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SUPPLEMENT 1

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

The prospect of stem cells as multi-faceted purveyors of immune modulation, repair and regeneration in multiple sclerosis

N Payne, G Sun, C McDonald, A Silvain, D Herszfeld, N Veron, R Shu, C Wei, C Siatskas, CCA Bernard
Multiple Sclerosis Research Laboratory, Australian Regenerative Medicine Institute, Monash University, Australia

A rational approach to the treatment and cure of autoimmune diseases such as multiple sclerosis (MS) requires incorporation of three fundamental processes: suppression of the inflammatory response, regeneration of the target cells or tissues, and restoration of self tolerance, whereby damaging white immune cells can be purged or silenced.

Given that stem cell transplantation has long been considered a promising regenerative therapy for a number of central nervous system (CNS) diseases, we have assessed the therapeutic potential of mesenchymal stem cells (MSCs) isolated from different tissues as well as neural stem cells (NSCs) generated from induced pluripotent stem cells lines of MS patients in an MS-like disease (experimental autoimmune encephalomyelitis [EAE]) in mice. Intravenous and intraperitoneal injections of green fluorescent protein (GFP) + nucleocapsids (NCs) and MSCs from the onset of the disease lead to a significant clinical improvement of this MS-like disease as compared to untreated EAE mice and those receiving autologous bone marrow cells and/or fibroblasts, used as control cell populations. The mechanism by which this suppressive effect is produced appears to be the result of immunoregulation and/or immunosuppression occurring in peripheral lymphoid organs, rather than a direct effect on autoreactive cells present in the CNS. Apart from their broad immunomodulatory properties, stem cells are capable of homing to sites of inflammation and therefore represent promising tools for the delivery of therapeutic molecules. We therefore studied the effect of human MSCs, engineered to overexpress anti-inflammatory cytokines in mice with chronic progressive EAE. MSCs transduced with a bicistronic lentiviral vector encoding human interleukin (IL)–10 or mouse IL-4 and enhanced GFP maintained their differentiation potential, cell surface phenotype and immunosuppressive properties. Transplantation studies revealed that IL10-MSCs could prevent or significantly delay the development of EAE when administered during the priming phase of disease, reducing T-cell proliferative responses and pro-inflammatory cytokine secretion. Co-culture studies demonstrated that Ad-IL10-MSCs could inhibit dendritic cell function, suggesting that the mechanism of action may involve inhibition of antigen presentation and T-cell activation. In contrast, transplantation of IL4-MSCs either at the priming or effector phase had little impact on disease severity. The therapeutic efficacy of NSCs derived from monozygotic twins discordant for MS was also assessed by transplantation into mice with EAE. While intravenous injection had no effect, intrathecal injection significantly attenuated clinical and pathological signs of disease. The successful generation of MS patient–specific NSCs with potential clinical applications represents an important step towards novel approaches for personalised regenerative therapies in MS.

Collectively, these findings further add to the armamentarium of non-toxic cell and gene-based strategies for the treatment of debilitating diseases such as MS.

Plenary 2

Perspective in the future management of hepatocellular carcinoma

AL Cheng
International Center of Excellence in Cancer Research, National Taiwan University College of Medicine, Taiwan

Sorafenib was approved for the treatment of hepatocellular carcinoma (HCC) in 2007. Since then, up to 80 compounds, mostly molecular targeted therapy (MTT), have been tested in more than 190 trials. Among these, anti-angiogenic multi-target tyrosine kinase inhibitors remain the mainstay of development. Although none of them showed efficacy superior to sorafenib, several others, particularly those with extra activity on fibroblast growth factor receptors (FGFR) or c-Met appeared promising in early trials and are currently undergoing pivotal phase III trials. Recent large-scale genomic studies have suggested Wnt/β-catenin mutation, JAK mutation, VEGF-A amplification, TSC-2 lost, FGF19/FGFR4 overexpression, and PI3KCA mutation may be important in HCC. A comprehensive approach by cluster studies to test these targets simultaneously are now under discussion.

Recently, immunotherapy has attracted vast attention. Early experience indicated that immune check-point inhibitors, such as anti-PD1 and anti-CTLA4, have modest but definitive effect on HCC. While the hype on immunotherapy is booming, it remains important to revive MTT and try to make the most out of both major modalities of treatment. For example, targeted therapy, including sorafenib, has significant effect on immune microenvironment. Combinations of sorafenib and several other MTTs with immunotherapy have been shown as highly synergistic in pre-clinical studies.

Looking back, identification of biomarkers to select HCC patients who may better respond to MTT has proven difficult. However, enrichment policy appears much more tangible at the era of immunotherapy. It is highly possible that further characterisation of the biologic features of HCC relevant either to oncogenic signatures or immunologic signatures will lead to identification of clinically meaningful biomarkers which may help improve both MTT and immune therapies.

Plenary 3

Big data and improving the outlook of lymphomas

D Sandeep
Department of Medicine, Duke University School of Medicine, United States

The advent of high throughput methods, particularly next generation sequencing, has revolutionised our approaches to measurement of molecular aberrations in cancer. In this talk, we will discuss the methodological underpinnings of these methods and the different considerations that are critical to big data. Through concrete examples, we will examine how these methods can be applied to identify new aspects of biology and new treatment options in different lymphomas. These considerations are equally applicable to other cancers and human diseases.

Engineering the cardiac muscle strips for atrioventricular node regeneration

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ZJ Cai, Z Chen, CW Siu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Atrioventricular node (AV node) is a group of specialised cardiac muscle that conducts the electrical impulse from atrium to ventricle. The structural damage of AV node caused by degenerative diseases or genetic disorder will lead to the advanced heart block with ventricular standstill or fibrillation. Apart from the traditional pacemaker therapy, this project aimed to apply the latest 3D bioprinting technology to engineer functional AV node for regeneration.

Methods: Cardiomyocytes were firstly differentiated from the induced pluripotent stem (iPS) cells. 2D and 3D cardiac muscle strips were generated by either seeding or embedding the cardiomyocytes on the bioprinted collagen I strips. The engineered strips were characterised in terms of cellular structure, contractility, cardiac gene expression, as well as the ability to conduct the electrical impulse.

Results: Siu1 iPS cell–derived cardiomyocytes were highly aligned and elongated along both 2D and 3D cardiac strips. Typical cardiac muscle structure of striation and filaments were shown in the cardiac strips by immunofluorescent staining of alpha-actinin and troponin T (TnT) antibodies. Spontaneous contraction could be observed approximately 7 days upon fabrication. The mRNA levels of the early and mature cardiac genes of 2D and 3D cardiac strips were quantified and compared by real-time polymerase chain reaction. The cardiomyocytes were maturing in 3D collagen environment as TnT, myosin heavy chain beta (MHC-β) level keep increasing from d7 to d14. The higher level of nkx2.5, TnT, and SERCA in 2D and 3D cardiac strips compared with cardiomyocyte clusters indicated that collagen-based engineered cardiac muscles were more ideal for cardiomyocytes differentiation, maturation, and calcium handling. Optical mapping technique was used to characterise the electrophysiology of the engineered AV node. For the 2D and 3D HL-1 strips, the electrical impulse propagated linearly along the direction of cell alignment with the activation time of around 80 ms and conduction velocity of 13.27 ± 1.323 cm/s and 9.123 ± 1.179 cm/s respectively, which was comparable to the native cardiac conduction tissues. The electrical conductions were also affected by the physiological stimulations such as isoprenaline and acetylcholine.

Conclusion: The engineered cardiac muscle strips resembled the native cardiac conduction tissue in cellular structure, gene expression, and electrophysiological function. The future work is to implant the engineered AV node to heart-block mice model to verify if the engineered tissue could provide an alternative conduit for the AV conduction.

Autoimmune encephalitis in Hong Kong Chinese patients: experience of a regional hospital

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KH Chan, KC Teo, KK Lau, WI Leung, SL Ho, RTF Cheung, RSK Chang, SYY Pang, JCY Lee, RCN Ng
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Autoimmune encephalitis includes a heterogeneous group of non-infectious neuroinflammation of the brain characterised by detection of autoantibodies targeting a wide variety of neuronal and synaptic antigens in serum and/or cerebrospinal fluid (CSF) of patients.

Methods: Records of patients with a diagnostic code of autoimmune encephalitis confirmed with detection of specific neuronal, glial, or synaptic autoantibodies cared in Queen Mary Hospital from January 2001 to October 2015 were studied retrospectively.

Results: A total of 11 patients had symptomatic autoimmune encephalitis confirmed with detection of specific autoantibodies. Eight (72.7%) had neuromyelitis optica spectrum disorders seropositive for aquaporin-4 autoantibodies and had encephalitis involving various brain regions: hypothalamus (2), periaqueductal grey matter (3), periventricular region around fourth ventricle (3), ventral midbrain (3), dorsal pons and cerebellar peduncles (4), medulla commonly in continuity with high cervical myelitis (6) and periventricular white matter in cerebral hemispheres (2) and corpus callosum (1). Two patients had voltage-gated potassium channel complex antibodies detected in sera—one presented with confusion, memory loss, and disorientation with typical magnetic resonance imaging (MRI) brain findings of limbic encephalitis; the other presented with marked weight loss, insomnia, haemodynamic instability, limb weakness, and fasciculation suggestive of central, autonomic, and peripheral hyperexcitability. A single young woman presented with confusion, disorientation, followed by status epilepticus and involuntary facial dyskinesia with normal MRI brain. She had anti-NMDAR antibodies detected in her CSF but not serum, confirming the diagnosis of anti-NMDAR encephalitis. She had no ovarian teratoma or other tumour found. She responded well to pulse steroid and intravenous immunoglobulin during acute phase, and had no relapse for a year while on mycophenolate mofetil.

Conclusion: Autoimmune encephalitis is a potentially severe but treatable neurological disorder. Detection of specific neuronal, glial, or synaptic autoantibodies greatly facilitates early diagnosis and prompt treatments.

Novel manifestations of anti-myelin–associated glycoprotein (MAG) antibodies neuropathy

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KH Chan, RSK Chang, SYY Pang, JCY Lee, KK Lau, KC Teo, RCL Ng
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Anti-myelin–associated glycoprotein (anti-MAG) antibodies–associated peripheral neuropathy (anti-MAG neuropathy) is detected in approximately 50% of patients presenting with demyelinating neuropathy and immunoglobulin (Ig) M monoclonal gammopathy. The typical presentation of anti-MAG neuropathy is that of distal, predominantly sensory large-fibre ataxic neuropathy progressive over years or decades. The rate of progression is variable.

Methods: We retrospectively studied clinical features of Chinese anti-MAG neuropathy patients diagnosed and cared for in Queen Mary Hospital (QMH) for the past 10 years' (January 2005 to December 2014) duration.

Results: During the study period, there were 18 patients with idiopathic chronic inflammatory demyelinating polyneuropathy (iCIDP)—six CIDP associated with monoclonal gammopathy of uncertain significance (MGUS), two POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes), two anti-MAG neuropathy, and one CIDP associated with myeloma, diagnosed and cared for in QMH. The clinical and electrophysiological features of the iCIDP, CIDP with MGUS, POEMS, and CIDP with myeloma were similar to that of reported series. The two patients with anti-MAG neuropathy had novel neurological presentations. One presented with acute rapidly progressive demyelinating polyneuropathies fulfilling diagnostic criteria of Guillain-Barré syndrome (GBS) that did not respond to intravenous Ig and progressed to severe generalised weakness with respiratory involvement with marked secondary axonal degeneration on nerve conduction study (NCS). The patient gradually recovered with prolonged therapy with azathioprine and steroid. The other patient presented with diplopia due to bilateral abducens and right facial nerve palsy with NCS confirmed demyelinating neuropathy associated with hyperviscosity from IgM monoclonal gammopathy. He completely recovered following therapy with fludarabine, dexamethasone, and mycophenolate mofetil.

Conclusion: Anti-MAG neuropathy in Hong Kong Chinese patients may present as GBS or multiple cranial neuropathies with good response to immunosuppressant therapies.

Gastrointestinal haemorrhage in atrial fibrillation patients: impact of quality of anticoagulation control

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PH Chan, WH Li, JJ Hai, KH Chan, HF Tse, BMY Cheung, WK Leung, IFN Hung, CW Siu
Department of Medicine, The University of Hong Kong, Hong Kong

Background: Gastrointestinal haemorrhage is a known complication of antiplatelet and anticoagulation therapy. Little is known about the impact of warfarin of different time in therapeutic range (TTR) on the risk of gastrointestinal haemorrhage in atrial fibrillation (AF) patients compared to aspirin.

Methods: This was an observational study.

Results: We studied 5426 Chinese AF patients (77.7 ± 10.7 years; female: 53.1%) with CHA_2DS_2-VASc score of ≥1—3832 (70.6%) patients were taking aspirin, 1594 (29.4%) patients were taking warfarin, while the remaining patients did not receive any anticoagulation. The mean baseline HAS-BLED score (hypertension, abnormal renal/liver function, stroke, bleeding history or predisposition, labile international normalized ratio, elderly, drugs/alcohol concomitantly) was 2.22 ± 0.93. Among those on warfarin, the median TTR was 39.2%. After a mean follow-up of 3.6 years (19 777 patient-years), 262 (4.83%) patients developed gastrointestinal haemorrhage requiring transfusion with an annual incidence of 1.32%. Annual incidences of gastrointestinal haemorrhage requiring transfusion among patients on aspirin and warfarin were 1.53% and 1.00%, respectively. For patients on warfarin, the incidence of gastrointestinal haemorrhage increased progressively with higher HAS-BLED scores, from 0.93% per year for those with HAS-BLED score of ≤1 to 1.68% per year for those with HAS-BLED score of ≥3, and decreased progressively with increasing TTR from 1.69% per year for patients in the lowest quartile of TTR to only 0.51% per year for those in the top quartile.

Conclusion: Overall, aspirin was associated with a higher risk of gastrointestinal bleeding compared with warfarin despite the suboptimal TTR in the study population. For patients on warfarin, HAS-BLED score at baseline prior to commencing anticoagulation positively correlated with gastrointestinal bleeding. Poor TTR was associated with a higher risk of gastrointestinal bleeding.

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Recalibration of CHA_2DS_2-VASc with an additional age category (age, 50-64 years) enhances stroke risk stratification in Chinese patients with atrial fibrillation: The Hong Kong Atrial Fibrillation Registry

PH Chan, JJ Hai, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Chinese patients with atrial fibrillation (AF) and low CHA_2DS_2-VASc score have a disproportionately high ischaemic stroke risk. Nonetheless, little is known about the impact of age on ischaemic stroke risk in this population. The purpose of this study was to examine the age-related ischaemic stroke risk in Chinese patients with non-valvular AF and no other risk factors for stroke.

Methods: This observational study used a hospital-based cohort of Chinese patients with AF.

Results: A total of 1198 Chinese patients with AF (mean age, 73.6 ± 16.5 years; male, 53.3%) were included in this analysis. The mean CHA_2DS_2-VASc and HAS-BLED scores were 1.81 ± 1.00 and 1.32 ± 0.77, respectively, and none were prescribed antiplatelet or anticoagulation therapy. After a mean follow-up of 2.95 years, there were 234 (19.5%) ischaemic strokes with an annual ischaemic stroke incidence of 6.62%. The overall annual ischaemic stroke risk was 0.43%, 5.87%, 7.49%, and 8.04% for age-groups of <50 years, 50-64 years, 65-74 years, and ≥75 years, respectively. There was a 10- to 20-fold gradient in ischaemic stroke risk that increased sharply after the age of 50 years. The hazard ratios were 1.0, 13.0, 19.3, and 21.6 for age-groups of <50 years, 50-64 years, 65-74 years, and ≥75 years respectively (P value for trend <0.0001). Similar trends were also observed in both male and female AF patients.

Conclusion: Chinese patients with AF and low CHA_2DS_2-VASc score were at a disproportionally high risk of ischaemic stroke. Chinese patients aged between 50 and 64 years are at a high risk for stroke despite a low CHA_2DS_2-VASc score and have low bleeding risk. Only patients of <50 years old are truly at low risk.

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Stroke prevention using dabigatran in the very elderly with atrial fibrillation

PH Chan, D Huang, WH Li, JJ Hai, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Little is known about the clinical benefit of a non-vitamin K antagonist oral anticoagulant (NOAC) compared with warfarin in elderly Chinese patients with atrial fibrillation (AF). We aimed to evaluate the clinical benefit of dabigatran in elderly (age ≥80 years) Chinese patients with non-valvular AF with regard to risk of ischaemic stroke and intracranial haemorrhage (ICH).

Methods: This was an observational study.

Results: We studied 571 Chinese patients (age, 84.8 ± 4.0 years; 58.1% female) with non-valvular AF. The primary outcome was hospital admission for ischaemic stroke, and secondary outcome was admission for ICH. The mean CHA_2DS_2-VASc and HAS-BLED score was 4.8 ± 1.6 and 2.4 ± 0.8, respectively. Of these, 129 (22.6%) patients were taking dabigatran 110 mg twice daily and the remaining were on warfarin. After a mean follow-up of 2.6 years (total, 1471 patient-years), ischaemic stroke occurred in 83 patients on warfarin (6.9%/year) compared with four patients on dabigatran (1.4%/year) [hazard ratio=0.22; 95% confidence interval, 0.23-0.67). There were eight incidences of ICH: seven in patients on warfarin (0.59%/year) and one patient on dabigatran (0.35%/year). Dabigatran was associated with a much lower ischaemic stroke risk (1.4%/year vs 5.4%/year) and similar ICH risk (0.35%/year vs 0.36%/year) when compared with warfarin with time in therapeutic range (TTR) of ≥55%.

Conclusion: In elderly Chinese patients with AF, dabigatran achieved superior stroke risk reduction and similar risk of ICH compared with warfarin with TTR of ≥55%. Dabigatran is preferable to warfarin in elderly AF patients for stroke prevention, particularly in those with a poor TTR.

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Net clinical benefit of dabigatran over warfarin in patients with atrial fibrillation stratified by CHA_2DS_2-VASc score and time in therapeutic range: The Hong Kong Atrial Fibrillation Project

PH Chan, D Huang, WH Li, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Although dabigatran is one of the preferred agents for stroke prevention in atrial fibrillation (AF), warfarin remains the mainstay of treatment in many publicly financed health care systems. Little is known about the net clinical benefit of switching patients on warfarin at different risk profiles and time in therapeutic range (TTR) to dabigatran. We aimed to investigate the net clinical benefit of switching warfarin to dabigatran in relation to CHA_2DS_2-VASc score and TTR in Chinese AF patients.

Methods: This was an observational study.

Results: We studied 2153 patients (mean age, 72.7 ± 12.2 years; CHA_2DS_2-VASc score, 3.65 ± 1.94). Of them, 1686 patients were on warfarin, and 467 on dabigatran. After 4.2-year follow-up, the incidence of ischaemic stroke among patients on warfarin and dabigatran was 4.25%/year and 1.89%/year, respectively. Among patients on warfarin, ischaemic stroke risk was positively correlated with CHA_2DS_2-VASc score and negatively with TTR. Using a regression analysis, it was found that for every 10% increase in TTR, the annual ischaemic stroke decreased by 0.74%/year (R^2=0.77, P=0.04). Patients with higher CHA_2DS_2-VASc scores had greater ischaemic stroke risk reductions per 10% TTR increment (ie for CHA_2DS_2-VASc score of ≤2, 3-4, and ≥5: -0.38%/year, -0.60%/year, and -0.84%/year, respectively). Similar trends were also observed in intracranial haemorrhage. While the net clinical benefit favoured switching warfarin to dabigatran for all patients, the best net clinical benefit for switching was found in those with high CHA_2DS_2-VASc score and poor TTR. The number needed to treat to prevent one adjusted intracranial event per year ranged from 14.2 for those with CHA_2DS_2-VASc score of ≥5 and TTR of <35%, to 21.7 for those with CHA_2DS_2-VASc score of ≥5 and TTR of 55-64%.

Conclusion: The combination of CHA_2DS_2-VASc score and TTR facilitates patient prioritisation for dabigatran. The best net clinical benefit for switching warfarin to dabigatran was found in those with both high CHA_2DS_2-VASc score and poor TTR.

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Time in therapeutic range and percentage of international normalised ratios in therapeutic range as measure of quality of anticoagulation control in atrial fibrillation patients

PH Chan, D Huang, WH Li, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Time in therapeutic range (TTR), albeit being the standard measure of quality of anticoagulation control for warfarin, is underutilised in everyday clinical practice due to its tedious calculation. In contrast, the percentage of international normalised ratio measurements in range (PINRR) is a convenient alternative. Our objective was to investigate the correlation between PINRR and TTR, and whether PINRR has clinical utility for prediction of ischaemic stroke and intracranial haemorrhage (ICH) in a 'real world' atrial fibrillation (AF) cohort.

Methods: This was an observational study.

Results: Among 1428 Chinese AF patients taking warfarin (mean age, 76.2 ± 8.7 years; mean CHA_2DS_2-VASc, 4.2 ± 1.6, and mean HAS-BLED, 2.3 ± 0.9), mean and median TTR values were 38.2 ± 24.4% and 38.8% (interquartile range [IQR], 17.9%-56.2%), respectively. Patients with TTR of ≥65% (14.8%) had a lower annual risk of ischaemic stroke (3.04%/year) than those with TTR of <65% (5.35%/year). The mean and median PINRR was 34.3 ± 17.1% and 34.2% (IQR, 22.7%-46.0%), respectively. TTR significantly correlated with PINRR in a linear fashion (r=0.81, P<0.0001). A cut-off of PINRR of ≤56.1% was a good discriminator of TTR of <65%, with a high sensitivity (98.3%) and a positive predictive value (91.9%). The annual ischaemic stroke risk in patients with PINRR of >56.1% was 2.56%/year, which was lower than that with TTR of ≥65% (3.04%/year). Patients with PINRR of >56.1% had an annual incidence of ICH comparable to those with TTR of ≥65% (0.49%/year vs 0.68%/year).

Conclusion: Among AF patients on warfarin, the PINRR is a user-friendly alternative to TTR, having a high sensitivity and positive predictive value in predicting TTR. As with TTRs, PINRR is associated with clinical adverse events, ie ischaemic stroke and ICH.

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Efficacy and safety of dabigatran, rivaroxaban, and warfarin for stroke prevention in patients with atrial fibrillation: The Hong Kong Atrial Fibrillation Project

WH Li, PH Chan, D Huang, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Little is known about the comparative effectiveness and safety of non-vitamin K antagonist oral anticoagulants (NOAC) compared to warfarin in Chinese atrial fibrillation (AF) patients. We aimed to compare ischaemic stroke risk reduction and incidence of intracranial haemorrhage (ICH) of warfarin in relation to quality of anticoagulation control (as reflected by time in therapeutic range [TTR]), and to dabigatran and rivaroxaban in a 'real-world' cohort of Chinese AF patients.

Methods: This was an observational study.

Results: Of 2099 patients (mean age, 73.1 ± 12.3 years; female, 44.6%; mean CHA_2DS_2-VASc 3.7 ± 1.9 and HAS-BLED 2.0 ± 1.0) with non-valvular AF, 963 (45.9%) patients were on warfarin (only 16.3% had TTR of ≥65%), 669 patients were on rivaroxaban, and 467 patients were on dabigatran. After a mean follow-up of 21.7 ± 13.4 months, there were 156 ischaemic strokes (annual incidence of 4.10%), with the incidence of ischaemic stroke being highest in patients on warfarin with TTR of <65% (5.24%/year), followed by those on rivaroxaban (3.74%/year) and those on warfarin with TTR of ≥65% (3.35%/year), while patients on dabigatran had the lowest incidence of ischaemic stroke (1.89%/year). The incidence of ICH was lowest in patients on dabigatran (0.39%/year) compared with those on rivaroxaban (0.52%/year) and warfarin, with TTR of <65% (0.72%/year) and TTR of ≥65% (0.50%/year).

Conclusion: In Chinese AF patients, the benefits of warfarin therapy for stroke prevention and ICH reduction depend on TTR. Of the treatments compared, dabigatran therapy was associated with lowest ischaemic stroke and ICH rates, while rivaroxaban was comparable to warfarin with TTR of ≥65% in terms of efficacy and safety.

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Azacytidine sensitises acute myeloid leukaemia cells to arsenic trioxide by up-regulating the arsenic transporter aquaglyceroporin 9

DHW Chau, K Ng, TSY Chan, YY Cheng, YL Kwong, E Tse
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The therapeutic efficacy of arsenic trioxide (As_2O_3) in acute myeloid leukaemia (AML) is partly limited by its intracellular uptake. As_2O_3 enters cells via the transmembrane protein aquaglyceroporin 9 (AQP9). In this study, we hypothesised that demethylating agent azacytidine may up-regulate AQP9 and enhance As_2O_3-mediated cytotoxicity in AML.

Methods: Arsenic-induced cytotoxicity, the expression of AQP9, and the intracellular uptake of As_2O_3 were determined in AML cell lines and primary AML cells with or without azacytidine pre-treatment. The mechanism of AQP9 up-regulation was investigated by examining the expression of transcription factors for *AQP9* gene and the methylation status of their gene promoters.

Results: As_2O_3-induced cytotoxicity in AML cells was significantly enhanced after azacytidine pre-treatment as a result of AQP9 up-regulation, leading to increased arsenic uptake and hence intracellular concentrations. Blocking AQP9-mediated As_2O_3 uptake with $HgCl_2$ abrogated the sensitisation effect of azacytidine. *AQP9* promoter did not contain CpG islands. Instead, azacytidine pre-treatment led to increased expression of HNF1A, a transcription activator of *AQP9*, through demethylation of *HNF1A* promoter. HNF1 knockdown abrogated azacytidine-induced *AQP9* up-regulation and almost completely blocked intracellular As_2O_3 entry.

Conclusions: Azacytidine sensitises AML cells to As_2O_3 treatment and our results provide proof-of-principle evidence that pharmacological up-regulation of AQP9 potentially expands the therapeutic spectrum of As_2O_3.

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Arsenic trioxide targets NPM1 mutant oncoprotein for degradation to induce myeloid cell differentiation and growth impairment in NPM1-mutated acute myeloid leukaemia cells

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

Introduction: Arsenic trioxide (As_2O_3) is a highly effective salvage therapy in acute promyelocytic leukaemia (APL). The unique sensitivity of APL cells to As_2O_3 is likely to be related to the As_2O_3-mediated degradation of the PML-RARα. In "Acute myeloid leukaemia (AML) with mutated nucleophosmin (*NPM1*)", the role of NPM1 cytoplasmic mutant (NPMc+) in contributing to leukaemogenesis has been previously described by a number of researches. In this study, we sought to identify if As_2O_3 targets NPMc+ for degradation and to investigate the subsequent effects of As_2O_3 in AML cells harbouring NPMc+.

Methods: Arsenic-induced cytotoxicity and dysregulated NPMc+ expression were determined in an AML cell line (OCI-AML3). The effect and mechanism induced from As_2O_3-mediated NPMc+ degradation were further explored in AML cells with *NPM1* gene mutated.

Results: As_2O_3 was found to selectively degrade NPMc+, but not the wild-type NPM1. As_2O_3 impaired cell colony growth, which was comparable to what has been observed in cells with NPM knock-down by siRNA. Arsenic treatment was also found to induce myeloid cell differentiation through induction of CD11b as well as *p53, p21*, and *C/EBPα* expression. Finally, proteasome inhibitor rescued the shortened half-life of NPMc+ due to As_2O_3 treatment, suggesting that As_2O_3 degraded NPMc+ through ubiquitin-proteasomal pathway.

Conclusions: The increased ubiquitylation of NPMc+ after As_2O_3 treatment indicates that NPMc+ degradation mediated by As_2O_3 is through ubiquitin-proteasome pathway. These results underscore the potential of targeting NPMc+ as therapy in AML with *NPM1* gene mutations.

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Peptidyl-prolyl isomerase (PIN1) attenuated the inhibitory effect of p27 on cyclin-dependent kinase 2 (CDK2) kinase activity

CW Cheng, KW Leong, YM Ng, YL Kwong, E Tse
Departments of Medicine, The University of Hong Kong, Hong Kong

Introduction: Peptidyl-prolyl isomerase (PIN1) interacts with and modulates functions of many phosphorylated proteins that are involved in cell cycle progression and oncogenesis. Previously, PIN1 was found to catalyse conformational changes of p27 and increase its protein stability. As p27 is a well-known tumour suppressor protein which suppresses tumour growth and prevents cell cycle progression by inhibiting cyclin A- or cyclin E-cyclin-dependent kinase 2 (CDK2) activities. Therefore, we hypothesised that PIN1-catalysed conformational change of p27 may affect oncogenesis by reducing its binding to and the subsequent inhibitory effect on CDK2 kinase activity.

Method: Protein expression of PIN1 and p27 was determined by western blotting. Cell cycle profile, S phase proliferating cell profile, and CDK2 kinase activity were determined by flow cytometry, BrdU pulse-chase assay and in-vitro fluorescence-based kinase assay, respectively. Co-immunoprecipitation (Co-IP) assays were performed to determine the binding between p27 and cyclin A- or cyclin E-CDK2.

Results: Western blot analysis demonstrated that wild type (WT) mouse embryonic fibroblasts (MEFs) had a higher endogenous p27 protein level as compared with *Pin1*-null MEFs. A double thymidine block was used to synchronise cells at G1/S phase for the cell cycle studies. Upon release from the double thymidine block, cell cycle profile analysis showed that WT MEFs progressed faster through S phase to G2/M phase as compared with *Pin1*-null MEFs. In pulse-chase BrdU labelling assay, WT MEFs showed a higher proportion of S phase proliferating cells as compared with *Pin1*-null MEFs. Using in-vitro fluorescence-based kinase assay, WT MEFs had shown higher total CDK2, cyclin A-CDK2 and cyclin E-CDK2 activities as compared with *Pin1*-null MEFs. Co-IP assays demonstrated that p27 immunoprecipitates bound less cyclin A or cyclin E in WT MEFs as compared with Pin1-null MEFs whereas in the reciprocal experiments, WT MEFs showed a lower level of p27 in either cyclin A or cyclin E immunoprecipitates. Finally, WT MEFs showed an enhanced binding of cyclin A or cyclin E to the PIN1-binding defective p27 mutant as compared with the wild type p27.

Conclusion: PIN1-expressing cells possess a higher CDK2 activity through reducing the binding between CDK inhibitor p27 and cyclin A- and cyclin E-CDK2 kinase.

Association of increased systolic blood pressure in children with obesity and the metabolic syndrome

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AJ Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Our previous study in British schoolboys suggested a strong relationship between systolic blood pressure (SBP) and obesity. To characterise this relationship further, we analysed the latest United States National Health and Nutrition Examination Survey (NHANES) data.

Methods: A total of 1981 participants (1007 boys, 974 girls) of NHANES 2011-12 aged <20 years (mean age, 13.0 ± 3.5 years) were included in the analysis. The protocol was approved by the National Center for Health Statistics Research Ethics Review Board. Blood pressure was measured by certified personnel, and blood samples were analysed by central laboratories. Lifestyle information was obtained using questionnaires.

Results: SBP and diastolic blood pressure (DBP) correlated with body weight (BW) stronger than body mass index or waist circumference (r=0.51 and 0.28 respectively, P<0.001). SBP correlated more strongly with BW in boys than girls (r=0.57 and 0.38, respectively, P<0.001; age-adjusted r=0.34 and 0.26 respectively, P<0.001). In boys, SBP also correlated with serum insulin, high-density lipoprotein, and triglycerides (r=0.22, 0.22, and 0.32, respectively; P<0.001), but these correlations became insignificant when adjusted for BW. There was no significant association between SBP or DBP with smoking, alcohol intake, quantity or quality of sleep, hours of television viewing, hours at computer, or amount or rigor of physical exercise.

Conclusions: Increased SBP in children is most strongly related to BW; in boys, it is also associated with components of the metabolic syndrome. Our results emphasise the importance of children's eating habits.

Blood level of fibroblast growth factor 21 and blood pressure

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BMY Cheung, YC Woo, CHY Fong, A Xu, KSL Lam
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Fibroblast growth factor 21 (FGF21) is a circulating peptide playing a key role in metabolism, obesity, and diabetes. An FGF21 analogue has been shown to reduce body weight and ameliorate lipid profile in man. We previously reported that elevated FGF21 blood level was associated with carotid atherosclerosis. We therefore sought to investigate its relationship with blood pressure (BP).

Methods: We measured FGF21 in the plasma of 1921 participants (891 men, 1030 women; mean age, 52 ± 12 years) of the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) taken at baseline using an enzyme-linked immunosorbent assay (Antibody & Immunoassay Services, The University of Hong Kong). The log of FGF21 level was analysed for relationship with systolic and diastolic BP at baseline and at follow-up (median, 5.4 years).

Results: Plasma FGF21 level was 224.3 ± 7.4 and 214.1 ± 7.1 pg/mL in men and women, respectively. It correlated significantly (P<0.001) with age (r=0.30), waist circumference (r=0.31), systolic BP (r=0.32), diastolic BP (r=0.22), triglyceride (r=0.41), high-density lipoprotein–cholesterol (r= –0.27), fasting blood glucose (r=0.27), and high-sensitivity C reactive protein (r=0.27). In multivariate analysis, FGF21 was independently related to systolic (β=0.076, P<0.001) and diastolic (β=0.074, P=0.001) BP at baseline and to diastolic BP at follow-up (β=0.06, P=0.025).

Conclusions: FGF21 blood level increases with age and is related to the components of the metabolic syndrome. It is related to systolic and diastolic BP, independent of age, obesity, lipids, and blood glucose. FGF21 may have a role in the pathophysiology of hypertension.

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Evaluation of cutoffs for low lean mass and slow gait speed in predicting death in the National Health and Nutrition Examination Survey 1999-2004

CL Cheung[1,2], KSL Lam[1], BMY Cheung[1]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong

Introduction: Sarcopenia is commonly defined as loss of muscle mass with limited muscle function or strength. Different cutoffs of low lean mass and slow gait speed have been proposed by different professional working groups. We compared the performance of different cutoffs of low lean mass and slow gait speed in predicting death.

Methods: We analysed data of participants aged 65 years or older from the continuous National Health and Nutrition Examination Survey 1999-2004 (n=2841), and the subsequent follow-up data on mortality up to 31 December 2006. For low lean mass, cutoffs based on appendicular lean mass (ALM) alone, ALM adjusted for body mass index (ALM_{BMI}), and ALM adjusted for height squared (ALM_{H2}) were evaluated. For slow gait speed, the cutoffs based on 0.8 and 1.0 m/s were evaluated. A cox-proportional hazard regression model with adjustment for multiple confounding factors was used for the association analyses.

Results: For low lean mass, the cutoffs based on ALM_{BMI} (<0.512 in women and <0.789 in men) showed the most significant association and highest hazard ratio with death (hazard ratio=1.72; 95% confidence interval [CI], 1.28-2.29). For slow gait speed, all cutoffs tested showed significant association with death in the full model (P<0.001), while the cutoff of 0.8 m/s showed the highest hazard ratio (2.32; 95% CI, 1.58-3.39).

Conclusions: Low lean mass defined by ALM_{BMI} showed the strongest association with death; while slow gait speed showed significant association with death, with the strongest association being observed for the cutoff of 0.8 m/s. Further studies validating the cutoffs are warranted before using them in clinical settings.

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Diabetes is associated with increased risks of low lean mass and slow gait speed only in the presence of peripheral vascular disease

CL Cheung[1,2], KSL Lam[1], BMY Cheung[1]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong

Introduction: Controversial findings have been reported between the association of diabetes with lean mass and gait speed. Such discrepancies could be due to the confounding effect of comorbidities such as peripheral arterial disease (PAD). The aim of the current study was to evaluate the independent relationship of diabetes and PAD with lean mass and gait speed.

Methods: This was a cross-sectional study of the US population in 1999 through 2004, including 4769 participants aged ≥40 years of the National Health and Nutrition Examination Survey 1999-2004. Appendicular lean mass divided by body mass index (ALM_{BMI}) and gait speed were analysed. Low lean mass was defined as ALM_{BMI} of <0.512 in women and <0.789 in men, whereas mobility impairment was defined as gait speed of <0.8 m/s.

Results: In the fully adjusted model, participants with both diabetes and PAD had a higher odds of low lean mass (odds ratio [OR]=2.21; 95% confidence interval [CI], 1.07-4.57) and mobility impairment (OR=4.8; 95% CI, 1.93-11.97) when compared with participants with neither diabetes nor PAD. No significant association of diabetes alone or PAD alone with low lean mass or mobility impairment was observed. Participants with diabetes and PAD had significantly lower ALM_{BMI} and gait speed when compared with all other participants.

Conclusions: People with both diabetes and PAD had a higher likelihood of low lean mass and mobility impairment; such association was not observed in people with either diabetes or PAD alone.

Randomised controlled trial of the effect of phytosterol-enriched low-fat milk on lipid profile in Chinese

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CL Cheung[1,2,3], KC Ho[1], CW Sing[1], MF Tsoi[2], KF Cheng[1], BMY Cheung[2,3]
[1]Department of Pharmacology and Pharmacy; [2]Department of Medicine; [3]State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong

Introduction: Phytosterols found naturally in plants are known to reduce cholesterol absorption in the gut. The Chinese diet typically contains many vegetables and not much meat. Therefore, we aimed to test if phytosterols are effective in reducing cholesterol absorption in the Chinese population.

Method: There were 221 (41 men, 180 women; aged 24-79 years) subjects who consented to participate in the study; the protocol of which was approved by the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster. Subjects were randomised to double-blind intake of a phytosterol-enriched low-fat milk or a conventional low-fat milk for 3 weeks. Every day before breakfast and lunch, they had a 273-mL serving. Active treatment contained 1.5 g of phytosterol per day. Blood samples were taken from fasting subjects before and at the end of the study for the measurement of lipid profile. Body weight, waist circumference, and blood pressure were also measured.

Results: Low-density lipoprotein (LDL)–cholesterol level decreased from 3.22 ± 0.08 to 3.06 ± 0.08 mmol/L in the phystosterol group and increased from 3.08 ± 0.08 to 3.20 ± 0.08 mmol/L in controls. Comparing treatment with control, the decrease in LDL-cholesterol was 9.5 ± 2.0% (P<0.001). There were no significant changes in high-density lipoprotein–cholesterol, triglycerides, body weight, or blood pressure. Five subjects (2.3%; 4 in treatment group) withdrew.

Conclusion: Consumption of a phytosterol-enriched low-fat milk led to a significant fall in LDL-cholesterol. This can be recommended as part of a healthy diet for people with mildly elevated cholesterol levels and not at high cardiovascular risk. Statins and ezetimibe remain the treatment of choice for those with high cholesterol levels or high cardiovascular risk.

Relationship between serum beta-2-microglobulin and cardiovascular risk factors

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CL Cheung[1], BMY Cheung[2]
Departments of [1]Pharmacology and Pharmacy, and [2]Medicine, The University of Hong Kong, Hong Kong

Objective: Serum beta-2-microglobulin (B2M) level reflects cellular turnover (especially lymphocytes) and renal tubular function. We previously reported its association with cardiovascular and all-cause mortality. We sought to explain this association in terms of cardiometabolic risk factors.

Methods: Data on 6474 participants (3114 men, 3360 women; mean age ± standard deviation, 44.7 ± 17.2 years) of the Third National Health and Nutrition Examination Survey were analysed using analysis of covariance. The relationship of B2M with age, body mass index (BMI), blood pressure, glycaemia, lipids, inflammation, and liver and renal functions were studied. Where appropriate, data were log-transformed.

Results: Serum B2M level was 1.92 ± 0.66 and 1.92 ± 0.77 mg/L in men and women, respectively (P>0.05). It correlated with age (r=0.57), BMI (r=0.15), systolic blood pressure (r=0.38), A1C (r=0.21), triglycerides (r=0.25), high-density lipoprotein (HDL; r=-0.14), aspartate aminotransferase (AST; r=0.18), alkaline phosphatase (ALP; r=0.29), C-reactive protein (r=0.28), and estimated glomerular filtration rate (eGFR; r= –0.66) [all P<0.001]. In the fully adjusted model, serum B2M remained positively associated with systolic blood pressure (β=0.11; 95% confidence interval [CI], 0.04-0.18), AST (β=0.14; 95% CI, 0.10-0.18), ALP (β=0.10; 95% CI, 0.07-0.13), and CRP (β=0.05; 95% CI, 0.04-0.07), and negatively associated with HDL (β= –0.11; 95% CI, -0.07 to -0.15) and eGFR (β= –0.65; 95% CI, -0.60 to -0.69) [all P<0.001].

Conclusions: The association of serum B2M level with Framingham risk factors as well as other risk factors of cardiovascular disease helps to explain why it is a good predictor of cardiovascular risk and mortality. This readily available blood test may be useful to identify high-risk patients and prompt the search for reversible causes.

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Compliance of disease-modifying anti-rheumatic drugs in rheumatoid arthritis patients: a longitudinal study

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

Introduction: Compliance of disease-modifying anti-rheumatic drugs (DMARDs) could affect the effectiveness of DMARDs for the treatment of rheumatoid arthritis in real-life clinical setting. Factors affecting compliance vary in different population and health care systems. This study aimed to identify the characteristics of patients with and the factors contributing to lower degree of compliance to DMARDs.

Methods: This study was conducted in Queen Mary Hospital. For inclusion, patients must be diagnosed with rheumatoid arthritis and treated with at least one DMARD(s). Levels of compliance and beliefs about medications were assessed using 19-item Compliance Questionnaire Rheumatology and the Beliefs about Medicine Questionnaire (BMQ) respectively. Demographic variables, disease activities, and health states were collected during patient visits. Results were analysed by using SPSS v22.0.

Results: A total of 385 patients were recruited into this study. Female sex and young age (56.7 ± 0.66 vs 60.5 ± 1.90; P=0.038) were associated with low levels of compliance. Patients with low levels of compliance had poorer patients' global health (36.0 ± 1.57 vs 25.8 ± 3.97; P=0.017), lower BMQ necessity score (17.6 ± 0.17 vs 21.5 ± 0.43; P<0.001) but higher BMQ concerns score (15.5 ± 0.15 vs 13.3 ± 0.53; P<0.001). There was no significant difference in terms of education levels, disease duration and activities, and number of DMARDs used as stratified by the level of compliance.

Conclusion: Low level of compliance is characterised with female sex, young age, poorer patients' global health, lower level of necessity, and higher level of concerns about DMARDs.

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Effect of TNF-alpha inhibitor on subclinical atherosclerosis in rheumatoid arthritis patients: a meta-analysis

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

Introduction: Tumour necrosis factor (TNF)–alpha inhibitors are a class of biological disease–modifying anti-rheumatic drug for the treatment of rheumatoid arthritis. It is well-known that patients with rheumatoid arthritis have higher cardiovascular risks. However, the effects of TNF-alpha inhibitors on subclinical atherosclerosis have not been confirmed in clinical studies due to contradictory results. Therefore, a meta-analysis is required to assess the overall effect of TNF-alpha inhibitors on subclinical atherosclerosis.

Methods: We searched for publications and recent rheumatology abstracts in PubMed, EMBase, Cochrane Database of Systematic Reviews, and ISI Web of Science. For inclusion, studies must report at least one of the variables of subclinical atherosclerosis, ie carotid intimal media thickness, pulse wave velocity, and augmentation index before and after treatment. Results were analysed by RevMan v5.3.5 using random effects model.

Results: A 24-week treatment with TNF-alpha inhibitors was not associated with improvements in carotid intimal media thickness (standardised mean difference [SMD], 0.28; 95% confidence interval [CI], 0.08-0.48), augmentation index (SMD, -0.05; 95% CI, -0.49 to -0.38) or pulse wave velocity (SMD, -0.30; 95% CI, -0.97 to -0.36). However, 52-week treatment of TNF-alpha inhibitors yielded a significant improvement in carotid intimal media thickness (SMD, 0.03; 95% CI, -0.41 to -0.48).

Conclusion: There was no significant improvement in subclinical atherosclerosis in patients with rheumatoid arthritis treated with TNF-alpha inhibitors, which could be due to short assessment period. Therefore, long-term studies are advised to further evaluate the effect of TNF-alpha inhibitors on subclinical atherosclerosis.

Evolution of neoadjuvant chemotherapy in locally advanced HER2-positive breast cancer over 10 years in Hong Kong

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JW Chiu[1], R Leung[1], V Tang[1], I Cheuk[2], J Lo[2], H Wong[1], G Kwok[1], D Suen[2], C Chen[2], KK Ma[2], L Chow[2], P Cheung[3], TT Wong[3], T Yau[1], A Kwong[2,3]
[1]Department of Medicine, Queen Mary Hospital; [2]Department of Surgery, Queen Mary Hospital; [3]Department of Surgery, Hong Kong Sanatorium and Hospital, Hong Kong

Introduction: Over the last 10 years, we witnessed major changes in treatment for locally advanced HER2-positive breast cancer. The emergence of multiple anti-HER2 therapies and recognition of pathological complete response (pCR) as the surrogate marker for survival are the major landmarks. The clinical benefit of this approach and effects on patient behaviour in Asian countries have not been reported.

Methods: This retrospective study summarises the changes in practice and outcome during the period 2005 to 2015 from our hospital. Patients were divided into three groups according to the neoadjuvant chemotherapy (NAC) they received: chemotherapy only (CH), chemotherapy plus trastuzumab (CH-H), and chemotherapy plus double anti-HER2 therapies (CH-DH).

Results: There were 177 patients captured during this period and significantly more pre-menopausal women were in the groups receiving anti-HER2 therapies (P<0.01). Rate of pCR was higher in patients who received anti-HER2 therapies compared with CH (24% and 58% vs 5%; P<0.01), with patients in CH-DH group had more pCR than those in CH-H group (58% vs 24%; P<0.01). This was accompanied by a trend in increased rate of breast conservation therapy (BCT) [CH-H 26% vs CH-DH 42%]. The estimated recurrent-free survival (censored) at 5 years was 57% (CH), 76% (CH-H), and 93% (CH-DH) with a mean follow-up of 4.2 years. Longer follow-up would be required for conclusive results.

Conclusion: Current approach of NAC using CH-H and CH-DH derive favourable pCR rate in Asian patients. The option of BCT after the enhanced tumour shrinkage achieved with NAC may explain the preference of NAC in HER-positive premenopausal women. Combination NAC with double anti-HER2 therapy is likely to become the preferred option to achieve higher BCT rates.

Collagen-gelatin scaffold for 3D printing in cardiac tissue engineering

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THA Choy, CW Siu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: The advance in induced pluripotent stem cell (iPSC) has provided a potentially unlimited source of cardiomyocyte for engineering of patient-specific cardiac tissue. Engineering by additive manufacturing (or 3D printing) would allow freeform control of tissue shape, interfaces, and spatial composition, which were crucial for creating complex tissue organisation. This study aimed to develop a combination of matrix material and cardiomyocyte which would support such fabrication process.

Methods: Cardiomyoctyes that were differentiated from human iPSC (iPSC-CM) were mixed with collagen and gelatin into a slurry solution. Line samples were made and stained for cell viability.

Results: Majority of cells were viable.

Conclusion: Using the combination of collagen and gelatin, iPSC-CM were encapsulated in engineered tissue construct and remained viable.

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The presence of serotonin in cigarette smoke—a possible mechanism in cigarette smoke–induced airway inflammation

YT Cui[1], WKW Lau[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

Introduction: Serotonin (5-hydrotrytamine [5-HT]) is a well-known neurotransmitter but recent evidences have suggested that it may also regulate pulmonary functions. Clinical study indicated increased 5-HT levels in the platelet of smokers, implicating a role of 5-HT in smoking-related diseases. Cigarette smoke has reported to inhibit monoamine oxidase (MAO)–A, which metabolises 5-HT, in line with the reduction of MAO-A levels in lung of smokers. Using human bronchial epithelial cells, we aimed to compare the effects of cigarette smoke and 5-HT on oxidative stress and inflammation and the signalling pathways.

Methods: We first prepared cigarette smoke medium (CSM) by bubbling smoke from two cigarettes through 20-mL phosphate-buffered saline, and measured levels of 5-HT in CSM using commercially available enzyme-linked immunoassay (EIA) kits. We then cultured BEAS-2B cells and incubated cells with serotonin or CSM. MAO activity was measured by the MAO fluorometric assay kit. Intracellular reactive oxygen species (ROS) was detected by the dichlorofluorescein diacetate assay. Protein expressions of p38, ERK1/2 MAPK, NADPH oxidase 2 (NOX2) were detected by Western blot. Release of pro-inflammatory mediators interleukin 8 (IL-8) was measured by ELISA.

Results: We detected the presence of 5-HT in CSM. In BEAS-2B cells, CSM significantly inhibited the activity of MAO-A and MAO-B. Exogenous application of 5-HT or CSM rapidly increased ROS generation, which was accompanied by increased protein expression of NOX2. 5-HT or CSM induced phosphorylation of ERK1/2 at 5 minutes and sustained up to 30 minutes, while activation of p38 was started at 2 minutes and sustained up to 60 minutes. Exposure to 5-HT or CSM caused elevation of IL-8 release, which was attenuated by pre-treatment of MAPK inhibitors U0126 (a selective ERK1/2 inhibitor) or SB203580 (a selective p38 MAPK inhibitor) as well as ROS scavenger TEMPOL.

Conclusion: Our data suggest that the presence of 5-HT in CSM and the CSM-induced inhibition of 5-HT metabolism may cause oxidative stress and inflammation via NOX2 and activation of ERK and p38 MAPK dependent pathways in bronchial epithelial cells. CSM and 5-HT responses share common characteristics, which suggest the importance of 5-HT–related pathways in CSM-induced epithelial cell injury.

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Duration of dual antiplatelet therapy after drug-eluting stent implantation: 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: Patients are recommended for 6 to 12 months of dual antiplatelet therapy (DAPT) after drug-eluting stents (DES) implantation. The optimal duration of DAPT has been debated. Therefore, this meta-analysis was conducted to evaluate safety and efficacy of different DAPT durations after DES implantation.

Methods: We searched the literature using MEDLINE, Scopus, EMBASE, ISI Web of Science, Cochrane Library, ClinicalTrials.gov, and recent conference proceedings. We included trials randomising patients to receive different durations of DAPT after DES implantation. Data were analysed with RevMan.

Results: Eleven randomised controlled trials with 23 559 patients were included for analysis with four trials comparing extended DAPT versus 12-month regimen and seven trials comparing short-term DAPT versus 12-month regimen. Compared to 12-month DAPT treatment, extended DAPT was significantly associated with lower frequency of myocardial infarctions (odds ratio [OR]=0.36; 95% confidence interval [CI], 0.24-0.55; P<0.00001) and stent thrombosis (OR=0.36; 95% CI, 0.24-0.55; P<0.00001) but the risks of major bleeding (OR=1.54; 95% CI, 1.22-1.96) and all-cause mortality (OR=1.43; 95% CI, 1.14-1.81) were substantially increased. There was no significant difference in preventing stroke, cardiac mortality, and repeat revascularisation. Compared to short-term DAPT, 12-month DAPT was associated with increased major bleeds (OR=1.98; 95% CI, 1.26-3.11). However, no significant alteration was found in the risk of other primary outcomes.

Conclusion: Extended DAPT beyond 12 months yields benefits in reducing myocardial infarction and stent thrombosis, but it increases risk of bleeding and all-cause mortality. Discontinuation of DAPT before 12 months decreases number of major bleeds with no apparent difference in other primary endpoints.

Neuroprotection of melatonin and/or calpeptin in a rat experimental cerebral ischaemia and reperfusion model

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Y Feng, RTF Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Melatonin is a potent antioxidant. Previously, we have demonstrated beneficial effects of pretreatment with melatonin in rodent models of focal cerebral ischaemia. Cerebral ischaemia increases intracellular concentration of calcium ion and activates several calcium-dependent proteases such as calpain. Calpeptin is a novel calpain inhibitor. The aim of this study was to investigate the neuroprotective effects of melatonin and/or calpeptin administered after onset of reperfusion in transient focal cerebral ischaemia.

Methods: Right-side middle cerebral artery occlusion was induced for 90 minutes in male Sprague Dawley rats weighing 260 to 280 g, and this was followed by reperfusion for 24 or 72 hours. Post-ischaemic treatment was delivered via an intracerebroventricular injection initiated about 10 to 15 minutes after the onset of the reperfusion. Regional cerebral blood flow was monitored using a laser Doppler flowmeter. Cerebral infarction volume was evaluated using tetrazolium staining and analysed by Image J software. Neurological behaviour was assessed using neurological deficit scoring system (NDSS). Protein for Western blot was extracted from brain tissue specimens obtained in the striatum and cortex. Coronal cryosections of 30 μm thick were collected from the brain, and three levels were selected for immunofluorescence or Fluoro-Jade to study neuronal survival.

Results: Three single doses of calpeptin, two single doses of melatonin, and two combination dosages were investigated. Treatment with either melatonin or calpeptin reduced infarction volume and NDSS score in a dose-dependent manner. Although calpeptin at 15 μg/kg and melatonin at 50 μg/kg did not show significant benefits when injected individually, the combination of the two treatments reduced the infarct volume and improved the neurological deficit at 24 hours after reperfusion.

Conclusion: We conclude that the combination of melatonin and calpeptin exerts synergistic effects.

Reliability of conventional tape measurement versus computerised measurement of waist circumference: a pilot study

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SSL Fung[1], SYN Shek[1], CK Yeung[1], JCY Chan[1], HHL Chan[1,2]
[1]Division of Dermatology, Department of Medicine, The University of Hong Kong, Hong Kong; [2]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, United States

Objective: Waist circumference is common and an essential measurement in clinical practice but prone to measurement error. A build-in analytical function of Vectra® 3D imaging (Canfield Scientific, Inc., Fairfield [NJ], US) stitches front and back images together by common anatomical landmarks to simulate one 360° image, which allows circumference analysis. The validity of this software function has not been studied yet. Thus the purpose of this study was to investigate the compatibility of tape measurement and computer-simulated measurement.

Methods: A convenient sample of 13 colleagues and friends was recruited in Hong Kong Dermatology and Laser Centre. Tape measurement and Vectra® 3D imaging to the trunk were collected by trained research assistants twice with standardised protocol. Analysis was based on three parameters: 2 cm above umbilicus (above-umbilicus), umbilicus, and 2 cm below umbilicus (below-umbilicus). The procedure was observed by another research assistant to ensure the proper technique was applied.

Results: Thirteen pairs of tape measurements and eight pairs of the computerised measurements were obtained. Intra-observer error of tape measurement ranged from 0 to 1 cm for above-umbilicus, 0 to 0.8 cm for umbilicus, and 0.1 to 0.6 cm for below-umbilicus; intra-observer error of computerised measurements ranged from 0.2 to 2.9 cm for above-umbilicus, 0.3 to 1.1 cm for umbilicus, and 0.4 to 1.9 cm for below-umbilicus. The average reliabilities of tape and computer circumference were 54.2% and 57.1%, respectively.

Conclusion: Computerised measurement had slight improvement on reliability compared to manual measurement. However 30.8% of simulation failure suggested the auto image cropping and stitching is highly dependent on the photographing technique. Sufficient flesh exposure and proper posture during photographing are the keys to improve the quality of raw images.

Incidence and outcomes of hospitalised heart failure—insight from a Chinese Registry

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JJ Hai, PH Chan, HF Tse, CW Siu
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Current epidemiological data for heart failure (HF) are derived primarily from Caucasian populations. Little is known for other ethnic groups. We sought to describe the incidence, risk factors, and long-term outcomes of HF in a Chinese population.

Methods: We conducted a retrospective, observational study on consecutive Chinese patients hospitalised for new-onset HF between 2005 and 2012 in our centre.

Results: Between January 2005 and April 2012, 1940 patients (mean age, 78.2 ± 11.8 years; 54.2% female) were hospitalised for new-onset HF. The overall annual incidence was 0.59 per 1000 population per annum, which increased with advancing age. The most common risk factors for HF were hypertension (69.8%) and coronary artery disease (29.3%). Overall, 59.8% of the 952 patients who had echocardiogram done during hospitalisation had left ventricular ejection fraction of ≥40% (ie heart failure with preserved ejection fraction (HFpEF]). A total of 95 (4.9%) patients died during the index hospitalisation. For those who survived, 30.3% were re-hospitalised for HF within 1 year. The all-cause mortality at 1 year, 2 years, and 5 years was 19.5%, 32.1% and 54%, respectively. No statistical difference in HF re-hospitalisation or all-cause mortality was observed between those with heart failure with reduced ejection fraction (HFrEF) and those with HFpEF.

Conclusion: In this study, we presented clinical characteristics and outcomes of HF patients among Chinese patients in Hong Kong. Despite advancement in the treatment for HF, both HFrEF and HFpEF were associated with similarly high rates of HF re-hospitalisation and poor long-term survival.

Predictors for stroke in heart failure patients without atrial fibrillation

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JJ Hai, PH Chan, HF Tse, CW Siu
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Heart failure (HF) is a risk factor for ischaemic stroke/transient ischaemic attack (TIA) among patients with atrial fibrillation (AF). Studies have shown that HF also increases the risk of stroke/TIA in patients without AF. We sought to describe the predictors for stroke/TIA among HF patients without AF.

Methods: We conducted a retrospective, observational study on consecutive Chinese patients hospitalised for new-onset HF between 2005 and 2012 in our centre. Predictors for ischaemic stroke/TIA among those without AF were studied in a multivariate Cox regression model.

Results: Between January 2005 and April 2012, 1940 patients were hospitalised for new-onset HF. Among them, 1234 patients had no documented AF at baseline (mean age, 77.3 ± 12.4 years; 51.7% female). After a mean follow-up of 39.5 ± 31.1 months, 116 (9.4%) of them developed ischaemic stroke/TIA. Univariate analysis revealed that age (hazard ratio [HR]=1.32 per decade [1.11-1.57]; P=0.002), hypertension (HR=1.79 [1.13-2.85]; P=0.01), vascular disease (HR=1.18 [1.02-3.23]; P=0.04), and severity of chronic kidney disease (HR=1.43 [1.18-1.74] for each stage; P<0.001) predicted stroke/TIA in HF patients without AF. In the multivariate model, age (HR=1.54 [1.25-1.89] per decade; P<0.001) and severity of chronic kidney disease (HR=1.38 [1.11-1.72] for each stage; P=0.004) remained independently predictive of stroke/TIA in HF.

Conclusion: In this study, we found that HF without AF is associated with a high incidence of ischaemic stroke/TIA. Old age and severe chronic kidney disease independently predicted a high-risk subset of patients who may benefit from early preventive measures.

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A case-control study on the genetic risks for development of lung adenocarcinoma in never-smoking Hong Kong population

L Han[1], CK Lee[2], JCM Ho[1]
[1]Division of Respiratory Medicine, Department of Medicine, The University of Hong Kong; [2]Hong Kong Red Cross Blood Transfusion Service, Hong Kong Red Cross, Hong Kong

Introduction: Epidermal growth factor receptor (EGFR)–mutated lung adenocarcinoma in never-smoker behaves differently from other molecular subtypes of lung cancer. We believe genetic susceptibility among such population is unique.

Methods: Patients with lung adenocarcinoma were recruited from Queen Mary Hospital. Healthy controls were recruited from Hong Kong Red Cross. Blood samples were taken for all subjects for single nucleotide polymorphism (SNP) MassARRAY. Questionnaires including environmental exposures were administered to all subjects. The associations between 51 SNPs and lung cancer were explored at SNP level, gene level, and pathway level. Gene-gene, gene-environment interactions at each pathway were characterised with multifactor dimensionality reduction model.

Results: Among 267 never-smoking lung adenocarcinoma and 453 healthy controls, significantly higher proportion of cases than controls had been exposed to environmental tobacco smoke. In genetic analysis restricted to 103 EGFR-mutated age- and gender-matched pairs and 45 EGFR wild-type age- and gender-matched pairs, six SNPs showed significant associations with EGFR-mutated lung adenocarcinoma. They were rs2069840 (odds ratio [OR]=3.51; 95% confidence interval [CI], 1.33-9.23) of interleukin-6 gene in inflammatory pathway; and rs238406 (OR=2.26; 95% CI, 1.08-4.70), rs238416 (OR=1.93; 95% CI, 1.01-3.66), rs1618536 (OR=2.26; 95% CI, 1.08-4.70) of *ERCC2* gene; rs2854508 (OR=2.24; 95% CI, 1.04-4.82), rs3213328 (OR=2.45; 95% CI, 1.21-4.92) of *XRCC1* gene in DNA repair pathway. Five different SNPs showed significant associations with EGFR wild-type lung adenocarcinoma. They were rs611624 (OR=2.97; 95% CI, 1.10-8.04) of *ATM* gene; rs2279017 (OR=3.92; 95% CI, 1.20-12.77) of *XPC* gene; rs50871 (OR=0.22; 95% CI, 0.05-0.98) of *ERCC2* gene in DNA repair pathway; rs2279744 (OR=0.35; 95% CI, 0.12-0.99) of *MDM2* gene in tumour suppressor pathway and rs2131877 (OR=2.85; 95% CI, 1.03-7.84) of *C2orf21* gene. Gene-gene interactions were observed in several pathways, wherein the multiple SNPs showed an excess risk than single SNP. Gene-environment interactions were also observed in several pathways in modulating lung cancer risk jointly.

Conclusions: The predisposition SNPs for EGFR-mutated never-smoking lung adenocarcinoma were different from those for EGFR wild-type. Moreover, gene-gene and gene-environment interactions were observed in several pathways in modulating lung cancer risk.

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The impact of musculoskeletal ultrasound on diagnosis and management of patients with undifferentiated/inflammatory arthritis

CTK Ho, CKY Yuen, TWL Li
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Musculoskeletal ultrasound (MSUS) has become widely used as a diagnostic imaging tool in the clinical management of inflammatory arthritis and clinical trials. The use of ultrasound to diagnose various articular and periarticular pathologies including synovitis, bony erosions, bursitis, enthesitis, and tenosynovitis has been well validated. MSUS has good correlation with magnetic resonance imaging (MRI) in regard to different pathologies. Grey-scale and power Doppler ultrasound examination is more sensitive and more reliable than clinical examination for synovitis.

Objectives: To evaluate the impact of using MSUS as a diagnostic imaging tool in managing undifferentiated/inflammatory arthritis.

Methods: Patients referred from rheumatology clinics for diagnosis and assessment of synovitis dated from January 2013 to September 2015 were recruited. A General Electric LOGIQ S7 ultrasound unit with a 5-15 MHz linear array transducer was used. MSUS database has been set up since January 2013 when MSUS clinic was set up in Queen Mary Hospital. Demographic data including sex, age, and diagnosis in referral letter were recorded. The sonographic diagnosis, change of management plan including reduction of disease-modifying anti-rheumatic drugs (DMARDs), augmentation of DMARDs or stopping pharmacological treatment were also recorded.

Results: There were 144 patients scanned for diagnosis in our database. Their mean age was 54.3 (range, 22-82) years; 115 (79.9%) were female and 29 (20.1%) were male. Of the patients, 112 (77.8%) had their diagnoses changed after MSUS; 32 (22.2%) had the same diagnosis before and after MSUS. Overall, 39 patients labelled as having rheumatoid arthritis clinically before scanning; only eight (20.5%) patients were agreed by MSUS. A total of 65 patients were having unclear arthritis before scanning—14 (21.5%) was diagnosed as rheumatoid arthritis, 43 (66.2%) was having osteoarthritis, and 8 (12.3%) remained unclear of inflammatory arthritis requiring MRI.

Conclusion: MSUS is an important non-invasive diagnostic tool for undifferentiated/inflammatory arthritis. It is especially important for early rheumatoid arthritis; MSUS offers a prognostic assessment.

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Increase in prescriptions for osteoporosis and reduction in hip fracture incidence in Hong Kong during 2005-2014

KC Ho[1], MF Tsoi[1], TT Cheung[1], CL Cheung[2], BMY Cheung[1]
Departments of [1]Medicine and [2]Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong

Aim: Ageing of the population makes osteoporosis and the consequent hip fractures a growing problem. In clinical trials, drugs for osteoporosis typically halved the risk of hip fractures. Generic bisphosphonates are thought to be cost-effective in reducing fractures and subsequent complications. Therefore, we studied the trends in the prescription of drugs for osteoporosis and the incidence of hip fractures.

Methods: We used the data from 2005 to 2014 of the computerised database of the Hong Kong Hospital Authority. Hip fracture rates were adjusted to the 2006 Hong Kong population structure.

Results: In 2014, 84% of the drugs prescribed for osteoporosis were bisphosphonates. The annual number of bisphosphonate prescriptions increased from 3888 in 2005 to 32 389 in 2014. The age-adjusted incidence of hip fracture in women aged ≥60 years decreased from 426.6 per 100 000 people in 2005 to 276.7 per 100 000 people in 2012, and levelled off in the range between 270.5 and 281.8 per 100 000 people in 2012-2014. The hip fracture incidence in men aged ≥60 years also decreased, from 197.4 in 2005 to 142.5 per 100 000 in 2009 and levelled off in the range of 130 to 150 in 2009-2014 per 100 000. There was a significant reciprocal relationship between the number of typical hip fractures and the number of bisphosphonate prescriptions (r=0.94, P<0.001 in both men and women).

Conclusion: In the past decade in Hong Kong, the age-adjusted incidence of hip fractures decreased, which is associated with increased use of bisphosphonates for osteoporosis.

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Activation of chaperone-mediated autophagy prevents accumulation of oligomeric α-synuclein in LRRK2 R1441G knockin mice of Parkinson's disease

PWL Ho, SYY Pang, HF Liu, CSC Lam, CT Leung, LF Li, MHW Kung, DB Ramsden, SL Ho
Division of Neurology, Department of Medicine, The University of Hong Kong, Hong Kong

Background: Leucine-rich repeat kinase-2 (LRRK2) mutations are the commonest genetic cause of Parkinson's disease (PD). α-Synuclein accumulation enhances formation of its toxic oligomers with ageing. Dysfunction of aberrant protein clearance by chaperone-mediated autophagy (CMA) was linked to PD. We explored effects of LRRK2 mutation and ageing on α-synuclein clearance and disease-modification strategy.

Methods: α-Synuclein levels in striatal lysates of $LRRK2^{R1441G}$ knockin (KI) mice and wild-type (WT) from 3 to 18 months of age were determined by immunoblotting. Primary cortical neurons were incubated with recombinant α-synuclein to determine its clearance rate. Levels of lysosomal multimeric lamp2a complexes in cortices of 3 and 18-month-old mice were assayed to determine CMA activity. α-Synuclein levels in neurons treated with AR7 (specific retinoic acid receptor alpha-antagonist and CMA activator) at 10 and 20 μM, for 72 hours or 12 days in culture were determined.

Results: Greater age-dependent increase of striatal oligomeric α-synuclein in KI mice compared with WT was apparent by 15 months, reaching significance at 18 months of age. KI neurons had higher monomeric α-synuclein levels with slower decline indicating impaired clearance. Aged KI mice showed abnormal accumulation of multimeric lamp2a complexes indicating impairment in CMA activity. AR7 decreased monomeric α-synuclein levels, and prevented oligomeric α-synuclein accumulation in KI neurons.

Conclusions: Age-related accumulation of striatal oligomeric α-synuclein was due to impaired clearance of α-synuclein in KI mice, associated with impaired CMA activity. CMA activation prevented oligomeric α-synuclein accumulation.

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Burden of upper gastrointestinal symptoms in patients prescribed dabigatran for stroke prevention

D Huang, PH Chan, JJ Hai, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Dabigatran, a non-vitamin K antagonist oral anticoagulant, has been shown to prevent stroke in patients with non-valvular atrial fibrillation (AF). Nonetheless studies show that 10% to 30% of those prescribed dabigatran experience dyspepsia that may eventually lead to discontinuation of therapy and loss of clinical benefit. We aimed to evaluate the gastrointestinal tolerability of dabigatran utilising a validated questionnaire as well as determining subsequent non-compliance and drug discontinuation.

Methods: This was an observational study. All patients were assessed by a validated questionnaire, Hong Kong Dyspepsia Index (HKDI), prior to drug prescription and again 4 weeks later.

Results: In this study, 115 patients with non-valvular AF (mean age, 74.6 ± 11.4 years; mean CHA_2DS_2-VASc score, 3.39 ± 1.59) were prescribed dabigatran. At baseline, the mean HKDI was 12.9 ± 1.6 and nine patients had significant dyspepsia (HKDI ≥16). Four weeks later, the mean HKDI was similar at 12.6 ± 1.9 (P=0.23). There was no change in HKDI after initiation of dabigatran in 59 (51.3%) patients, and improvement in 37 (32.2%). Only 19 (16.5%) patients had worsening of HKDI and only one (0.9%) patient discontinued dabigatran due to significant dyspepsia.

Conclusion: Worsening of dyspepsia with dabigatran 110 mg twice daily was uncommon with correct drug administration and clear instructions provided. Systematic assessment of dyspeptic symptoms using a validated questionnaire (ie HKDI) before and after treatment initiation allows a more objective comparison of dyspeptic symptoms.

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Oestradiol induces apoptosis via activation of miRNA-23a and p53: implication for gender difference in liver cancer development

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

Introduction: Oestrogen (E2) has been suggested to have a protective role in attenuating hepatocellular carcinoma (HCC) development. miRNAs have great potential as biomarkers and therapeutic agents owing to their ability to control gene expression. We sought to determine the effects of E2 on apoptotic miRNAs expression and explore the possible mechanisms underlying apoptosis in HCC.

Methods: Differential expression of apoptotic miRNAs in E2-treated cancer cells was measured by miRNA polymerase chain reaction (PCR) array. miRNAs and mRNAs expression were determined by quantitative real-time PCR. Protein expression was analysed by Western blotting. Caspase-3/7 activity was measured by luminescent assay. Cell apoptosis was detected by flow cytometry and Hoechst 33258/propidium iodide double staining.

Results: Using miRNA PCR array, more than 2-fold alteration was observed in 25 upregulated and 10 downregulated apoptotic miRNAs in E2-treated cells. Among these miRNAs, expression of miR-23a was related to p53 functional status in the male-derived liver cell lines. We demonstrated that E2 via ERαtranscriptionally activated miR-23a and p53 expression, and thus enhanced p53 activation of miR-23a expression. Moreover, we found miR-23a expression correlated inversely with the expression of target gene X-linked inhibitor of apoptosis protein (XIAP), but positively with the caspase-3/7 activity. Decreasing of XIAP might contribute to caspase-3 activity and cell apoptosis.

Conclusion: Our findings reveal a novel E2-signalling mechanism in regulating miRNAs expression for controlling apoptosis in liver cells. Delineating the role of E2 in regulating the activation of p53 and miR-23a expression in HCC is crucial to the understanding of the sex difference observed in HCC.

Adipose-derived fibroblast growth factor 21 is required for adaptive thermogenesis by promoting the browning of subcutaneous white adipose tissue in mice

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Z Huang, TH Lee, Y Wu, CM Wong, A Xu, KSL Lam
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Fibroblast growth factor 21 (FGF21) is a potent hormone with multiple metabolic benefits on obesity and diabetes. Recently, a number of studies showed that FGF21 is also involved in white adipose tissue browning which holds great therapeutic potential against obesity and metabolic syndromes. Although the liver has been suggested to be the major production site of FGF21, several studies also demonstrate that the expression of FGF21 in white adipose tissues is dramatically increased under cold challenge. Therefore, this study aimed to investigate whether FGF21 derived from the liver or adipose tissue regulates thermogenic activity of adipose tissues in mice.

Methods: Liver and adipose-specific FGF21 knockout mouse strains were generated by using LoxP-cre–based strategy. Liver and adipose-specific FGF21 knockout mice and their wild-type littermates were exposed to cold temperature to induce thermogenesis. The browning phenotypes of different adipose depots were analysed by immunocytochemistry and immunoblotting analyses. The thermogenic activity of adipose tissues was measured by the Seahorse metabolic analyser.

Results: Chronic cold exposure led to a selective elevation of FGF21 mRNA in brown and subcutaneous white adipose tissues, but not in the liver. Mice with the loss of adipose-derived FGF21, but not liver-derived FGF21, showed lower body temperature and impaired browning of subcutaneous white adipose tissue in response to cold exposure. Further analysis found that chronic cold-induced mitochondrial biogenesis and activation was markedly attenuated in adipose-specific FGF21 knockout mice, but not in mice with the loss of hepatic FGF21, therefore leading to impaired white adipose tissue browning.

Conclusions: Adipose-derived FGF21 is a key mediator for cold-induced browning of white adipose tissue and adaptive thermogenesis, potentially by mediating mitochondrial biogenesis and activity. Therefore, it will be important to develop novel therapeutics to increase local concentrations of FGF21 in white adipose tissues to combat metabolic diseases by increasing browning of white adipose tissues and thus thermogenesis.

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Adaptor protein APPL1 attenuates beta cell loss and inflammation in type I diabetes

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X Jiang, Z Chen, KKY Cheng, A Xu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: The adaptor protein APPL1 regulates both secretion and actions of insulin, thereby maintaining glucose homeostasis. Our recent data indicated that APPL1 expression is dramatically decreased in pancreatic β-cells of mouse models with type 1 diabetes (T1D), suggesting that this adaptor may involve in β-cell mass maintenance. In this project, we aimed to test whether APPL1 protects β-cells from inflammation and apoptosis, two hallmarks of T1D, by using APPL1 knockout (KO) and β-cell specific APPL1 overexpression mouse models.

Methods: 10-week-old male APPL1 KO mice and their wild-type (WT) littermates as well as C57 BL6/N lean mice injected with adeno-associated virus encoding human APPL1 or green fluorescent protein controls were subjected to streptozotocin (STZ, 50 mg/kg) injection to induce β-cell apoptosis and subsequent T1D. The parameters related to glucose metabolism, β cell mass, and insulin content were examined.

Results: APPL1 KO mice exhibited exacerbated STZ-induced hyperglycaemia and glucose intolerance, which were due to increased β cell loss and reduced pancreatic insulin content. Immunohistochemical analysis revealed that β cell apoptosis and proliferation were increased and decreased, respectively, in STZ-treated APPL1 KO mice when compared to its WT counterparts. In contrast, β-cell specific overexpression of APPL1 partially reversed the detrimental effects of STZ on glucose metabolism and β-cell mass. In both isolated pancreatic islets and INS-1E cells, APPL1 deficiency markedly enhanced cytokine cocktails (tumour necrosis factor–α, interleukin-1β and interferon-γ), the major cytokines involved in the pathogenesis of β-cell dysfunction in T1D, induced inflammatory and apoptotic response, as evidenced by increased activation of nuclear factor kappa B, inducible nitric oxide synthase and caspase 3.

Conclusion: We conclude that APPL1 protects β cell from apoptosis and inflammation in T1D, and APPL1 can be a potential target for T1D therapy.

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Prevalence and clinical impact of white matter hyperintensities, cerebral microbleeds, and medial temporal lobe atrophy in Alzheimer's disease

J Kwan, G Kai, B Ng, R Hui, K So, R Azman, H Mak
Department of Medicine, The University of Hong Kong, Hong Kong

Background: This study examined the prevalence and clinical impact of white matter hyperintensities (WMH), cerebral microbleeds (CMB), and medial temporal lobe atrophy (MTLA) in patients with Alzheimer's disease (AD).

Methods: We analysed the data for 70 patients with AD and 42 age-matched cognitively normal controls recruited from Memory and Geriatric Medicine Clinics, Queen Mary Hospital. The controls had no history of dementia, stroke, Parkinson's disease, head injury, seizures, cancers within 5 years, end-stage organ failure, excessive alcohol or drug use, or psychiatric disease. 3T magnetic resonance imaging (MRI) brain images were retrospectively examined for the severity and location of WMH (Fazekas score 0-3, >1 abnormal), CMB (Microbleed Anatomical Rating Scale score 0-3, >0 abnormal), and MTLA (Scheltens score 0-4, >1 abnormal).

Results: The mean (± standard deviation) ages were similar for AD patients (76.5 ± 8.5 years) and controls (74.1 ± 6.6 years). Gender and history of diabetes, hypertension, hyperlipidaemia, and ischaemic heart disease were similar. AD patients were more likely to be dependent (P=0.037), and live in nursing homes (P=0.05). Controls were more likely to have atrial fibrillation (P=0.007) and take antiplatelets (P=0.02) or anticoagulants (P=0.009). In the AD group, 19% of patients had a history of stroke or transient ischaemic attack, and the mean Mini-Mental State Examination (MMSE) was 18.9 ± 4.9. Comparing AD with the control groups, the prevalence of: (a) abnormal periventricular WMH was 26% vs 19% (P=0.42); (b) abnormal subcortical WMH was 40% vs 74% (P=0.001); (c) CMB was 39% vs 36% (P=0.76); and (d) abnormal MTLA was 61% vs 33% (P=0.004). Severity grades of MRI abnormalities were significantly different between AD and control groups for periventricular WMH (worse for AD group, P<0.001), subcortical WMH (worse for the control group, P=0.002), and MTLA (worse for AD group, P=0.026), but not CMB. Regression models confirmed that MMSE was independently predicted by nursing home residency (P=0.03), but not age or vascular risk factor. Presence of WMH, CMB, or MTLA did not provide additional independent predictive power.

Conclusion: MRI evidence of small vessel disease and MTLA are prevalent among AD patients and age-matched cognitively normal controls. Higher rates of atrial fibrillation and antithrombotic use may have accounted for more subcortical WMH lesions among normal controls. Severity grades of MRI abnormalities differ significantly between AD and controls, but do not independently predict cognitive function. These findings may indicate complex overlaps of neurodegenerative pathologies in AD and cerebral ageing. Further studies are needed.

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Prevalence and clinical impact of white matter hyperintensities, cerebral microbleeds, and medial temporal lobe atrophy on short- and long-term outcomes after stroke rehabilitation

J Kwan, SF Mak, R Hui, K So, A Har, R Azman, H Mak
Department of Medicine, The University of Hong Kong, Hong Kong

Background: The influence of cerebral structural abnormality on stroke rehabilitation outcome is unclear. This study examined the prevalence and clinical impact of white matter hyperintensities (WMH), cerebral microbleeds (CMB), and medial temporal lobe atrophy (MTLA) on short- and long-term outcome after stroke rehabilitation.

Methods: We analysed the data for 377 consecutive patients with confirmed stroke admitted to the stroke rehabilitation unit at Tung Wah Hospital between 1 March 2008 and 23 May 2014. 3T magnetic resonance imaging (MRI) brain images were retrospectively examined for the severity and location of WMH (Fazekas score 0-3, >1 abnormal), CMB (Microbleed Anatomical Rating Scale score 0-3, >0 abnormal), and MTLA (Scheltens score 0-4, >1 abnormal). Stroke rehabilitation outcomes were assessed by comparing functional status between admission and discharge. For 208 patients who were admitted between March 2008 and May 2012, we also retrieved data on clinical outcomes (death or any vascular events, post-stroke dementia) at 2 years.

Results: Mean (± standard deviation) age was 70 ± 12 years. Of the patients, 94% had ischaemic strokes, and the overall stroke severity was mild to moderate according to the baseline functional scores. Overall, 12.6% of the patients had abnormal periventricular WMH, 48% had abnormal subcortical WMH, 52% had CMB, and up to 33% had abnormal MTLA. Stroke subtypes and functional status on admission, but not MRI abnormalities, independently predicted functional improvement during stroke rehabilitation. At 2-year follow-up, 11% had died or any vascular events. Functional status (Barthel index) on admission, but not MRI abnormalities, independently predicted this 2-year outcome. Furthermore, age, WMH, and MTLA independently predicted the development of post-stroke dementia at 2 years.

Conclusion: Among Chinese stroke patients with mild-to-moderate stroke severity, MRI evidence of small vessel disease and MTLA is highly prevalent. These MRI abnormalities do not appear to predict short-term functional improvement during stroke rehabilitation, or 2-year mortality or vascular events. However, WMH and MTLA may predict development of post-stroke dementia at 2 years. Future larger prospective studies are warranted to confirm these findings.

Frailty status influences the attitudes towards end-of-life care among Chinese centenarians in Hong Kong

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J Kwan, BHP Lau, KSL Cheung
Department of Social Work and Social Administration & Sau Po Centre on Ageing, The University of Hong Kong, Hong Kong

Introduction: As the society rapidly ages, planning for health care towards the end-of-life is important for older people and their families. This study examined the attitudes towards end-of-life care among near-centenarians and centenarians, and the influence of frailty status on these attitudes.

Methods: Data for 131 participants (76% female; mean age, 97.5; age range, 95-108 years) were extracted from the Hong Kong Centenarian Study. Overall, 80% of the participants were community-dwelling (49% with family, 31% alone), whilst the remaining 20% were institutionalised. Participants were asked to indicate their agreement on three items: (a) the society's acceptance on advance directives; (b) the society's acceptance on euthanasia; and (c) the idea that under no circumstance should anybody seek to hasten death. We assessed frailty status using a multidimensional model with 44-item including physical health, psychological well-being, socio-familial conditions, environmental barriers to social activities, and economic conditions. Multiple regressions were performed to examine the relationship between frailty status and attitudes towards end-of-life care.

Results: In this study, 69% and 60% of the 131 participants believed that the society did not accept advance directives and euthanasia, respectively; and 89% believed that under no circumstance should one hasten death. Centenarians with higher levels of frailty were more likely to believe in acceptance of advance directives (beta=0.23), euthanasia (beta=0.26) and under no circumstance should anybody seek to hasten death (beta=0.21), after controlling for the effects of gender, age, and living arrangement.

Conclusion: Acceptance of advance directives and euthanasia is low among community-dwelling extremely old adults in Hong Kong, and the vast majority do not believe in hastening death under any circumstances. However, these attitudes are significantly influenced by their frailty status. Further studies are needed to compare with the attitudes among younger-old adults. Greater efforts in raising public awareness about the end-of-life care options are warranted to facilitate choices and autonomy in the final phase of their lives.

PD-L1 as the bull's eye of lung cancer stem cell

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

Introduction: Lung cancer is the top killer among all cancers. Mutation in epidermal growth factor receptor (*EGFR*) is one of the oncogenic driver mutation as well as druggable target in lung cancer. *EGFR*-mutant lung cancer patients could demonstrate remarkable clinical response with upfront targeted therapy. However, acquired resistance is a challenging issue faced by clinicians, highlighting the urgent need for novel therapeutic strategies. Cancer cells employ several mechanisms to evade immune surveillance by expressing cell surface protein that maintain immune tolerance in peripheral tissues, such as PD-Ligand-1 (PD-L1, B7-H1, CD274), which binds with its cognate receptor programmed cell death-1 (PD-1, CD279) on T-lymphocyte. Therefore, PD-1/PD-L1 blockade may play a key role as an adjuvant treatment in lung cancer. Clinical results obtained with immune-checkpoint inhibitors demonstrated remarkable durable tumour responses when compared with classical chemotherapy. However, it remains unknown on the mechanism of this long-lasting effect. The purpose of this study was to characterise the expression pattern and the functional role of PD-L1 in lung cancer stem cells.

Methods: Expression of PD-L1 in various *EGFR* wild-type or mutant was determined by Western blot analysis and real-time polymerase chain reaction. Lung cancer stem cells were isolated from lung cancer cell lines derived from local patients.

Results: Preliminary data suggested that the protein as well as the mRNA level of PD-L1 were higher in *EGFR* mutant cell lines than in wild-type.

Conclusion: *EGFR* mutation may contribute to the immune suppression within the tumour microenvironment. Further study will be conducted to investigate the PD-L1 expression level in lung cancer stem cells.

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Suppression of c-Jun N-terminal kinase-mediated inflammation in visceral adipose tissue protects against atherosclerosis in mice

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

Introduction: Obesity, an independent risk factor for atherosclerosis, exhibits adipose tissue inflammation mediated by c-jun N-terminal kinase (JNK), which secretes pro-inflammatory cytokines and contributes to systemic inflammation. This study investigated whether selective inactivation of JNK in adipose tissue can protect against atherosclerosis, which is a chronic inflammatory disease.

Methods: Lean ApoE-/- mice received sham operation, or subcutaneous transplantation of visceral fat for 4 weeks. Diet-induced obese donors for visceral fat were either wild-type (WT) mice or transgenic mice expressing adipose-specific dominant-negative form of JNK (dnJNK). Recombinant adipocyte fatty-acid binding protein (rA-FABP) was delivered by osmotic pump. Recipient ApoE-/- mice were subjected to assessment for atherosclerosis development.

Results: ApoE-/- mice that received fat transplantation from obese WT mice (WT/ApoE) displayed exacerbated atherosclerosis, as evidenced by increased macrophage, smooth muscle cell, and collagen content within the atherosclerotic plaque. WT/ApoE mice also had elevated circulating levels of pro-inflammatory cytokines, including AFABP. Recipients carrying fat graft from dnJNK donors (dnJNK/ApoE) were protected from this accelerated atherogenesis and augmented systemic inflammation. However, the continuous infusion of rA-FABP in dnJNK/ApoE mice largely compromised these beneficial effects. There was no difference in glucose and lipid metabolism in recipient mice.

Conclusion: Local adipose tissue inflammation promotes atherosclerosis development at the vasculature in part by distant crosstalk through the JNK/A-FABP pathway.

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In-vitro and in-vivo study of arginase in treatment of malignant pleural mesothelioma

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

Introduction: Malignant pleural mesothelioma (MPM) is the commonest form of mesothelioma. Asbestos and erionite fibre exposure are notable causes of MPM. Combination of pemetrexed and cisplatin is the cornerstone chemotherapy in clinical management of MPM with a median overall survival of 12 months, which is far from satisfactory. Arginine is a semi-essential amino acid in tumours, but non-essential in normal cells. Arginine-degrading enzymes (arginase and arginine deiminase) have been used to treat different cancers with inability to synthesise arginine. BCT-100 is a pegylated arginase, which is currently undergoing formal phase I/II clinical trials in treatment of hepatocellular carcinoma at Queen Mary Hospital with encouraging results. The effect of BCT-100 has not been investigated in MPM. The aim of this study was to investigate the effect of BCT-100 in MPM in vitro and in vivo.

Methods: A panel of MPM cell lines (H28, 211H, H226, H2052, and H2452) was used. The effect of BCT-100 on cell viability and protein expression was studied by Crystal Violet assay and Western blot, respectively. The in-vivo effect of BCT-100 was investigated with nude mice xenograft models.

Results: BCT-100 reduced cell viability with IC_{50} values ranges from 13 to 22 mU/mL in different MPM cell lines after 72 hours' incubation. BCT-100 suppressed tumour growth in H226 and 211H nude mice xenografts in a dose-dependent manner. The median survival was significantly increased accompanied with induction of apoptosis and cell cycle arrest in BCT-100 treatment group when compared with control group in both xenografts.

Conclusion: BCT-100 (pegylated arginase) reduced cell viability in vitro. BCT-100 also suppressed tumour growth and increased median survival which is partially mediated by apoptosis and cell cycle arrest in vivo.

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Effect of epigenetic memory on differentiation and function of cardiac-induced pluripotent stem cells

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YM Lau, KM Ng, YK Lee, WH Lai, CW Siu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Induced pluripotent stem cells (iPSCs) are a potentially unlimited source for the generation of cardiomyocytes (iPSC-CMs). However, it remains unclear whether iPSC-CMs generated from various cell sources of iPSCs are molecularly and functionally similar. This study aimed to assess the effect of epigenetic memory on differentiation and function of iPSC-CMs generated from skin and cardiac fibroblasts (SF-&CMF-iPSCs).

Methods: Skin fibroblasts (SFs) and cardiac fibroblasts (CMFs) from the same adult human donors were reprogrammed into iPSCs and then differentiated into iPSC-CMs for DNA methylation profiling study.

Results: A significantly high percentage of contracting CMs could be observed in CMF-iPSC-CMs than in SF-iPSC-CMs group on day 21 of differentiation. CMF-iPSC-CMs expressed a significantly higher level of MESP1 and GATA4 during differentiation. To examine DNA methylation status in different lines, the genome-wide DNA methylation was performed using Illumina's 450K Infinium methylation. Quantitative scores of DNA methylation levels were obtained as β values. As assessed by unsupervised hierarchical clustering analysis and scatter plot of DNA methylation, human iPSCs could be clearly discriminated from their parent cells. Comparison of differentially methylated site (DMSs) between SF- and CMF-iPSCs showed slight but significant differences. Intracellular Ca^{2+} handling properties of both SF- and CMF-iPSC-CMs were also examined. The data showed functional variations between the iPSC-CM from different cell sources.

Conclusion: Our results suggested that iPSCs retain an epigenetic memory of their somatic cells of origin. These observations may influence ongoing attempts to use iPSCs for disease modelling and could also be exploited in potential therapeutic applications to enhance differentiation into desired cell lineages.

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A search for biomarkers in systemic lupus erythematosus and lupus nephritis

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CCP Lee, PH Li, AKW Chan, VSF Chan, CS Lau
Division of Rheumatology, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease that involves many organs. Among those, nephritis is one of the most common and severe clinical manifestations. So far, the only and most reliable tool for diagnosing and monitoring lupus nephritis (LN) is renal biopsy. However, renal biopsy cannot be done to some LN patients because of its risks or contraindications. Therefore, a non-invasive alternative is needed. Since cytokines and chemokines are pivotal in SLE and LN pathogenesis, the cytokines/chemokines profile between the LN and non-LN groups in SLE patients may be different so the profile can be used to diagnose and monitor LN.

Methods: Multiplex is a cost-effective and bead-based immunoassay for measuring multiple cytokines/chemokines simultaneously in each sample. Serological concentrations of 26 cytokines/chemokines from four modules were compared between the LN and non-LN groups of SLE patients in this study. The four modules of cytokines/chemokines were B-cell proliferation, T-cell secretion, inflammation, and recruitment of immune cells.

Results: In this cross-sectional and pilot study of 39 SLE patients, it was proposed that the concentration of chemokines, for example, macrophage inflammatory protein-1α (MIP-1α) and macrophage inflammatory protein-1β (MIP-1β) might have significant difference ($P<0.05$) between LN group and non-LN group of SLE patients. Also, most of the high-IL-18 producers (top 25% in IL-18 level) were from the LN group.

Conclusion: Certain cytokines and chemokines may have associations with LN. Further study with a larger cohort is needed for the validation of our findings.

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Serum high-sensitivity C-reactive protein levels are predictive of both the development and progression of nephropathy in Chinese patients with type 2 diabetes mellitus

CH Lee[1], YC Woo[1], WS Chow[1], CHY Fong[1], MMA Yuen[1], A Xu[1,2], KSL Lam[1,2]
[1]Department of Medicine; [2]State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong

Introduction: Elevated serum high-sensitivity C-reactive protein (hsCRP), a marker of low-grade systemic inflammation, has been associated with diabetic nephropathy. Recently, hsCRP levels were reported to predict the development of microalbuminuria in a 1-year prospective study of Japanese patients with type 2 diabetes and normoalbuminuria. We investigated whether, with longer-term follow-up, serum hsCRP was predictive of both development and nephropathy progression among Chinese type 2 diabetes patients.

Methods: Baseline serum hsCRP levels were measured in 1101 Chinese type 2 diabetic subjects recruited from the Hong Kong West Diabetes Registry. The role of serum hsCRP in predicting decline in estimated glomerular filtration rate (eGFR) over a median follow-up of 5 years was analysed using Cox regression analysis.

Results: Among 1101 subjects with baseline eGFR of ≥30 mL/min/1.73 m^2, serum hsCRP levels were significantly higher in those with eGFR decline during follow-up (n=254) than those without decline (n=847): 1.8 mg/L (0.9-4.1) vs 1.3 (0.5-3.2); P<0.001. On multivariable Cox regression analysis, baseline serum hsCRP levels were independently associated with eGFR decline (hazard ratio [HR]=1.14; 95% confidence interval [CI], 1.03-1.28; P=0.016). In 632 subjects without diabetic nephropathy at baseline, serum hsCRP levels were higher in those who developed diabetic nephropathy (1.26 mg/L [0.59-3.29] vs 1.04 [0.39-2.39]; P=0.012). In multivariable Cox regression analysis, baseline serum hsCRP also independently predicted nephropathy development (HR=1.11; 95% CI, 1.01-1.22; P=0.029).

Conclusions: Serum hsCRP could be a useful biomarker for predicting both the development and progression of diabetic nephropathy in Chinese patients with type 2 diabetes mellitus.

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Deciphering the role of the orphan G protein-coupled receptor GPR110 in hepatic energy metabolism

JTH Lee, Z Huang, CM Wong, KSL Lam, A Xu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Obesity is a risk factor for numerous co-morbidities, including type 2 diabetes, cardiovascular diseases, and certain types of cancers. In this study, we investigated the biological significance of an orphan G protein-coupled receptor (GPC) GPR110 in hepatic lipid uptake and glucose metabolism by both molecular and physiological approaches.

Methods: GPR110 antibody was raised. The expression pattern of GPR110 mRNA and protein in various tissues were examined by quantitative polymerase chain reaction and Western blotting analyses, respectively. GPR110KO mice were generated and fed with high-fat diet (HFD) to comprehensively evaluate the effects of hepatic GPR110 on energy metabolism. Their metabolic fitness was examined by glucose tolerance test, insulin tolerance test, and pyruvate tolerance test. Indirect calorimetry method was performed to examine the change of energy expenditure. The signalling pathways regulated by GPR110 were explored by in-vitro assays.

Results: GPR110 was a liver-specific GPCR and its expression was tightly regulated by nutrient availability. Deletion of GPR110 expression could significantly improve metabolic phenotypes, including glucose tolerance and insulin sensitivity, and increase energy expenditure in diet-induced obese mice. In addition, replenishment of GPR110 expression in liver by adenovirus gene delivery system promoted the development of fatty liver in mice fed with HFD. GPR110 would induce gluconeogenesis and lipogenesis via PKA signalling pathway.

Conclusion: Significant decline in GPR110 expression in hepatocytes might be a protective mechanism to delay the pathogenesis of fatty liver and insulin resistance. Antagonists against GPR110 will be new therapeutic target to combat metabolic diseases.

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Low-frequency intermittent hypoxia reduces endothelium-dependent contraction in C57 mice with diet-induced obesity

MYK Lee, JCW Mak, MSM Ip
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Obstructive sleep apnoea (OSA) is associated with elevated systemic oxidative stress and inflammation, resulting in endothelial dysfunction. Obesity, a major risk factor for the development and severity of OSA, also modulates contractile and relaxing responses in the blood vessels.

Objective: To study the impact of intermittent hypoxia (IH), a hallmark feature of OSA, on endothelium-dependent contraction and relaxation in lean and obese mice.

Methods: Male C57BL/6N mice were fed with normal (Std) or high-fat high-cholesterol (HFHC) diet for 6 weeks before exposing to normoxia (Air) or chronic IH (IH) treatment (10 hypoxic events/hour [O_2 10% at nadir; 6 h/d]) for 6 weeks. All mice were then sacrificed and endothelial function was assessed ex vivo, using the wire myograph device. Quiescent carotid arteries were used to evaluate endothelium-dependent contraction to acetylcholine, in the presence of L-nitro-arginine methyl ester (L-NAME; a non-selective inhibitor of nitric oxide synthase). To investigate the role of oxidative stress in mediating the endothelium-dependent contraction, half of the carotid arterial rings were pre-incubated with apocynin, an inhibitor for superoxide-generating enzyme, NADPH oxidase, together with L-NAME, before acetylcholine-induced contraction. Pre-contracted aortae were used to assess the endothelium-dependent relaxation to acetylcholine.

Results: In the presence of L-NAME, cumulative addition of acetylcholine induced a basal level of endothelium-dependent contraction in carotid artery of Std+Air group. The contraction was significantly increased when the mice were fed with HFHC diet (HFHC+Air) in comparison to Std+Air group. However, treatment with apocynin significantly abolished the augmented response in HFHC+Air. After IH exposure, the HFHC-induced endothelium-dependent contraction was significantly reduced in comparison to HFHC+Air group. There was comparable level of endothelium-dependent contraction between Std+Air and Std+IH groups. On the contrary, treatment with HFHC diet, IH, or their combination did not affect the endothelium-dependent relaxation in aorta compared to Std+Air.

Conclusion: The data suggest the involvement of superoxide in HFHC-induced endothelium-dependent contraction in carotid artery without affecting the endothelium-dependent relaxation in obese mice. The reduction of HFHC-induced endothelium–dependent contraction on low-frequency IH exposure suggests a defensive mechanism on endothelial function.

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PIN1 expression level is controlled by a negative feedback loop through the regulation of c-Myc and miRNAs

KW Leong, CW Cheng, YL Kwong, EWC Tse
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: PIN1 is a peptidyl-prolyl-isomerase (PPIase) that binds to specific motifs (serine/threonine preceding a proline) in proteins and catalyses cis/trans isomerisation of the peptide bond between the phosphorylated serine/threonine and proline. As a result of such conformational changes, the stability, sub-cellular localisation, functional activity as well as the protein-protein interactions of the PIN1-bound proteins may be altered. PIN1 has been demonstrated to play an important role in controlling various cellular processes such as cell proliferation, apoptosis and migration, and conceivably its expression or level has to be tightly controlled. Previously, we found that two miRNAs, miR-296-5p and miR-874-3p, regulate PIN1 expression. However, the mechanism(s) regulating these two miRNAs remain(s) to be defined.

Methods: The online database-UCSC Genome Bioinformatics (NCBI/hg18) was used to search for the upstream regulator of miR-296-5p and miR-874-3p. Luciferase reporter assay was conducted to examine the effect of the identified-regulator on the promoter regions of miR-296 and miR-874-3p. RT-qPCR was used to detect the expression levels of miR-296-5p and miR-874-3p. Protein expression levels of the identified-regulator and PIN1 were examined by western immunoblotting.

Results: By screening the online database-UCSC, c-Myc was identified as a potential transcription factor for the genes encoding miR-296-5p and miR-874-3p. As demonstrated by luciferase reporter assay, expression of c-Myc activated the promoter of miR-296-5p and miR-874-3p. Using reverse-transcription quantitative real-time polymerase chain reaction, we confirmed that c-Myc up-regulated the expression levels of miR-296-5p and miR-874-3p while knock-down of c-Myc decreased their expression. In addition, protein level of PIN1 was suppressed by c-Myc over-expression. Interestingly, c-Myc is a known PIN1 binding protein and its expression correlated with PIN1. We, therefore, hypothesised that PIN1 expression was controlled via a feedback loop involving c-Myc, miR-296-5p, and miR-874-3p. Consistent with this hypothesis, we showed that up-regulation of PIN1 increased the expression levels of c-Myc, and miR-296-5p and miR-874-3p by immunoblotting and RT-qPCR, respectively. Furthermore, up-regulation of miR-296-5p and miR-874-3p decreased PIN1 and c-Myc expression levels.

Conclusion: Taken together, our results suggested that the expression level of PIN1 is controlled by a negative feedback loop through the regulation of c-Myc, miR-296-5p, and miR-874-3p.

Potential use of melatonin as a therapy for intracerebral haemorrhage

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JWH Leung, RTF Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Intracerebral haemorrhage (ICH) is a devastating form of stroke and is characterised by rupture of blood vessels within the brain parenchyma. Patients with ICH may suffer from severe neurological sequels like motor deficits and cognitive impairment. We aimed to study the neurological outcomes of ICH. The potential therapeutic effects of melatonin on ICH were also investigated.

Methods: ICH was induced in the rat by an intrastriatal injection of collagenase type IV. Haematoma size and neurological deficits were studied 24 hours later. To study the beneficial effect of melatonin on ICH, repetitive intraperitoneal injections of melatonin were given to the rats at 2, 24, and 48 hours after ICH; they were sacrificed at 72 hours. In addition, the neuroprotective role of melatonin was further investigated in vitro.

Results: At 24 hours after the ICH induction, haematoma was found in the brain parenchyma. The rats also manifested neurological deficits. Repetitive intraperitoneal injections of melatonin at 50 mg/kg achieved significant improvement in the rotarod test at 72 hours after ICH when compared to 24 hours. The expression of ED-1 (a marker of activated microglia) in the peri-hematomal tissue was decreased in 50 mg/kg melatonin-treated group when compared to the vehicle-treated group. In the in-vitro study, the SH-SY5Y neuronal cells were challenged with red blood cell lysate, and the cell viability was reduced in a dose-dependent manner. Melatonin at optimal concentrations could increase the neuronal viability.

Conclusion: Our present results reveal some potential neuroprotective effects of melatonin in a rat model of ICH and may provide a new insight to the future drug development for this devastating disease.

Use of neuromuscular electrical stimulation in treating sub-acute post-stroke dysphagia

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KP Leung[1], K Chiu[2]
[1]Department of Medicine, The University of Hong Kong, Tung Wah Hospital; [2]Department of Speech Therapy, Tung Wah Hospital, Hong Kong

Introduction: Dysphagia is a common problem after stroke with incidence ranged from 19% to 45%. This study investigated the usefulness of a 2-week neuromuscular electrical stimulation (NMES) programme on top of usual care in treating patients with post-stroke dysphagia.

Methods: Prospective patients admitted from September 2014 to September 2015 with significant dysphagia within 6 months after stroke were recruited. Patients with pre-existing dysphagia due to other medical or surgical conditions were excluded. All received 2 weeks of NMES on top of usual dysphagia intervention. Dysphagia was assessed either radiologically or endoscopically using the Royal Brisbane Hospital Outcome Measure for Swallowing (RBHOMS) scale before and after intervention.

Results: Twenty patients were recruited with 16 males and 4 females. Their age ranged from 50 to 90 years with a mean of 64.9 years. Fourteen patients who were not able to eat orally resumed partial (n=7) or normal oral diet (n=7) after treatment. Four patients who were taking modified diet could upgrade their diet to optimal or normal diet. Two patients who could not eat orally remained the same after intervention. The overall response rate was 18 (90%) out of 20 in which 14 (87.5%) out of 16 non-oral patients could resume partial or normal oral feeding.

Conclusion: NMES is highly effective in treating post-stroke dysphagia. However, the study was limited by its small sample size and non-blind nature and the masking effect of spontaneous recovery. Future randomised controlled study is warranted to explore the true potential of NMES.

51 The impact of the Oncotype DX® Breast Cancer Assay on treatment decisions for women with oestrogen receptor–positive, node-negative breast carcinoma in Hong Kong

RCY Leung, TCC Yau, MCM Chan, SWW Chan, TWC Chan, YYY Tsang, TT Wong, C Chao, C Yoshizawa, IS Soong, WH Kwan, CCH Kwok, JSJ Suen, RKC Ngan, PSY Cheung
Department of Medicine, Queen Mary Hospital, Hong Kong

Introduction: The Oncotype DX® Breast Cancer Assay is a validated multigene molecular test designed to assess risk of distant recurrence and likelihood of chemotherapy (CT) benefit in ER+ early stage breast cancer (ESBC) patients in various populations. In Hong Kong, over 80% of breast cancers are ESBC and >60% of these women receive CT. This prospective decision impact study measured changes in CT type and recommendations, as well as physician impression of assay impact in a homogeneous Chinese population.

Methods: Consecutive patients with ER+, T1-3 N0-1mi M0 ESBC were offered enrolment. After surgery, physicians discussed treatment options with patients, then ordered the assay, and reassessed treatment recommendation considering assay results. Changes in treatment recommendation, CT utilisation, physician confidence, physician rating of influence on their treatment recommendation were measured.

Results: A total of 146 evaluable patients received pre- and post-testing treatment recommendations. CT recommendations (including changes in type intensity of CT) were changed for 34 (23.3%) patients (95% confidence interval [CI], 16.7%-31.0%); change in intensity occurred only in seven (4.8%). There were 27 (18.5%) changes in treatment recommendations of adding or removing CT altogether (95% CI, 12.6%-25.8%). CT recommendations decreased from 52.1% to 37.7%, a net absolute reduction of 14.4% (P<0.001 by McNemar's test; 27.6% net relative reduction). At pre-assay stage, 95% of physicians agreed/strongly agreed that they were confident in their treatment recommendation. At post-assay stage, 91% of physicians agreed/strongly agreed with the same statement. Overall, 30% of physicians agreed/strongly agreed that the test had influenced their recommendation, similar to the proportion of changed recommendations.

Conclusions: The Oncotype DX Assay appears to influence physicians' ESBC adjuvant treatment recommendations in Hong Kong.

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52 Vitamin binding protein, 25-hydroxyvitamin D and bioavailable vitamin D status in the Hong Kong Chinese population

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: The association between serum 25-hydroxyvitamin D [25(OH)D] and chronic diseases like diabetes, hypertension, and cardiovascular diseases is controversial. Recently, racial differences in bone mineral density between white and black Americans were explained by the levels of bioavailable vitamin D, which suggested that it might be a better marker of vitamin D status than 25(OH)D. We aimed to investigate if bioavailable vitamin D was a better vitamin D marker than 25(OH)D in the Hong Kong Chinese population.

Methods: Serum 25(OH)D and vitamin D binding protein (DBP) levels were measured in 2674 participants (592 men, 2082 women; mean [± standard deviation] age, 49.2 ± 16.2 years) in the Hong Kong Osteoporosis Study. The levels of free and bioavailable vitamin D were calculated using the equation developed by Vermeulen et al. Their associations with serum calcium, phosphorus, and parathyroid hormone (PTH) concentrations were evaluated using ANCOVA with adjustment for age and sex.

Results: The mean levels of 25(OH)D and DBP were 50.3 nmol/L ± 13.7 nmol/L and 178.8 μg/mL ± 74.0 μg/mL, respectively, and calculated free and bioavailable vitamin D levels were 8.79 pg/mL ± 4.23 pg/mL and 3.50 ng/mL ± 1.67 ng/mL, respectively. Serum 25(OH)D level was significantly associated with serum calcium, phosphorus, and PTH levels (P<0.05). In contrast, bioavailable vitamin D levels were only associated with serum calcium, while free vitamin D levels were only associated with serum phosphorus.

Conclusion: Bioavailable vitamin D and free vitamin D correlate less well with mineral metabolism markers than 25(OH)D in the Hong Kong Chinese population. Whether genetic variations affecting 25(OH)D and DBP play a role in the association remains to be determined.

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Efficacy of disease-modifying drugs in neuromyelitis optica spectrum disorders

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SST Leung, RSK Chang, SYY Pang, JCY Lee, KK Lau, KC Teo, KH Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Neuromyelitis optica spectrum disorders (NMOSD) are central nervous system (CNS) inflammatory disorders predominantly characterised by relapsing myelitis, optic neuritis, and encephalitis. NMOSD are probably due to autoimmunity against CNS aquaporin-4.

Methods: The response of Chinese patients to azathioprine, mycophenolate mofetil (MMF), and rituximab was retrospectively studied.

Results: A total of 45 patients (87% female; mean onset age, 44.3 [range, 20-71] years; median disease duration, 72.0 [range, 12-384] months) were studied. Of them, 38 were treated with azathioprine, but withdrawn early in eight (deranged liver function, leukopenia, and pancreatitis). Among 30 treated with azathioprine for 6 months or longer (mean, 55.3; range, 8-276 months), 25 (83.3%) had annualised relapse rate (ARR) reduced by 50% or more and 17 (56.7%) remained relapse-free. The median ARR decreased from 1.57 pretreatment to 0 during therapy (P=0.000). Nineteen (63.3%) had Expanded Disability Status Scale (EDSS) score improved or stabilised. Of the 45 patients, 10 (22.2%) were treated with MMF for 3 months or longer (mean, 29.1; range, 7-96 months). Eight (80%) had ARR decreased by 50% or more and six (60%) remained relapse-free. The median ARR decreased from 0.8 to 0 (P=0.028). Six (60%) had EDSS score improved or stabilised. Seven (13.7%) patients were treated with rituximab (mean duration, 27.3; range, 6-74 months) without intolerance. Six (85.7%) had ARR reduced by 50% or more and three (42.9%) became relapse-free. The median ARR decreased from 2.40 to 0.33 (P=0.018). Five (71.4%) had EDSS score improved or stabilised. Overall, three (6.7%) died from NMOSD and/or treatment complications.

Conclusion: We concluded that azathioprine, MMF, and rituximab are effective for prevention of relapse and disability progression in the majority of NMOSD patients.

Efficacy of beta-interferon in relapsing multiple sclerosis among Hong Kong Chinese

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SST Leung, RSK Chang, SYY Pang, JCY Lee, KK Lau, KC Teo, KH Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Multiple sclerosis (MS) is the most common central nervous system inflammatory demyelinating disorder characterised by relapsing neuroinflammation affecting different regions of the brain, cord, and optic nerves. Beta-interferon (β-IFN) is a first-line disease-modifying drug for MS.

Methods: We retrospectively studied the response to β-IFN of Chinese MS patients followed up in Queen Mary Hospital (QMH).

Results: A total of 92 Chinese MS patients who had been followed up by the Neurology Clinic of QMH with follow-up data available were studied. Their mean age at MS onset was 28.8 (range, 12-56) years, mean disease duration 163.3 (range, 6-468) months, and 71 (77.2%) were female. Of the 92 patients, 49 (53.3%) were treated with β-IFN and 41 (44.6%) received β-IFN for 12 months or longer. These 49 patients were studied in detail. Their median duration of β-IFN therapy was 32 (range, 5-204) months and median time from clinical onset to initiation of β-IFN therapy was 39 (range, 2-348) months. The mean annualised relapse rate (ARR) of the 49 patients decreased significantly from pre-treatment 1.19 to post-treatment 0.37; P=0.001 (median, 0.75 to 0.00). Of the 49 patients, 34 (69.4%) had ARR reduction by 30% or more. The median modified Rio score, a measure of clinical relapse and MRI disease activity, was 0.71 at 12 months of β-IFN therapy. Concerning disability, the mean Expanded Disability Status Scale (EDSS) score before β-IFN and after β-IFN therapy/at latest follow-up were 1.8 and 3.3 respectively (median, 2.0 and 2.5, respectively). Overall, 34 (37%) patients had EDSS score of 6 or more at the latest follow-up. Kaplan-Meier analysis reviewed the median time to EDSS score 6 of these 34 patients was 100 months, and there was no difference in median time to EDSS score 6 between those with and without β-IFN therapy for 12 months or longer (85 months vs 100 months; P=0.937 by log-rank test).

Conclusion: β-IFN therapy for 12 months or longer is moderately effective for prevention of clinical relapse but may not prevent/slow down disability progression in relapsing MS among Hong Kong Chinese.

Cognitive impairment in multiple sclerosis and neuromyelitis optica among Hong Kong Chinese

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SST Leung, CY Lee, KK Lau, RSK Chang, SYY Pang, RCN Ng, KH Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD) are important central nervous system inflammatory demyelinating disorders. Cognitive impairment is increasingly recognised in both MS and NMOSD.

Methods: We studied cognitive functions of Chinese MS and NMOSD patients followed up in Queen Mary Hospital by using a Chinese version of Rao's Brief Repeatable Battery of Neuropsychological Tests (BRBN). The BRBN consisted of five groups of tests: (1) Symbol Digit Modalities Test (SDMT) and (2) Paced Auditory Serial Addition Test (PASAT) for sustained attention, concentration and speed of information processing; (3) Selective Reminding Test (SRT) for verbal immediate and delayed recall memory; (4) 10/36 Spatial Recall Test (SPART) for spatial immediate and delayed recall memory; and (5) Word List Generation Test (WLG) for verbal fluency on semantic stimulus; yielding a total of 9 test scores. An abnormal score is defined as that below the fifth percentile of the normative value derived from healthy subjects. Patients with abnormal scores in three or more tests were diagnosed as having cognitive impairment.

Results: A total of 30 healthy subjects (16 men; mean age, 37.3; range, 21-56 years), 17 NMOSD patients (15 women; mean age, 47; range, 28-69 years), and 21 MS patients (14 women; mean age, 40.5; range, 18-59 years) were studied. The mean disease duration was 80.5 months, the mean latest Expanded Disability Status Scale (EDSS) score was 2.7, 13 (76.5%) were AQP4 antibody positive and 10 (58.8%) had magnetic resonance imaging brain abnormalities for the NMOSD patients. The mean disease duration was 141.7 months and the mean latest EDSS score was 3.5 for the MS patients. Among the 17 NMOSD patients, five (29.4%) had impaired SDMT, six (35.3%) had impaired SRT, seven (41.2%) had impaired SPART, 7 (41.2%) had impaired PASAT, and 7 (41.2%) had impaired WLG. Overall, eight (47.1%) NMOSD patients had cognitive impairment. Among the 21 MS patients, 15 (71.4%) had impaired SDMT, 10 (47.6%) had impaired SRT, 9 (42.9%) had impaired SPART, 14 (66.7%) had impaired PASAT, and 14 (66.7%) had impaired WLG. Overall, 15 (71.4%) MS patients had cognitive impairment.

Conclusion: Cognitive impairment is common among NMOSD and MS patients.

The effects of age and back pain duration on the clinical and radiological changes in patients with spondyloarthritis: results from a Chinese cohort

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P Li[1], HY Chung[1], CS Wong[2], HHL Tsang[1], CS Lau[1]
[1]Division of Rheumatology and Clinical Immunology, Queen Mary Hospital; [2]Department of Radiology, The University of Hong Kong, Hong Kong

Objective: To study the effects of age and back pain duration on the clinical and radiological manifestations in patients with spondyloarthritis (SpA).

Methods: A total of 126 SpA patients were recruited from the rheumatology clinics of Queen Mary Hospital and Pamela Youde Nethersole Eastern Hospital. Clinical data, tender/swollen joint counts (44 joints count), blood, modified Stoke Ankylosing Spondylitis Stroke Score (mSASSS), Spondyloarthritis Research Consortium of Canada (SPARCC) magnetic resonance imaging (MRI), apparent diffusion coefficient (ADC) values of diffusion-weighted imaging were compared between those aged >40 and ≤40 years in a cross-sectional manner. Variables with significant differences in univariate analyses were used as dependent variables in multivariate linear models. Back pain duration was also analysed independently using univariate and multivariate analyses.

Results: Multivariate analyses showed that increase in age was associated with increased tender (B=0.05; P=0.03)/swollen joint count (B=0.04; P=0.002), higher Bath Ankylosing Spondylitis Metrology Index (BASMI; B=0.03; P=0.003), Bath Ankylosing Spondylitis Functional Index (BASFI; B=0.04; P=0.02), and mSASSS (B=0.41; P=0.004). It was negatively associated with maximum sacroiliac (SI) joint ADC values (B= –0.01; P=0.02) and tendency to associate negatively with SPARCC MRI SI joints (B= –0.20; P=0.054). Back pain duration was associated positively with SPARCC MRI spine scores (B=0.21; P=0.04), mean spinal ADC values (B=0.01; p=0.05), and tendency with maximum spinal ADC values (B=0.01; P=0.07) in mutivariate linear models. It was also negatively associated with mean (B= –0.01; P=0.01) and maximum (B= –0.01; 0.04) SI joints ADC values.

Conclusion: Age is independently associated with increased radiological damages, worsened functional status and spinal mobility. Back pain duration is associated with increased extent of MRI-detected spinal inflammation as well as intensity. Both parameters are associated with less MRI-detected SI joint inflammation intensity. These findings are consistent with the traditional belief of 'ascending' axial inflammation in SpA.

A nested case-control study on Z-drug usage and risk of dementia

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TT Chan, WCY Leung, WKY Wong, WM Chu, KC Leung, YZ Ng, GYM Kai, V Li, YF Shea, RSK Chang, SL Ho
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: Benzodiazepines and Z-drugs (zaleplon, zolpidem, zopiclone) are commonly prescribed medications for the treatment of insomnia. While benzodiazepine use is shown to be associated with increased long-term risk of dementia, the association of Z-drug use and risk of developing dementia is unclear.

Methods: Chinese patients (182 controls, 91 cases) with more than 5 years of follow-up were recruited in a retrospective case-control study. Gender, age categories, and follow-up duration were matched. Data for Z-drug usage, benzodiazepine usage, and co-morbidities were collected. The primary outcome of interest was to investigate whether Z-drug ever use was associated with subsequent development of dementia.

Results: There was no significant difference in terms of ever-usage of Z-drugs between dementia subjects and controls (52.7% vs 45.6%; P=0.27). Multivariate logistic regression analysis adjusted for confounders and known risk factors of dementia showed no significant association between Z-drug ever use and dementia risk (adjusted odds ratio=1.34; 95% confidence interval [CI], 0.75-2.38). Univariate logistic regression analysis for Z-drug ever use also showed no significant association between Z-drug ever use and dementia risk (odds ratio=1.33; 95% CI, 0.80-2.21).

Conclusion: Z-drug ever usage is not significantly associated with increased risks of dementia.

Impact of antithrombotic therapy in atrial fibrillation on the presentation of coronary artery disease

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PH Chan, WH Li, JJ Hai, L Lam, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Background: Little is known about whether atrial fibrillation is a presentation of coronary disease. There is a paucity of knowledge about their causal relationship and also the impact of different antithrombotic strategies on the subsequent presentation of symptomatic coronary disease.

Methods: This was an observational study.

Results: We studied 7526 Chinese patients diagnosed with non-valvular atrial fibrillation and no documented history of coronary artery disease. The primary endpoint was the new occurrence of coronary artery disease—either stable coronary artery disease or acute coronary syndrome. After a mean follow-up of 3.2 ± 3.5 years (24 071 patient-years), a primary endpoint occurred in 987 (13.1%) patients. The overall annual incidence of coronary artery disease was 4.10%. No significant differences in age, sex, and mean CHA_2DS_2-VASc score were observed between patients with and without the primary endpoint. When stratified according to the antithrombotic strategies applied for stroke prevention, the annual incidence of coronary artery disease was 5.49%, 4.45%, and 2.16%, respectively in those prescribed no antithrombotic therapy, aspirin, and warfarin. Similar trends were observed in patients with acute coronary syndromes. Diabetes mellitus, smoking history, and renal failure requiring dialysis were predictors for primary endpoint in all antithrombotic therapies.

Conclusion: In patients with non-valvular atrial fibrillation, there is a modest association with coronary artery disease. Patients prescribed warfarin had the lowest risk of new-onset coronary artery disease.

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Use of the SAMe-TT2R2 score to predict good anticoagulation control with warfarin in Chinese patients with atrial fibrillation: relationship to ischaemic stroke incidence

PH Chan, WH Li, JJ Hai, L Lam, HF Tse, CW Siu
Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: The efficacy and safety of warfarin therapy for stroke prevention in atrial fibrillation (AF) depends on the time in therapeutic range (TTR). We aimed to assess the predictive ability of SAMe-TT_2R_2 score in Chinese AF patients on warfarin, whose TTR is notoriously poor.

Methods: This was a single-centre observational study. Patients with non-valvular AF on warfarin diagnosed between 1997 and 2011 were stratified according to SAMe-TT_2R_2 score, and TTR was calculated using Rosendaal method. The predictive power of SAMe-TT_2R_2 scores for good TTR (ie >70%) was assessed.

Results: We included 1428 Chinese patients (mean age, 76.2 ± 8.7 years; 47.5% male) with non-valvular AF on warfarin. TTR decreased progressively with increasing SAMe-TT_2R_2 score (P=0.016). When the cut-off value of SAMe-TT_2R_2 score was set to 2, the sensitivity and specificity to predict TTR of <70% was 85.7% and 17.8%, respectively. The corresponding positive and negative predictive values were 10.1% and 92.0%. After a mean follow-up of 4.7 ± 3.6 years, 338 patients developed an ischaemic stroke (4.96%/year). Patients with TTR of ≥70% had a lower annual risk of ischaemic stroke of 3.67% compared with than those with TTR of <70% (5.13%).

Conclusion: The SAMe-TT_2R_2 score correlates well with TTR in Chinese AF patients, with a score of >2 having high sensitivity and negative predictive values for poor TTR. Ischaemic stroke risk increased progressively with increasing SAMe-TT_2R_2 score, consistent with poorer TTRs at high SAMe-TT_2R_2 scores. The majority of Chinese AF patients with SAMe-TT_2R_2 score of >2 should be considered for non-vitamin K antagonist oral anticoagulants rather than warfarin, allowing better stroke prevention opportunities.

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Induced-pluripotent stem cell–derived mesenchymal stem cells attenuate cigarette smoke–induced mitochondrial dysfunction and apoptosis in airway smooth muscle cells

X Li[1,2], C Michaeloudes[1], YL Zhang[2], QZ Lian[2], PK Bhavsar[1], MS Ip[2], KF Chung[1], JCW Mak[2]
[1]Airway Disease, National Heart & Lung Institute, Imperial College London, London, United Kingdom; [2]Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Cigarette smoking is the primary cause of chronic obstructive pulmonary disease (COPD). Oxidative stress-mediated mitochondrial dysfunction is believed to contribute to lung inflammation and remodelling. Mesenchymal stem cells were reported to attenuate lung damage in various animal models of COPD. The mechanisms are still unclear. We investigated the effect of human induced–pluripotent stem cell–derived mesenchymal stem cells (iPSC-MSCs) on cigarette smoke medium (CSM)–induced mitochondrial damage in primary airway smooth muscle cells (ASMCs). Mitochondrial transfer and paracrine effect were examined.

Methods: ASMCs were pre-stained with CellTrace Violet and were cultured alone or in the presence of iPSC-MSCs stained with the mitochondrial-specific dye MitoTracker. After treatment with CSM, mitochondrial transfer was determined by flow cytometry or fluorescence microscopy. In ASMCs, we determined mitochondrial reactive oxygen species (ROS) levels, mitochondrial membrane potential (MMP), and apoptosis by MitoSOX, JC-1, and Annexin V staining, respectively. To study paracrine effects, the co-cultured cells were separated by Transwell inserts.

Results: CSM (25%) increased mitochondrial ROS levels, reduced MMP, and increased apoptosis in ASMCs. Co-culture with iPSC-MSCs attenuated CSM-induced mitochondrial ROS levels (4-fold vs 6.71-fold; P<0.05) and apoptosis (29.8% vs 40.6%; P<0.01), whilst it partially reversed the reduction in MMP (0.78-fold vs 0.52-fold; P<0.01). CSM treatment increased the percentage of ASMCs positive for iPSC-MSC-derived (MitoTracker-stained) mitochondria (75.5% vs 18.8%; P<0.001). Mitochondrial transfer was also confirmed by observation of iPSC-MSC–derived mitochondria in ASMCs by fluorescence microscopy. Co-culture using the Transwell system also led to attenuation of CSM-induced mitochondrial ROS in ASMCs. However, the CSM-induced drop in MMP and increased apoptosis were not attenuated by the Transwell co-culture.

Conclusions: Co-culture of iPSC-MSCs and ASMCs leads to protection of ASMCs against CSM-induced mitochondrial dysfunction and apoptosis. Mitochondrial transfer from iPSC-MSCs to ASMCs may be a mechanism mediating this protective effect. Paracrine regulation may also be a contributing factor. Our findings illustrate the potential of iPSC-MSCs as a novel therapy for airway diseases and demonstrate that their action might be driven by multiple mechanisms.

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Paracrine effect of human mesenchymal stem cell on cigarette smoke medium–exposed human AC16 cardiomyocytes

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YM Liang[1], YL Zhang[1], MSM Ip[1,4], HF Tse[1], 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: Cigarette smoking is the major risk factor for cardiovascular disease (CVD). Mesenchymal stem cells (MSCs) are emerging as a promising therapeutic agent for tissue regeneration, especially in CVD. The biologically active molecules secreted from MSCs have been found to exert the paracrine actions in many processes of cardiac repair, including cardiac regeneration, neovascularisation, inflammation, apoptosis, remodelling, contractility, and metabolism. The aim of this study was to investigate the effects of secretions from MSCs in a cigarette smoke medium (CSM)–exposed AC16 cardiomyocytes model in vitro.

Methods: The AC16 cardiomyocyte cell line was cultured in DMEM/F12 containing 12.5% fetal bovine serum, in a CO_2 incubator at 37°C. When cells reached 70% confluence, the medium was replaced with medium consisting of 1% fetal bovine serum 24 hours before treatment. CSM and conditioned medium (CdM) from bone marrow–derived mesenchymal stem cell (BM-MSC) or induced pluripotent stem cell–derived mesenchymal stem cell (iPSC-MSC) were added into cells and incubated for 24 hours.

Results: CdM from iPSC-MSC had a superior effect than that from BM-MSC in attenuating CSM-induced reactive oxygen species production via the reversal of total antioxidant capacity, and enzyme activities of superoxide dismutase and catalase. CdM from iPSC-MSC but not from BM-MSC inhibited CSM-induced NF-κB and p38 MAPK activation. Furthermore, CdM from iPSC-MSC inhibited cell apoptosis caused by CSM exposure along with significant reversal of Bax/Bcl-2 ratio.

Conclusion: These data suggest that iPSC-MSCs mediate direct protective effects on cardiomyocytes by inhibiting cell injury through the paracrine action of unknown secretory factor with anti-oxidative and anti-apoptotic properties, via the inhibition of NF-κB and p38 MAPK signalling pathways in vitro.

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Higher susceptibility of LRRK2 R1441G knockin mice to rotenone

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HF Liu[1], PWL Ho[1,3], GCT Leung[1], CSC Lam[1], LF Li[1], MHW Kung[1], ZJ Zhou[2], DB Ramsden[4], SL Ho[1,3]
Departments of [1]Medicine; [2]Biochemistry; [3]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong; [4]Department of Clinical and Experimental Medicine, University of Birmingham

Introduction: LRRK2 is linked to mitochondria functions. However, the pathogenic mechanism of LRRK2 mutation in striatal dopamine (DA) homeostasis and mitochondria dysfunction is unknown. We aimed to assess the susceptibility of LRRK2 R1441G knockin mice against (1) striatal DA uptake deficit, (2) locomotor inactivity, and (3) neuronal cell death, as induced by rotenone (mitochondrial complex-I inhibitor).

Methods: Cell viability of primary DA and cortical neurons from R1441G knockin (KI) mice and wild-type (WT) littermates were compared by immunohistochemistry or MTT assay after rotenone exposure. [3H]-DA uptake in isolated striatal synaptosomes from young (3 months old) KI mice incubated with rotenone was compared with WT littermates. Locomotor activity in open-field test after chronic (20 weeks) oral gavage of rotenone was also assessed.

Results: Without rotenone, R1441G mutant mice showed no overt phenotype. However, synaptosomes from young (3 months old) mutant mice exhibited lower DA uptake when incubated with rotenone (100 nM), when compared with WT controls. Number of DA neurons and cortical neurons, and intracellular ATP of cortical neurons in mutant culture with rotenone exposure was significantly lower. Also, chronic exposure to rotenone (5 mg/kg, twice per week orally) for 20 weeks caused significantly lower locomotor activity in mutant mice compared with the WT controls.

Conclusion: Despite the apparent lack of abnormal phenotype in our *LRRK2*$^{R1441G/R1441G}$ KI mice, current results showed that a differential functional susceptibility can still exist. Primary neurons and locomotor activity appeared normal in these KI mice under normal condition. However, as what we have shown recently, they are more liable to DA depletion and locomotor inactivity. This differential susceptibility against rotenone toxicity of the mutant mice suggested that LRRK2 R1441G mutation may be a predisposing genetic factor in synaptic energy deficiency leading to early striatal synaptic dysfunction, and later nigrostriatal DA cell death in LRRK2-associated Parkinson's disease. How pre-synaptic defects, abnormal DA homeostasis, and related mitochondria dysfunction lead to neuronal cell death associated with LRRK2 mutations require further investigations.

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Age difference in responses to cigarette smoke–induced injury in a rat model

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KWK Liu[1,3], YT Cui[1], 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: Cigarette smoking is the major cause of chronic obstructive pulmonary disease. Recent theories suggest that older individuals may be more prone to the effects of cigarette smoking. This study aimed at studying the effects of ageing on inflammatory responses using a rat model of passive smoking.

Methods: Male Sprague-Dawley rats of 4 months old (young) or 8 months old (grown-up adult) were exposed to air as control or cigarette smoke (CS; 4%) twice daily for 1 hour each for 7 consecutive days. Lung function was measured using whole-body plethysmograph (DSI) before the commencement of CS exposure and after the last exposure. On the day of sacrifice, bronchoalveolar lavage fluid (BALF) was collected, total cells were counted, and cytospins were prepared. Levels of CINC-1 and MCP-1 in BALF were measured using ELISA.

Results: Short-term CS exposure led to an increase in cell number in BALF (total, neutrophils and macrophages), accompanied by an increase in CINC-1 and MCP-1 levels compared to control treatment. There was a trend of a heightened response in the older group compared with the young group. In addition, there was a reduction in tidal volume in the older rats after 7 days of CS exposure, which was not found in the young rats.

Conclusion: We conclude that the age of the animals could affect the inflammatory responses to short-term cigarette smoking.

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Loss of MDM2 triggers adipocyte apoptosis leading to lipodystrophy and metabolic disorders

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Z Liu, A Xu, KKY Cheng
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Adipocyte apoptosis is increased in human and rodents with obesity and lipodystrophy; both chronic diseases are associated with a cluster of cardiometabolic dysfunctions. The MDM2-p53 pathway plays an important role in regulating cell survival and apoptosis, yet their role in adipocyte biology remains unclear. In this study, we employed adipocyte-specific MDM2 knockout (A-MDM2KO) mice to investigate the physiological role of MDM2 in adipose tissue functions.

Methods: A-MDM2KO mice were generated by crossing $MDM2^{floxed}$ mice with transgenic mice expressing Cre recombinase under the control of adiponectin promoter, which was only expressed in mature adipocyte. Male A-MDM2KO mice and its wild-type littermates fed with standard chow were used. The parameters related to glucose, energy, and lipid metabolism were measured during the 24-week monitoring period.

Results: A-MDM2KO mice displayed increased food intake and body weight, accompanied with reduction in serum levels of leptin and adiponectin, which are two major hormones secreted from adipocytes. Genetic deletion of MDM2 in adipocyte caused diabetes, fatty liver disease, hyperlipidaemia, and impaired adaption to cold environment and starvation. Further analysis revealed that white and brown adipose tissues were progressively diminished in A-MDM2KO mice, suggesting that they were lipodystrophy. In-vitro experiment indicated pre-adipocyte isolated from A-MDM2KO mice underwent normal adipogenesis. In contrast, adipocytes lack of MDM2 displayed increased apoptosis, perhaps due to expression of the pro-apoptotic protein p53. These data suggested that lipodystrophy in A-MDM2KO mice was due to increased adipocyte apoptosis but not impaired adipogenesis. We will further explore whether the actions of MDM2 on adipocyte is mediated via a p53-dependent pathway using a MDM2-p53 double knockout mouse model in our future study.

Conclusion: Our results indicated that MDM2 is a key player in maintaining adipocyte tissue function by regulating adipocyte survival and apoptosis.

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Expression and functional roles of large tumour suppressor 2 (LATS2) in lung adenocarcinomas

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SY Luo, DCL Lam
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Being first identified within the *Hippo* pathway, large tumour suppressor 2 (*LATS2*) kinase has played a key role in multiple biological processes known to be important in carcinogenesis. In some tumours, such as malignant mesothelioma, colorectal cancer as well as breast cancer, decreased *LATS2* expression has been associated with increased tumour growth, suppressed apoptosis and/or metastasis. However, *LATS2* was reported to be overexpressed and promote tumourigenesis in certain cancers, for example, nasopharyngeal carcinoma and acute myeloid leukaemia. The expression status and the functional roles of *LATS2* kinase in lung adenocarcinomas (AD) have not been explored.

Methods: The expression levels of *LATS2* mRNA in primary lung AD tissues were assessed by real-time polymerase chain reaction. Transient and stable in-vitro silencing of *LATS2* has been established by transfecting cells with *LATS2*-specific siRNA duplexes and viral particles with *LATS2*-specific shRNA, respectively. The percentage of cells undergoing apoptosis was determined by Annexin-V staining assay, and the invading ability of cells was evaluated by Matrigel invasion assay. The expression status of different regulators was measured by Western blotting.

Results: Decreased *LATS2* mRNA expression was an independent predictor for shorter disease-free survival and overall survival in patients with resected pulmonary AD. In-vitro study revealed that the regulation of *LATS2* kinase on apoptosis and cancer cell invasion was cell context dependent: in low *LATS2*-expressed epidermal growth factor receptor (*EGFR*) wild-type lung AD cells and mesothelioma cells, *LATS2* exhibited tumour suppressive roles by promoting apoptosis and suppressing cell invasiveness. Nevertheless, in *EGFR* wild-type lung AD cells with high *LATS2* expression, this kinase showed oncogenic functions by diminished apoptosis as well as enhancing the invasive phenotypes.

Conclusion: *LATS2* mRNA expression is a significant predictor of survival in resected lung AD patients. *LATS2* plays roles in regulation of apoptosis and cell invasion dependent on the nature of the tumour cell.

White matter hyperintensities, cerebral microbleeds, and medial temporal lobe atrophy in high-risk non-demented adults: prevalence and progression over 2 years

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J Kwan, SF Mak, A Har, R Azman, H Mak
Department of Medicine, The University of Hong Kong, Hong Kong

Background: Magnetic resonance imaging (MRI) markers of small vessel disease (white matter hyperintensities [WMH]) and cerebral microbleeds [CMB]) and medial temporal lobe atrophy (MTLA) are frequent findings in patients with stroke and dementia. This was a hospital-based study to examine the prevalence of these MRI abnormalities among high-risk non-demented adults, and the risk factors for progression over 2 years.

Methods: We analysed the data for 29 older non-demented adults (mean ± standard deviation age was 73.8 ± 8.5 years; 66% male) with high baseline vascular risk. Each patient had undergone two brain MRI scans with a mean interval of 2 ± 0.9 years. 3T MRI brain images were retrospectively examined for the severity and location of WMH (Fazekas Score 0-3, >1 abnormal), CMB (Microbleed Anatomical Rating Scale score 0-3, >0 abnormal), and MTLA (Scheltens Score 0-4, >1 abnormal). Comparisons were made between the first and second MRI for each patient.

Results: Among our 29 participants, 38% were current/ex-smokers, 83% had hypertension, 35% had diabetes mellitus, 21% had coronary heart disease, 65% had previous stroke, 62% had hyperlipidaemia, and 7% had atrial fibrillation (AF). For medications, 35% were on statins, 69% on antiplatelet agents, and 10% on oral anticoagulants. MRI at baseline showed that 100% had subcortical WMH, 83% had periventricular WMH, 57% had CMB, and 38% had MTLA. During a mean interval of 2 years, 33% had progression of WMH, 32% had higher numbers of CMB (16% had worse CMB grades), and 38% had progression of MTLA. Patients with AF were more likely to experience WMH progression (P=0.038), and patients with diabetes were more likely to experience MTLA progression (P=0.01). Age, other vascular risk factors, and prior antithrombotics and statin use were not significantly correlated with progression of MRI abnormalities. Multiple logistic regression analyses found no independent predictive factors.

Conclusion: Among older non-demented adults with high cardiovascular risk, MRI markers of small vessel disease and MTLA are highly prevalent. One in three patients demonstrate rapid progression in WMH, CMB, and MTLA within 2 years. AF and diabetes may exacerbate cerebral ageing and progressive vascular damage, but their independent risks are unclear. Future trials should examine the effectiveness of vascular preventive interventions (eg use of non-vitamin K anticoagulants for AF) on cerebral protection.

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Prevalence and clinical impact of white matter hyperintensities, cerebral microbleeds, and medial temporal lobe atrophy in transient global amnesia

J Kwan, B Ng, G Kai, R Hui, K So, R Azman, H Mak
Department of Medicine, The University of Hong Kong, Hong Kong

Background: Transient global amnesia (TGA) is a transitory, short-lasting neurological disorder characterised by a sudden onset of antero- and retrograde amnesia. The pathophysiology of TGA remains unclear. This study examined the prevalence and clinical impact of white matter hyperintensities (WMH), cerebral microbleeds (CMB), and medial temporal lobe atrophy (MTLA) in TGA patients versus normal controls.

Methods: We analysed the data for 13 patients with TGA and 11 age-matched cognitively normal controls recruited from Memory and Geriatric Medicine Clinics of the Department of Medicine, Queen Mary Hospital. The control subjects had no history of dementia, stroke, Parkinson's disease, head injury, seizures, and cancers within 5 years, and end-stage organ failure, excessive alcohol or drug use, or psychiatric disease. 3T magnetic resonance imaging (MRI) brain scans were retrospectively examined for the severity and location of WMH (Fazekas Score 0-3, >1 abnormal), CMB (Microbleed Anatomical Rating Scale score 0-3, >0 abnormal), and MTLA (Scheltens Score 0-4, >1 abnormal).

Results: Mean (± standard deviation) ages were similar for TGA patients (63.8 ± 5.5 years) and controls (66.4 ± 1.7 years). Gender, statin use, and history of diabetes, hypertension, hyperlipidaemia, and ischaemic heart disease were similar. TGA patients were more likely to be smokers (P=0.009). Controls were more likely to have atrial fibrillation (P=0.04) and take anticoagulants (P=0.04). In the TGA group, only 1 of 13 patients had a history of stroke or TIA. Comparing TGA with the control groups, the prevalence of (a) abnormal periventricular WMH was 0% vs 9% (P=0.27); (b) abnormal subcortical WMH was 23% vs 73% (P=0.015); (c) CMB was 15% vs 64% (P=0.15); and (d) abnormal MTLA was 8% vs 18% (P=0.44). Severity grades of MRI abnormalities were significantly different between TGA and control groups for periventricular WMH (TGA worse, P<0.001), subcortical WMH (controls worse, P=0.02), CMB (controls worse, P=0.046), but not MTLA.

Conclusion: Overall, TGA patients have a low prevalence of small vessel disease and MTLA on MRI, as compared to age-matched cognitively normal controls. Higher rates of atrial fibrillation and anticoagulant use may have accounted for more severe subcortical WMH and CMB among controls. Higher rate of grade 1 severity of periventricular WMH among TGA patients may possibly be a result of smoking exposure. Future prospective studies are warranted to examine the role of MRI abnormalities in understanding the pathophysiology of TGA.

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The difference of expression level of alpha 7 nicotine acetylcholine receptors in the mutant and wild-type non–small-cell lung cancer

ZY Ning, HH Kwok, KH Chan, DCL Lam
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Lung cancer is the leading cause of cancer mortality worldwide. Epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitors (TKI) could be used upfront to treat advanced-stage non–small-cell lung cancer (NSCLC) patients with sensitising *EGFR* mutations. However, the NSCLC patients with *EGFR* wild-type are resistant to the TKI treatment and the *EGFR*-mutant patients would eventually become resistant to TKI because of secondary mutations. The activation of nicotine acetylcholine receptors (nAChRs) plays a role in the lung carcinogenesis via several pathways. There is also evidence that the nAChRs have a transactivation effect onto the *EGFR* pathways. We investigated the difference of expression level of alpha 7 nicotine acetylcholine receptors in the *EGFR* mutant and wild-type NSCLC.

Methods: We used real-time polymerase chain reaction to detect the RNA expression level of alpha 7 nAChRs in 12 cell lines. These 12 cell lines consisted of four *EGFR* wild-type cell lines, six *EGFR* mutant cell lines including the 19 deletion and L858R, one mesothelioma cell line, and one lung squamous–cell cancer cell line.

Results: The RNA expression level of alpha 7 nAChRs in lung squamous–cell cancer cell line was the highest, followed by the *EGFR*-mutant lung adenocarcinoma cell line than in *EGFR* wild-type lung adenocarcinoma.

Conclusion: These results indicate that activation of nAChRs may modulate the EGFR growth signalling pathways in the NSCLC.

Inhibition of autophagy by CLEC16A through modulation of mTOR nutrient sensing pathway

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RCY Tam, YT Pang, CS Lau, VSF Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The human gene *CLEC16A* has been shown to genetically link to autoimmune diseases such as multiple sclerosis and systemic lupus erythematous in a number of genome-wide association studies. There have been studies demonstrated the protein function of CLEC16A in regulating autophagy, an evolutionally conserved cellular event to remove unwanted material for degradation or recycle. The Drosophila ortholog of CLEC16A, EMA, has been reported for its physiological role in the regulation of endosomal trafficking and autophagy. A recent study reported that CLEC16A silencing leads to autophagy dysfunction in thymic epithelial cells, which resulted in T cell hyporeactivity that may give rise to disease protection in non-obese diabetic mouse model. Yet the underlying mechanism of how CLEC16A directly exerts effect on autophagy is still largely unknown. Here we aimed to dissect the physiological role of CLEC16A in the regulation of autophagy in mammalian cells.

Methods: Mammalian CLEC16A-overexpressing cells underwent starvation to access autophagic activity. Western blotting was performed to measure relative amounts of proteins upstream to the autophagy pathway to determine how CLEC16A may modulate autophagy.

Results: Upon nutrient deprivation, autophagic flux was significantly reduced in cells over-expressing 16a compared to the control. Results from immunoblotting further revealed that 16a over-expressing cells showed an increased basal level of phosphorylated form of mammalian target of Rapamycin (mTOR), which is a key protein involved in nutrient-sensing pathway, and proteins that are downstream to mTOR, including p60S6 kinase and 4E-binding protein 1.

Conclusion: Our results suggest that CLEC16A negatively regulates starvation-induced autophagy by modulating the mTOR nutrient-sensing pathway.

A 1-year prospective study on cognitive decline in patients on peritoneal dialysis and their clinical outcomes

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

Introduction: Cognitive impairment (CI) may have a negative impact on the outcome of patients on peritoneal dialysis (PD), including the risk of peritonitis or exit-site infection (ESI). We reported that 13.9% of local PD patients showed CI according to the Cantonese version of Mini-Mental State Examination (CMMSE).

Objective: We examined the longitudinal change in CMMSE score of PD patients and investigated the risk factors associated with a decline in cognitive function and its potential impact on peritonitis and ESI.

Methods: This was a 1-year prospective single-centre cohort study. CMMSE was performed in patients newly started on PD between July 2011 and July 2014, and repeated 1 year later. Demographic and clinical data including co-morbidities, medications, peritonitis, and ESI episodes were collected. CI was determined by locally defined CMMSE cut-off.

Results: A total of 123 patients were included, and 91 (74%) patients underwent the second CMMSE assessment. The prevalence rate of CI was 10.6% at baseline and 10.8% at 1 year. Overall, 46 patients showed cognitive decline over 1 year. Age of ≥65 years (46.7% vs 26%; P=0.04) and a lower residual renal function at baseline (3.9 ± 3.0 vs 5.2 ± 3.5 mL/min; P=0.05) were associated with cognitive decline. Only age of ≥65 years remained a significant risk factor for cognitive decline after 1 year in multivariate analysis (odds ratio=2.56; 95% confidence interval, 1.03-6.37; P=0.04). In patients who performed PD exchanges themselves, those who showed or did not show cognitive decline (n=36 in each group) had similar rates of PD-related peritonitis (P=0.77), ESI (P=0.28), and hospitalisation (P=0.38).

Conclusion: PD patients of ≥65 years are at increased risk of cognitive decline. Cognitive decline was not associated with altered risks of peritonitis, ESI, or hospitalisation during the 1-year observation period.

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A systematic review of familial Alzheimer's disease during 1991-2014: differences in presenting clinical features among three mutated genes and potential ethnic differences

YF Shea, LW Chu, AOK Chan, J Ha, Y Li, YQ Song
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: There are great diversities of clinical phenotypes among the familial Alzheimer's disease (FAD) families.

Methods: We systematically reviewed all the previously reported cases of FAD and performed comparisons between Asian and Caucasian patients. We collected individual-level data from 658 pedigrees.

Results: Patients with presenilin 1 (PSEN1) mutations had the earliest age of onset (AOO) (43.3 ± 8.6 years; P<0.001) and were more commonly affected by seizures, spastic paraparesis, myoclonus and cerebellar signs (P<0.001, P<0.001, P=0.003, and P=0.002, respectively). PSEN2 mutations had a delayed AOO with longest disease duration and presented more frequently with disorientation (P=0.03). Amyloid precursor protein (APP) mutations presented more frequently with aggression (P=0.02) and APP duplication presented more frequently with apraxia (P=0.03). PSEN1 mutations in codon preceding 200 had an earlier AOO than those above codon 200 (41.4 ± 8.0 vs 44.7 ± 8.7; P<0.001). Since 42.9% of the mutations reported are novel, the mutation spectrum and clinical features in Asian FAD families could be different from Caucasian patients. Asian patients with PSEN1 mutations presented more frequently with disorientation (P=0.02) and personality change (P=0.01), but less frequently with atypical clinical features. Asian patients with APP mutations presented less frequently with aphasia (P=0.02).

Conclusion: Clinical features could be modified by underlying mutations. Asian FAD patients may have different clinical features when compared with Caucasians.

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Impact of ^{18}FDG-PET and PiB PET brain imaging in the diagnosis of Alzheimer's disease and other dementias in a regional memory clinic

YF Shea, J Ha, SC Lee, LW Chu
Department of Medicine, Queen Mary Hospital, Hong Kong

Introduction: Pathological and functional brain biomarkers are feasible in improving the diagnosis of dementia subtypes. There has not been any study on the impact of functional imaging with or without pathological imaging on the diagnoses of dementia subtypes among Chinese in Memory clinic setting.

Objective: To investigate the improvement in the accuracy of diagnoses of dementia subtypes among Chinese dementia patients who received positron emission tomography (^{18}FDG-PET) with or without Pittsburgh Compound B (collectively as PET±PIB).

Methods: This was a retrospective cohort study. Data including age, sex, education level, Mini-Mental State Examination scores (MMSE), clinical dementia rating (CDR), neuropsychiatric inventory score, neuroimaging report, initial clinical diagnoses and final diagnoses after taking into account neuroimaging were collected from medical record. The frequency of accurate initial clinical diagnoses was analysed by descriptive statistics. The agreement between the initial and post PET±PIB dementia diagnoses was analysed by the Cohen's kappa (*k*) statistics.

Results: We recruited 109 subjects (56.9% female) who received PET±PIB between July, 2007 and December 2014. The overall accuracy of initial clinical diagnoses of dementia subtypes was 63.3%, and subsequently 36.7% of subjects received a change in diagnosis after PET±PIB. The rates of accurate initial clinical diagnosis (compared to the final post-imaging diagnosis) were 81.5%, 44.4%, 14.3%, 42.9%, 44.4% and 0% for Alzheimer's disease (AD), Dementia of Lewy Body (DLB), frontotemporal dementia (FTD), vascular dementia (VAD), other dementia and mixed dementia respectively. The agreement between the initial and final post-imaging dementia subtype diagnoses was only fair, with a Cohen's αof 0.297 (95% CI, 0.138 to 0.456). For the 22 subjects with PiB PET imaging, 17.4% of the AD subjects (PIB positive) were initially diagnosed to have non-AD dementia. For 7 patients with amnestic mild cognitive impairment, 2 out of 3 with imaging risk factors developed deterioration to dementia longitudinally while 4 out of 4 without imaging risk factors did not deteriorate.

Conclusions: PET±PIB brain imaging helps to improve the accuracy of dementia subtypes in 36% of our patients with underlying AD, DLB, VAD and FTD.

755-nm Picosecond laser for skin rejuvenation in Chinese

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SYN Shek[1], CK Yeung[1], JCY Chan[1], HHL Chan[1,2]
[1]Division of Dermatology, Department of Medicine, The University of Hong Kong, Hong Kong; [2]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, United States

Background and objective: It has been reported that the 755-nm picosecond laser is effective for tattoo removal. The newer focus lens array 755-nm handpiece can be used for non-ablative skin rejuvenation resulting in collagen and elastin production. The objective of the study was to assess the efficacy of the 755-nm picosecond laser for skin rejuvenation.

Methods: A total of 20 patients aged 35 to 60 years with signs of photodamage were recruited. A total of six treatment sessions were performed on each subject. Topical anaesthetic was applied for at least 30 minutes and the treatment parameters were 0.4 J/cm^2, 8-mm spot size, pulse rate of 5 Hz, and four passes. After treatment, a cool pad was provided if necessary. Standardised photographs were taken at baseline, treatment visits, and 1-3 months' follow-up visits. Two trained physicians assessed the photographs independently. Any adverse effects were documented during the study.

Results: Nine subjects underwent the study with up to five treatment sessions received. With reference to the latest global improvement assessment, 67% had a slight improvement in overall skin condition. Most experienced mild erythema after treatment, which subsided spontaneously. The mean satisfaction score on visual analogue scale at present was 6.8 and average pain score was 3.4. There were no adverse effects.

Conclusion: There is slight improvement in skin texture with the 755-nm picosecond laser with focus lens. Other than mild erythema, there are no adverse effects.

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The efficacy in reducing abdominal subcutaneous fat by a non-contact radiofrequency device in Chinese: a retrospective study

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SYN Shek[1], CK Yeung[1], JCY Chan[1], HHL Chan[1,2]
[1]Division of Dermatology, Department of Medicine, The University of Hong Kong, Hong Kong; [2]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, United States

Background and objective: A case study showed an increase in apoptotic index after treatment with a focused field radiofrequency device (Vanquish, BTL Industries Ltd). A safety study carried out by Pumprla et al showed that this treatment is safe and most efficient in moderate abdominal overweight with lower insulin resistance. The objective of this study was to assess the efficacy of this non-contact radiofrequency device for reducing abdominal fat and abdominal circumference.

Method: Thirteen healthy adult subjects with abdominal fat bulge and/or love handles who received treatment with the non-contact radiofrequency device in the past 12 months were included. The medical records of the subjects were reviewed. All the subjects received a total of six treatments with a 7- to 10-day interval. The duration of each session was 30 minutes. The initial power was 200 W and a tuning of ≥75% must be achieved throughout the treatment. Clinical photographs, abdominal circumference, and weight were measured at baseline, 1 week, 1 month, 2 months, and 3 months after the last treatment.

Results: Among 12 female and 1 male subjects, the mean age was 35 years with a normal mean body mass index of 23.8 kg/m^2. An average tuning power of at least 86% was reached for each session and a mean temperature of 40°C was achieved at the area treated. Ultrasound measurements of subcutaneous fat thickness showed statistically positive reduction in the flanks at all follow-up visits (P=0.012, 0.004, 0.016, 0.002) but not at the abdomen. There was reduction in waist circumference at all visits but it was not statistically significant. Overall, 78% experienced mild erythema and 13.5% moderate erythema, while 5.8% had mild oedema. In terms of adverse events, there were two (3.8%) incidents of blistering and two (3.8%) incidents of induration experienced.

Conclusion: The non-contact radiofrequency device showed a significant reduction in fat thickness at the flanks and a mild improvement in waist circumference.

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The efficacy and safety of a dual emission (1540 nm and 10 600 nm) fractional laser system for the treatment of acne scars in Chinese

SYN Shek[1], CK Yeung[1], JCY Chan[1], HHL Chan[1,2]
[1]Division of Dermatology, Department of Medicine, The University of Hong Kong, Hong Kong; [2]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, United States

Background and objective: Previous studies have shown that non-ablative and ablative fractional lasers are individually efficacious for the treatment of acne scars. While fractionated ablative lasers produce better results, they are associated with higher risk of adverse effects and longer downtime. The objective of this study was to assess the efficacy and safety of the dual emission approach with lower energy ablation and increased depth of coagulation.

Methods: Twenty healthy subjects aged 18 to 65 years with facial acne scar were recruited. Two treatments, 1 month apart, were provided. Prior to treatment, topical anaesthetic was applied for 30 minutes and locoregional nerve block to supraorbital nerve and facial nerves were administered. Two passes in sequential mode 1540 nm followed by 10 600 nm were applied. Standardised photographs were taken at baseline, 1, 3, and 6 months post-treatment. Two independent physicians analysed the photographs. Clinical scores were based on acceptable scale for acne scarring and for overall global improvement. Subjective improvement and satisfaction were also recorded.

Results: Six subjects completed the study, five subjects are being followed up, and one subject had completed one treatment. Preliminary results showed that nine out of 12 subjects rated moderate-to-good improvement at their last follow-up visit. In terms of physician assessment, 10 subjects completed 1-month follow-up, seven of them had mild-to-moderate improvement. Six of the eight subjects who completed 3-month follow-up had mild-to-good improvement. Six subjects completed 6-month follow-up and five had mild-to-good improvement.

Conclusion: Majority of subjects has various degrees of improvement in acne scar with no adverse effects.

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Optimal cut-offs of homeostasis model assessment of insulin resistance to identify dysglycaemia and diabetes

AZL Shih, OY Leung, CH Lee, CHY Fong, YC Woo, BMY Cheung, KSL Lam
Department of Medicine, The University of Hong Kong, Hong Kong

Objective: The homeostasis model assessment of insulin resistance (HOMA-IR) has been used extensively as an index of insulin resistance in clinical research. However, the normal reference value in Chinese has not been defined. This study aimed to establish HOMA-IR cut-offs for identifying dysglycaemia and type 2 diabetes mellitus (T2DM) in Southern Chinese, based on the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS), a population-based cohort study with long-term follow-up.

Methods: Data were analysed from 2779 Hong Kong Chinese subjects aged 25 to 74 years from CRISPS 1 (1995-1996) as baseline and followed up in CRISPS 4 (2010-2012). Normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and T2DM were defined according to the 1998 World Health Organization criteria. In our study, dysglycaemia referred to IFG, IGT, or T2DM. Super-control was defined as subjects who were NGT both at baseline and at CRISPS 4 (n=872). The optimal HOMA-IR cut-offs for dysglycaemia and T2DM were determined by the Youden index on the receiver operating characteristic (ROC) curve. Their sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were also calculated.

Results: The optimal HOMA-IR cut-offs to identify dysglycaemia and T2DM at baseline were 1.37 (area under the ROC [AUC]=0.735; 95% confidence interval [CI], 0.713-0.758; Se, 65.6%; Sp, 71.3%; PPV, 44.9%; NPV, 85.4%) and 1.97 (AUC=0.807; 95% CI, 0.777-0.886; Se, 65.5%; Sp, 82.9%; PPV, 29.8%; NPV, 95.6%), respectively. These cut-offs corresponded closely to the 75th (1.44) and 90th (2.03) percentiles, respectively, of HOMA-IR in the super-controls.

Conclusion: HOMA-IR cut-offs, derived from this long-term follow-up study, of 1.4 and 2.0 discriminated dysglycaemia and T2DM respectively from NGT in Southern Chinese. These cut-off values can serve as useful references in clinical research involving the assessment of insulin resistance.

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Adipocyte fatty acid–binding protein mediates adaptive thermogenesis by inducing intracellular activation of thyroid hormones

LL Shu, XP Wu, Y Pan, PC Lee, LY Cheong, KSL Lam, A Xu, RLC Hoo
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Adaptive thermogenesis is a defense mechanism that organism dissipates energy in the form of heat protecting against hypothermia or obesity. Adipocyte fatty acid–binding protein (A-FABP) is an adipokine that regulates fatty acid trafficking and signalling. A-FABP deficiency alleviates metabolic dysfunction while exacerbates high fat diet (HFD)–induced obesity in mice. Here, we explored the role and potential mechanism whereby A-FABP modulates adaptive thermogenesis.

Methods: (1) A-FABP knockout (KO) mice and their wide-type (WT) littermates were fed with standard chow or HFD for 24 weeks. (2) WT or KO mice with or without rA-FABP replenishment by osmotic pump were treated with thyroxine (T4) or 3,3,5-triiodothyronine (T3). Thermoregulatory ability of mice was assessed by measurement of energy expenditure and cold tolerance test. Fatty acid uptake in brown adipose tissue (BAT) was determined by in-vivo tracing of 3H-palmitate. Serum and BAT were harvested for measurement of thyroid hormone concentrations. BAT recruitment was assessed by biochemical analysis.

Results: Circulating A-FABP and its expression in adipose tissue were increased upon cold exposure or HFD feeding. A-FABP deficiency impeded HFD- and cold-induced energy expenditure in mice. Free fatty acid (FFA) uptake in BAT was attenuated in A-FABP KO mice. Moreover HFD- or cold-induced T4 to T3 conversion was significantly decreased in A-FABP KO mice. Restoration of rA-FABP markedly increased the FFA uptake in BAT and significantly improved thermogenesis by promoting BAT recruitment in A-FABP KO mice. The induction of type II deiodinase (D2), the key enzyme responsible for T4 to T3 conversion, was abolished in BAT of KO mice while rA-FABP replenishment reversed the condition and restored T4-induced energy expenditure in A-FABP KO mice.

Conclusion: A-FABP promotes adaptive thermogenesis by enhancing intracellular T4 to T3 conversion in BAT through its regulation on D2 activity and expression. A-FABP also facilitates the transportation of FFAs from white adipose tissue into circulation and enhances FFA uptake by BAT, further induces the expression and activation of UCP-1 for thermogenesis.

Acknowledgement
This study was supported by the National Key Basic Research Development Program-973Program (2015CB553603), RGC/ Collaborative Research Fund (C7055-14G), General Research Fund (HKU 766812M) and Shenzhen Basic Research Grant (JCYJ20140903112959965).

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Long-term survival after upfront matched sibling allogeneic haematopoietic stem cell transplant for multiple myeloma: a retrospective study at a single transplant centre in Hong Kong, 1991-2014

JPY Sim, AKW Lie
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: Induction with novel agents followed by autologous haematopoietic stem cell transplant (HSCT) is the current recommended upfront treatment for newly diagnosed transplant-eligible multiple myeloma (MM) patients. However, with this approach, the disease remains incurable. Allogeneic HSCT offers the prospect of long-term cure by immune-mediated graft-vs-myeloma effect but the expected advantage is cancelled out by its higher risk of transplant-related mortality (TRM). Reduced intensity conditioning (RIC) though has reduced TRM, has also increased risk of relapse. Studies from ABMTRR and Swiss-Blood-SCT have recently shown that patients who received transplant upfront versus late after relapse had significantly better outcome.

Methods: We retrospectively reviewed the long-term outcomes of all MM patients who underwent upfront (without prior autologous HSCT) allogeneic HSCT from matched siblings in our centre.

Result: A total of 45 patients (M:F=27:18) were identified. The median age at transplant was 49 (range, 35-63) years. All received matched sibling donor grafts (bone marrow, 15; peripheral blood stem cells, 30). Myeloablative conditioning (MAC) was used in 16 patients (busulfan plus cyclophosphamide, 13; total body irradiation and melphalan, 3). At the time of analysis, 34 (76%) patients had died, with a median follow-up of 124 (range, 16-240) months among surviving patients. The cause of death was progressive/relapsed disease in 25 (74%) patients. Patients aged ≤40 years receiving MAC (n=10) had significantly longer overall survival (OS) and progression-free survival (PFS) than older patients having MAC or RIC transplants (median OS, 90.8 vs 40.1 months, P=0.022; median PFS, 90.8 vs 12.5 months, P=0.005).

Conclusions: Our current study suggested that upfront matched sibling allogeneic HSCT using MAC can offer long-term cure to young newly diagnosed MM patients with tolerable toxicities. Though a very selected group, given the ultimately inevitable relapse after auto-HSCT, this strategy is worth exploring even in the era of novel agents especially among young (age ≤40 years) MM patients who have matched sibling donor.

Rifaximin for the treatment of functional dyspepsia: a double-blind randomised placebo-controlled trial

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VP Tan, KSH Liu, FYK Lam, IFN Hung, MF Yuen, WK Leung
Department of Medicine, The University of Hong Kong, Hong Kong

Objective: Gut dysbiosis may contribute to pain and bloating in patients with functional gastro-intestinal disease. We tested whether treatment with rifaximin would improve the symptoms of functional dyspepsia (FD).

Methods: Consecutive subjects with a diagnosis of FD as per the ROME III criteria were randomised to receive rifaximin 400 mg or placebo, all taken 3 times daily for 2 weeks. The investigators and study subjects were blinded to the allocation. Subjects were followed up for 8 weeks for symptoms and hydrogen breath tests performed. The primary endpoint was adequate relief of global dyspeptic symptoms (GDS). The secondary endpoint was relief of individual dyspeptic symptoms.

Results: A total of 86 subjects were recruited. At 8 weeks, there were significantly more subjects in the rifaxmin than in the placebo group who experienced adequate relief of GDS (77.5% vs 52.2%; P=0.02). A similar trend favouring rifaximin group was also noted in the preceding 4 weeks. Rifaximin was also superior to placebo in providing adequate relief of belching and postprandial bloating (PPB) in subjects at 4 weeks. Subgroup analysis revealed that female subjects had more significant response to rifaximin treatment (adequate relief of GDS at 4 weeks: 75.9% vs 41.7%, P=0.006; at 8 weeks: 79.3% vs 47.2%, P=0.008), as well as improvements in their belching and PPB at 4 weeks. The incidences of adverse effects were similar in both groups.

Conclusions: Treatment with 2 weeks of rifaximin led to adequate relief of GDS, belching, and PPB in subjects with FD. The difference was particularly marked in female subjects.

Adapting the low FODMAP diet to special populations: functional dyspepsia

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VP Tan
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Functional dyspepsia (FD) is a common disorder where subjects experience bloating, belching, epigastric pain, and discomfort. A subset of patients with FD experience triggers exclusively related to meals, known as postprandial distress syndrome in the Rome III guidelines. There is significant overlap of symptoms with another common functional gastro-intestinal disorder, irritable bowel syndrome (IBS). This review aimed to examine the current role of diet in the management of FD and FD/IBS overlap in East Asia, with an exploration of the likely efficacy and mechanisms of action of the low FODMAP diet in these subjects.

Methods: PubMed, EMBASE, and Google Scholar were screened for original studies and reviews examining the role of diet in the management of subjects with FD or FD/IBS overlap in East Asia. Mapping of the known mechanisms of action of the low FODMAP diet to current pathophysiological mechanisms suspected to contribute to symptoms in FD and IBS was undertaken.

Results: FD and FD/IBS overlap was common in the East Asia region. In particular, it was evident that a proportion of subjects with IBS may have been misdiagnosed with FD due to the predominance of upper gastro-intestinal symptoms in IBS in this region. Dietary manipulation was widely employed in the management of FD and FD/IBS overlap in East Asia but this occurred without the input of dieticians or in a structured fashion. In terms of the low FODMAP diet a reduction in delivery of highly fermentable substrates to the distal small intestine and large colon was likely to reduce the sensation of bloating and epigastric discomfort/pain in subjects with FD and FD/IBS overlap.

Conclusions: Dietary manipulation is widely employed by patients and clinicians in East Asia to manage FD and FD/IBS overlap. The low FODMAP diet appears to address some of the mechanisms implicated in the genesis of symptoms in FD and FD/IBS and holds great promise as a treatment modality.

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Blood lead level in the US population: analysis of the US National Health and Nutrition Examination Survey 1999-2012

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

Objective: Blood lead level is associated with increased mortality. Therefore, we analysed the updated trends in blood lead level in the US population.

Methods: Data were extracted from the US National Health and Nutrition Examination Survey (NHANES) in 1999 to 2012. For inclusion, participants must include blood lead measurements in the record. They were stratified according to sampling year and age. Data were analysed using SPSS version 22 complex sample module.

Results: There were 7970, 8946, 8373, 8407, 8266, 8793, and 7920 participants in NHANES 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, 2009-2010, and 2011-2012, respectively. Blood lead levels were (geometric mean [95% confidence interval]) 1.77 [1.72-1.81], 1.57 [1.54-1.61], 1.52 [1.48-1.55], 1.41 [1.38-1.44], 1.38 [1.35-1.41], 1.23 [1.21-1.25], and 1.09 [1.06-1.21] for adults aged 20 years or above, and 1.43 [1.39-1.47], 1.16 [1.13-1.19], 1.18 [1.15-1.21], 0.99 [0.96-1.01], 0.98 [0.96-1.01], 0.81[0.80-0.83], 0.66 [0.64-0.68] for children, respectively. Both decreasing trends were significant (P<0.001). Compared to children aged 7 years or above, children aged 6 years or below had significant higher blood lead levels (1999-2000: 1.24 [1.20-1.28] vs 2.08 [2.01-2.26]; 2001-2002: 1.03 [1.00-1.06] vs 1.65 [1.57-1.73]; 2003-2004: 1.03 [1.01-1.06] vs 1.69 [1.61-1.78]; 2005-2006: 0.86 [0.84-0.88] vs 1.41 [1.35-1.48]; 2007-2008: 0.85 [0.83-0.88] vs 1.46 [1.40-1.54]; 2009-2010: 0.72 [0.70-0.74] vs 1.15 [1.10-1.19]; 2011-2012: 0.59 [0.57-0.61] vs 0.93 [0.88-0.99]; P<0.001).

Conclusion: There is continuous decreasing trend in blood lead level in the US population during the period 1999-2012. The blood lead level in children aged 6 years or below is of concern.

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Inorganic mercury poisoning due to cosmetic cream in Hong Kong: a case series

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

Introduction: Physicians might prescribe corticosteroid in addition to chelation therapy in patients with inorganic mercury poisoning with the presence of proteinuria. However, there is controversy over the efficacy of corticosteroid in treating these patients. Therefore, there is a need to study a case series to evaluate patient characteristics and treatment outcome of inorganic mercury poisoning due to cosmetic cream for skin whitening.

Methods: Cases in 2008 to 2015 were identified and reviewed using the electronic database of the Hong Kong Poison Information Centre. For inclusion, patients must have documented inorganic mercury toxicity, use of cosmetic cream, and proteinuria. Patients were classified into two groups (dimercaptosuccinic acid chelation or combination therapy of dimercaptosuccinic acid and prednisolone) according to their treatment received during period of mercury toxicity. The primary outcome of the study was the duration to proteinuria remission. Data were analysed using SPSS statistics version 22.

Results: Seventeen patients were included in this study: 15 (88.2%) patients were female and 13 (76.5%) patients were Southeast Asians. Of the patients, 14 (82.4%) were treated with chelation, prednisolone, or combination therapy. Comparing patients with combination therapy to chelation only, there was no significant difference in the duration to achieve proteinuria remission (67.3 ± 4.91 vs 46.8 ± 12.5 days; P=0.307). Baseline blood mercury level correlated with duration to achieve proteinuria remission (r=0.79; P=0.004).

Conclusion: Inorganic mercury in certain skin-whitening creams can cause mercury poisoning and nephrotic syndrome. The duration of proteinuria is related to baseline blood mercury level and is not significantly affected by the addition of corticosteroid to chelation therapy.

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APPL2 in ventromedial hypothalamus regulates adaptive thermogenesis by promoting browning of subcutaneous white adipose tissue

B Wang, X Li, A Li, PWL Ho, Z Liu, KKL Wu, X Jiang, Y Zhou, L Jin, KSL Lam, A Xu, KKY Cheng
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Induction of browning in white adipose tissue (WAT), which is defined as transformation from white adipocytes to brown adipocytes, not only promotes adaptive thermogenesis but acts as a defensive mechanism to protect against obesity and its related metabolic disorders. Browning is tightly controlled by the cross-talk between hypothalamus and WAT, yet the underlying mechanism is unclear. In this study, we investigated whether the adaptor APPL2, a key player in adiponectin and insulin signalling, controls browning by mediating the cross-talk between hypothalamus and WAT.

Method: $APPL2^{flox/flox}$ mice were crossed with rat insulin promoter Cre transgenic mice or locally injected (in ventromedial hypothalamus) with adenovirus-associated virus (AAV) encoding Cre to generate hypothalamus and pancreatic β cell–specific APPL2 knockout (RIP-APPL2-KO) mice and hypothalamic-specific APPL2 KO mice (Hypo-APPL2-KO), respectively. Basic metabolic parameters were monitored for 24 weeks. Cold challenge was employed to induce browning in the above mice.

Results: RIP-APPL2KO mice displayed increased adiposity accompanied by a dramatic reduction of energy expenditure. These KO mice were cold sensitive in response to acute cold challenge. Browning program in subcutaneous WAT (sWAT) of RIP-APPL2-KO mice was almost absent during chronic cold acclimation, whereas no obvious change appeared in their brown adipose tissue. The impaired browning in RIP-APPL2-KO mice was due to decreased neuronal activation in raphe pallidus and subsequent diminished sympathetic outflow to sWAT. Likewise, AAV-mediated knockout of APPL2 in ventromedial hypothalamus recapitulated the phenotypes of RIP-APPL2-KO mice.

Conclusion: Our study uncovers a novel role of hypothalamic APPL2 in maintaining energy homeostasis by controlling browning in WAT.

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Macrophage polarisation plays a role in intermittent hypoxia (IH)–impaired subcutaneous adipogenesis in an IH-exposed mouse model

Y Wang[1], MYK Lee[1], JCW Mak[1,2,3], MSM Ip[1,2]
Departments of [1]Medicine and [3]Pharmacology and Pharmacy, [2]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong

Introduction: Obesity is associated with majority of obstructive sleep apnoea (OSA)–related morbidities including cardiovascular and metabolic disorders. Impairment of adipogenic capacity in pre-adipocytes may promote adipocyte hypertrophy and increase the risks of insulin resistance and type 2 diabetes. Recent research findings have suggested an inhibitory role of macrophage polarisation in adipogenesis. However, the impact of intermittent hypoxia (IH), as a characteristic pathophysiologic feature in OSA, on adipogenesis is unknown. This study investigated the impact of IH on adipogenesis and further explored the role of macrophage polarisation in IH-regulated adipogenic process.

Method: C57BL/6N male mice were exposed to either IH or room air (RA) for 6 weeks. The profile of IH was cycles of approximately 240 seconds of 10% O_2 and 120 seconds of 21% O_2, ie 10 cycles/hour. Epididymal adipose tissue (AT) and abdominal AT were isolated to represent visceral (VIS) and subcutaneous (SUB) AT, respectively. Stromal vascular fraction (SVF) from depot-specific AT was isolated, cultured, and underwent two differentiation cycles in vitro. Macrophage-conditioned media from cultured RAW264.7 cell line were also incubated with SUB SVF and VIS SVF during adipogenic differentiation. Oil Red O staining was carried out for identification of oily droplets. Assessments of differentiation-associated markers, adipogenic transcriptional factors, and macrophage markers were achieved by real-time polymerase chain reaction. Morphological change and M1 macrophage infiltration were detected by H&E and immunofluorescence staining on formalin-fixed and paraffin-embedded AT sections (10 μm), respectively.

Result: After adipogenic differentiation in vitro, oily droplets of SUB SVF from IH-exposed mice were significantly down-regulated in comparison with that derived from RA mice, whilst the inhibitory effect of IH on oily droplets was not observed in VIS SVF. In agreement with the production of oily droplets, differentiation-associated markers and adipogenic transcriptional factors were also suppressed in SUB SVF from IH-exposed mice. IH exposure caused hypertrophic adipocytes in SUB AT but not in VIS AT. Furthermore, IH exposure increased infiltration of preferentially M1 macrophages in SUB AT, in line with the reduction of M2 phenotype and increment of M1 phenotype markers in SUB SVF. In support, conditioned-medium from RAW264.7 (M1 macrophage dominant) significantly inhibited the adipogenesis of SUB SVF, suggesting the involvement of macrophage polarisation in IH-inhibited adipogenesis.

Conclusion: Macrophage polarisation may be a potential mechanism responsible for IH-impaired subcutaneous adipogenesis.

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Massive population screening of atrial fibrillation with camera-based mobile phone application Cardiio Rhythm™

CK Wong[1], PH Chan[1], MZ Poh[3], YK Poh[3], MY Wong[2], DW Chu[2], CW Siu[1]
[1]Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong; [2]Department of Family Medicine and Primary Health Care, Hong Kong East Cluster, Hospital Authority, Hong Kong; [3]Cardiio, Inc., California, United States

Introduction: There is a need for massive population screening of asymptomatic atrial fibrillation (AF) to reduce embolic stroke. The objective of this study was to assess the performance of mobile phone application Cardiio Rhythm™ in screening AF. It operates by detecting photoplethysmography with mobile phone camera to deduce pulse regularity. It requires no extra hardware beyond a camera-equipped mobile phone. Head-to-head comparison against Food and Drug Administration–cleared and CE-marked handheld single-lead electrocardiography device, AliveCor® Heart Monitor, was performed.

Method: Patients of age 65 years or above, with diabetes mellitus or hypertension were recruited from primary care clinic in Hong Kong. Both Cardiio Rhythm™ and AliveCor® Heart Monitor were employed to screen for AF. Two cardiologists independently gave electrocardiography diagnosis. Sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) for AF screening of the two tools were then calculated.

Results: From May 2015 to June 2015, 1013 patients (mean age, 68.4 years) completed the study. AF and atrial flutter were found in 2.72% and 0.10%, respectively of the patients. For the Cardiio Rhythm™ and AliveCor® Heart Monitor, their respective sensitivity was 92.9% and 71.4%, specificity 97.8% and 99.4%, and AUC 0.95 (0.90-1.00) and 0.85 (0.75-0.96).

Conclusion: Cardiio Rhythm™ has accuracy comparable to AliveCor® Heart Monitor. The high sensitivity and low cost of Cardiio Rhythm™ have the potential of enabling massive population screening of AF.

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Serologic activity, intrahepatic virology and histology in subjects with occult hepatitis B infection

DKH Wong[1], J Fung[1], CK Lee[2], WK Seto[1], J Leung[2], FY Huang[1], CK Lin[2], CL Lai[1], MF Yuen[1]
[1]Department of Medicine, The University of Hong Kong; [2]Hong Kong Red Cross Blood Transfusion Service, Hospital Authority, Hong Kong

Background: We studied the virologic and histologic activities as well as serology, in subjects with occult hepatitis B infection (OBI).

Methods: Forty Chinese OBI subjects were compared with 40 age- and sex-matched chronic hepatitis B (CHB) patients. Intrahepatic hepatitis B virus (HBV) DNA, covalently closed circular DNA (cccDNA), and pregenomic RNA (pgRNA) were measured by real-time polymerase chain reaction. Serum hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) were measured using the Lumipulse G HBsAg-Quant (10 times more sensitive than conventional HBsAg assays) and HBcrAg assays, respectively.

Results: OBI subjects had median necroinflammation and fibrosis scores of 1 and 0, respectively. None had fibrosis score of ≥2. HBsAg (by HBsAg-Quant), HBcrAg, intrahepatic total HBV DNA, cccDNA, and pgRNA were detectable in 9 (23%), 4 (10%), 30 (77%), 1 (3%), and 5 (13%) OBI subjects, respectively. HBV genotype B were found in 52.5% of OBI subjects compared with 23% of CHB patients (P=0.014). OBI subjects with detectable HBsAg (by HBsAg-Quant) had higher median serum and intrahepatic HBV DNA than those with undetectable HBsAg (P=0.02 and 0.017, respectively).

Conclusions: OBI subjects had nearly normal liver histology and low serological and intrahepatic HBV replicative activity. HBV genotype B was associated with OBI.

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Down-regulation of renal tubular Wnt/beta-catenin signalling induces tubular cell death in proteinuric nephropathy

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

Background: Persistent proteinuria from any cause is closely associated with chronic kidney disease (CKD) progression. Excessive transglomerular protein trafficking impacts on renal tubular cell injury by triggering tubular cell death. Studies on the role of Wnt/beta-catenin signalling in different forms of CKD have yielded discrepant results.

Methods: Beta-catenin expression was measured in control/human serum albumin (HSA)–treated human kidney 2 (HK-2) cells and kidney cortical lysates of protein-overloaded mice given 4- or 8- week BSA injection by Western blotting and immunohistochemistry (IHC) staining. Genetic knockdown of beta-catenin in HK-2 cells was achieved using siRNA transfection. Apoptotic phenotypes were evaluated by quantitative polymerase chain reaction, Western blotting, IHC staining/activity assay, and TUNEL assay.

Results: Upon 4-day HSA stimulation, protein levels of active nuclear beta-catenin in HK-2 cells declined by 67% ± 0.04 ($P<0.05$ vs control) coincided with up-regulation of Bax/Bcl-2 gene expression. HSA treatment with or without beta-catenin siRNA transfection in HK-2 cells up-regulated Bax/Bcl-2 gene expression ratio by 116% ± 0.2 ($P<0.05$) and 52% ± 0.2 ($P<0.05$), respectively. Similarly, TUNEL and caspase-3 activity was also further increased by silencing beta-catenin. In protein-overloaded mice, dynamic expression of tubular beta-catenin was observed, with up-regulation by 88% ± 0.2 vs control animals ($P<0.05$) at the early stage (4-week BSA injection) and abrogation (24% ± 0.2; $P<0.05$) in the late phase (8-week BSA injection). Urine albumin-to-creatinine ratio decreased during renal tubular beta-catenin overexpression but increased thereafter as tubular beta-catenin levels came down. Elevated apoptotic phenotypes were evident in the later phase and associated with up-regulation of NGAL and KIM-1 genetic expressions.

Conclusions: Protein-overload promotes renal tubular apoptosis via abrogation of Wnt/beta-catenin signalling in vitro and in vivo.

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Association of benzodiazepine use in Chinese patients and risk of dementia: a retrospective cohort study

TT Chan, WCY Leung, KY Wong, WM Chu, KC Leung, YZ Ng, GYM Kai, V Li
Division of Neurology, Department of Medicine, The University of Hong Kong, Hong Kong

Background: Benzodiazepine has been associated with dementia. There has been no local study performed in Hong Kong investigating the association of benzodiazepine use and long-term risk of dementia.

Methods: A retrospective case-control study of 273 Chinese patients (91 cases, 182 controls) with at least 6 years of follow-up was carried out. Patients were matched on sex, age-group, and duration of follow-up. Data for benzodiazepine usage and co-morbidities were collected. The primary objective was to investigate whether benzodiazepine ever-use and the cumulative doses of benzodiazepine were related to future development of dementia.

Results: The cumulative benzodiazepine dosages of ≥1096 prescribed daily doses was more common in dementia subjects than controls (59.3% vs 44%; $P=0.02$). There was no difference in terms of ever-usage of benzodiazepine ($P=0.14$). Dementia subjects were more common to have a history of cerebrovascular accidents (CVA) and depression than controls (51.6% vs 29.1%; $P<0.001$ and 38.5% vs 24.7%; $P=0.02$, respectively). Multivariate logistic regression analyses showed that independent risk factors for dementia include cumulative benzodiazepine dosages (adjusted odds ratio=1.71; 95% confidence interval, 1.02-2.89) and CVA (2.47, 1.46-4.18).

Conclusion: High cumulative dosages of benzodiazepine may be associated with development of dementia in long term.

Clinical and biochemical characteristics of subjects with diabetes suffering from low bone mass and prevalent fragility fracture

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YC Woo[1], CHN Loong[1], CHY Fong[2], VWK Chau[2], JKY Lam[1], MMA Yuen[1], CH Lee[1,2], ACH Lee[1], KSL Lam[1,2], KCB Tan[1,2]
[1]Department of Medicine, Queen Mary Hospital; [2]Department of Medicine, University of Hong Kong, Hong Kong

Objectives: Patients with low bone mass and a history of low trauma fractures are at increased risk of future fracture. Type 2 diabetes is itself a risk factor for fracture as well. The aim of this study was to evaluate the clinical and biochemical characteristics of subjects with low bone mass and prevalent fragility fracture, who also suffer from type 2 diabetes, in comparison to those without diabetes.

Methods: All patients with low bone mass and prevalent fragility fractures underwent a standardised assessment protocol and had their bone mineral density (BMD) measured by dual-energy X-ray absorptiometry scan. Patients with a known history of diabetes assessed from 2008 to 2014 were recruited for analysis (DM group). Age- and gender-matched subjects without diabetes were selected as control (non-DM group). The variables were compared between groups by one-way analysis of variance for continuous data, Chi-square test for categorical data whichever appropriate.

Results: A total of 251 subjects with diabetes (mean age, 74.5 ± 7.9 years; 81.7% female) and 502 age- and gender-matched control (mean age, 74.5 ± 7.8 years; 81.7% female) were included in this report. Compared to non-DM control, DM patients had higher body mass index (P<0.001), fasting glucose (P<0.001), a higher BMD at spine (P<0.001) and at neck of femur (P=0.018). There was no difference in the history of fall in the two groups (P=0.283). Fewer subjects in the DM group experienced back pain (P=0.011) and their daily calcium intake were significantly lower than the non-DM control subjects (P=0.035).

Conclusion: For subjects with low bone mass and diabetes present with fracture despite having a higher BMD. They have a lower dietary calcium intake which may reflect a deficiency in the general knowledge on bone health. As they are less likely to be symptomatic (back pain), their awareness and alertness to the risk of fracture may even be lower. Hence, subjects with diabetes should be reminded to have adequate dietary calcium during their diabetes dietary education sessions. In addition, fracture risk assessment, as well as BMD measurement in indicated cases, should also be considered in diabetes complication screening programme.

Inhibition of MDM2 alleviates endotoxin-induced inflammation in macrophages

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KKL Wu, Z Liu, A Xu, KKY Cheng
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Macrophages sense and quickly respond to pathogens and inflammatory stimuli such as endotoxin lipopolysaccharide (LPS), and polarise to the pro-inflammatory M1 phenotype. However, unrestrained inflammatory response causes diseases such as sepsis, diabetes, and atherosclerosis. The E3 ubiquitin ligase murine double minute-2 (MDM2) is a negative regulator of tumour suppressor p53. Pharmacological inhibition of MDM2 reduces sterile inflammation in acute kidney injury, while p53 governs metabolic balance and its activation inhibits inflammation. The aim of this project was to investigate whether MDM2 plays a role in LPS-induced M1 macrophage polarisation and inflammatory response.

Methods: Primary peritoneal macrophages isolated from C57BL/6N mice were treated with the MDM2 inhibitor nutlin-3a, followed by LPS stimulation (100 ng/mL) for 20 hours. Markers related to inflammation were examined. Myeloid-specific haplodeletion of MDM2 (Mye-MDM2KO) mice were generated using Cre-LoxP system with lysozyme driven Cre transgene. LPS 10-mg/kg was injected into Mye-MDM2KO mice and its wild type (WT) littermates. Glucose level, core body temperature, body weight, and inflammatory markers were monitored for 24 hours.

Results: Expression of MDM2 is significantly elevated in primary macrophages upon LPS stimulation. Inhibition of MDM2 by nutlin-3 repressed LPS-induced inflammatory response, as evidenced by reduced expression of pro-inflammatory cytokines, including interleukin-1β and monocyte chemoattractant protein-1 and the M1 marker inducible nitric oxide synthase and its product nitric oxide. Furthermore, genetic deletion of MDM2 in myeloid cells partially alleviated LPS-induced hypoglycaemia, weight loss, and nitric oxide production in mice. Other inflammatory markers and tissue inflammation in the LPS-treated Mye-MDM2KO mice and WT controls will be measured, and the underlying mechanism by which MDM2 controls inflammatory response will be delineated in the future study.

Conclusion: MDM2 regulates immune response in endotoxin-induced inflammation and its inhibition can be a potential therapeutic strategy in attenuating inflammation.

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The role of adipocyte fatty acid–binding protein in the pathogenesis of alcoholic liver fibrosis in mice

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XP Wu, LL Shu, L Cheong, SL Lam, A Xu, LC Hoo
Department of Medicine, State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong

Introduction: Adipocyte fatty acid–binding protein (A-FABP) is an adipokine that associated with various cardiometabolic diseases including liver diseases. Circulating A-FABP level is positively related to the stages of non-alcoholic liver fibrosis. Pharmacological inhibition of A-FABP attenuates the expression of hepatic fibrogenic markers in diet-induced obese mice. Here, we investigated the role of A-FABP in the pathogenesis of alcoholic liver fibrosis.

Methods: A-FABP knockout mice and their wild-type littermates received carbon tetrachloride were fed with either alcoholic liquid diet or maltodextrin liquid diet (control diet) for 6 weeks. Liver injury was assessed. For in-vitro studies, primary macrophages and primary hepatic stellate cells (HSCs) were treated with ethanol for 48 hours.

Results: A-FABP deficiency attenuated ethanol-induced hepatic mRNA expression of pro-collagen alpha 1 as well as collagen formation and alleviated HSCs activation in mice. Consistently, in-vitro study demonstrated that treatment with ethanol increased the expression of A-FABP both in the primary macrophages and primary HSCs.

Conclusion: These data implicated that A-FABP plays a pathogenic role in the development of alcoholic liver fibrosis.

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The effect of arginase on small-cell lung cancer

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

Introduction: Small-cell lung cancer (SCLC) accounts for about 15% of all lung cancer cases. SCLC is characterised by easy to relapse, and current treatment lacks tumour specificity. BCT-100 is a pegylated arginase which shows anticancer activity in human melanoma, hepatocellular carcinoma, and acute myeloid leukaemia. One of the resistance mechanisms is overexpression of argininosuccinate synthetase (ASS1) and ornithine transcarbamylase (OTC). The aim of this study was to determine the effect of BCT-100 on SCLC.

Methods: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to detect cell viability of different SCLC cell lines after BCT-100 treatment. Western blotting was employed to evaluate the protein expression. Knockdown of OTC was performed using specific siRNA.

Results: After incubation of BCT-100 for 72 hours, the IC_{50} values of H69, DMS79, H187, H209, H526, H841, and SW1271 cells were 462.9 ± 112.2, >1000, 24.9 ± 6.4, 8.6 ± 0.8, 10.1 ± 0.7, >1000, and 49.2 ± 7.4 mU/mL, respectively. Overexpression of ASS1 in H69 and DMS79 cells and OTC in H841 cells might account for the resistance to BCT-100. Furthermore, knockdown of OTC increased sensitivity of BCT-100 in H841 cells partially via apoptosis. Moreover, co-incubation of N-acetylcysteine (an antioxidant) with BCT-100 reversed BCT-100–induced cell death via inhibition of apoptosis in H526 cells.

Conclusion: The SCLC cell lines with low expression of ASS and OTC were responsive to BCT-100 treatment partially via ROS-induced apoptosis. BCT-100 showed potential anticancer activity in lung cancer research.

Symptoms of attention deficit hyperactivity disorder in patients with systemic lupus erythematosus

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G Yang, L Yi, MY Mok
Division of Rheumatology and Clinical Immunology, Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong

Objectives: Cognitive function and mood disturbance are common in patients with systemic lupus erythematosus (SLE). This study aimed to examine whether SLE patients have more features of adult attention deficit hyperactivity disorder (ADHD) and their relation to anxiety and depressive symptoms.

Methods: Symptoms and clinically significant items of the inattention and hyperactivity/impulsivity domains of ADHD were examined in Part A and Part B by the screening instrument of ADHD Self-Reported Scale, respectively. Anxiety and depressive symptoms were measured by HADS-A and HADS-D, respectively.

Results: There were no differences in symptom scores of inattention and hyperactivity/impulsivity between inactive SLE patients (n=117) and age- and sex-matched controls (n=64). However, SLE patients had more clinically significant items in the inattention domain compared with controls (P=0.006), particularly among those who had previous cerebral involvement (P=0.004). Patients who had psychiatric diseases had more clinically significant items of the hyperactivity/impulsivity domain (P=0.006). Possible ADHD was found in 7.7% of SLE and 6.3% of healthy subjects (P=1.00) by the screening tool. Patients with higher inattention symptom scores were more likely to be unemployed but not for duration of education and smoking habit. Anxiety and depressive symptoms correlated with ADHD symptoms. HADS-A was an independent predictive factor for clinically significant symptoms of inattention (P<0.001) and hyperactivity/impulsivity (P=0.04) by logistic regression.

Conclusion: Inactive SLE patients, particularly those who had previous cerebral lupus, had more clinically significant symptoms of inattention but not hyperactivity/impulsivity reflecting underlying cognitive impairment. Anxiety and depressive symptoms were common confounders for ADHD-like symptoms.

Evaluation of cognitive function by electrophysiological study in systemic lupus erythematosus patients with previous neuropsychiatric involvement

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G Yang[1], RTF Cheung[1], JL Gao[1], EYY Lau[2], JHY Wan[2], MY Mok[1]
Departments of [1]Medicine and [2]Psychology, Queen Mary Hospital, The University of Hong Kong, Hong Kong

Objective: Previous studies on cognitive dysfunction evaluated by electrophysiological test in patients with systemic lupus erythematosus (SLE) who had previous neuropsychiatric involvement (NPSLE) were inconsistent. This study aimed to evaluate P300 as an electrophysiological marker of cognitive function in NPSLE patients who were diagnosed to have cognitive impairment by standard neuropsychological tests.

Methods: Event-related potentials were assessed by the auditory and visual oddball paradigms. Amplitude and latency of P300 at the frontal (Fz), central (Cz), and parietal (Pz) regions were determined and compared to controls.

Results: Sixteen patients with previous NPSLE were identified to have cognitive impairment, defined as one or more tests below 2 standard deviations of demographically normative data, among 20 patients recruited for comprehensive neuropsychological tests. P300 detection was performed in NPSLE patients with cognitive impairment (n=9), matched SLE patients without previous NPSLE (non-NPSLE; n=9), and healthy controls (n=15). Auditory oddball task did not show any P300 abnormality between groups. Visual oddball task revealed reduced amplitude of P300 over Fz (P=0.002) and Cz (P=0.009) electrodes in NPSLE patients compared to healthy controls and among those who had predominant memory deficit (P=0.01 at Fz). Abnormal P300 was also observed in non-NPSLE patients at Fz and Cz.

Conclusion: P300 elicited by auditory oddball paradigm was not a sensitive electrophysiological marker for cognitive impairment in NPSLE patients. Using visual oddball paradigm, abnormal P300 was found in NPSLE patients over Fz and Pz regions compared to normal controls but was not discriminative from possible subclinical disease in non-NPSLE patients.

Risk factor for renal relapse of lupus nephritis in the era of effective immunosuppressive treatments

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DY Yap, TM Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Repeated renal flares compromise the long-term renal outcomes in lupus nephritis (LN) patients. This study investigated the risk factors for relapse in the era of effective immunosuppressive treatments.

Methods: All LN patients who were followed up in Queen Mary Hospital during the period of 1 January 1983 to 31 December 2013 were reviewed. Episodes of renal relapses were identified and the risk factors for relapse were analysed. The risk of relapse was also analysed according to the era before (1983-1997) and after (1998-2013) the availability of mycophenolic acid (MPA) treatment in our centre.

Results: A total of 346 episodes of renal flares occurred in 184 patients (mean follow-up duration, 195.3 ± 94.4 months). Multivariate analysis showed that age (odds ratio [OR]=0.97; 95% confidence interval [CI], 0.943-0.998; P=0.038), higher serum creatinine on presentation (OR=0.992; 95% CI, 0.985-0.999; P=0.019), use of MPA as maintenance treatment (OR=0.322; 95% CI, 0.0109-0.953; P=0.041), and achievement of complete remission after induction therapy (OR=0.394; 95% CI, 0.175-0.886; P=0.024) were factors associated with lower risk of renal relapse. The choice of induction therapy, proteinuria, and serological parameters on presentation did not affect the risk of subsequent flares (all P>0.05). Rates of renal relapse were 0.044 and 0.024 relapse per patient-year before and after the availability of MPA treatment (P<0.001). Patients who received prednisolone and MPA maintenance showed better relapse-free survival than those on prednisolone and azathioprine (83% vs 68%, 66% vs 42%, and 62% vs 37% at 5, 10, and 15 years; P=0.048).

Conclusion: The overall risk of flare is low in the era of effective immunosuppressive treatments and the MPA maintenance is associated with a lower long-term risk of LN flare as compared to azathioprine.

Pre-emptive intensification of immunosuppressive treatments prevents renal flare in lupus nephritis patients

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DY Yap, TM Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Whether lupus nephritis (LN) patients with asymptomatic serological flares (ASF) should receive pre-emptive intensification of immunosuppressive treatments remains undefined.

Methods: All episodes of ASF (defined as increase in anti-dsDNA >100 IU/mL, with or without decrease in serum complement levels, and with no renal/systemic lupus manifestations) which occurred during 2005-2015 were reviewed. Pre-emptive intensification in immunosuppressive treatments referred to increase in prednisolone to 0.4-0.5 mg/kg/day, and increase in mycophenolate mofetil to 1 g/day or azathioprine to 75 mg/day (if patients were already on these agents).The outcomes of pre-emptive increase in immunosuppression were analysed.

Results: A total of 138 episodes of ASF occurred in 98 LN patients (56 episodes received pre-emptive treatment; 82 episodes received no treatment). The mean dose of prednisolone used for pre-emptive treatment of ASF was 19.0 ± 5.3 mg/day. Pre-emptive treatment significantly reduced the risk of renal relapse in 1 year (3.6% vs 29.3%; P<0.001). Patients who received pre-emptive treatment also had better estimated glomerular filtration rate after 5 years (92.7 ± 21.1 mL/min/1.73^2 vs 80.1 ± 28.1 mL/min/1.73^2; P=0.028) but no difference in the renal survival rate at 5 years (97.45 vs 96.7%; P=0.884). The pre-emptive treatment group showed significant improvement in anti-dsDNA (210.2 ± 114.4 IU/mL to 80.9 ± 70.0 IU/mL; P<0.001) and C3 levels (56.2 ± 20.9 mg/dL to 66.3 ± 23.2 mg/dL; P=0.001) after 12 months. There was no impact of pre-emptive treatment on non-renal flares (15.9% vs 16.1%; P=0.973).

Conclusion: Pre-emptive intensification of immunosuppressive treatments for ASF effectively reduced the risk of renal flare in the subsequent 1 year, and was associated with better long-term renal function.

The effect of rapamycin in regulatory T cells in lupus nephritis

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DY Yap, P Lee, S Yung, TM Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Rapamycin (RAPA) can induce allograft tolerance via promotion of regulatory T cells (Treg) proliferation and survival, and is an effective immunosuppressive agent for prevention of rejection in organ transplantation recipients. Preliminary animal and human data also showed its efficacy in the treatment of lupus nephritis (LN).

Methods: We compared the circulating Treg and its associated cytokines/inhibitory molecules in LN patients who received low-dose prednisolone plus either (1) RAPA or (2) mycophenolate mofetil (MMF) and azathioprine (AZA). The immunological effects of RAPA in LN was also investigated using NZB/W F1 lupus mouse model.

Results: Preliminary data included 10 LN patients and two healthy subjects. RAPA-treated patients were associated with higher percentage of circulating Treg than MMF- or AZA-treated patients, but the percentage was similar to healthy controls (3.23% ± 0.53% vs 1.20% ± 0.44%, 0.13% ± 0.1%, and 4.27 ± 1.21%, respectively; P=0.302, 0.013, and 0.001 for RAPA vs healthy individuals, MMF, and AZA, respectively). Moreover, Treg isolated from RAPA-treated patients showed numerically higher mRNA expression of FOXP3, IL-10 and CD73, but lower TGF-β expression. RAPA treatment for 12 weeks in NZB/W F1 mice was associated with increased FOXP3+Treg infiltration in the glomeruli and interstitium as compared to control mice.

Conclusion: RAPA can potentially increase circulating Treg and intra-renal Treg infiltration, which might confer better treatment response and long-term disease stability in LN patients.

Novel biomarkers for the prediction of hepatorenal syndrome in patients with advanced cirrhosis

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DY Yap, WK Seto, J Fung, G Chan, MF Yuen, TM Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Hepatorenal syndrome (HRS) is a life-threatening complication of cirrhosis and prediction of its occurrence is difficult.

Methods: We prospectively recruited Child-Pugh B or C cirrhotic patients with normal serum creatinine (Cr), and followed them for 12 weeks for the development of HRS. Serum cystatin C (CysC), serum neutrophil gelatinase-associated lipocalin (NGAL), serum interleukin (IL)–18, urine kidney injury molecule-1 (KIM-1), and urine liver-type fatty acid–binding protein (LFABP) were measured at recruitment (baseline), and their relationship with subsequent HRS investigated.

Results: Overall, 43 patients were included; 12 (27.9%) developed HRS at 7.3 ± 5.1 weeks from baseline. Patients who developed HRS showed higher baseline serum CysC (1.42 ± 0.51 mg/L vs 1.07 ± 0.50 mg/L; P=0.021), serum NGAL (129.10 ± 68.66 ng/mL vs 72.84 ± 48.91 ng/mL; P=0.025), serum IL-18 (759.91 ± 477.05 pg/mL vs 358.13 ± 153.00 pg/mL; P=0.001), urine KIM-1 and LFABP (3.64 ± 3.34 ng/mL vs 1.18 ± 1.68 ng/mL and 10.17 ± 8.37 ng/mL vs 3.28 ± 4.20 ng/mL; P=0.023 and 0.035, respectively) when compared to patients who did not develop HRS. Logistic regression revealed that baseline serum NGAL and IL-18 and urinary KIM-1 had significant correlation with subsequent HRS (odds ratio [OR]=1.017, 95% confidence interval [CI], 1.001-1.033, P=0.035; OR=1.007, 95% CI, 1.002-1.013, P=0.012; OR=1.503, 95% CI, 1.009-2.237, P=0.045, respectively). The cut-off values for baseline serum NGAL, serum IL-18, urinary KIM-1 to predict HRS were 90.47 ng/mL, 442.82 pg/mL, and 1.499 ng/mL (area under the curve=0.76, 0.86, 0.78; P=0.025, 0.001, 0.008, respectively).

Conclusion: Serum NGAL and IL-18 and urinary KIM-1 could serve as early biomarkers to predict HRS in Child-Pugh B or C cirrhotic patients whose serum Cr were within normal range.

Blood mycophenolic acid levels and clinical outcomes in lupus nephritis

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DY Yap, CH Tam, MY Mok, SH Wong, KY Yuen, MK Ma, CS Lau, S Yung, TM Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The dosing regimen of mycophenolate mofetil (MMF) in the treatment of lupus nephritis (LN) is adopted from experience in renal transplantation. The role of therapeutic drug monitoring of mycophenolic acid (MPA) level in managing LN remains undefined.

Methods: We prospectively studied LN patients on maintenance treatment with prednisolone and MMF. Blood MPA levels at 1, 2, 4, 8, 10, and 12 hours (ie C1, C2, C4, C8, C10, and C12) after MMF administration were assayed. C12 MPA blood level was measured every 6 months for 24 months, and at occurrence of clinically significant events, to investigate their clinical correlations.

Results: In this study, 51 LN patients who received prednisolone (6.2 ± 1.8 mg/d) and MMF (1284 ± 493 mg/d) during disease remission were recruited. C1, C2, and C12 MPA levels were 9.9 ± 8.7 mg/L, 8.6 ± 6.2 mg/L, and 1.9 ± 1.4 mg/L, respectively, and they correlated with AUC 0-12 (r=0.521, 0.852, and 0.765; P=0.004, <0.001, and <0.001, respectively). C12 correlated negatively with haemoglobin level, white cell count, platelet count, and the level of total immunoglobulin (Ig), IgG, and IgA (r= –0.359, –0.226, –0.198, –0.411, –0.368, and –0.267; P=0.001, 0.010, 0.024, 0.001, 0.003, and 0.036, respectively). No consistent association was detected between C1 or C2 with clinical or serological parameters. Clinical events included infection (n=2), gastro-intestinal upset (n=3), renal flare (n=3), significant anaemia (n=10), and leukopenia (n=7). C12 MPA level at the time of these events were 1.7 ± 1.6 mg/L, 2.6 ± 1.7 mg/L, 2.1 ± 0.9 mg/L, 2.9 ± 1.5 mg/L, and 2.6 ± 1.4 mg/L, respectively.

Conclusion: While C1, C2, and C12 blood MPA level correlate with drug exposure, C12 level may be more relevant to clinical events.

Dendritic cells display abnormalities during onset of systemic lupus erythematosus

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ALY Yim, S Yan, CCP Lee, BYT Pang, 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 causing multi-organ damages. Plasmacytoid dendritic cells (pDCs) are potent type I interferon (IFN) producers and myeloid dendritic cells (mDCs) are professional antigen-presenting cells. Clinically, the level of serum interferon alpha (IFNa) correlates with the disease severity, suggesting that pDCs may play a role in SLE pathogenesis. Moreover, mDCs from SLE patients also display activated phenotypes. These observations suggested that different DC subsets may contribute to the pathogenesis of SLE.

Methods: Using the New Zealand black/white F1 (BWF1) murine lupus model, this study evaluated the phenotypical and functional properties of purified pDCs and mDCs in vitro using flow cytometry, ELISA, quantitative polymerase chain reaction, and allogenic T-cell proliferation assay.

Results: Symptomatic mice had a lower frequency but similar number of splenic pDCs when compared with pre-symptomatic mice. Expression levels of CD40, CD80 and MHC II on pDC upon Toll-like receptor (TLR) 7 or TLR9 stimulation and the level of IFNa produced upon TLR9 stimulation were also comparable between symptomatic and pre-symptomatic mice. For splenic mDCs, both the frequency and number were higher in symptomatic mice. Surprisingly, ex-vivo mDCs from symptomatic mice displayed a less-activated phenotype with lower levels of CD80 and MHC II but the ability of mDCs to induce allogeneic CD4+ T cell proliferation was comparable between symptomatic and pre-symptomatic mice. On the other hand, the basal expressions of TLR7 and TLR9 on mDCs from symptomatic mice were higher than its pre-symptomatic counterparts which suggested that TLR signalling may contribute to SLE pathogenesis in BWF1.

Conclusion: mDCs in BWF1 had displayed abnormality upon disease onset. More studies should be performed to investigate whether mDCs contribute to SLE pathogenesis via aberrant TLR signalling.

Inhibition impairments in temporal lobe epilepsy patients: electroencephalography evidence from a Go/Nogo study

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HM Yu, JL Gao, RSK Chang, W Mak, RT Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Temporal lobe epilepsy (TLE) is a common type of epilepsy that easily run an intractable course. It can harm cognitive inhibition function, an essential executive function that enables us to suppress inappropriate actions in a given context at different levels. The aim of this study was to investigate whether TLE also affects related Go/Nogo-potentials related to the inhibition using high-resolution electroencephalography (EEG) technology.

Methods: Participants were recruited in epilepsy clinics in Queen Mary Hospital, including refractory TLE patients (n=16), well-controlled TLE patients (n=11), and healthy control subjects (n=10). A Go/Nogo task was designed in which subjects were instructed to rapidly push a button in response to stimulus presentation. EEG data were collected by 128-channel Neuroscan system. The data were analysed by Neuroscan software.

Results: We chose frontal for N2 detection in Go/Nogo task, central for Nogo P3 detection, and parietal for Go P3 detection. Analysis of variance showed that N2 amplitude in the Nogo condition was different among the three groups (P=0.048). Post-hoc analysis showed that the mean amplitude in the refractory TLE group (–0.73 ± 2.02 μV) was smaller compared with healthy people (-2.91 ± 1.44 μV), whereas the group effect was not significant in the Go condition (P=0.48). The P3 amplitude in the Nogo condition was significant (P=0.042). Post-hoc analysis indicated P3 amplitude in the refractory TLE patients (1.40 ± 1.86 μV) was smaller than healthy people (3.92 ± 4.12 μV). The group effect was also significant in the Go-P3 (P=0.038), healthy people (2.89 ± 2.05 μV) had larger mean amplitude over refractory TLE patients (0.75 ± 1.51 μV).

Conclusion: The event-related potential data suggest that there is selective impairment of inhibitory function in TLE. Impaired inhibitory executive function may lie in the frontal lobe.

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Association of periodontitis with subclinical myocardial dysfunction in patients with diabetes

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Z Zhen, Y Chen, JH Liu, I Lai, LJ Jin, KH Yiu
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: In patients with type 2 diabetes mellitus (T2DM), the association between periodontitis and cardiovascular disease (CVD) has been established recently. However, the association between periodontitis and subclinical cardiac dysfunction, especially exercise-induced cardiac alterations in T2DM patients is not clear. In the present study, our aim was to investigate the relationship between periodontitis and subclinical cardiac dysfunction by (1) basic dental examination and (2) detailed cardiac assessment using resting and exercise echocardiography.

Methods: A total of 52 T2DM patients without histories of CVD were enrolled, and all received dental examination, and resting and exercise transthoracic echocardiography. Echocardiographic images were analysed in detail with the following parameters: (1) left ventricular ejection function (LVEF) was evaluated by Simpson's method–derived ejection fraction and speckle tracking–derived global longitudinal strain (GLS), (2) diastolic function was assessed by tissue Doppler derived E′ and E/E′ ration. Dental measurement parameters included tooth loss number (TL) and probing depth (PD).

Results: The mean age of the recruited participants were 64.8 ± 8.7 years; 50% of them were male. Even though LVEF did not have significant relationship with dental parameters, TL was significantly correlated with resting (r=0.40, P=0.01) and exercise (r=0.44, P=0.01) GLS, as well as E′ after exercise (r= –0.38, P<0.01). PD had significant association with resting (r=0.41, P=0.02) and exercise (r=0.52, P<0.01) GLS, resting (r= –0.33, P=0.03) and exercise (r= –0.46, P<0.01) E′. Furthermore, multivariate analysis demonstrated TL and PD were independent predictors of impaired resting GLS (B=0.09, CI=0.01-0.18, P=0.04 and B=1.08, CI=0.05-2.10, P=0.04, respectively). In addition, PD was independently related with exercise E′ (B= –0.01, CI= –0.020 to –0.001, P=0.03) and E/E′ (B=1.28, CI=0.14-2.42, P=0.03) after multivariate analysis.

Conclusion: The present study demonstrated an independent relationship between the severity of periodontitis and myocardial systolic and diastolic dysfunction both at rest and exercise status. The findings suggest that patients with periodontitis requires a detailed clinical assessment to detect subclinical myocardial dysfunction in order to prevent adverse cardiac outcome.

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