Results: A total of 568 CHB patients (mean age, 57.3 years; 73.2% male; median NA therapy duration, 76.7 months; 90.5% hepatitis B e-antigen negative) were recruited, with 142 matched fibrotic patients and 426 controls. There was no difference in the proportion of patients with undetectable HBV DNA between the groups (92.3% vs 92.5%; P=0.938). Fibrotic patients, when compared to controls, had higher CAP (255 vs 240 dB/m; P<0.05), HbA1c (6.0% vs 5.7%; P<0.001) and body mass index (BMI) (25.0 vs 23.8 kg/m^2; P<0.001), and lower platelet count (135 vs 192 x 10^9 /L; P<0.001). Fibrotic patients were also more likely to have metabolic syndrome, central obesity, and raised fasting glucose than controls (all P<0.05). In multivariate analysis, CAP, raised fasting glucose, and BMI were independent predictors of significant fibrosis (odds ratio=1.005, 2.233, and 1.116 respectively; all P<0.05), while platelet count was inversely associated with fibrosis (odds ratio=0.982; P<0.001).

Conclusion: Steatosis and metabolic abnormalities were independent predictors of significant fibrosis, implying that they could contribute to fibrosis persistence in CHB patients on NA therapy. Identification and management of NAFLD and metabolic conditions in on-treatment CHB patients may potentially assist regression of fibrosis.

26

A prospective randomised controlled trial to compare the safety and immunogenicity of intradermal and intramuscular influenza vaccines in patients with inflammatory bowel disease

IFN Hung, KSH Liu, M Cheung, D But, WK Seto, SY Wong, KH Chan, WK Leung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Poor influenza vaccine immunogenicity has been reported in inflammatory bowel disease (IBD) patients. The new intradermal (ID) influenza vaccine has been shown to be equally effective as intramuscular (IM) vaccine in adults.

Methods: We conducted a prospective open-label randomised trial to compare the immune responses and safety of ID and IM trivalent influenza vaccine (TIV) on adult patients with IBD. Patients were stratified according to the diagnosis of Crohn's disease (CD) or ulcerative colitis (UC), and randomly assigned to ID or IM TIV. We measured the hemagglutination inhibition (HAI) and geometric mean titres (GMT) against A and B at baseline, 21-day, and 6-month post-vaccination.

Results: Between 2013 and 2014, we recruited 127 IBD patients and randomised to receive the ID (n=63) and IM (n=64) vaccine. There were 65 (51.2%) UC and 62 (48.8%) CD patients. The baseline demographics and disease severity-score were similar between the two vaccine groups. Immunogenicity at baseline and day 21 for all three vaccines' strains were comparable. At 6 months, the immunogenicity was significantly higher in the ID group for the A(H3N2) and B strains (P<0.05). Local reactions of redness and swelling were more common in the ID group with no difference in systemic adverse events. There were also no significant changes in IBD disease activity after the two vaccines.

Conclusions: Both IM and ID TIV confer acceptable immune responses to IBD patients with no significant adverse effect. The 6-month immunogenicity against the A(H3N2) and B influenza was significantly higher in the ID vaccine.

Dual influenza and pneumococcal vaccination protects smokers against hospitalisation

27

IF Hung, P Li, KK To, KH Chan, KY Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: Chronic smokers are at risk of premature death associated with underlying pulmonary or cardiovascular diseases. Dual influenza and pneumococcal vaccination has been shown to prevent death and hospitalisation secondary to pulmonary or cardiovascular diseases in elderly persons. Its effect in chronic smokers remained unknown.

Methods: This was a prospective randomised open-label trial conducted from April 2010 to March 2013, comprising adult patients who were chronic smokers and aged less than 50 years. Subjects were randomly assigned into four groups. Group 1 (study group) received both trivalent influenza vaccine (TIV) and the 23-valent polysaccharide pneumococcal vaccine (PPV), and three control groups. Group 2 received the TIV only. Group 3 received the PPV only. Group 4 did not receive any vaccines. The TIV used was the Vaxigrip® (Sanofi Pasteur, France) and the PPV used was the Pneumovax®23 (Merck, USA). All enrolled patients were followed up for 24 months post vaccination. Patient demographics, Charlson comorbidity index, medications, hospitalisation, diagnosis, and mortality were recorded.

Results: A total of 1006 subjects were enrolled and completed the study (group 1, n=250; group 2, n=254; group 3, n=250; group 4, n=259). The baseline demographics and Charlson comorbidity index were similar among subjects in the four groups. The median age was 48 years and 85.9% were male. Significantly fewer subjects who received the dual vaccination (group 1) were hospitalised (P<0.001), with shorter mean length of stay (P<0.001), and less frequent hospitalisation (P<0.001) for cardiovascular or respiratory diseases than no vaccination (group 4) or single vaccination (groups 2 and 3). Multivariate analysis demonstrated that dual vaccination with PPV + TIV was the only independent factor associated with reduced risk of hospitalisation (P<0.001; relative risk=0.288; 95% confidence interval, 0.101-0.154). There was no difference in mortality rate among the groups. Both vaccinations were well tolerated and no serious adverse events were reported.

Conclusion: Dual influenza and pneumococcal vaccination prevented chronic smokers against hospitalisation secondary to pulmonary or cardiovascular causes. Annual influenza and a single pneumococcal vaccination should be promoted among chronic smokers.

Absolute cardiovascular and mortality benefits of empagliflozin in type 2 diabetes

28

CR Kumana, BMY Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The benefits (and harm) of therapeutic interventions on outcomes should be assessed in both relative and absolute terms. We therefore undertook such an assessment for empagliflozin treatment in type 2 diabetes, based on the published data from the EMPA-REG trial,[1] in which all participating patients also received standard treatment for cardiovascular risk factors in diabetes.

Methods: The study recruited 4687 and 2333 patients randomised to empagliflozin and placebo, respectively. The median patient observation time was 3.1 years. As described previously,[2] we computed unadjusted critical end-point risk ratio (RR) and number-needed-to-treat (NNT)/year values and corresponding 95% confidence intervals (CIs) for empagliflozin treatment, based on pooled data for 10 or 25 mg/day users.

Results: Our findings are summarised below. The only significant adverse outcome was genital infection for which RR and 'number-needed-to-harm'/year values were 3.57 (2.37-4.96) and 67 (83-56), respectively.

Endpoint	RR (95% CI)	NNT/year (95% CI)
Cardiovascular death, non-fatal MI or stroke	0.86 (0.74 to 1.00)	189 (95 to 22007)
Cardiovascular death	0.62 (0.50 to 0.79)	141 (93 to 286)
Death from any cause	0.68 (0.57 to 0.83)	120 (79 to 249)
Non-fatal MI (excluding silent-MI)	0.88 (0.70 to 1.10)	483 (–675 to 178)
Non-fatal stroke	1.24 (0.92 to 1.69)	–493 (–212 to 1516)
Silent-MI	1.29 (0.70 to 2.37)	–862 (–263 to 676)

Abbreviation: MI = myocardial infarction

Conclusions: Statistically significant benefits were mainly driven by reductions in mortality; no significant non-fatal event rate changes imply conversion of fatal to non-fatal outcomes coupled with prevention of some non-fatal events. The NNT/year for preventing death was 120. This absolute benefit was about half that for preventing fatal and non-fatal coronary events enjoyed by high-risk patents in the 4S trial (in whom the NNT/year was 63).[2] Moreover, such benefit appears additional to that derived from standard anti-lipidaemic, anti-hypertensive, and other interventions offered to these diabetic patients.

References

1. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-28.
2. Kumana CR, Cheung BM, Lauder IJ. Gauging the impact of statins using number needed to treat. JAMA 1999;282:1899-907.

PD-L1 expression is associated with stem cell markers in non–small-cell lung cancer

29

HH Kwok, DCL Lam
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Lung cancer is the top cancer killer globally and it is one of the few cancers possessing high mutational burden. Even with the advances in treatment of lung cancer with chemotherapy and targeted therapy, advanced stage disease usually presents late and cannot be cured. The success of cancer immunotherapy using immune checkpoint inhibitors such as monoclonal antibodies targeting programmed cell death 1 (PD-1, CD279) or programmed death-ligand 1 (PD-L1, CD274) could be useful in patients with advanced-stage NSCLC. The aim of this project was to study the associations of cancer stem cell characteristics with the expression of PD-L1 in non–small-cell lung cancer (NSCLC).

Methods: Twenty-two NSCLC cell lines harbouring different oncogenic mutations were cultured in differentiated condition and in tumour spheroid condition. The mRNA expression of PD-L1 together with the expressions of cancer stem cell surface markers CD44 and CD133, stem cell transcription factors SOX2, OCT4 and NANOG were examined by real-time reverse-transcription polymerase chain reaction. The protein expression of PD-L1 was determined by immunoblotting. Fluorescent immunohistochemistry was used to study the co-expression of cancer stem cell marker with PD-L1 on tumour biopsy.

Results: Cancer cells grown in tumour spheroid condition displayed significant increased expression of cancer stem cell surface markers and transcription factors, and with concomitant increase in PD-L1 expression in some cell lines. The expression of PD-L1 was associated with the expression of cancer stem cell markers in EGFR wild-type and squamous NSCLC cells grown in spheroid condition.

Conclusion: Targeting of cancer stem cells population as guided by the presence of PD-L1 expression and cancer stem cell markers may contribute to the therapeutic effects of PD-1/PD-L1 blockade.

Activation of c-Jun N-terminal kinase in adipose tissue favours visceral fat inflammation that is associated with accelerated atherogenesis in apoE$^{-/-}$ mice

30

KHM Kwok, KKY Cheng, RLC Hoo, D Ye, A Xu, KSL Lam
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: The c-Jun N-terminal kinase (JNK) is activated in adipose tissues in obesity, an independent risk factor for atherosclerosis. It promotes insulin resistance and the secretion of pro-inflammatory cytokines in adipose tissues that contribute to systemic inflammation. This study investigated the effect of adipose-selective inactivation of JNK on fat inflammation and atherosclerosis.

Methods: ApoE$^{-/-}$ mice were crossbred with transgenic mice expressing adipose-selective dominant negative form of JNK (dnJNK) to generate the double-mutant apoE$^{-/-}$/dnJNK (ADJ) mice. ApoE$^{-/-}$ and ADJ mice were subjected to 10-week high-fat-high-cholesterol (HFHC) diet treatment. Basic metabolic parameters including body weight, fat mass, glucose tolerance, and lipid profile were measured. Adipose tissue and systemic inflammation, and atherosclerotic plaque development were examined.

Results: ApoE$^{-/-}$ and ADJ mice treated with HFHC diet did not show any difference in body weight, fat mass, and glucose tolerance. The serum levels of triglyceride, and high- and low-density lipoprotein cholesterol were similar in both groups. In contrast, the expression levels of pro-inflammatory markers—including MCP-1, TNF-α, adipocyte fatty acid binding protein (A-FABP) and cyclooxygenase 2 (Cox2)—were markedly lower in visceral fat but not subcutaneous fat from ADJ mice compared with apoE$^{-/-}$ controls. ADJ mice had alleviated systemic inflammation, which correlated with significantly reduced atherosclerotic plaque size, as evidenced by histological analysis of the aortic root and tree.

Conclusion: Suppression of JNK activity in adipose tissues alleviated inflammation selectively in visceral fat, and conferred protective effects on the development of atherosclerosis without affecting glucose and lipid metabolism. Our data have highlighted the importance of visceral fat inflammation in mediating the adverse effect of HFHC diet on atherogenesis.

Acknowledgement
This study was supported by the Hong Kong Research Grant Council (GRF769410).

Exit-site granuloma after percutaneous endoscopic gastrostomy insertion in Chinese patients

31

TK Kwok
Division of Rehabilitation, Department of Medicine, Tung Wah Hospital, Hong Kong

Introduction: Granuloma is one of the common complications of long-term percutaneous endoscopic gastrostomy (PEG) user observed in Chinese patients. It causes contact bleeding, discharge, and leaking from the exit site. However there are not many studies trying to review this complication.

Methods: We collected demographic and clinical data from Tung Wah Hospital PEG clinic from January 2010 to January 2016. We tried to identify the factors associated with development of granuloma by using logistic regression analysis.

Results: A total of 182 Chinese patients were identified. The development of granuloma at the PEG exit site was highly associated with patients with a history of nasopharyngeal carcinoma (NPC) [odds ratio=12.87; P=0.0006). Other demographic data and clinical data did not have statistical significant association with granuloma development at the exit site.

Conclusion: Patients with NPC have very high risk of developing granuloma after PEG insertion at the exit site. Exit site care should include this complication as a standard for this group of patients. Self-initiated treatment for granuloma should be taught to these patients upon discharge.

An evaluation of the impact of potentially inappropriate medications on all-cause mortality

32

MPS Lam[1,2], BMY Cheung[1], ICK Wong[2]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong

Introduction/Objective: Potentially inappropriate medications (PIMs) can lead to adverse drug events that resulted in hospitalisations and death. Older adults are at greater risk due to multiple chronic comorbidities and multiple medications prescribed. Criteria such as Beers or STOPP (Screening Tool of Older Person's Prescriptions) on medication usage have been developed to assess prescribing appropriateness. The objective of this study was to investigate the hazard of all-cause mortality among older adults with PIMs.

Methods: A retrospective, observational study was carried out. Patient's characteristics including medications list and death record were retrieved. The PIMs were assessed by both criteria.

Results: A total of 500 patients (246 males and 254 females; mean age ± standard deviation [SD], 81.45 ± 8.61 years) were recruited. Overall, 3997 medication items (mean number of medications per person ± SD, 7.99 ± 4.53) were reviewed. Prevalence of PIM was 38.6% and 31.6% according to Beers 2012 and STOPP criteria, respectively. Of note, 212 (42.4%) patients and 356 (71.2%) patients died at 1-year and 3-year follow-up, respectively. In the unadjusted Cox proportional analyses, there was an increased risk of all-cause mortality in patients with PIMs when compared to those without PIMs, regardless of criteria used (all $P<0.05$). However, no statistically significant difference was found between patients with or without PIMs for both 1-year and 3-year mortality in the adjusted models. Looking at the Kaplan-Meier curves, statistically significant differences were observed between those with PIMs and without PIMs (all log-rank $P<0.05$). When PIM was categorised in groups, an even more distinctive pattern was observed. Progressive increase in hazard of mortality was shown for those with more PIMs than those with fewer PIMs (all log-rank $P<0.05$).

Conclusion: This is the first local study that examined the impact of PIM on mortality among Hong Kong older adults. Significant difference in mortality was observed between patients with and without PIM. Although the association was no longer significant after adjusting for other variables, the study was able to show that increased trend of mortality risk was observed with increased number of PIM.

Inhibition of ornithine decarboxylase facilitates pegylated arginase treatment in lung adenocarcinoma xenograft models

33

SK Lam, S Xu, JC Ho
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Arginine depletion has shown anticancer effects among arginine auxotrophic cancers. Pegylated arginase (BCT-100) depletes arginine by converting arginine to ornithine. In this study, BCT-100 inhibited cell growth in a panel of lung adenocarcinoma cell lines while stimulated tumour growth in most lung adenocarcinoma xenograft models. Furthermore, ornithine decarboxylase (ODC) was induced by BCT-100 in two solid xenograft models with tumour growth stimulating effect. We postulated that accumulated ornithine was used to produce polyamines by ODC which promoted tumour growth, and ODC inhibition might rescue the therapeutic effect of BCT-100 treatment in lung adenocarcinoma.

Methods: A panel of seven lung adenocarcinoma cell lines (H23, H358, HCC827, H1650, H1975, HCC2935, and HCC4006) was used to study the in-vitro and in-vivo effects of BCT-100. Protein expression, arginine level, and apoptosis were investigated by Western blot, ELISA, and TUNEL assay, respectively.

Results: BCT-100 reduced in-vitro cell viability across different cell lines and HCC4006 xenograft model while paradoxical growth stimulation was observed in H358, HCC827, H1650, and H1975 xenograft models. Upon BCT-100 treatment, ODC was induced in two solid tumour xenograft models (H1650 and H1975), while unaltered in cystic tumour xenograft models (H358 and HCC827) and the remaining solid tumour (HCC4006) xenograft model. In both H1650 and H1975 xenografts, combined α-difluoromethylornithine (DFMO, an ODC inhibitor) and BCT-100 significantly suppressed tumour growth compared with control or single-arm treatments. In HCC4006 xenograft model, the tumour suppression effect of BCT-100 arm and DFMO/BCT-100 arm was similar. The tumour suppression effect was partially mediated by arginine and polyamines depletion resulted in apoptosis.

Conclusion: Inhibition of ODC by DFMO is essential in BCT-100 (pegylated arginase) treatment in lung adenocarcinoma.

Adenoma recurrence rates after curative resection for right-side or left-side colonic cancer

34

YF Lam[1], WK Seto[1], T Tong[1], O Lo[2], WL Law[2], WK Leung[1]
Departments of [1]Medicine and [2]Surgery, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Patients with a history of colorectal cancer (CRC) are at increased risk of developing metachronous lesions including adenoma and cancer. We aimed to determine the rates of colorectal polyp and adenoma recurrence on surveillance colonoscopy in patients after colonic resection for right-side (R-CRC) and left-side CRC (L-CRC).

Methods: Consecutive patients with CRC who had undergone surgical resection in our hospital between January 2001 and December 2004 were identified from our colorectal cancer database. Patients were included only if they had undergone curative surgical resection and had a clearing colonoscopy performed either before or within 6 months after the operation. Patients with familial colorectal cancer syndrome (familial adenomatous polyposis, hereditary non-polyposis colorectal cancer syndrome), subtotal or total colectomy, and inflammatory bowel syndrome were excluded. Findings of surveillance colonoscopy performed up to 5 years after colonic resection were included in the analysis. Patient's baseline characteristics, tumour locations, type of surgical intervention, and surveillance colonoscopy findings were retrieved. In this study, R-CRC was defined as cancer at and proximal to splenic flexure and L-CRC included all other distal cancers.

Results: A total of 863 patients underwent curative surgical resection for CRC during the study period and 473 patients (172 patients with R-CRC and 301 with L-CRC) fulfilled our inclusion criteria. Among them, 107 (62.2%) patients with R-CRC and 220 (73.1%) patients with L-CRC had at least one surveillance colonoscopy, with a total of 474 colonoscopies performed. The proportion of patients who had polyp and adenoma detected on surveillance colonoscopy was higher for those who had surgery for L-CRC compared to those who had surgery for R-CRC (polyp 30.9% vs 19.6%, P=0.03; adenoma 25.5% vs 13.1%, P=0.01). The mean number of adenoma on surveillance colonoscopy was also higher for patients with L-CRC compared to R-CRC (0.52, 95% confidence interval [CI] 0.37-0.68 vs 0.22, 95% CI 0.08-0.35; P<0.01). On multivariate analysis, increasing age, male gender, longer follow-up time, and L-CRC were independent predictors of adenoma detection on surveillance colonoscopy after curative surgical resection for CRC.

Conclusion: Patients who had surgery for L-CRC have a higher chance of developing metachronous polyps and adenoma than those with R-CRC. Our findings may imply a need to have a different surveillance strategy for patients with L-CRC or R-CRC.

Validation of a novel definition of low disease activity state in systemic lupus erythematosus

35

ALH Lee, T Cheung, HY Chung, CTK Ho, TWL Li, PH Li, MY Mok, H Tsang, RWS Wong, CKY Yuen, CS Lau
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: While a 'treat-to-target' principle is widely advocated in the management of systemic lupus erythematosus (SLE), there is currently not an internationally agreed definition of low disease activity in lupus. In 2015, the Asia-Pacific Lupus Collaboration presented a novel consensus definition of a safe state in lupus, the 'Lupus Low Disease Activity State' (LLDAS).

Methods: This was a prospective study to determine whether LLDAS predicted lower flare-ups, damage, and mortality. A total of 339 SLE patients were recruited and followed up for 30 months. Multivariable binomial regression was used to determine the factors associated with LLDAS and Cox proportional hazard model to determine whether prior higher percentage of days in LLDAS would be associated with lower future flare-ups.

Results: The mean patient age was 48.1 years and mean disease duration 19.6 years. Female-to-male ratio was 16:1. Overall, 79 flare-ups were documented; 92.6% of patients had ever achieved LLDAS during the study period and 62.1% of patient-days were in LLDAS. No major demographic or prior disease presentations were found to be associated with the attainment of LLDAS. Patients with prior higher percentage of days in LLDAS were having lower hazard of lupus flare-ups (hazard ratio=0.420; P=0.015) after adjustment for gender and age. The numbers of patient damage and death were insufficient for analysis.

Conclusion: In this study, LLDAS is an independent construct achievable by most patients of different history or background. Our preliminary study shows LLDAS can predict the risk of future flare-ups although further studies are needed to determine whether it is associated with less lupus-related damage and lower mortality.

Diagnostic performance of different clinical criteria for familial hypercholesterolemia in Hong Kong Chinese patients

36

AC Lee[1], M Chan[1], CL Cheung[2], DC Siu[1], JY Leung[3], TW Wong[3], HK Pang[4], KC Tan[1]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong
[3]Department of Medicine and Geriatrics, Ruttonjee & Tang Shiu Kin Hospitals, Hong Kong
[4]Department of Medicine, Pamela Youde Nethersole Eastern Hospital, Hong Kong

Introduction: Familial hypercholesterolemia (FH) is the most common inherited disorder of lipid metabolism resulting in high levels of low-density lipoprotein cholesterol and increased risk of premature atherosclerotic cardiovascular disease. Although genetic testing remains the gold standard for diagnosis, it is not widely available. Different sets of clinical criteria have been used in screening of FH and guiding the selection of patients for genetic testing. However their performance has not been comprehensively evaluated among Hong Kong Chinese patients. The aim of this study was to compare the performance of three sets of FH clinical criteria, namely Dutch Lipid Clinic Network criteria (DLCN), Modified DLCN criteria for Chinese developed in China (M-DLCN), and Japanese criteria for FH (JC) among Hong Kong Chinese patients.

Methods: A total of 94 index patients with suspected FH were recruited from Queen Mary Hospital, Ruttonjee Hospital, and Pamela Youde Nethersole Eastern Hospital. Genomic DNA was isolated from peripheral blood leukocytes and mutations were identified by Sanger sequencing of the coding regions of LDL-receptor (LDLR), apolipoprotein B (APOB), or proprotein convertase subtilisin/kexin type 9 (PCSK9) genes. Diagnostic performance of each set of clinical criteria was validated against the mutation status.

Results: Overall, 67 and 82 patients had probable or definite FH by DLCN and M-DLCN, respectively. Of note, 43 patients were diagnosed to have FH by JC; 64 (68%) patients carried pathogenic mutations. Upon validation against genetic testing, DLCN demonstrated the best overall performance in diagnosing FH (sensitivity 82.8%, specificity 53.3%, positive predictive value 79.1%, and negative predictive value 59.3%). Although M-DLCN had the highest sensitivity (93.8%), it was not specific (specificity, 26.7%). On the other hand, JC was the most specific (specificity, 66.7%), but it was not sensitive (sensitivity, 51.7%).

Conclusion: This study demonstrated that DLCN had superior efficacy in diagnosing FH in Hong Kong Chinese patients, when compared with criteria developed by other Asian countries.

37

The role of serum fibroblast growth factor 21 levels in the prediction of incident coronary heart disease in patients with type 2 diabetes without prevalent cardiovascular disease

CH Lee[1,2], YC Woo[1], WS Chow[1], CYY Cheung[1], CHY Fong[1], MMA Yuen[1], A Xu[1,2,3], HF Tse[1,2], KSL Lam[1,2,3]
[1]Department of Medicine; [2]Research Centre of Heart, Brain, Hormone and Aging; [3]State Key Laboratory of Pharmaceutical Biotechnology; The University of Hong Kong, Hong Kong

Introduction: Serum fibroblast growth factor 21 (FGF21) levels have been associated with coronary heart disease (CHD) in cross-sectional studies. Prospective studies, however, have been lacking especially among subjects without prevalent cardiovascular disease (CVD). Here we investigated prospectively the role of serum FGF21 levels in the prediction of incident CHD in subjects with type 2 diabetes without prevalent CVD.

Methods: Baseline serum FGF21 levels were measured in 5746 Chinese subjects with type 2 diabetes recruited from the Hong Kong West Diabetes Registry. The role of baseline serum FGF21 levels in predicting incident CHD over a median follow-up period of 3.8 years was analysed using Cox regression analysis.

Results: Among 3528 recruited subjects without prevalent CVD, 147 (4.2%) developed CHD, with a cumulative incidence of 10.1 per 1000 person-years. Baseline serum FGF21 levels were significantly higher in those who had incident CHD than those who did not (222.7 pg/mL [92.8-438.4] vs 151.1 pg/mL [75.6-274.6]; P<0.001). On multivariable Cox regression analysis, baseline serum FGF21 levels independently predicted the development of CHD (hazard ratio=1.22; 95% confidence interval, 1.03-1.43; P=0.019) after adjustment of conventional cardiovascular risk factors at baseline including gender, age, smoking status, duration of diabetes, HbA1c, hypertension, estimated glomerular filtration rate, and dyslipidaemia.

Conclusion: Baseline circulating FGF21 levels might serve as a useful marker for cardiovascular risk stratification in predicting incident CHD among subjects with type 2 diabetes.

38

Performance of different creatinine-based estimated glomerular filtration rate equations in the prediction of all-cause mortality in Chinese subjects with type 2 diabetes

CH Lee[1,2], AZL Shih[1], YC Woo[1], CHY Fong[1], MMA Yuen[1], WS Chow[1], KSL Lam[1,2,3]
[1]Department of Medicine; [2]Research Centre of Heart, Brain, Hormone and Healthy Aging; [3]State Key Laboratory of Pharmaceutical Biotechnology; The University of Hong Kong, Hong Kong

Introduction: In Chinese, ethnicity-based and/or diabetes-specific modifications of the Modification of Diet in Renal Disease (MDRD) and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations have been developed for determining estimated glomerular filtrate rate (eGFR). This study aimed to compare the performance of five different creatinine-based eGFR equations in predicting all-cause mortality among Chinese subjects with type 2 diabetes (T2DM).

Methods: A total of 6739 Chinese subjects with T2DM were included. Their eGFR was calculated using the MDRD, CKD-EPI, their respective modified equations for Chinese, and the diabetes-specific CKD-EPI Chinese T2DM equations. Multiple Cox regression analysis was used to evaluate the associations of eGFR with all-cause mortality. C-statistics, category-free net reclassification index (NRI), and integrated discrimination index (IDI) were applied to assess the discrimination and reclassification of each eGFR equation in predicting mortality outcome.

Results: Over a follow-up of 5.7 years, the incidence of all-cause mortality was 12.9% (n=867). The CKD-EPI equation discriminated all-cause mortality better than the MDRD equation (C-indices: 0.714 vs 0.689; P<0.0001), and Chinese modification of their respective equations did not improve discrimination. Among the five eGFR equations evaluated, the CKD-EPI Chinese T2DM equation provided the best discrimination in predicting all-cause mortality among Chinese subjects with T2DM, and was the only equation providing a significantly positive NRI and IDI relative to the CKD-EPI equation.

Conclusion: Among Chinese subjects with T2DM, our findings suggested that the CKD-EPI Chinese T2DM equation best predicted all-cause mortality, and relative to the CKD-EPI equation, conferred improved discrimination and reclassification.

39

Differential brainstem atrophy in multiple sclerosis and neuromyelitis optica spectrum disorders among Hong Kong Chinese

CY Lee[1], HKF Mak[2], PW Chiu[2], HC Chang[2], F Barkhof[3,4], KH Chan[1]
Departments of [1]Medicine and [2]Diagnostic Radiology, The University of Hong Kong, Hong Kong
[3]Department of Radiology and Nuclear Medicine, VU University Medical Centre, The Netherlands
[4]Institutes of Neurology and Healthcare Engineering, University College London, United Kingdom

Introduction: Multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD) are the most common inflammatory demyelinating disorders of central nervous system in Hong Kong. Brainstem is commonly involved in both disorders. We aimed to study the regional brainstem atrophy of local MS and NMOSD patients.

Methods: Semi-automated segmentation and volumetric measurement of brainstem were performed and compared among MS, NMOSD, and healthy controls (HC). Clinical symptoms and severity were graded using Expanded Disability Status Scale (EDSS) and Kurtzke Functional System Scores (FSS). Associations between the volumes of interest (VOIs) and clinical disability scores were assessed by partial correlation and multiple regression analyses.

Results: Baseline characteristics were comparable across the three groups, without significant difference in disease duration and severity between MS and NMOSD subjects. Normalised whole brainstem, midbrain, and pons volumes were significantly smaller in MS subjects compared to HC (-5.2%, P=0.027; -8.3%, P=0.000; and -5.9%, P=0.048; respectively) while only the normalised medulla volume was significantly smaller in NMOSD subjects compared to HC (-8.5% vs HC, P=0.024). Normalised midbrain volume was significantly smaller in MS compared to NMOSD subjects (-5.0%, P=0.014) while normalised medulla volume was significantly smaller in NMOSD compared to MS subjects (-8.1%, P=0.032). Smaller normalised whole brainstem, pons, and medulla oblongata volumes were associated with greater disability on EDSS, FSS-brainstem, and FSS-cerebellar in NMOSD patients.

Conclusion: Our findings revealed different patterns of brainstem atrophy between MS and NMOSD patients. This can be related to different underlying pathologies and pathophysiological mechanisms.

40

Investigation in the role of Lamin A (*LMNA*) deficiency in heart block and dilated cardiomyopathy using human induced pluripotent stem cell derived cardiomyocytes

YK Lee, X Ran, Y Jiang, HF Tse
Cardiology Division, Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Lamin A/C is an essential component in nuclear matrix for maintenance of nuclear architecture. Clinical manifestations indicate that cardiac laminopathy was associated with prognosis and an early onset of atrial fibrillation, AV blocks, and dilated cardiomyopathy (DCM). We aimed to investigate the role of *LMNA* in the above-mentioned disease progression using human induced pluripotent stem cell (hiPSC)-cardiomyocytes (CMs).

Methods: We relied on previously generated hiPSC of a patient bearing $LMNA^{R225X/WT}$ and a lentiviral shRNA *LMNA* knockdown (shLMNA)-hiPSC as a source of CMs for disease modeling.

Results: The loss of nuclear integrity was observed in sh*LMNA* cell as a typical phenotype of laminopathy. sh*LMNA*-hiPSC-CMs showed disorganised sarcomeric structure, thus affecting force generation. Regarding electrical-contraction coupling, the diastolic intracellular calcium was remarkably elevated, together with the significantly depleted calcium handling kinetics with a corresponding impaired contraction force generation. Multielectrode arrays analysis indicated the sh*LMNA*-hiPSC-CMs were refractory to beta-adrenergic stimulation.

Conclusion: Loss of lamina integrity deteriorates connection of nucleus to intermediate filament and sarcomere, thus further reduced nuclear integrity and impaired force generation. In fact, the high diastolic calcium in sh*LMNA*-hiPSC-CMs elevated resting membrane potential far from the depolarisation threshold, thus reducing the excitability of cells to propagate action potential. Accelerated heart block and DCM progression in cardiac laminopathy is possibly due to mechanical stress on nuclear architecture. The sarcoplasmic reticulum linked with nuclear laminar presents impaired calcium handling properties and elevated threshold for cell excitability.

Acknowledgement
This work was supported by grants from the Hong Kong Research Grant Council, Theme-Based Research Theme (T12-705/11 to Prof. Tse and Dr. Siu); Strategic Priority Research Program of the Chinese Academy of Sciences (XDA01020106, to MAE).

Relationship between serum 25-hydroxyvitamin D and parathyroid hormone in Hong Kong Chinese population

41

RYH Leung[1], BMY Cheung[1], AWC Kung[1], KCB Tan[1], CL Cheung[2]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong

Introduction: Serum 25-hydroxyvitamin D [25(OH)D] level is generally used as the indicator to determine vitamin D status. However, there is still no consensus on the threshold level for vitamin D deficiency. We aimed to evaluate the break-point of 25(OH)D level that maximally suppresses parathyroid hormone (PTH) level as the optimal level for bone health.

Methods: Serum 25(OH)D and PTH levels were measured in 5276 participants (1583 men, 3693 women; mean age ± standard deviation [SD], 55.4 ± 15.6 years) of the Hong Kong Osteoporosis Study. The break-points of 25(OH) D level on PTH were estimated by three-phase segmented regression.

Results: In our study population, the mean levels ± SD of 25(OH)D and PTH were 54.1 ± 16.3 nmol/L and 3.9 ± 1.7 pmol/L, respectively. The prevalence of vitamin D deficiency (<50 nmol/L) was 43.8% and that of insufficiency (<75 nmol/L) or deficiency was 90.1%. The estimated first and second breakpoints of 25(OH)D on PTH expression were 32 nmol/L (95% confidence interval [CI], 29-35) and 89 nmol/L (95% CI, 77-101), respectively. After adjustment for the factors affecting bone and mineral metabolism, the estimated first and second break-points of 25(OH)D were 27 nmol/L (95% CI, 24-30) and 47 nmol/L (95% CI, 37-56), respectively.

Conclusion: Our estimated break-points of 25(OH)D level were significantly different from those suggested threshold levels for vitamin D deficiency in literature. Further investigation is needed on the standardisation of methodology in determination of the universal optimal level of vitamin D.

Acknowledgement
This research was supported by a HMRF grant (12132451).

25-Hydroxyvitamin D and the risk of stroke in Hong Kong Chinese population

42

RYH Leung[1], Y Han[2], CW Sing[2], BMY Cheung[1], ICK Wong[2,3], KCB Tan[1], AWC Kung[1], CL Cheung[2]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong
[3]Research Department of Practice and Policy, UCL School of Pharmacy, London, United Kingdom

Introduction: Although evidence suggested that low vitamin D level was linked to the risk of cardiovascular diseases, the association between vitamin D with stroke remains inconclusive. In this study, we aimed to evaluate the association between serum 25-hydroxyvitamin D [25(OH)D] and risk of stroke in Hong Kong Chinese population.

Methods: A total of 3458 participants aged ≥45 (1310 men, 2157 women; mean age ± standard deviation [SD], 63.2 ± 10.2 years) from the Hong Kong Osteoporosis Study were included in this study. They had a baseline examination in 1995-2010 and the occurrence of stroke was determined using electronic medical records. The relevant ICD-9 codes were used for defining Ischemic and haemorrhagic stroke.

Results: The mean levels ± SD of 25(OH)D was 56.5 ± 16.9 nmol/L. Quintiles 1 and 4 of 25(OH)D were significantly correlated with increased risk of stroke when compared to the highest quintile (quintile 1: hazard ratio [HR]=1.78; 95% confidence interval [CI], 1.16-2.74 and quintile 4: HR=1.61; 95% CI, 1.07-2.43) using multivariable Cox-proportional hazard regression. A significant association was also observed in the subgroup with ischaemic stroke. There was a reverse J-shape association between serum 25(OH)D and risk of stroke in penalised regression spline, with the lowest risk of stroke at 25(OH)D levels between 70 and 80 nmol/L.

Conclusion: A low vitamin D level is associated with higher risk of ischaemic stroke. Further study is needed to evaluate whether high vitamin D level is also associated with increased risk of stroke.

Acknowledgement
This research was supported by a HMRF grant (12132451).

43 Localisation and functional study of synaptic vesicle protein synaptogyrin-3 (SYNGR3) on dopaminergic neuronal system

LF Li[1], PWL Ho[1], HF Liu[1], CSC Lam[1], GCT Leung[1], MHW Kung[1], DB Ramsden[2], SL Ho[1]
[1]Division of Neurology, Department of Medicine, The University of Hong Kong, Hong Kong
[2]School of Biosciences, University of Birmingham, United Kingdom

Introduction: SYNGR3 is an integral synaptic vesicle protein in the synaptogyrin family. Among the three isoforms of synaptogyrin (SYNGR1-3), SYNGR3 is specifically expressed in brain. However, the physiological function of SYNGR3 in neurons is unknown. Previous studies have shown that SYNGR3 can physically interact with DAT protein. Intracellular dopamine (DA), if not properly sequestered, will undergo auto-oxidation leading to oxidative stress. Therefore, we hypothesise that SYNGR3 has a functional role in dopaminergic neurons to facilitate DA uptake for vesicle packaging and recycling.

Methods: Subcellular localisation of SYNGR3 and DAT in mouse brain striatum was visualised by immunogold electron microscopy (EM). Protein-protein interaction between SYNGR3 and DAT was determined by immunoprecipitation and Western blotting. The effects of SYNGR3 expression on DA uptake was determined by [3H]-dopamine uptake assay in human DAT-positive SH-SY5Y neuroblastoma cells after overexpressing SYNGR3.

Results: Immunogold EM revealed that SYNGR3 was co-localised in close proximity with DAT in the striatal synaptic termini. Immunoprecipitation of DAT using anti-DAT antibody resulted in co-precipitation of SYNGR3 from mouse striatal lysates, and vice versa. Overexpression of SYNGR3 in SH-SY5Y cells caused significant increase in cellular DA uptake activity as compared with empty-vector controls.

Conclusion: Our findings demonstrated that overexpressing SYNGR3 in neuronal cells increased DA uptake efficiency, possibly via strengthening interaction between synaptic vesicles and DAT on the plasma membrane. Striatum is enriched with dopaminergic innervations from the midbrain and cortex. Co-localisation of SYNGR3 and DAT in striatal synapses may have functional significance to maintain DA homeostasis for normal motor movement and cognitive functions.

Acknowledgement
This study was supported by the Henry G. Leong Professorship in Neurology (SLH); the Donation Fund for Neurology Research (SLH).

44 Regulatory role of autoimmune-associated gene *CLEC16A* in inflammasome activity in human macrophages

MWM Li, CS Lau, VSF Chan
Division of Rheumatology and Clinical Immunology, Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: C-type lectin domain family 16 member A (CLEC16A) has been associated with autoimmune diseases such as systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes in various genome-wide association studies. Subsequent characterisation studies of mouse/human CLEC16A and its Drosophila homolog endosomal maturation defective isoform A revealed their regulatory role in different aspects of autophagy, the regulated degradation of cellular components that are in excess or dysfunctional. Crosstalk between autophagy and inflammasome activity of innate immune responses has been reported while inflammasomes are activated in various autoimmune diseases. We thus sought to investigate the role of CLEC16A in inflammasome pathway in this study.

Methods: Functional genetic studies of CLEC16A in NLRP3 and AIM2 inflammasome pathways using monocyte-derived macrophages isolated from peripheral blood mononuclear cells of healthy individuals were performed.

Results: During induction of NLRP3 inflammasome pathway by nigericin, a knockdown of CLEC16A using specific siRNAs downregulated secretion of interleukin-1β (IL-1β), an inflammasome pathway effector. Its secretion during AIM2 inflammasome induction using intracellular dsDNA poly(dA:dT) however was not affected by the knockdown of CLEC16A. The induction of NLRP3 mRNA level upon nigericin stimulation was abolished in the siCLEC16A group. No significant changes in mRNA levels were observed in other selected genes of NLRP3 inflammasome pathway, namely the adaptor protein ASC, interleukin-1 converting enzyme caspase-1, and precursor pro-IL-1b.

Conclusion: These data suggest that CLEC16A may regulate the NLRP3 inflammasome pathway and affect the maturation and secretion of IL-1b. The mechanism involved and its association with autoimmune diseases such as systemic lupus erythematosus remains to be elucidated.

Immunoglobulin G4–related disease in Hong Kong

45

PH Li[1], SCW Chan[1], KL Ko[2], CTK Ho[1], L Lau[3], RKY Tsang[3], TT Cheung[4], WK Leung[2], CS Lau[1]
[1]Division of Rheumatology and Clinical Immunology; [2]Division of Gastroenterology and Hepatology, Department of Medicine; [3]Division of Head and Neck Surgery; [4]Division of Hepatobiliary, Pancreatic Surgery and Liver Transplant, Department of Surgery; Queen Mary Hospital, Hong Kong

Introduction: Immunoglobulin G4–related disease (IgG4-RD) remains an under-recognised disease and local data are lacking. We conducted this study to review the clinical features, treatment practices, and factors associated with more extensive disease involvement in Hong Kong.

Methods: We retrospectively evaluated all patients with IgG4-RD over the past 13 years in our centre and combined this with patient data extracted from previous local publications. We analysed the clinical features, treatment practices, and factors associated with the number of organ systems involved.

Results: A total of 149 patients (55 from our centre and 49 from existing literature) were identified. Patients were predominantly older men (61.9 years; male-to-female ratio=3:1) and 94.4% had elevated pre-treatment serum IgG4 levels. Hepatobiliary and pancreatic (40.4%), salivary gland (33.6%), lymphadenopathy (29.8%), and ophthalmic manifestations (19.2%) were the most common systems involved. Use of glucocorticoids was associated with lymphadenopathy (odds ratio [OR]=2.65; P=0.034) but negatively associated with central nervous system involvement (OR=0.12; P=0.044). Surgical intervention was negatively associated with lung involvement (OR=0.07; P=0.017). Pre-treatment serum IgG4 levels correlated with the number of involved organ systems (ß=0.347; P=0.004), and specifically with salivary gland involvement (mean, 1109 mg/dL vs 598.6 mg/dL; P=0.012).

Conclusion: We describe the clinical features and treatment modalities of the largest cohort of IgG4-RD in our region thus far. We identified pre-treatment serum IgG4-RD to be associated with more extensive disease, especially with salivary gland involvement. Increased physician awareness and multidisciplinary efforts are required for optimal management of this masquerading disease.

Induced pluripotent stem cell–derived mesenchymal stem cells exert growth differentiation factor-15–dependent paracrine effect in cigarette smoke medium-induced cardiomyocyte injury

46

YM Liang[1], YL Zhang[1], MSM Ip[1,4], HF Tse[1,4], QZ Lian[1,2,4], JCW Mak[1,3,4]
Departments of [1]Medicine, [2]Ophthalmology, [3]Pharmacology and Pharmacy; [4]Research Centre of Heart, Brain, Hormone and Healthy Aging; The University of Hong Kong, Hong Kong

Introduction: Mesenchymal stem cells (MSCs) are emerging as a potential cell-based therapy for cardiovascular diseases (CVDs), in which cigarette smoking is the major risk factor. Our previous findings demonstrated that the secretions from human induced pluripotent stem cell–derived mesenchymal stem cells (iPSC-MSCs) had a superior effect over bone marrow–derived MSCs (BM-MSCs) in attenuating cigarette smoke medium (CSM)–induced cardiomyocytes injury. Secretions of iPSC-MSCs contain higher level of growth differentiation factor-15 (GDF-15) than BM-MSCs,[1] which has been regarded as a cardioprotective protein against cell apoptosis. The aim of this study was to investigate whether GDF-15 is responsible for the superior therapeutic effects of secretions from iPSC-MSCs.

Methods: The human AC16 cardiomyocyte cell line was cultured in DMEM/F12 containing 12.5% fetal bovine serum. The CSM and conditioned medium (CdM) from iPSC-MSCs or recombinant human GDF-15 (rhGDF-15; same concentration as detected in CdM from iPSC-MSC) were added into cells and incubated for 24 hours. Cells were harvested to perform H2DCF-DA and MitoSox Red assays to determine cellular reactive oxygen species (ROS) and mitochondrial superoxide by flow cytometry. Apoptotic cells were measured with Annexin V apoptosis detection kit. JC-1 assay was conducted on cells to measure mitochondrial membrane potential.

Results: CdM from iPSC-MSC had a superior effect on the inhibition of CSM-induced cell apoptosis than that from BM-MSCs, along with reduced cleaved caspase 3 and cleaved PARP. CdM from iPSC-MSCs or rhGDF-15 significantly reduced CSM-induced ROS, mitochondrial superoxide production and cell apoptosis, respectively. Recombinant hGDF-15 partially restored mitochondrial function by maintaining mitochondrial membrane potential compared to CdM from iPSC-MSCs.

Conclusion: These data suggest that iPSC-MSCs-mediated protective effects on cardiomyocytes by inhibiting cell apoptosis and oxidative stress and attenuating mitochondrial dysfunction are likely to be GDF-15-dependent paracrine action in vitro.

Reference

1. Zhang Y, Liang X, Liao S, et al. Potent Paracrine Effects of human induced Pluripotent Stem Cell-derived Mesenchymal Stem Cells Attenuate Doxorubicin-induced Cardiomyopathy. Sci Rep 2015;5:11235.

Acknowledgement
This research was supported by National Natural Science Foundation of China (No. 81370140).

Differential inflammatory responses resulted from cellular senescence induced by doxorubicin and antimycin in airway epithelial cells

47

KWK Liu[1,3], YM Liang[1], MSM Ip[1,3], JCW Mak[1,2,3]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy; [3]Centre of Heart, Brain, Hormone and Healthy Aging; The University of Hong Kong, Hong Kong

Introduction: Recent theories have implicated a role for senescence in contributing to the worsening of inflammatory responses as implicated in chronic obstructive pulmonary disease (COPD). Nevertheless, in airway epithelial cells, established models are not well-defined. It is also unclear whether the method of senescence induction (ie via DNA damage by doxorubicin, or through eliciting mitochondrial stress by antimycin) affects the inflammatory responses involved. This study aimed at establishing a cellular model of senescence to investigate the inflammatory responses involved.

Methods: In order to establish senescence, human bronchial epithelial cells (BEAS-2B) were incubated with doxorubicin (10-50 nM) or antimycin (1-10 μM) for up to 3 days. Growth kinetics was assessed by the MTT assay. Senescence over time was scored using a senescence associated beta-galactosidase (SA-b-GAL) staining kit. Levels of interleukin (IL)–8 in cell supernatant were measured using ELISA.

Results: Doxorubicin and antimycin both inhibited cell growth as measured by MTT. In doxorubicin-treated cells, senescence was found as early as 2 days post-exposure; but for antimycin, cells typically established senescence upon 3 days post-exposure. Compared with the untreated control, an increase of IL-8 release was found in doxorubicin treatment, but a suppression of IL-8 release was found in antimycin-treated cells.

Conclusion: We conclude that the method of senescence induction can affect the inflammatory response involved.

Acknowledgement
This research was supported by Small Project Grant from the University of Hong Kong and matching fund from the Centre of Heart, Brain, Hormone and Healthy Aging.

Inhibition of adipocyte fatty-acid-binding protein attenuates cerebral ischaemia injury via alleviating MMP-9 mediated blood brain barrier disruption

48

B Liao, KSL Lam, A Xu, RLC Hoo
Department of Medicine, State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong

Introduction: Ischaemic stroke is one of the major causes of death and permanent disability in the worldwide. Adipocyte fatty-acid-binding protein (A-FABP) is an adipokine, mainly expressed in adipocytes and macrophages. Previous clinical studies reported that serum A-FABP is significantly increased in patients with ischaemic stroke and positively correlated with the severity of stroke outcomes. However, the direct role of A-FABP in pathogenesis of ischaemic stroke is unknown.

Methods: Focal cerebral injury in adult male A-FABP KO mice and their wild type (WT) littermates was induced by middle cerebral artery occlusion (MCAO) surgery. The infarct volume, neurological score, and blood brain barrier (BBB) disruption were assessed after 23 hours of reperfusion. The serum and cerebral A-FABP were measured by ELISA and immunohistochemical staining at different time points after surgery. The BBB tight junction–related protein (ZO-1 and Occludin), Matrix metallopeptidase 9 (MMP-9) and JNK/c-Jun activities were assessed by Western blot and gelatine zymography. On the other hand, adenovirus mediated overexpression of A-FABP (Ad-AFABP) in brain cortex was performed to determine the role of A-FABP supplementation in outcomes of ischaemia stroke.

Results: Serum A-FABP was significantly increased at 24 hours after MCAO surgery. The MCAO-induced cerebral injury and BBB disruption were alleviated in A-FABP KO mice compared with WT littermates. MCAO-induced upregulation of MMP-9 and degradation of ZO-1 and Occludin were protected by A-FABP deficiency. Cerebral JNK/c-Jun activity was upregulated higher in WT mice after MCAO compared with KO littermates.

Conclusion: A-FABP deficiency protects mice from MCAO-induced cerebral ischaemia injury by suppressing MMP-9 activity and BBB disruption. A-FABP enhances MMP-9 activity during ischaemic stroke possibly through JNK/c-Jun pathway.

49

Screening proteins profiles associated with spontaneous hepatitis B surface antigen seroclearance in chronic hepatitis B patients using proteomics

F Liu, WK Seto, DK Wong, FY Huang, R Sharma, KS Cheung, MF Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: In the natural history of chronic hepatitis B (CHB) infection, some patients may undergo spontaneous seroclearance of hepatitis B surface antigen (HBsAg). However, the underlying molecular mechanism of HBsAg seroclearance remains elusive. We aimed to identify proteins that may associated with HBsAg seroclearance.

Methods: The plasma proteomic profiles of 20 patients with spontaneous HBsAg seroclearance and 20 age- and sex-matched CHB patients were compared using a 4plex iTRAQ and SCX-LC-MS/MS technique (experimental group was labelled with 116 and 117 tags; control group was labelled with 114 and 115 tags). The differential proteins were analysed with ingenuity pathway analysis (IPA).

Results: A total of 298 proteins were identified, of which 50 proteins were found to be differentially expressed (43 up-regulated [116:114, 116:115, 117:114, and 117:115 abundance ratio >1.2] and 7 down-regulated [abundance ratio <0.8]). On mapping of the differentially expressed proteins to cellular pathways and biological functions via IPA, we observed that nearly 70% proteins were involved in immunological disease, cellular movement, inflammatory response, and cell-to-cell signalling and interaction. Taken together, six candidate proteins (CD44, APCS, ANAX1, CAT, FN1, and A2M) were identified as the key nodes in the IPA analysis.

Conclusion: Candidate proteins preliminarily discovered in this study may be involved in spontaneous HBsAg seroclearance and might potentially lead to the establishing interventions with the aim of achieving the 'functional cure' of HBV. The results will be validated in a larger patient cohort.

50

Defective ubiquitinated mitochondria accumulation in aged parkinsonian LRRK2R1441G knockin mice

HF Liu[1], PWL Ho[1], GCT Leung[1], CSC Lam[1], LF Li[1], MHW Kung[1], DB Ramsden[2], SL Ho[1]
[1]Division of Neurology, Department of Medicine, The University of Hong Kong, Hong Kong
[2]Institute of Metabolism and Systems Research, University of Birmingham, United Kingdom

Introduction: *LRRK2* (leucine rich repeat kinase 2) mutation is the commonest genetic risk of Parkinson's disease (PD). Genetic susceptibility and ageing are two major factors contributing to mitochondrial dysfunction in PD. *LRRK2* mutations were linked to dysregulation of mitochondrial autophagy. Defective mitochondria are ubiquitinated via Parkin/PINK1 pathway for degradation via mitophagy. We hypothesise that *LRRK2* mutation may contribute to accumulation of defective mitochondria in aged brain leading to mitochondria dysfunction.

Methods: In this study, 24-month-old LRRK2R1441G knockin mice and their age-matched wild-type (WT) controls were cardiac perfused and fixed for examination of mitochondria morphology in dorsal striatum using electron microscopy. The total number of mitochondria and those under fission were quantified and compared with WT controls (20 randomised photomicrographs x 3 animals). Degree of ubiquitination in total mitochondria pool was determined by flow cytometry after immuno-labelling. The respiratory chain reactions in isolated mitochondria were assayed using Clark-type oxygen electrodes.

Results: The total number of mitochondria in aged LRRK2R1441G knockin mouse striatum was higher, whereas the average cross-section area of each mitochondrion was smaller as compared with the WT controls. The number of mitochondria under fission was significantly higher in these knockin mice. The amount of mitochondria, which were simultaneously labelled by ubiquitin antibody and Mitotracker, was significantly higher in knockin mice. Oxygen consumption assay showed a trend of decrease in state-IV respiration in isolated mitochondria extracted from knockin mice.

Conclusion: Our study showed higher level of ubiquitinated mitochondria accumulation in aged LRRK2R1441G knockin mice, indicating that *LRRK2* mutation adversely affected normal mitochondrial function and recycling. These findings also suggested that LRRK2R1441G knockin mouse is a useful experimental model to explore genetic-ageing interactions in the pathogenesis of PD.

Acknowledgement
This study was supported by the Henry G. Leong Professorship in Neurology (SLH), the Donation Fund for Neurology Research (SLH).

Deletion of uncoupling protein 1 (UCP1) leads to mitochondria dysfunction in brown adipose tissues

51

Q Liu, Y Gao, TS Feng, XY Hui, AM Xu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Brown adipose tissue (BAT) is the adipose tissue specialised to generate heat by uncoupling oxidative phosphorylation with ATP production. This is achieved by the mitochondria inner membrane protein uncoupling protein 1 (UCP1) which functions as non-selective proton channel. However, whether and how it maintains normal functioning of thermogenic mitochondria is unclear.

Methods: The histology of the interscapular BAT was examined by H&E staining and electron microscopy. The mitochondria were isolated from BAT by differential centrifugation. The activity of electron transport chain reactions were examined by biochemical assays and Western blotting. The superoxide content was quantified by staining with MitoSox.

Results: Interscapular BAT from UCP1 knockout mice were paler and had enlarged lipid vacuole. Electron microscopy imaging revealed that mitochondria in UCP1-deficient BAT exhibited significantly impaired cristae formation. Western blotting showed that the expression of subunits in electron transport chain complexes I-IV was almost completely diminished upon deletion of UCP1, while expression of complex V was elevated. Consistently, the activities of complexes I-IV were suppressed whereas that of complex V was enhanced in UCP1-null mitochondria. Furthermore, UCP1 deletion caused superoxide accumulation in BAT. Additionally, using 1-dimensional gel analysis and mass spectrometry, a number of candidate mitochondrial proteins which showed altered expression have been identified.

Conclusion: UCP1 is not only necessary for thermogenesis, but plays an essential role in maintaining normal structure and function of mitochondria in BAT. The underlying mechanism warrants further investigation.

Expression of larger tumour suppressor 2/large tumour suppressor 1 (*LATS2/LATS1*) influences chemotherapy responses in non–small-cell lung cancer

52

SY Luo, HH Kwok, ZY Ning, DCL Lam
Division of Respiratory Medicine, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: LATS family protein (*LATS1* and *LATS2*) are core members of the mammalian Hippo pathway which is believed to play a role in control of organ size and in tumorigenesis. *LATS1* and *LATS2* were first discovered as tumour suppressors and played important roles in tumour progression through multiple signalling pathways including Hippo, p53, and Wnt. However, recent findings suggested that LATS kinases, especially *LATS2*, may exhibit tumour-promoting functions in some cancers. Therefore, the tumour-suppressive effects of LATS kinases remained controversial. Although there are new-generation chemotherapeutic drugs for treatment of advanced-stage non–small-cell lung cancer (NSCLC), platinum-based chemotherapy is still the standard-choice treatment for patients with advanced NSCLC. In this study, we aimed to study the roles of LATS kinases in modulation of chemo-sensitivity in NSCLC cells.

Methods: In NSCLC cell lines with low *LATS2/LATS1* ratios (CL1-0 and CL83), we over-expressed *LATS2* by transfecting cells with specific plasmids, while in NSCLC cells with high *LATS2/LATS1* ratio at baseline (H2023), we used *LATS2*-specific shRNA to silence *LATS2* expression. Changes in drug sensitivity towards cisplatin were measured by MTT assays. Cisplatin-induced apoptotic levels were determined by Annexin V assay. Intracellular distribution of LATS kinases was visualised with immunofluorescence.

Results: Over-expression of *LATS2* made cells more resistant to cisplatin as indicated by less apoptotic cells after cisplatin exposure; at the same time, *LATS2* knockdown in high-*LATS2/LATS1*-ratio cells sensitised cells to cisplatin treatment and promoted cisplatin-induced apoptosis. With altered expression of *LATS2*, there was concomitant alteration of the intra-cellular distribution of *LATS1*. *LATS2* downregulation was associated with translocation of *LATS1* from cytosol to nucleus, which might help to facilitate cisplatin-induced apoptosis.

Conclusion: The expression status of *LATS2* would influence *LATS1* activity by shifting the cellular distribution of *LATS1*. The observed interaction between *LATS2* and *LATS1* might affect cisplatin sensitivity in advanced NSCLC and the detail mechanisms warrant further investigations.

53 Health care resource utilisation in inflammatory bowel disease patients in the initial two years following diagnosis: a population-based registry study

LY Mak[1], SC Ng[2], OL Wong[3], KK Li[4], FH Lo[5], TL Wong[6], CM Leung[7], WC Tsang[8], KH Chan[9], SS Fung[10], HS Shan[11], CY Lam[12], AJ Hui[13], WK Leung[1]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]Institute of Digestive Diseases, The Chinese University of Hong Kong, Hong Kong
[3]School of Public Health, The University of Hong Kong, Hong Kong
[4]Tuen Mun Hospital, Hong Kong
[5]United Christian Hospital, Hong Kong
[6]Princess Margaret Hospital, Hong Kong
[7]Pamela Youde Nethersole Eastern Hospital, Hong Kong
[8]Tseung Kwan O Hospital, Hong Kong
[9]North District Hospital, Hong Kong
[10]Queen Elizabeth Hospital, Hong Kong
[11]Caritas Medical Centre, Hong Kong
[12]Kwong Wah Hospital, Hong Kong
[13]Alice Ho Miu Ling Nethersole Hospital, Hong Kong

Background: There are scanty data on the health care utilisation from Asia where the incidence of inflammatory bowel disease (IBD) is rising rapidly. We aimed to determine the health utilisation and costs in the first 2 years of diagnosis in an IBD cohort from Hong Kong and the factors associated with high cost outliers.

Methods: This was a retrospective study of patients who were newly diagnosed with IBD in a territory-wide, population-based IBD registry. Patients' clinical information, hospitalisation records, investigations, and IBD treatments were retrieved for up to 2 years following diagnosis of IBD.

Results: A total of 435 newly diagnosed IBD patients were included in this analysis—198 with Crohn's disease (CD) and 237 with ulcerative colitis (UC). Total direct medical expenditure for this cohort in the 2 years after the IBD diagnosis was US$7 072 710—hospitalisations (33%), 5-aminosalicylic acid (5-ASA; 23%), imaging and endoscopy (17%), outpatient visits (10%), surgeries (8%), and biologics (6%). Mean medical costs per patient-year were significantly higher for CD ($9918) than UC ($6634) [P=0.001]. The total health care cost decreased significantly after transition to the second year for all IBD patients (P<0.01). High-cost (>90th percentile) outliers for CD were associated with penetrating disease (odds ratio=6.03; 95% confidence interval, 2.21-16.41; P<0.01) and surgery leading to diagnosis (5.45; 1.90-15.62; P=0.003).

Conclusion: Hospitalisation and 5-ASA accounted for 56% of total medical costs in the first 2 years of newly diagnosed IBD patients. Health care costs were higher in the first-year compared to the second-year of diagnosis. Patients with CD who had penetrating disease and surgery leading to diagnosis predicted high-cost outliers.

54 Predictors for extremely low serum infliximab trough concentration in patients with Crohn's disease

LY Mak, T Tong, ELJ Chen, M Ko, E To, KSH Liu, IFN Hung, WK Leung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: Although infliximab (IFX) is effective as maintenance therapy for patients with Crohn's disease (CD), loss of response happens in 40% of patients, mainly due to low serum IFX trough concentration (ITC). We aimed to determine factors associated with extremely low ITC in CD patients receiving maintenance IFX.

Methods: This was a retrospective cohort study of CD patients who were on 5 mg/kg maintenance IFX infusion every 8 weeks. Disease phenotypes were classified according to the Montreal classification. Therapeutic range of ITC was defined as concentrations of 3-7 μg/mL, whereas extremely low ITC was defined as concentrations in the lowest quartile.

Results: A total of 44 CD patients were included. The median age was 36 years, with male-to-female ratio of 35:9. The median number of IFX infusion was 13.5. Of the patients, 40 (90.9%) were on azathioprine and eight of them had reduced dose due to leukopenia. Among these patients, only 10 (22.7%) achieved a therapeutic range of ITC. Median ITC of this cohort was 1.8 μg/mL (range 0.4-5.2 μg/mL) and the lowest quartile of ITC was ≤1.2 μg/mL. Median faecal calprotectin was 352 μg/g (range 30-1000 μg/g) and the levels were correlated with C-reactive protein (CRP) [r= –0.43; P=0.003], but not with ITC (P=0.84). On univarate analysis, extremely low ITC was associated with fistulating (B3) disease (hazard ratio [HR]=5.5; 95% confidence interval [CI], 1.3-23.6), non-ileocolonic (L3) location (HR=20.0; 95% CI, 2.3-176.8), IFX duration ≥2 years (HR=6.1; 95% CI, 1.1-32.8), albumin ≤37 g/L (HR=12.0; 95% CI, 1.1-131.2), and CRP >0.4 mg/dL (HR=7.9; 95% CI, 1.5-42.6). The use of azathioprine, regardless of dose, was not protective against extremely low ITC. Multivariate analysis showed that non-L3 location (HR=125.8; 95% CI, 2.6-6215; P=0.015) and IFX treatment duration ≥2 years (HR=39.3; 95% CI, 1.6-959.3; P=0.024) were independent variables associated with extremely low ITC. However, normal CRP (HR=12.9; 95% CI, 1.5-113.3) and L3 ileocolonic disease (HR=20.9; 95% CI, 15.7-877.8) were associated with higher percentage of patients achieving therapeutic ITC.

Conclusion: Majority of our CD patients on 5 mg/kg maintenance IFX every 8 weeks did not achieve therapeutic ITC range despite the use of azathioprine. Non-L3 disease location and treatment duration ≥2 years were associated with extremely low ITC.

55

Adipose tissue microRNA-34a deficiency improves metabolic benefits through enhancing M2 macrophage polarization and proliferation in dietary-induced mice

Y Pan[1,2], XY Hui[1,2], TS Feng[1,2,3], P Gu[1,2], Q Liu[1,2], Y Gao[1,2], KSL Lam[1,2], AM Xu[1,2,3]
[1]State Key Laboratory of Pharmaceutical Biotechnology, [2]Department of Medicine, [3]Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong

Introduction: Adipose tissues play critical role in obesity-associated cardio-metabolic abnormalities. Previous studies identified that microRNA-34a (miR-34a) was elevated in obese fat tissue, but whether miR-34a participates in metabolic disorders is unclear.

Methods: Adipose tissue miR-34a knockout (KO) mouse model was established for determining its role in diet-induced metabolic disorders. The clinical correlation between miR-34a and metabolic parameters was measured in 53 Chinese overweight/obese or lean subjects.

Results: After high-fat diet (HFD) treatment, the increasing miR-34a exaggerated dietary-induced glucose intolerance and insulin insensitivity in mice. KO mice exhibited susceptible to obese, but improvement of HFD-induced glucose intolerance, insulin insensitivity, hyperlipidaemia, and steatohepatitis. Additionally, we identified miR-34a KO decreased serum levels of inflammatory cytokines, and reduced crown-like structure and inflammatory M1 macrophage in adipose tissues, but increased anti-inflammatory M2 macrophage. Furthermore, the metabolic benefits in KO mice depended on macrophages, and miR-34a regulated macrophage polarization via binding to transcriptional factor Kruppel like factor (Klf) 4. Gain-of-function and loss-of-function analyses of Klf4 confirmed miR-34a/Klf4 axis played a critical role in macrophage polarization and proliferation. More importantly, clinical findings supported the positive correlation between miR-34a levels and metabolic parameters in visceral fat from overweight/obese human subjects.

Conclusion: These results demonstrated that adipose tissue miR-34a deficiency improved metabolic benefits through enhancing M2 macrophage polarization and proliferation.

56

Burden of rare variants in amyotrophic lateral sclerosis genes influences disease progression and survival in familial and sporadic ALS

SYY Pang[1], JS Hsu[2], KC Teo[1], Y Li[2], MHW Kung[1], M Li[2], PC Sham[2], SL Ho[1]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]Centre for Genomic Sciences, The University of Hong Kong, Hong Kong

Introduction: Amyotrophic lateral sclerosis (ALS) is heterogeneous with a possible oligogenic inheritance. It is unclear whether the burden of rare variants in potential causative genes has an effect on disease progression and survival.

Methods: We performed whole-genome sequencing on eight familial ALS (FALS) patients with *SOD1* mutation and whole-exome sequencing on 46 sporadic ALS (SALS) patients. Rare variants in the exons of 40 known ALS genes were examined. Survival analysis and rare variant association tests were performed.

Results: FALS patients with additional rare missense variants had shorter survival than those without. Combining FALS and SALS patients, 67% had at least one rare variant; 22% had two or more. Rare variant burden was significantly associated with survival—patients with two or more rare variants had lower probability of survival than patients with zero or one variant (P=0.001). After adjusting for age, site of onset, gender, mode of inheritance (familial versus sporadic) and treatment with riluzole, each additional rare variant increased the risk of requiring ventilatory support or death by 60% (P=0.0098). Compared with control subjects, presence of rare variant was significantly associated with risk of ALS (odds ratio=1.91; 95% confidence interval, 1.03-3.61; P=0.03). Patients with ALS had significantly higher rare variant burden than controls (Madsen-Browning, P=0.004).

Conclusion: Our findings support an oligogenic basis with burden of rare variants in causative genes affecting the development and progression of ALS. Elucidation of pathogenic pathways involving these rare variants will clarify the pathogenic mechanisms of ALS and identify potential therapeutic targets for disease modification.

Acknowledgement
This study was supported by Liu Po Shan/Dr Vincent Liu Endowment Fund for Motor Neurone Disease, Henry G. Leong Professorship in Neurology, the Donation Fund for Neurology Research.

Patient-specific induced pluripotent stem cell as model for hypertrophic cardiomyopathy study

57

X Ran, YK Lee, HF Tse
Department of Medicine, The University of Hong Kong, Hong Kong

Objective: Hypertrophic cardiomyopathy (HCM) is a highly prevalent monogenic cardiac disease. Numerous HCM-associated mutations were identified in over 20 genes, while the molecular pathways lead to the HCM phenotype are still not understood. We aimed to establish the patient-specific human induced pluripotent stem cell (iPSC) as a platform to elucidate the role of the identified HCM gene mutation as well as to develop the individualised therapeutic strategies.

Methods: Whole-genome EXOME sequencing was utilised to identify HCM-associated mutations, followed by generation of the patient-specific iPSC-derived cardiomyocytes (iPSC-CMs). The cellular size and myofilament organisation were determined by immunofluorescence staining. The calcium handling properties were investigated by fluorescence imaging and video edge detection. The electrophysiological properties were recorded by whole cell patch clamp.

Results: A missense mutation (Arg186Gln) was identified in cardiac troponin I gene (*TNNI3*) from two HCM probands. HCM iPSCs-CMs exhibited enlarged cellular size and higher frequency of sarcomere disorganisation. Additionally, the irregular calcium transients and elevated diastolic calcium level were observed in HCM iPSCs-CMs. The HCM iPSCs-CMs displayed prolonged action potential duration and higher ratio of early afterdepolarisations arrhythmic waveforms, which indicated high arrhythmic risk.

Conclusion: This study demonstrates that the iPSC-CMs bearing *TNNI3* mutation recapitulated HCM phenotypes, which could be utilised as a promise platform for exploring molecular mechanisms and the therapeutic strategies.

Novel presenilin 1 mutation (p.F386I) in a Chinese family with early-onset Alzheimer's disease

58

YF Shea, AOK Chan, LW Chu, SC Lee, CY Law, CH See, KL Yiu, PKC Chiu
Department of Medicine, Queen Mary Hospital, Hong Kong

Introduction: Autosomal dominant familial Alzheimer's disease (FAD) accounts for 0.5% of all Alzheimer's disease.

Methods: A FAD Chinese family, with seven affected family members, underwent *PSEN1* screening in three affected family members. DNA was extracted from patients' blood after obtaining informed consent from their caregivers. All coding exons including intron-exon boundaries of the *PSEN1* gene were amplified by polymerase chain reaction and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit on a 3500 Genetic Analyzer (Applied Biosystems).

Results: The three affected family members, with age of onset between 45 and 60 years old, presented with amnesia and progressive cognitive impairment. A heterozygous novel missense mutation in the *PSEN1* gene c.1156T>A, altering phenylalanine to isoleucine at codon 386, was identified. In-silico mutational analysis using SIFT, Polyphen-2, and MutationTaster predicted damaging, probably damaging and disease causing effects, respectively. The change also occurred in conserved domains of *PSEN1*.

Conclusion: p.F386I in *PSEN1* may have a mutagenic and probably pathogenic effect.

A descriptive study of Lewy body dementia with functional imaging support in Chinese: a preliminary study

59

YF Shea, SC Lee, LW Chu
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Lewy body dementia (LBD) includes dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD). There have been limited clinical studies among Chinese patients with LBD.

Methods: We retrospectively reviewed 23 patients with LBD supported by functional imaging. Baseline demographics, presenting clinical and behavioural symptoms, functional and cognitive assessment scores, and complications (including fall, dysphagia, aspiration pneumonia, pressure sores, and death) were collected. Patients with DLB were further classified to have Alzheimer's disease (AD) imaging pattern if the functional imaging demonstrated bilateral temporoparietal hypometabolism or hypoperfusion with/without precuneus and posterior cingulate gyrus hypometabolism or hypoperfusion.

Results: The pre-imaging accuracy of clinical diagnosis was 52.2%; 83.3% suffered from behavioural and psychological symptoms of dementia. Fall, dysphagia, aspiration pneumonia, pressure sores, and death happened in 69.6%, 52.2%, 26.1%, 26.1%, and 30.4%, respectively with event rates per 1000 person-years being 15.2, 7.4, 7.8, 3.5, and 4.3, respectively. Patients with aspiration pneumonia were more likely to have dysphagia (100% vs 35.3%; P=0.01). Patients with LBD who died scored higher in Clinical Dementia Rating (1 [interquartile range, 1-2] vs 0.5 [0.5-1.0]; P=0.01), lower in Barthel index (13 ± 7.0 vs 18 ± 3.5; P=0.04), and more likely to be using levodopa (85.7% vs 31.3%; P=0.03). Patients with LBD with AD pattern of functional imaging had an earlier age of presentation (73.4 ± 5.6 vs 79.6 ± 6.0; P=0.02) and scored lower MMSE at one year (15 ± 8.1 vs 22 ± 5.9; P=0.05).

Conclusion: Fall, dysphagia, aspiration pneumonia, and pressure sores were common among LBD patients. Patients with LBD with AD pattern of functional imaging had an earlier age of onset and lower 1-year Mini–Mental State Examination scores.

One-year interval Montreal Cognitive Assessment and risk of peritonitis in self-care peritoneal dialysis patients

60

YF Shea, MSC Lee, MYM Mok, MF Lam, LW Chu, FHW Chan, TM Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Cognitive impairment (CI) may have a negative impact on the outcome of patients on peritoneal dialysis (PD), especially on the risk of peritonitis.

Methods: We conducted a 2-year prospective single-centre cohort study. Hong Kong Montreal Cognitive Assessment (HK-MoCA) was performed in patients newly started on PD between July 2011 and August 2014, and repeated 1 year later. Demographics and clinical data including co-morbidities, medications, and peritonitis were collected. CI was determined by locally defined HK-MoCA cut-off. Patients were classified as 'cognitively impaired' (Ci) if they remained CI or became CI at 1 year; otherwise patients were classified as 'cognitively preserved' (Cp).

Results: A total of 104 patients were included. An age older than 65 years was an independent risk factor for CI (odds ratio=3.37; 95% confidence interval [CI], 1.31-8.65; P=0.01). Ci patients showed a higher PD peritonitis rates (1 episode per 22 patient-months vs 1 episode per 73 patient-months; P=0.01) and longer median duration of admissions (11 [interquartile range, 6-25] days vs 0 [0-6] days; P<0.001) than the Cp group. Low baseline serum albumin level and classification as Ci were independent risk factors for PD-related peritonitis (hazard ratio=0.91; 95% CI, 0.84-0.99; P=0.02 and 2.13; 95% CI, 1.06-4.3; P=0.03, respectively) using cox regression model analysis. Also, Ci status was associated with shorter peritonitis-free survival (P<0.001).

Conclusion: CI increases with age, and is a significant risk factor for peritonitis and prolonged hospitalisation in self-care PD patients.

Fractional radiofrequency for improving skin texture in Chinese

61

SYN Shek[1], CK Yeung[1], KY Kung[1], MMH Chung[1], T Kono[2], HHL Chan[1,3]
[1]Division of Dermatology, Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Plastic Surgery, Tokai University School of Medicine, Japan
[3]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, United States

Introduction: Fractional radiofrequency devices were shown to improve skin texture such as smoothness, rhytides, brightness as well as atrophic acne scars by increasing dermal thickness, dermal collagen content, and dermal fibrillin content. Majority of clinical experiences with fractional skin rejuvenation were focused on Caucasian subjects. The objective of the study was to assess the efficacy and adverse effects of this device on Asian skin.

Methods: A total of 20 Chinese subjects aged 21 to 60 years with irregularities of skin texture, rhytides, and acne scars were recruited. Subjects received six treatments at 2-4–week intervals. Treatment is initiated with maximum energy tolerated and was adjustable during treatment if subject felt excessive discomfort. A total of two passes were delivered at each session. Physician assessment and standardised photographs were taken at baseline, all treatment visits, and 1-, 2-, and 6-month long-term follow-up.

Results: The study is still ongoing with 18 subjects recruited. One subject withdrew after the first treatment due to overreaction to local anaesthesia. 10 out of 17 subjects completed treatment phase and entered the follow-up phase. Assessment was made before each treatment and on follow-up visits. At 1-month follow-up, 70% of the patients were satisfied and 30% were very satisfied while treatment physician reported various degrees of improvement based on the global assessment scale in 80% of the subjects. Anticipated side-effects including erythema, oedema, pinpoint bleeding, and acne flare up were recorded but there were no serious adverse effects.

Conclusion: Fractional radiofrequency improves skin texture in Chinese. No serious adverse effect has been recorded.

Acknowledgement
This study was supported by Venus Concept.

Safety and efficacy of the 755-nm picosecond laser with diffractive lens for the treatment of facial melasma in Chinese

62

SYN Shek[1], CK Yeung[1], KY Kung[1], MMH Chung[1], T Kono[2], HHL Chan[1,3]
[1]Division of Dermatology, Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Plastic Surgery, Tokai University School of Medicine, Japan
[3]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, United States

Introduction: Due to the recurrent and refractory nature of melasma, management remains a challenge. The use of various lasers to treat melisma including Q-switched Nd:YAG, Q-switched alexandrite, pulsed dye laser, and various fractional lasers have all been explored. The objective of this study was to investigate the efficacy of a 755-nm picosecond laser with diffractive lens for the treatment of facial melasma in Chinese.

Methods: Overall, 13 Chinese subjects with facial melasma were recruited. A total of 6 sessions at 4-week intervals were delivered. Treatment parameters were 0.71 J/cm^2, 6 mm, 10 Hz, total 4 passes, and clobetasone 0.05% cream BD for 3 days post-treatment. Standardised photos with Canfield® System were taken at baseline, prior to each treatment session, and at 1-, 2-, and 3-month follow-up. Two independent physicians evaluated the photos and the operator rated the global aesthetic improvement post-third treatment onwards. Subjective evaluation and pain score were collected.

Results: A total of 49 sessions were performed and the mean pain score was 4.4/10. At 1-month post third treatment, 57% had improvement in pigment reported by the operator by means of the global aesthetic improvement. The Melasma area severity index score will be evaluated by two independent physicians.

Conclusion: Some improvement is observed in the treatment of melasma, by the 755-nm picosecond laser with diffractive lens in Chinese.

Acknowledgement
This study was supported by Cynosure Inc.

Hip fracture incidence and mortality in 2005-2013 in Hong Kong: a population-based study

63

CW Sing[1], DKC Ho[2], BMY Cheung[2], CL Cheung[1,2]
Departments of [1]Pharmacology and Pharmacy and [2]Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Background: Hip fractures result in huge burdens on health care system as well as poor quality of life in patients. A recent decline in the incidence of hip fracture has been reported worldwide. This study examined the trend of hip fracture incidence and mortality in Hong Kong.

Methods: Data were obtained from the electronic databases in the Hospital Authority of Hong Kong. Patients aged over 50 years and admitted with a new diagnosis of hip fracture (International Classification of Diseases, 9th edition, 820.XX) from 2005 to 2013 were included. The crude and age-standardised incidence rates, and all-cause mortality after 1 year of hip fracture were calculated. Variations across gender, age, and treatment of hip fracture were evaluated.

Results: A total of 48 719 incident hip fractures were identified, with an increase of 10.3% (men 20.1%, women 6.0%), from 2005 to 2013. The crude incidence rate (per 100 000 populations) decreased from 256.6 in 2005 to 207.2 in 2013. The age-standardised incidence rate dropped from 258.6 (95% confidence interval [CI], 251.5-265.9) to 201.9 (95% CI, 196.6-207.4). A significant decreasing trend was observed in both men (P=0.001) and women (P<0.001). The overall 1-year all-cause mortality was 18.9%. The mortality in men (26.9%) was significantly higher (P<0.001) than women (15.2%). After hip fracture, 8.6% of the patients were treated with anti-osteoporosis drugs and the mortality decreased with an odds ratio of 0.43 (95% CI, 0.37-0.51), adjusted for age and sex, compared to patients without the treatment. The trend of mortality remained steady throughout the study period in all groups.

Conclusion: The incidence of hip fracture has declined in Hong Kong. However, with a growing number of old people, the absolute number of cases is expected to increase. More efforts in health care policy and clinical management to prevent hip fracture are needed.

Serum beta-2 microglobulin level in patients with acute coronary syndrome

64

FCC Tam, CL Cheung, EYT Chan, SH Tsui, SW Lee, BMY Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Beta-2 microglobulin (B2M) is a marker of renal dysfunction and immune activation. In our previous study, serum B2M was found to be related to age, renal function and cardiovascular risk factors, and was strongly related to cardiovascular mortality and all-cause mortality. Acute coronary syndrome (ACS) is a common cause of hospital admission and is associated with increased risk of myocardial infarction and mortality. We tested in a case-control study the hypothesis that the serum level of B2M is elevated in ACS.

Methods: We recruited 167 Chinese patients (118 men and 49 women; mean age ± standard deviation, 64 ± 13 years), of whom 88 had ACS and 79 were controls matched for age and sex. Serum B2M was measured in an accredited laboratory using a latex-enhanced immunoassay (intra-assay coefficient of variation 3.4%).

Results: The mean serum B2M level was 2.27 ± 0.12 μg/mL. The upper reference level of serum B2M in our laboratory was 1.42 μg/mL. Thus, only 24 (14%) subjects had a serum B2M within the reference range. Serum B2M levels (mean ± SE) were 2.52 ± 0.21 in ACS patients and 1.99 ± 0.06 μg/mL in controls (P=0.02). There was no correlation between serum B2M and troponin level, suggesting no association between serum B2M level and the extent of acute myocardial damage. Serum B2M correlated with age (Spearman's ɑ=0.52, P<0.001), serum creatinine (ɑ=0.62, P<0.001), and male gender (ɑ=0.19, P=0.01).

Conclusion: Serum B2M level is increased in ACS patients although not related to the troponin level. In contrast, it is related to age, creatinine, and male gender. Thus, serum B2M seems to be a cardiovascular risk marker in Chinese as it is in westerners. Long-term study of outcomes is warranted to investigate if the serum B2M level in ACS patients predicts prognosis and mortality.

Acknowledgement
This study was supported by a Seed Funding for Basic Research Grant from the University of Hong Kong.

Early manometry and pH studies may enable accurate diagnosis and broaden treatment options in subjects with upper gastrointestinal symptoms: a case series

65

VPY Tan[1], HS Chu[1], D Tong[2], SW Law[2]
Departments of [1]Medicine and [2]Surgery, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: In subjects who present with upper gastrointestinal (GI) symptoms including heartburn, acid regurgitation, and epigastric pain/discomfort, diagnostic options complementing clinical history include empiric drug treatment (such as a proton pump inhibitor trial) and/or upper endoscopy. Diagnosis utilising high-resolution manometry (HRM) and pH studies appear late in the management algorithm. This case series aimed to study the impact of the introduction of HRM and pH studies on the diagnosis and management of subjects with gastro-esophageal reflux disease (GERD) clinic in Hong Kong SAR, China.

Methods: A manometry and pH/impedance database was established when the new HRM/pH/Impedance system was introduced in 2013. All manometry and pH data between 2013 and 2015 were analysed. Demographic data, initial diagnosis, final diagnosis, delay to diagnosis, and initial versus final treatment were recorded.

Results: A total of 111 manometry and pH studies subjects were recruited; 34 (30.6%) were male and 111 (100%) had an upper endoscopy. Of the subjects, 70 (63.1%), 26 (23.4%), 12 (10.8%) , 1 (0.9%), and 2 (1.8%) had an initial diagnosis of GERD, functional dyspepsia, dysphagia, non–cardiac chest pain, and globus sensation, respectively. A total of 69 (62.2%) subjects had a revision of their initial diagnosis. Of the subjects originally diagnosed as GERD, 32 (45.7%) confirmed to be GERD; and 23 (32.9%) were confirmed to be a motility disorder, 6 (8.6%) were diagnosed with functional dyspepsia, 8 (11.4%) were diagnosed with functional heartburn, and 1 (1.4%) was diagnosed with eosinophilic esophagitis. Finally in subjects with a final diagnosis of GERD, 18 (42.8%) had their treatment unaltered or only minor alternations. A total of 24 (57.2%) had significant changes to their GERD treatment including offer of fundoplication surgery. Of the 46 (41.8%) subjects who were diagnosed with a motility disorder, 6 (13%) had no or only minor changes to their treatment regimen, 40 (86.9%) of subjects had significant alterations to their management regimen inclusive of options of endoscopy with balloon dilatation and/or surgical myotomies.

Conclusion: Clinical and endoscopic-based diagnosis of upper GI conditions is inaccurate. Over 60% of subjects had a revision to their diagnosis after HRM and pH studies. This case series suggest that early manometry and pH studies may enable accurate diagnosis and broaden treatment options in subjects with upper GI symptoms.

Aetiology, clinical presentation, and outcome of encephalitis in Hong Kong

66

KC Teo, CY Lee, SK Chang, SL Ho, KH Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Autoimmune encephalitis is now recognised as an important cause of encephalitis. On the other hand, encephalitis of unknown aetiology remained a diagnostic and management challenge. We aimed to study the clinical presentation and outcome of encephalitis of different aetiologies.

Methods: Immunocompetent patients aged 18 years or above with encephalitis, who were admitted from January 2010 to September 2016, were recruited in this single-centre cohort study. Systemic laboratory testing was utilised to identify the aetiology of the encephalitis. The 6-month outcome was assessed using the Modified Rankin Scale (mRS). Good outcome was defined as mRS 0-2 and poor outcome was defined as mRS 3-6.

Results: We identified 26 patients with encephalitis. Herpes simplex encephalitis was the most common identified aetiology (n=6; 23%), followed by anti-NMDA receptor encephalitis (n=3; 12%). There were six patients with encephalitis of unknown aetiology. Patients with autoimmune encephalitis had a lower frequency of fever at presentation (25.0% vs 88.9%; P=0.001) and cerebrospinal fluid protein level elevation (25.0% vs 70.6%; P=0.032), and a longer duration of symptoms before presentation (median 19.5 days, interquartile range 8.5-30 vs 1 day, interquartile range 1-3; P=0.030). The aetiology of the encephalitis was the major determination factor for neurological outcome. Autoimmune encephalitis was associated with good neurological outcome (hazard ratio=8.8; P=0.064). Encephalitis of unknown aetiology was associated with poor neurological outcome (hazard ratio=11.7; P=0.040) with the 6-month mortality rate of 50%.

Conclusion: Autoimmune encephalitis has a distinct clinical characteristic and was associated with a good neurological outcome. Further research is required for the management of encephalitis of unknown aetiology, which has a much guarded prognosis.

Treatment of calcium channel blocker overdose: a case series

67

MF Tsoi, TT Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Calcium channel blocker (CCB) is a class of anti-hypertensive medication. However, CCB overdose may cause hypotension, shock, and death. There has been limited evidence on efficacy of high-dose insulin (HDI) as a supportive therapy for CCB-overdose patients. Therefore, we characterised and studied the survival of CCB-overdose patients treated with HDI.

Methods: The Poison Information and Clinical Management System was used to identify patients with CCB overdose. For inclusion, patients must be documented with CCB overdose and presence of hypotension despite the use of intravenous calcium salt infusion. Patients were stratified into two groups: with or without HDI treatment. The outcome of this study was the all-cause mortality. Results were analysed by SPSS version 23.0.

Results: A total of 17 patients were included in this study. Of them, 15 (88.2%) patients were diagnosed with hypertension. Four (23.5%) and five (29.4%) patients had diagnosis of psychiatric disorder and diabetes mellitus, respectively. Six (35.3%) patients had overdose of other anti-hypertensive medication. The mortality rates of patients were 38.5% and 0% in patients with or without HDI treatment, respectively (P=0.152). There was no significant difference in time-to-event relationship in mortality (P=0.594).

Conclusion: Overdose of CCB can be fatal; HDI did not improve survival in patients with CCB overdose.

Network meta-analysis on efficacy of biologic disease-modifying anti-rheumatic drugs (bDMARDs) in bDMARDs-naïve psoriatic arthritis Patients

68

MF Tsoi, Y Fei, TT Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Biologic disease–modifying anti-rheumatic drugs (bDMARDs) and target-specific DMARDs (tsRMARDs) have demonstrated their better efficacy over placebo for treating patients with psoriatic arthritis (PsA). However, few trials directly compared different bDMARDs and tsDMARDs. Therefore, we performed a network meta-analysis to obtain indirect comparisons between different bDMARDs and tsDMARDs in bDMARDs-naïve PsA patients.

Methods: The literature was searched using ISI Web of Science, Scopus, Medline, Cochrane library, Clinicaltrials.gov, and EMBase. For inclusion, randomised controlled trials must report the proportion of bDMARDs-naïve PsA patients achieving ACR20 response as a study outcome. This outcome was analysed with reference to placebo and etanercept using R statistics version 3.3.1 with netmeta version 0.9.1.

Results: A total of 17 studies with altogether 4607 patients were included in this network meta-analysis. Compared to placebo, all bDMARDs and tsDMARDs were superior to placebo. With reference to etanercept, apremilast and ustekinumab were associated with fewer patients achieving ACR20 response (odds ratio [95% confidence interval]: 20 mg apremilast: 0.18 [0.07-0.48]; 30 mg apremilast: 0.24 [0.09-0.62]; 45 mg ustekinumab: 0.26 [0.09-0.73]; 90 mg ustekinumab: 0.32 [0.11-0.90]). Secukinumab, ixekizumab, adalimumab, certolizumab, golimumab, and infliximab did not differ from etanercept in ACR20 response (odds ratio [95% confidence interval]: 150 mg secukinumab: 0.64 [0.22-1.86]; 300 mg secukinumab: 0.77 [0.22-2.74]; ixekizumab Q2w: 0.43 [0.14-1.38]; ixekizumab Q4w: 0.38 [0.12-1.20]; adalimumab: 0.40 [0.15-1.06]; certolizumab 0.42 [0.13-1.36]; golimumab: 0.93 [0.26-3.32]; infliximab: 0.10 [0.04-0.22]).

Conclusion: Compared to etanercept, there was no significant difference in efficacy of secukinumab, ixekizumab, adalimumab, certolizumab, golimumab, and infliximab. Etanercept has a better efficacy than apremilast and ustekinumab.

Low-intensity intermittent hypoxia promotes subcutaneous adipogenic differentiation

69

Y Wang[1], JCW Mak[1,2], MYK Lee[1], AM Xu[1,3], MSM Ip[1,2]
[1]Department of Medicine; [2]Research Centre of Heart, Brain, Hormone and Healthy Aging; [3]State Key Laboratory of Pharmaceutical Biotechnology; The University of Hong Kong, Hong Kong

Introduction: Obstructive sleep apnoea (OSA), characterised by intermittent hypoxia (IH), is highly associated with obesity. Depot-specific adipogenic differentiation, an important physiological mechanism in maintaining adipose tissue homeostasis, could be regulated by intracellular transcriptional factors, extracellular signalling pathways, and inflammation. However, the impact of IH on adipogenesis is unclear. This study aimed to investigate the role of IH in adipogenic differentiation in vivo and in vitro.

Methods: The Sprague Dawley rats were subjected to intermittent normoxia (IN) or IH (each cycle: 240 seconds for 10% O_2 and 120 seconds for 21% O_2) for 6 weeks. Human subcutaneous preadipocytes (HPAs) underwent six differentiation cycles into mature adipocytes. Each differentiation cycle consisted of two sequential procedures of differentiation (for 3 days) and maintenance (for 2 days). During each 3-day differentiation, HPAs were subjected to IH (IH; 1% for 10 min and 21% O_2 for 5 min per cycle; 5% CO_2) or IN treatment. The degree of adipogenic differentiation was evaluated by the measurements of adipogenic transcriptional factors (CEBPα, PPARγ CEBPδ, and CHOP) and adipocyte-specific proteins (FABP4 and GLUT4) using Q-PCR and/or Western blots. The production of oily droplets in HPAs was detected by Oil Red O staining. The analysis of insulin-like growth factor 1 receptor (IGF-1R)/Akt pathway in HPAs was achieved by incubation of IGF-1R selective kinase inhibitor NVP-AEW541 (0.1 μmol/L) using Western blots. ELISA was applied to detect adiponectin in tissue-conditioned media and pro-inflammatory markers (interleukin [IL]-6 and monocyte chemoattractant protein [MCP]-1) in HPAs-conditioned media.

Results: The up-regulation of pro-adipogenic markers (CEBPα, PPARγ, FABP4) and down-regulation of anti-adipogenic markers CHOP were found in IH-exposed subcutaneous adipose tissue (SAT) but not visceral adipose tissue (VAT). In addition, IH exposure facilitated the release of adiponectin in SAT- but not VAT-conditioned media. In line with in-vivo results, IH accelerated the accumulation of oil droplets in HPAs. During differentiation, IH caused elevation of adipogenesis-associated markers (FABP4, GLUT4, CEBPα, and PPARγ) compared to cells exposed to IN. Moreover, the reduction in expression of CEBPαwas prevented by IH exposure with adipogenic development of HPAs. The pro-adipogenic role of IGF-1R/Akt activation in IH-exposed HPAs was significantly attenuated by IGF-1R kinase inhibitor NVP-AEW541. In addition, IH also induced elevated levels of IL-6 and MCP-1 in the conditioned media during the process of HPAs differentiation.

Conclusion: Low-frequency IH exposure could promote the adipogenic differentiation of SAT and subcutaneous preadipocytes (HPAs) via regulating transcriptional factors, IGF-1/Akt signalling pathway and inflammation.

Acknowledgement
This study was supported by HKU Stanley Ho Matching Grant.

Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA

70

DKH Wong, WK Seto, FY Huang, J Fung, CL Lai, MF Yuen
Department of Medicine and State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong

Introduction: Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) is responsible for viral persistence in chronic hepatitis B patients. We aimed to explore whether serum hepatitis B core-related antigen (HBcrAg), a novel HBV marker, could be a surrogate marker for cccDNA.

Methods: We analysed 305 liver biopsies and the corresponding sera collected from 138 nucleos(t)ide analogues-treated patients. Of the patients, 124 had paired liver biopsies at baseline and 1-year post-treatment, and 43 had a third biopsy after 6 to 12 years of treatment. Serum HBcrAg, serum HBV DNA, and intrahepatic total HBV DNA and cccDNA were measured.

Results: HBcrAg strongly correlated with cccDNA (r=0.70), intrahepatic total HBV DNA (r=0.67), serum HBV DNA (r=0.69) [all P<0.0001]. Serum HBV DNA were undetectable (<20 IU/mL) in 130 samples, in which HBcrAg was detectable in 101 (78%) samples. HBcrAg still correlated positively with cccDNA in these 130 samples (r=0.42, P<0.0001). After ≥6 years of treatment, the median logarithmic reduction of HBcrAg was 2.7 log kU/mL, the magnitude of which was comparable to that of cccDNA reduction. Of the 43 patients with long-term treatment, 21 patients had undetectable cccDNA (<0.005 copies/cell) after ≥6 years of treatment, in whom 15 (71%) had detectable HBcrAg.

Conclusion: Serum HBcrAg is a reliable surrogate marker for intrahepatic cccDNA content even when serum HBV DNA became undetectable. Even in cases when cccDNA levels were below the detection limit of assays upon long-term treatment, HBcrAg could be a potentially marker for persistence of disease.

Acknowledgement
HBcrAg measurement was supported by Fujirebio Inc., Japan.

Serum Mac-2 binding protein glycosylation isomer levels correlated with necroinflammation and fibrosis activities in chronic hepatitis B patients

71

DKH Wong, WK Seto, J Fung, CK Chong, CL Lai, MF Yuen
Department of Medicine and State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong

Introduction: Mac-2 binding protein glycosylation isomer (M2BPGi) is a novel non-invasive marker for liver fibrosis/cirrhosis. We aimed to examine the relationship between M2BPGi and histologic activities in chronic hepatitis B (CHB) patients.

Methods: Serum M2BPGi levels, expressed as cut-off index (COI) were measured in 325 treatment-naïve CHB patients with liver biopsies. Correlations between M2BPGi levels and the degree of necroinflammation (based on Knodell histologic activity index [HAI]; range 0-18) and fibrosis (based on Ishak score; range 0-6) were investigated.

Results: M2BPGi level correlated with necroinflammation grades (P=0.003) and fibrosis stages (P<0.0001). Patients with moderate/severe necroinflammation (HAI ≥9) had a higher median M2BPGi level (0.43 COI) than patients with mild/minimal (HAI <9) necroinflammation (0.32 COI; P=0.0003). There were significant differences in M2BPGi levels between patients with different fibrosis stages (advanced fibrosis/cirrhosis [Ishak score ≥4] vs moderate fibrosis [Ishak 2-3], P<0.0001; moderate [Ishak 2-3] vs no/minimal fibrosis [Ishak 0-1], P=0.012). A cut-off of 0.455 COI predicted advanced fibrosis/cirrhosis with an area under the receiver operating characteristic curve of 0.731.

Conclusion: Serum M2BPGi levels correlated well with histologic findings. It may be a useful non-invasive marker for necroinflammation and fibrosis activities in patients with CHB infection.

Acknowledgement
M2BPGi measurement was supported by Sysmex Corp., Japan.

Is serum fibroblast growth factor 21 the better adipokine in prediction of incident diabetes?

72

YC Woo, CH Lee, CHY Fong, A Xu, AWK Tso, BMY Cheung, KSL Lam
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: High circulating levels of fibroblast growth factor 21 (FGF21) are found in type 2 diabetes, suggesting FGF21 resistance. Serum FGF21 predicts incident diabetes but its performance, compared to established predictors, is not known. We aimed to study the performance of FGF21 in diabetes prediction, relative to other adipokines and established risk factors including 2-hour plasma glucose (2hG) during the oral glucose tolerance test (OGTT).

Methods: We studied 1380 non-diabetic subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study using the second visit (2000-2004) as baseline when serum levels of FGF21 and other adipokines were measured. Glycaemic status of the participants was assessed by OGTT.

Results: A total of 123 participants developed diabetes over 9.0 years (median). On multivariable logistic regression analysis, FGF21 (P=0.003), adipocyte-fatty acid binding protein (P=0.003), and adiponectin (P=0.035) were independent predictors of incident diabetes. FGF21 had the best change in log likelihood when added to a diabetes prediction model (DP) based on age, family history, smoking, hypertension, body mass index, dyslipidemia, and FG. It also improved the area under receiver operating characteristic curve (AUROC) of DP from 0.797 to 0.819 (P=0.0072), rendering its performance comparable to the 'DP + 2hG' model (AUROC=0.838, P=0.19).

Conclusion: As a predictor for diabetes, serum FGF21 appeared to be superior to other adipokines and, on its own, could be considered as an alternative to the OGTT.

Obstructive sleep apnoea and incident diabetes mellitus in a Chinese population

73

PH Xu, CKM Hui, DYT Fong, MSM Ip
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Although clinical and epidemiological studies have reported an association between obstructive sleep apnoea (OSA) and diabetes mellitus (DM), the results remain inconsistent. We hence propose to evaluate the role of OSA on the incidence of DM in a Chinese cohort.

Methods: The eligible cohort was drawn from those who received sleep studies between 1 January 2007 and 31 December 2010 in the Department of Medicine, Queen Mary Hospital. Only those without DM at the time of polysomnogram were included. Follow-up data were drawn from the Hospital Authority Clinical Management System up to the day of data entry in this study. Apnoea-hypopnoea index (AHI) from automated scoring of polysomnogram was used in the Cox regression model, controlling for age, gender, body mass index (BMI), daytime sleepiness, and comorbidities.

Results: Overall, 101 (14.5%) of 698 subjects experienced DM over a median follow-up of 82 months, giving an incident rate of 2.2 per 100 person-years. In fully adjusted models, AHI was significantly and independently associated with incident DM (hazard ratio=1.006; 95% confidence interval, 1.001-1.011), as were age and BMI. This analysis has not yet taken into account the influence of OSA treatment and manually scored sleep data.

Conclusion: AHI, as a measure of OSA severity, predicted incident diabetes, independent of obesity.

Obstructive sleep apnoea and cardiovascular events in a Chinese population

74

PH Xu, CKM Hui, DYT Fong, MSM Ip
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Although several clinical and epidemiological studies have reported an association between obstructive sleep apnoea (OSA) and cardiovascular (CV) events, the results and magnitude remain unclear and inconsistent. We hence propose to evaluate the role of OSA on the incidence of composite CV events in a Chinese cohort.

Methods: The eligible cohort was drawn from those who received sleep studies between 1 January 2007 and 31 December 2010 in the Department of Medicine, Queen Mary Hospital. Follow-up data were drawn from the Hospital Authority Clinical Management System up to the day of data entry in this study. Apnoea-hypopnoea index (AHI) from automated scoring of polysomnogram was used in the Cox regression model, controlling for age, gender, waist circumference, daytime sleepiness, and comorbidities.

Results: Overall, 158 (14.4%) of 1099 subjects experienced composite CV outcomes over a median follow-up of 85 months, giving an incident rate around 2.1 per 100 person-years. In both univariate and multivariate models, there was no significant association between AHI and CV events. While waist circumference was an independent and significant predictor, which indicated that obesity is always in the close partnership between OSA and CV events. This analysis has not yet taken into account the influence of OSA treatment and manually scored sleep data.

Conclusion: Beyond the obesity, OSA per se would not aggravate the CV outcomes. Other OSA-related variables should be considered in further studies.

75

In-vitro effects of pegylated arginase in small-cell lung cancer

S Xu, SK Lam, JC Ho
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Small-cell lung cancer (SCLC) accounts for 15% of all lung cancer cases. It is notoriously difficult to treat with high relapse rate and the current standard treatment remains chemotherapy. Arginine is an important amino acid in normal human cells that can be replenished through urea cycle, but some tumours are arginine-auxotrophic due to deficient argininosuccinate synthetase (ASS1) and/or ornithine transcarbamylase (OTC). BCT-100 is a pegylated arginase, which converts arginine to ornithine, has demonstrated anticancer activity in human many cancers. We aimed to determine the in-vitro effects of BCT-100 in SCLC.

Methods: A panel of seven SCLC cell lines was obtained from ATCC. Cell viability and protein expression were detected by MTT assay and Western blot, respectively. Knockdown of OTC was performed using siRNA. Mitochondrial membrane depolarisation and cell cycle arrest were analysed by flow cytometry.

Results: Over-expression of ASS1 in H69 and DMS79 cells, and OTC in H841 cells were associated with resistance to BCT-100. Knocking down of OTC increased sensitivity of BCT-100 in H841 cells partially via apoptosis. H526 cells (BCT-100-sensitive) was selected for mechanistic study. Mitochondrial membrane depolarisation was observed in BCT-100 treatment accompanied by cytochrome c and SMAC release from mitochondria to cytosol. Hydrogen peroxide and superoxide were upregulated in BCT-100 treatment and N-acetylcysteine (reactive oxygen species scavenger) could significantly reversed apoptosis induced by BCT-100. Besides, cyclin A2, cyclin B1 and CDK7 were downregulated by BCT-100 in a time-dependent manner. G1/S arrest was found in BCT-100 treatment by flow cytometry. The expression of p-AKT and p-mTOR was increased after exposure while RAS/RAF/ERK cell signalling pathway was inhibited with BCT-100 treatment.

Conclusion: SCLC cell lines with low ASS1 and OTC expression were sensitive to BCT-100 which was partially mediated through oxidative stress, cell cycle arrest, and apoptotic pathway.

76

Identifying the earliest sign of pathological cognitive decline by using magnetic resonance imaging structural brain connectivity analysis

X Xu, ES Hui, HKF Mak, J Kwan
The University of Hong Kong, Hong Kong

Introduction: With the increasing understanding of subjective cognitive decline (SCD) as a possible precursor of mild cognitive impairment (MCI) and Alzheimer's disease (AD), the early identification of those with truly pathological SCD, and distinguish them from normal aging, is of great clinical importance. We investigated this prodromal stage by studying the structural brain connectivity of subjective cognitive impairment (SCI) patients as compared to MCI and healthy subjects.

Methods: Patients with SCI (n=29) or MCI (n=33), and healthy controls (n=6) underwent cognitive testing and 3Tesla magnetic resonance imaging (MRI). We performed diffusion tensor imaging–based tractography and brain connectivity analysis on SCI, MCI, and healthy controls. The network topology, network efficiency, and the characteristics of individual brain regions were investigated, then compared between three cohorts. The relations between network metrics and cognitive assessments were also studied.

Results: Global network measures did not differ between SCI and healthy controls. However, SCI patients showed significant regional changes in varies brain regions, including orbitofrontal gyrus, rolandic operculum, hippocampus, lingual gyrus, middle occipital gyrus, inferior parietal gyrus, precuneus, superior temporal gyrus, lingual gyrus, inferior parietal gyrus, precuneus, and superior temporal gyrus. More importantly, those regional changes were significantly associated with cognitive assessment scores including Mini-Mental State Examination and Montreal Cognitive Assessment.

Conclusion: Our results suggested that cognitive decline–related pathological changes occurred in SCI stage, and MRI structural brain network analysis may be useful for early diagnosis of AD.

The long-term efficacy and tolerability data of sirolimus treatment in lupus nephritis

77

DYH Yap, TM Chan
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: There are preliminary clinical and animal data regarding the use of sirolimus in the treatment of lupus nephritis (LN). Long-term human efficacy and tolerability data, however, are lacking.

Methods: We retrospectively reviewed the long-term efficacy and tolerability data of LN patients who received corticosteroids and sirolimus either as induction or maintenance immunosuppression during 2007-2016.

Results: A total of 16 class III/IV/V LN patients were included (duration of sirolimus treatment: 27.2 ± 19.6 months). Of them, 10 received sirolimus due to intolerance to standard therapies while six due to a history of neoplasm. Five patients received sirolimus during active nephritis, which resulted in significant improvement in proteinuria (2.8 ± 1.9 g/day and 0.1 ± 0.1 g/day at baseline and 36 months; P=0.011 compared to baseline), anti-dsDNA (107.7 ± 91.9 IU/mL and 37.0 ± 55.4 IU/mL at baseline and 36 months; P=0.145) and C3 (54.8 ± 26.1 mg/dL and 86.3 ± 18.6 mg/dL at baseline and 36 months; P=0.084). Overall, 11 patients received sirolimus during maintenance phase, and demonstrated significant improvement in C3 (90.4 ± 18.1 mg/dL and 117.7 ± 25.1 mg/dL at baseline and 36 months; P=0.025), stable glomerular filtration rates (58.5 ± 25.2 mL/min and 56.7 ± 29.0 mL/min at baseline and 36 months; P=0.199), and urine protein excretion (0.8 ± 0.7 g/day and 0.7 ± 0.7 g/day at baseline and 36 months; P=0.263). Renal relapse occurred in one patient after 36 months. Sirolimus was discontinued in five patients including one with leukopenia. Four patients had worsening of lipid profile but were adequately controlled with statins.

Conclusion: Sirolimus showed favourable long-term efficacy and acceptable side-effects profiles, and thus can serve as an alternative therapeutic option for LN.

The effects of nucleoside/tide analogue treatments on renal tubular epithelial cells

78

DYH Yap, P Lee, TMY Lee
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Clinical observations showed that nucleoside/tide analogues (NA) have differential effects on renal function. While adefovir (ADV) and tenofovir (TDF) have showed clinical nephrotoxicity, lamivudine (LAM) and entecavir (ETV) have neutral effects on the kidneys. Preliminary data also suggest telbivudine (TBV) treatment might be associated with improvement in GFR. The mechanisms of these clinical observations remain unclear.

Methods: We investigate the effects of ADV (1.2 μM), TDF (21.3 μM), TBV (0.2 μM), LAM (37 μM), and ETV (3.75 nM) treatment on a renal tubular epithelial cell line (HK-2 cells). The cell viability of HK-2 cells was assessed by MTT assay and the mRNA of different apoptotic (GRP-78, ATF-4, caspase-9, and caspase-12) and pro-/anti-inflammatory markers (interleukin [IL]-6 and IL-10) were measured by qPCR.

Results: ADV- and TDF-treated cells showed the highest percentage of cell death after 72 hours of treatment when compared to LAM, ETV, and TBV (46%, 14%, 2%, <1%, and 2%, respectively). ADV-treated cells demonstrated an increase in GRP-78, ATF4, and caspase-9 expression. TDF-treated cells were associated with an elevation of caspase-9 and caspase-12 expression but no effects on GRP-78 and ATF-4. TBV-treated cells revealed a decrease in caspase-9 and caspase-12 expression and also increased IL-10 expression. LAM- and ETV-treated cells showed no significant effects in the expression of apoptotic and pro-/anti-inflammatory markers in HK-2 cells.

Conclusion: These in-vitro findings correlate with clinical observations of the different NAs on kidney function.

Validating diabetes prediction risk scores in Hong Kong Chinese patients

79

MY Yeung, YC Woo, CH Lee, CHY Fong, AWK Tso, BMY Cheung, KSL Lam
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: A number of diabetes risk scores, based on long-term prospective data in other populations, have been developed for the prediction of incident diabetes. Here we evaluated three existing risk scores based on follow-up durations comparable to that of the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS), to assess whether they could be usefully employed for diabetes risk prediction in Hong Kong Chinese. These risk scores were developed in the United States (San Antonio Heart Study; SAHS), Thailand (Diabetes Risk Score, DRS), and Finland (Finnish Diabetes Risk Score, FINDRISC).

Methods: Data from 1410 Hong Kong Chinese patients (aged 30-81 years), followed up for a median of 9.1 years (CRISPS 2-4), without a history of diabetes at baseline, were used to evaluate the risk scores. Glycaemic status was assessed by the oral glucose tolerance test (OGTT) or the use of anti-diabetes medications. Area under the receiver operating characteristics curve (AUROC) was used to evaluate the prediction performance of the three risk scores, baseline fasting glucose (FG), or 2-hour glucose during OGTT (2-hG). Optimal cut-off was determined in the model with the best AUROC using Youden J criterion.

Results: A total of 124 participants developed diabetes. The AUROC of FINDRISC, SAHS, and DRS were 0.783, 0.731, and 0.715, respectively, with FINDRISC being the best prediction model of diabetes ($P<0.05$ vs SAHS or DRS). It also showed an enhanced prediction compared to FG (AUROC=0.723; P=0.021) and was comparable to 2-hG (AUROC=0.790; P=0.70). An optimal cut-off at 7 out of 17 in FINDRISC demonstrated sensitivity of 75.8%, specificity of 68.8%, positive predictive value of 19.0%, and negative predictive value (NPV) of 96.7% for diabetes prediction in the CRISPS cohort.

Conclusion: FINDRISC was demonstrated to be the best model for diabetes prediction in the CRISPS cohort with satisfactory sensitivity and specificity as well as high NPV. It was superior to FG and comparable to 2-hG in terms of prediction performance and is therefore an attractive tool for assessing diabetes risk in our population in view of its low cost, simplicity, and non-invasiveness.

Dendritic cells display aberrant Toll-like receptor responses in systemic lupus erythematosus

80

ALY Yim, S Yan, CCP Lee, AWK Chan, CS Lau, VSF Chan
Department of Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease that causes multi-organ damages. Plasmacytoid dendritic cells (pDCs) are potent in producing type I interferon (IFN) and myeloid dendritic cells (mDCs) are professional in antigen presentation. Clinically, the level of serum IFN-alpha (IFNα) is correlated with disease severity. Moreover, mDCs from patients also display activated phenotypes. These observations suggest that pDCs and mDCs may mediate the pathogenesis of SLE.

Methods: The properties of pDCs and mDCs from the murine lupus model New Zealand Black/White F1 (BWF1) were compared before and after disease onset using flow cytometry, ELISA, and qPCR.

Results: The abundance of splenic pDCs in symptomatic and pre-symptomatic mice was comparable. The induction of CD40, CD80, and MHC II on pDCs upon Toll-like receptor (TLR) 7 or TLR9 stimulation and the level of IFN-alpha produced by pDCs upon TLR9 stimulation were also similar in symptomatic and pre-symptomatic mice. In contrast, splenic mDCs were expanded in symptomatic mice. These mDCs expressed lower levels of CD80 and MHC II but their ability in inducing allogenic T cell proliferation was not hampered when compared with mDCs from pre-symptomatic mice. On the other hand, TLR7 and TLR9 expressions in mDCs were higher than mDCs from age- and sex-matched parental NZW controls. Upon TLR7 or TLR9 stimulation, the amount of IL-10 and CXCL13 produced by mDCs from symptomatic mice was also higher than its pre-symptomatic counterparts.

Conclusion: Myeloid dendritic cells displayed heightened TLR7 and TLR9 responses after disease onset. More work should be done to further elucidate the role of mDCs in mediating SLE pathogenesis.

Acknowledgement
This work was supported by the Hong Kong Research Grant Council General Research Fund (770213).

81

Spleen tyrosine kinase inhibitor ameliorates tubular inflammation in immunoglobulin A nephropathy

WH Yiu, DWL Wong, HJ Wu, KW Chan, Y Liu, LYY Chan, JCK Leung, KN Lai, SCW Tang
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: It has been recently demonstrated that inhibition of spleen tyrosine kinase (Syk) has renoprotective effects in antibody mediated glomerulonephritis and the protein plays a pathogenic role in mediating inflammatory responses in human mesangial cells in immunoglobulin A (IgA) nephropathy (IgAN). However, the therapeutic potential of Syk inhibition in tubulointerstitial damage in IgAN remains unknown.

Methods: Proximal tubular epithelial cells were stimulated with conditioned medium prepared from human mesangial cells incubated with IgA (IgA-HMC) from patients with IgAN or healthy control subjects. The effect of Syk inhibitor R406 on the cytokine expression was detected by real-time qPCR and ELISA, and signal transduction in activated cells were determined by Western blotting. Expression of phosphorylated Syk protein was also examined on renal biopsies from patients with IgAN and normal control by IHC.

Results: Expression of IL-6, IL-8, and ICAM-1 were upregulated after incubating with IgA-HMC conditioned medium from IgAN patients. Pre-treatment with R406 significantly suppressed IgA-HMC conditioned medium-induced cytokine production and also attenuated the phosphorylation of NFκB p65 subunit in the activated PTECs. Phosphorylated level of Syk was increased in renal tubules of patients with IgAN compared to that of the healthy controls.

Conclusion: Syk mediates inflammatory responses in tubular cells, suggesting a role for this kinase in tubulointersitial damage of IgAN. Our data supported a therapeutic potential of Syk inhibitor in treating IgAN.

Acknowledgement
Hong Kong Society of Nephrology and Hong Kong Kidney Foundation Research Grant (2015).

82

Arsenic trioxide targets B-cell lymphoma 6 (BCL6) oncoprotein for degradation to induce growth inhibition and apoptosis in BCL6-dependent diffuse large B-cell lymphoma cells

LM Yue, DHW Chau, WY Piao, E Tse, YL Kwong
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: B-cell lymphoma 6 (BCL6), a hallmark oncoprotein in diffuse large B-cell lymphoma (DLBCL), represses various genes, such as *ATR*, *TP53* and *CDKN1A*, which are involved in DNA repair and cell proliferation. Arsenic trioxide (As_2O_3) is a Food and Drug Administration–approved drug that has been used in treating various malignancies, particular in acute promyelocytic leukaemia. Various publications have been documented that As_2O_3 targets oncogenic protein degradation, such as PML-RARA, NPMc+ and NPM-ALK, thereby inducing apoptotic cell death, in leukaemia and lymphoma. The current study focuses on elucidating whether BCL6 is a target for As_2O_3 in DLBCL and the subsequent effects in vitro.

Methods: Arsenic-induced cytotoxicity and dysregulated BCL6 expression were determined in DLBCL cell lines. The effect of As_2O_3 on DLBCL cell proliferation and apoptosis were analysed by cell proliferation assay and Annexin V-7AAD straining. The mechanistic degradation of BCL6 mediated by As_2O_3 was further explored in DLBCL cells.

Results: As_2O_3 was found to downregulate BCL6 expression and increase mRNA expression of BCL6 downstream targets in DLBCL cell lines, including *PRDM1*, *CD44*, and *CD69* and leading to lymphoma cell differentiation. As_2O_3 induced growth inhibition and apoptotic cell death, analogous to the effect of BCL6 inhibitor on the DLBCL cells. Proteasome inhibitor treatment rescued the As_2O_3-induced BCL6 degradation indicated that induce BCL6 degradation through ubiquitin-proteasome pathway.

Conclusions: As_2O_3 is a potent therapeutic agent for treating DLBCL.

Combination of bupropion XR / naltrexone for weight reduction: first experience in Hong Kong

83

MMA Yuen, CHY Fong, OY Leung, WS Chow, KSL Lam
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Obesity is a growing problem globally and Hong Kong is no exception. With the withdrawal of sibutramine from the market in 2010, individuals suffering from obesity in Hong Kong are left with very few treatment options, consisting of orlistat, phentermine, or bariatric surgery. Orlistat does not target food addiction and hyperphagia often observed in obesity and is laden with occasionally very socially disturbing gastrointestinal side-effects. Phentermine is only approved for short-term treatment and is far from a long-term solution. Bariatric surgery is invasive and irreversible and is not readily acceptable to some patients. Contrave® is a combination drug containing bupropion XR and naltrexone that is approved by both the Food and Drug Administration in the United States and the European Medicines Agency. Our centre has started using this drug since January 2016. This retrospective study shares our experience with this drug.

Methods: Data at baseline, 3, 6 and 12 months from subjects prescribed with Contrave® were analysed. The primary endpoint was percentage in weight reduction. The secondary endpoints were changes in percentage body fat (%BF), glycaemic status, blood pressure (BP), and tolerability.

Results: A total of 23 patients (12 men, 11 women; mean age 42.6 ± 9.0 years, range 30-60 years; mean body mass index 37.5 ± 3.94 kg/m^2) who were prescribed with self-financed Contrave® were included in this analysis. The drug was stopped at the 3-month time point in six patients due to a lack of efficacy (ie weight reduction of ≤5%) and in five patients due to poor tolerance to side-effects. One patient defaulted follow-up. Three-month data were available for 20 patients, 6-month data were available for 7 patients, and 9-month data were available for 3 patients. The median weight reduction from baseline at 3, 6, and 12 months were 5.1 kg (interquartile range [IQR], 2.3-7.7), 7.8 (6.2-13.0), and 8.2, respectively. Patients who achieved >5% weight reduction from treatment with Contrave® all had improvement in their %BF, glycaemic status and BP.

Conclusion: Contrave® offers a pharmacotherapeutic option for patients with obesity. Longer follow-up and further analysis of our data would help to identify patient characteristics that can help to predict response and tolerance.

Mouse beta-defensin 4 limits A(H1N1)09 influenza virus replication in respiratory tract of senescent mice

84

SH Zhu, A Zhang, KY Yuen, IF Hung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: Mouse beta-Defesins (mBDs) are small molecules of cationic peptides with broad spectrum of anti-microbial properties and immune modulatory functions, which plays important roles against respiratory viral infections. The expression and function of mBDs in response to influenza infection has not been fully elaborated.

Methods: The expression of mBD4 in mouse lung epithelial cell line LA4 and C57 BL/6 mouse respiratory tissues following A(H1N1)09 pandemic influenza virus infection were studied by quantitative RT-PCR, and also by immunofluorescence or immunohistochemistry staining. Virus replication and virus induced cytokines (tumour necrosis factor–alpha [TNF-α] and interleukin-6 [IL-6]) responses were studied and correlated with the induction of mBDs.

Results: Firstly, mBD2, mBD3, and mBD4 were quickly increased in LA4 cells at 6 hours after inoculation with A(H1N1)09 virus and remained upregulated until 24 hours post infection. The induction of mBD4 was positively correlated with initial virus inoculation doses, with significantly higher expression induced in the cells inoculated with multiplicity of infection (MOI) of 10, comparing to the cells infected by MOI of 1. The time course for the induction of mBD4 correlated with the upregulation of inflammatory cytokine IL-6 and TNF-α. The expression of mBD4 in mouse respiratory tissue was studied and compared between young (6-8 weeks) and aged (72 weeks) mice. The results showed mBD4 were not detectable by immunohistochemical staining of formalin fixed young mice trachea and lung tissues, but showed there was stronger expression of mBD4 in epithelial cells lining trachea and bronchioles in aged mice, which indicated a higher basal expression of mBD4 in aged mice respiratory tissues. Upon infection with A(H1N1)09 influenza virus, a quick induction of mBD4 in young mice trachea tissue were observed at 12 hours p.i. and maintained at this level until day 4 p.i.. However, despite the higher basal level, there was no further induction of mBD4 in aged mice trachea tissues. For the lung tissue, delayed induction of mBD4 was observed in aged mice following A(H1N1)09 infection, but no increase was observed in the young mice lung tissues. Accordingly we also observed a lower viral load and cytokine levels in young mice. After giving the fusion mBD4 protein (0.126mg/kg) after infection of A(H1N1)09 in aged mice, we saw a reduced viral load in respiratory tissues.

Conclusion: A(H1N1)09 influenza virus infection induces the expression of mBD4 in vitro and in vivo. Aged and young mice showed different pattern of change in mBD4 induction after infected by A(H1N1)09 influenza virus. The treatment of H1N1 infection in aged mice with fusion mBD4 could improve the outcome, which indicated mBD4 may play important roles in influenza infection.

Administration of mBD4 reduced A(H1N1)09 virus replication in obese mice respiratory tract

85

SH Zhu, AJX Zhang, ACY Lee, C Li, IF Hung, KY Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: Mouse beta-Defesin 4 (mBD4) is mainly expressed by epithelial cells and is thought to be the first line of defense. A(H1N1)09 pandemic influenza virus caused severe respiratory tract infection and led more death in obese population. The mBD4 should play important roles but its expression and function in response to influenza virus infection has not been fully elaborated.

Methods: The expression of mBD4 in mouse lung epithelial cell line LA4 and C57 BL/6 mouse respiratory tissues following influenza virus infection were studied by quantitative real-time polymerase chain reaction (PCR) and also by immunofluorescence or immunohistochemistry staining. Viral replication in mouse respiratory tissues were studied by plaque assay. Virus induced cytokines (TNF-α and IL-6) responses were studied by ELISA.

Results: Firstly, we showed that after inoculation with 10 multiplicity of infection (MOI) A(H1N1)09 virus or H7N9(AH1) virus, mBD4 was significantly induced on mRNA level at an early stage. Immunofluorescence staining showed there was higher mBD4 expression in H7N9(AH1) virus–infected LA4 cells at 24 hours post infection. Higher mBD4 induction resulted in lower viral load in LA4 cell lysates when comparing H7N9(AH1) virus–infected cells to A(H1N1)09 virus–infected cells. The expression of mBD4 in mouse respiratory tissue was studied and compared between lean and obese mice. Quantitative real-time PCR showed significantly higher basal level of mBD4 in obese mice's lung tissues but not in trachea tissues compared with lean mice. Immunohistochemical staining of formalin-fixed mice lung tissues showed there was stronger expression of mBD4 in epithelial cells lining bronchioles in obese mice which indicated a higher basal expression of mBD4 in obese mice lung tissues. Upon infection with A(H1N1)09 influenza virus, a quick induction of mBD4 in lean mice trachea tissue were observed at 12 hours p.i. while there was no induction of mBD4 in obese mice trachea tissues even at day 4 p.i.. For the lung tissues, induction of mBD4 was observed in lean mice following A(H1N1)09 infection at day 4 p.i., but a dramatic decrease in mBD4 was observed in obese mice lung tissues. Accordingly we also observed a lower viral load and cytokine levels in lean mice. After giving the recombinant mBD4 protein(6mg/kg) after infection of A(H1N1)09 in obese mice, we observed a reduced viral load in respiratory tissues.

Conclusion: Influenza virus infection could induce the expression of mBD4 in vitro and in vivo. Lean and obese mice showed different pattern of change in mBD4 induction after A(H1N1)09 influenza virus infection. The treatment of H1N1 infection in obese mice with recombinant mBD4 could reduce viral load in respiratory tract which indicated mBD4 may play important roles in this infection.

AUTHOR INDEX

Acknowledgement

The Organising Committee of the Medical Research Conference would like to extend their gratitude to the following sponsors (in alphabetical order) for their support and generous contribution.

AbbVie Limited

AstraZeneca Hong Kong Limited

Gilead Sciences Hong Kong Limited

Merck Sharp & Dohme (Asia) Ltd

Novartis Pharmaceuticals (HK) Ltd

Pfizer Corporation Hong Kong Ltd