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Abstracts of Papers

AN ADVENTURE IN RAPID IMMUNODIAGNOSTICS

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Immunodiagnostic tests have withstood the test of times and the challenge from newer tests to remain useful in clinical medicine and public health. Antibody detection tests, for example, are still the mainstay of diagnostic tests for many infectious diseases and autoimmune disorders. Recent years have witnessed a surge of interest in the development of rapid immunodiagnostics that can yield results within 15 minutes and which can be used for point of care testing in the field or by the bedside. The market growth of these diagnostics has been estimated to be 15% and for influenza alone, the market is valued at US$150 million. The method most commonly employed in these tests is immunochromatography, which was first applied to pregnancy testing. The scope and the immunology, including the shortcomings, of this system will be discussed. Another rapid test system exploited thus far for typhoid detection only and which is the focus of this talk is TUBEX. Here, antibodies are rapidly detected by their ability to inhibit the binding between antibody-coated indicator particles and antigen-coated magnetic particles. The development of this home-grown technology will be traced and the immunology of the system discussed, particularly in relation to these unusual features: (1) IgM antibodies are detected but not IgG, although the latter can aid the detection of the former, and (2) antigen can also be detected at the same time. These attributes make TUBEX a unique and an ideal system for detecting acute infections in endemic environments.

INHERITED THROMBOCYTOPENIA SYNDROMES

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Inherited causes of thrombocytopenia are far less common that secondary or acquired disorders especially immune thrombocytopenic purpura (ITP). Clinical vigilance is needed for correctly diagnosing these conditions, as the treatment approach would be different from ITP. Inherited forms of thrombocytopenia are usually syndromal disorders that show non-haematological or somatic features such as congenial malformations that may give a clue to the diagnosis. Moreover, changes in platelet size and morphology, in particular macrothrombocytopenia, is frequent and careful examination of peripheral blood film cannot be over-emphasized. The correct enumeration of large to giant platelets may pose a challenge to automated haematological analyzers. Current approaches to automated platelet counting based on impedance, optical or fluorescence techniques will be discussed. More importantly, recent advances in deciphering the molecular basis of these disorders not only contribute to understanding their pathogenesis but also provide an avenue for rapid diagnostic confirmation by mutation detection and genetic counseling of family members. Many of these genes are shown to play pivotal roles in normal haemopoietic development. These points will be illustrated by the haematological and genetic characterization of *MYH9*-related disorder in a Chinese family.

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THE POTENTIAL AND BARRIERS OF STEM CELLS APPLICATIONS IN CLINICAL MEDICINE : A REVIEW OF THE CURRENT STATUS

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Use of stem cells to replace damage tissues or organs has long been proposed since several decades ago but whether this can be materialized into reality in the near future remains controversial. The debates are multifaceted but the concept of "stem cells" has to be clarified. There are different kinds of stem cells currently under intense researches and they have different potential and barriers for future applications. The two main groups of stems cells are embryonic stem cells (ESC) and adult stem cells (ASC). ESC has the genuine self-renewal and totipotent differentiating capability. It is therefore more versatile in the bioengineering aspect. However, use of human ESC has been bound by the legal and ethical barriers for it involves the manipulation of human embryo. Technically, it is also restricted by the HLA immune compatibility. The possibility of tumour formation in the form of teratoma is another concern. Due to all these reasons, research on ESC currently involves animal studies only. On the other hand, ASC in the form of haematopoietic stem cells (HSC) (i.e. bone marrow transplantation) have been used clinically for more than 3 decades. Unlike ESC, most ASC (i.e. HSC, muscle stem cells, etc.) are lineage specific and has limited renewal capacity. However, careful selection of appropriate stem cells to be used with proper ex-vivo expansion can solve these problems. While some ASC may encounter ethical challenge (i.e. neural stem cells derived from fetal hippocampus), most ASC can be derived from autologous and allogeneous adult tissues without inducing excessive harms on the donors. It is therefore widely accepted legally and ethically. Except for mesenchymal stem cells (MSC), most ASCs also require HLA compatibility match but it will not form tumour. The possibility of applying different kinds of ASC clinically is very high. Recently, human trials of MSCs for various indications have been started in several developed countries. Our team has been involved in the study of the biology of MSC and its potential uses both in immunological and bioengineering aspects. Some of these aspects will be discussed.

LEAN SIX SIGMA IN QUALITY MANAGEMENT : MYTH OR MUDA

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Quality of laboratory service is difficult to define. The definition of quality requires definition of the needs which a medical laboratory has to satisfy (Customer Perception and Satisfaction). Quality must be designed from the front end, not tested on the back end. Lean Six Sigma combines the two most important improvement trends of our time: making work better (using Six Sigma) and making work faster (using Lean principles). The concept of Lean can give you the tools to identify and eliminate waste and quality problems in your own work area. On the contrary, the concept of Six Sigma quality management is rather vague. Generally, its goal has been defined as "business excellence". Lean Six Sigma are methods for looking strategically at how a business can create value. Earlier pioneers have discovered that lessons learned in manufacturing can be applied to other corporate processes, such as research & development and even in pharmaceutical companies. The term, Lean Six Sigma, however, needs some explanation if applied to medical laboratories. Lean Six Sigma is just what the name would suggest: the marriage of Lean principles and Six Sigma methodologies. The first principle of Lean Six Sigma is to delight your customers with quality. The second principle says to improve process flow and speed. Lean Six Sigma emphasizes that speed is directly tied to excellence. It can give you the ideas of how to improve you workflow, meet your goals, and better serve your customers. The international standards ISO 15189 and corresponding checklist documents for accreditation of medical laboratories have been published. Although these new standards are found useful in certain areas particularly in the pre- and post-examinational phases they are considered to some extent to be insufficient in the assessment of the efficiency of the laboratory service. The laboratory must audit the quality of its service. The internal audit program is designed primarily to detect those deficiencies in the workflow and control analytical process. Laboratory project managers must address defect rates for quality improvement and remove inefficiencies to get the job done faster. The keys for Lean Six Sigma are evidence-based laboratory medicine, speed and quality that work together for maximum productivity. The Lean Sigma Metric may provide new insight into the management skills needed for a measurement process in a laboratory. The laboratory managers of today need Lean and Six Sigma for balance. However, what are the unique challenges that can arise in applying the Lean Sigma Metric in defining acceptable laboratory quality: Myth or Muda? That is the question.

STEM CELL STRATEGIES IN BURNS AND WOUND CARE

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There is a growing interest in the potential roles of stem cells in the modulation of cutaneous wound repair and regeneration. The hypothesis that we are exploring is that mesenchymal stem cells can be placed into a pathological wound environment such as the chronic ulcer or non-healing burn; they will undertake an assessment of the physiological deficiencies in terms of matrix composition and cytokine milieu and correct these by producing the appropriate wound healing modulators. Bone marrow, peripheral blood and umbilical cord blood have all been used in chronic wounds to modulate the healing response. Of particular interest is the effect of topical applications of autologous bone marrow derived cells on chronic wounds. Although the early experience is limited, the consistent theme is that a chronic wound changes its nature to become an acute wound that heals or becomes healthy and can be closed with a skin graft. The management of an extensive burn wound is another very challenging process. Bone marrow suppression has been well documented to be associated with this type of burn. Two major problems exist: the compromised drive to dermal healing resulting from lack of mesenchymal stem cell (probably due to burn-related marrow failure) and a compromised or even absent source of epithelial regeneration. There is a growing interest in the potential of using human umbilical cord blood (HUCB) and cord derived cells to address these two challenges. HUCB is a free, natural resource with low incidence of graft versus host reaction. Cord lining stem cells have the potential to differentiate into keratinocytes under in vitro conditions. The potential of systemic infusion of ABO compatible HUCB and/or the topical application of such blood and/or cord-derived cells to burns and other wounds opens up the possibility of providing accessible burns and wound care to more of the world's neediest patients. This presentation gives a background to stem cell strategies in burns and wound care, and identifies actual or prospective applications which, collectively, will change wound care throughout the world. More research needs to be conducted to determine the types and/or fraction of stem cells, dose optimization, frequency and route of administration. These will all be fundamental in successful stem cell strategies in wound management.

DIFFERENTIATION OF EMBRYONIC STEM CELLS TO HEMATOPOIETIC LINEAGE IN THE PRESENCE OF SOLUBLE FACTORS OF FOETAL MICRO-ENVIRONMENTS

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Our previous study demonstrated that stromal supporting cells, primary mouse aorta-gonad-mesonephros (AGM) cells and C17.2 neonatal neural progenitor cell line, supported *ex vivo* expansion of enriched cord blood CD34+ cells to multi-lineage progenitors (*Li et al*., Leukemia 2005). These two micro-environments also expressed a panel of hematopoietic, bone morphogenetic and neural factors. We proposed that these soluble factors might exert promoting effects on early hematopoiesis. The objective of this study was to investigate the effects of stromal cell-conditioned media (CM) on the derivation of hematopoietic cells from embryonic stem cell (ESC) line ESE14tg2a. The ESC line was maintained undifferentiated on mitomycin C-treated primary mouse embryonic fibroblasts (MEF) in the presence of leukemia inhibitory factor (LIF). Undifferentiated ESC were characterized by positive immunostaining of ESC specific marker, SSEA-1. CM used in the study were collected daily from confluent, mitomycin-C treated AGM or C17.2 cells for 5 days. Hematopoietic differentiation of ESC was induced in (1) methylcellulose-based culture with stem cell factor (SCF) and (2) liquid suspension culture made up of (a) KO DMEM containing SCF (control), (b) AGM CM and (c) C17.2 CM for 8 days. Our results demonstrated that typical hematopoietic colonies, including CFU-GEMM, BFU/CFU-E and CFU-GM, were derived from ESC. Hematopoietic colonies formation in methylcellulose-based cultures (75.213.7 per 310^4 cells, n=6) was higher than that in liquid suspension cultures. This efficiency was similar with that in AGM CM (p=0.11) but significantly higher than those in control cultures (p=0.03) and C17.2 CM (p=0.04). The presence of AGM CM had a trend to promote the hematopoietic colonies formation (p=0.16), whereas C17.2 CM had no significant promoting effect, when compared with control cultures. AGM CM could be developed for use in inducing ESC to hematopoietic stem cells.

CELL THERAPY FOR ISCHAEMIC STROKE USING EMBRYONIC STEM CELL-DERIVED NEURAL CELL PRODUCTS

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Pluripotent embryonic stem (ES) cells can *in-vitro* differentiate into all cells of the body and ES cell-derived cell products emerge as a promising cell source for neuro-regeneration. However, there is little report on the efficacy of cellular therapy for neurodegenerative diseases, especially ischaemic stroke. With the hypothesis of the neural inducing activity from neural precursor cells, we investigated the stromal cell-derived inducing activity (SDIA) and conditioned medium (CM) of C17.2 neural precursor cells to differentiate ES cells D3 and E14TG2a. Reverse transcription-polymerase chain reaction demonstrated mRNA of neurotrophins and neuro-protective factors were expressed in C17.2 cells, suggesting the neurotrophic and neuroprotective potential. The SDIA was remarkable in both co-cultures and CM-supplemented cultures, despite the readouts derived from half-diluted CM was a bit lesser. A proportion of 75% colonies displayed neuro-epithelial stem cell intermediate filament (nestin) in co-cultures of D3 and C17.2. The immuno-reactivity of nestin, beta-tubulin class III, microtubule-associated protein 2, tyrosine hydroxylase, glial fibrillary acidic protein, and myelin binding protein in the ES cell-derived cell products suggested the growth of a cell population in hierarchy of the neural lineage. Molecularly, the expression of *Pax6, Otx1* and *Nurr1* (neural lineage cell-related genes), but not *Brachyury, Myf5* and *Nkx2.5* (mesodermal genes) and α-*fetoprotein* and *GATA-4* (endodermal genes), indicated neuro-ectodermal precursors, neural stem cells, neurons, astrocytes and oligodendrocytes in ES cell-derived cell products. Nevertheless, weak expression of *Oct-4* was noted. *In-vivo* studies of the SDIA-induced cell products were conducted in the mouse model of brain ischaemia induced by transient bilateral common carotid artery occlusion and reperfusion. Behavioural assessment of ischaemic mice after intra-cranial implantation of ES cell-derived cell product onto the putamen was conducted in the water maze system. Results demonstrated a significant improvement in spatial learning and memory ability in ischaemic mice having undergone cell therapy as compared to ischaemic mice receiving sham operation. Tracking of bromodeoxyuridine-labelled cell products revealed that mostly implanted cells were localized along the needle track of injection in the brain parenchyma, whereas some migrated to the contra-lateral hemisphere. An episode (1/22) of teratoma was noted. Data of this study suggested that a hierarchical population of neural lineage cells could be *in-vitro* derived from ES cells through the SDIA of neural precursor cell line C17.2. The clinical relevance was evident in ischaemic mice. However, there is still rooms for improvement to over-ride the hurdle of teratoma development before ES cell-derived neural lineage cells be used in clinics.

STUDY OF ATTENTION-DEFICIT / HYPERACTIVITY DISORDER USING FUNCTIONAL MAGNETIC RESONANCE IMAGING

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Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder mainly characterized by age-inappropriate inattention, impulsiveness, and hyperactivity. Spontaneously hypertensive rats (SHR) have been reported to represent a genetic animal model for ADHD. Over the past decade functional magnetic resonance imaging (fMRI) has superseded radionuclide-imaging techniques and blossomed into a widely used neuroscience research tool. Functional MRI is also beginning to realize its potential as an important mediator between genes and phenotypes, and may thus contribute to a better understanding of the pathophysiology of major neuropsychiatric diseases. In the present studies, we used SHR as an animal model of ADHD to demonstrate a differential response following the application of tail stimuli. Two groups of animals, SHR (n=5) and age- and gender-matched genetic control Wistar-Kyoto rats (WKY, n=5), were placed in a custom built plastic holder to keep them from moving, a light anesthesia (chloral hydrate 0.7%, 0.5ml/100g) was injected into each animal. A stimulus was applied to the tail of each mouse by placing a weight on top of the tail. Two different weights (250g and 500g) were used as light and heavy stimuli, respectively. While there was an enhanced response with the weight increased in SHR and WKY, the fMRI studies revealed that there was an increase in the area of blood oxygenation level-dependent (BOLD) images of the five week old SHR, compared with WKY. In conclusion, SHR was more sensitive to the stimulus than WKY. Therefore fMRI that we obtained provides new information that may be relevant to understanding ADHD in man.

REAL-TIME QUANTITATIVE GENE EXPRESSION ANALYSIS FOR INFECTION AND IMMUNITY RESEARCH

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The outbreak of SARS in 2003 and the current alert on avian influenza have stimulated an increasing interest on infection and immunity research in Hong Kong. Many assays for immunological functions involve the use of live cells and common laboratory procedures generate aerosols that may contain infectious materials. It is at times difficult to select a safe and appropriate assay for immunological studies. One remedy is to focus on gene expression using total RNA extracted from infected cells. Different viruses may cause distinct cytokine and chemokine responses. We have previously demonstrated an immune escape model in SARS-CoV infection and an immune activation model in avian influenza infection. This talk aims to facilitate the sharing of experience in using real time quantitative gene expression assays for infection and immunity research.

PUBLIC HEALTH, PATIENT CARE STANDARDS AND LABORATORY MEDICINE

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Experience with emerging infections such as SARS and H5N1 in the last decade has highlighted their potentials to produce tremendous disruption to the normal operation of modern societies. As a result, there is renewed interest in development of diagnostic methods, vaccines and drug-resistance tests to facilitate their recognition and containment. With the increasing availability, affordability and popularity of molecular and biological techniques, many once sophisticated tests which were luxuries in renowned research laboratories, have now become part of the daily routine in many service laboratories. At the same time, there is increased emphasis on clinical applications and a movement of scientific research from the bench to the bedside. This has strengthened ties and transfer of technology between research institutes, service laboratories and public health authorities. One notable benefit of such changes is that rapid diagnostic tests are now available for guiding many patient care and public health decisions. In Hong Kong, rapid tests for tuberculosis, CMV disease, influenza, dengue, and norovirus are examples that have found their way into the daily routine. Importantly, recent studies have confirmed that rapid test-based approaches could lead to measurable benefits: cost-saving, less antibiotic use, shorten hospital stay, and earlier initiation of therapy. Beside speed, molecular diagnostics (e.g. those for N. gonorrheae, C. trachomatis), by allowing use of non-invasive specimens, may enhance patient comfort and acceptability of testing. Furthermore, the epidemiologic evaluation of an outbreak may be monitored "real-time" using molecular techniques that track the relatedness of isolates. Examples of how laboratory medicine is modifying patient care and public health standards will be discussed in the talk. However, the modern biomedical scientists are faced with new challenges. For instances, laboratory scientists may be required to provide rapid test service for newly identified diseases such as SARS-CoV and H5N1 while the tests are still in the developmental stage and being evaluated. The rapid development of the field has also lead to difficulty in test standardizations and in running quality assurance programs.

ANALYTICAL EVALUATION OF RAPID INFLUENZA A ANTIGEN DETECTION TEST KITS FOR DETECTION OF COMMON CURRENT HUMAN INFLUENZA A AND SUBTYPES H5N1

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Recently, avian influenza A subtype H5N1 has spread to affect poultry in many countries across 3 continents. In 1997, avian H5N1 influenza A viruses were first evidence of direct transmission to human in Hong Kong with 42% mortality. At present, 228 human cases with 130 deaths have been confirmed. Rapid diagnosis for avian influenza virus is urged needed. It is not only important in the successful treatment of the disease but also important for hospital infection control particular to isolate the infected patients. In some clinical studies, existing rapid antigen detection tests for influenza A detection were found to have poor clinical sensitivity for diagnosis of patients with H5N1 disease. Therefore, several influenza A rapid antigen detection kits were sought to compare the analytical sensitivity for the detection of contemporary cultured influenza A viruses of subtype H5N1 and human influenza A subtype H1N1 and H3N2. It was found that each test kit detected the H5N1 virus have roughly comparable analytical sensitivity for detecting conventional human viruses H1N1 or H3N2. Therefore, apparently poor clinical sensitivity for diagnosis of H5N1 infection in patient is not related to a differentially lower sensitivity of the H5N1 virus. This may be due to inadequate specimen collection, age (adults have lower viral loads in upper respiratory tract than children), or inherent limitations in the sensitivity of these tests for detecting patients with influenza A infection. An alternative possibility is that the viral load of avian influenza A subtype H5N1 in the upper respiratory tract is lower than is found with human influenza A subtype H1N1 or H3N2.

THE NEW ERA FOR SPECIFIC PROTEIN ASSAY TESTING

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The human body contains numerous plasma proteins with diagnostic utility associated with immune defense, transport, acute phase protein response, cell death, blood clotting abnormalities. Quantitation of these specific proteins has evolved greatly from early techniques using serum electrophoresis. Over the past decades, numerous technicological advances in both engineering and immunology have lead to immunoassay methods that quantify individual specific proteins with a high degree of reliability, accuracy, and efficiency. Improved techniques of protein purification have lead to identification of monoclonal antisera specific to individual proteins with both high affinity and avidity. Immunonephelometric technologies emerged initially and provided precise and accurate assay results but required dedicated instrumentation and personnel. Immunoturbidmetric technologies using enhanced latex particles and high resolution instrument optics have now catalyzed the consolidation of specific protein testing on fully automated routine chemistry analyzers such as the Abbott Architect c8000. Using a review of published customer evaluations, this workshop will describe these advances along with associated reaction detection algorithms that provide confidence in quality results for specific protein testing across a spectrum of pathological conditions. Assay performance, laboratory workflow and implementation of specific protein testing into routine chemistry testing will be covered. Analysis of specific proteins has now graduated into the fast lane of core laboratory testing.

RECENT DEVELOPMENTS OF BIOCHEMICAL MARKERS IN CLINICAL PRACTICE

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New cardiac markers, on which this lecture is focused, have evolved over the past decade amongst which the cardiac troponins, viz., cardiac troponins T and I, have assumed a critical role in the diagnosis of acute myocardial infarction (AMI) an risk stratification in acute coronary syndrome (ACS).

Evaluation of patients with chest pain is one of the most clinically challenging and difficult problems a clinician faces. In the pre-troponin era, acute ischaemic heart disease was regarded as a binary phenomenon, AMI or non-AMI, using the WHO recommendations that include fulfillment of at least two of three well known diagnostic criteria: a history of acute, severe, and prolonged chest pain, presence of the characteristic ECG changes, and a rise to at least twice the upper reference limit of either total creatine kinase (CK) and its MB isoenzyme or total CK and one of aspartate aminotransferase (AST) or lactate dehydrogenase (LDH) within 72 hours of onset acute event. Unfortunately, chest pain is an unreliable indicator, and unless ST elevations are found at presentation, the ECG can be inconclusive in half to two-third of cases. The rather poor sensitivity and specificity of the traditional enzyme markers for myocardial injury is also well known. The advent of cardiac troponin tests in the early 90s has revolutionized the diagnosis and risk stratification of chest pain patients and they have been proved extremely valuable and sensitive in the diagnosis of myocardial necrosis. Furthermore, the troponins are valuable in risk stratification of both low- and high-risk patient populations. Troponin release kinetics mimic CK-MB, but elevated levels persist for days to weeks after AMI owing to the breakdown of the contractile apparatus over this period. It therefore has the advantage over CK-MB in diagnosing a recent myocardial infarction even when the patient presents late.

Cardiac troponin T (cTnT) has longer history than Troponin I (cTnI), both are extreme sensitive and specific biochemical markers for myocardial injury. Most major studies and analyses have confirmed that cardiac troponins I and T both can identify patients at risk for adverse cardiac events. However, the main issues of cardiac troponins surround: (1) clinically valid cut-off values for cardiac troponin I; and (2) appropriately defining the time of chest pain onset in the context of presentation to the emergency department. Initial studies on troponin risk stratification on patients with known ACS showed a statistically significant increase in mortality amongst those with cTnI levels > 0.4 ng/mL, and ACS patients with elevated baseline cTnT levels have up to 4 times higher mortality than those with normal values. On the analytical side, in vitro modifications of cTnI, its relative instability in vitro and the lack of a well characterized primary standard for assay have hampered a wider acceptance of the troponin assay despite its arguably slightly better diagnostic specificity over cTnT for myocardial injury.

Following the lead of troponins in ACS, the field of new cardiac markers has exploded in the past few years and expanded into areas such as congestive heart failure (CHF), pulmonary embolism and others. B-type (brain-type) Natriuretic Peptide

(BNP) came out of Japan and has had an impact in diagnosing congestive heart failure. BNP has been seen in several recent studies, including OPUS-TIMI 16 and TACTICS-TIMI 18, to be a powerful predictor of death and/or CHF in patients with ACS. One of the newest markers is N-terminal pro-brain natriuretic peptide (NT-proBNP), which has recently been developed as a laboratory test for the diagnosis of CHF. A recent prospective, multi-centre study of 1218 patients admitted with non-ST elevation ACS (NSTE-ACS) showed that patients with the highest quartile of NT-proBNP had a 4-fold increase in the risk of death or MI at 180 days. The association was present in both troponin-positive and troponin-negative patients, and amongst lower- and higher-risk patients using ST *vs* T wave changes on the ECG. It seems that NT-proBNP is a sensitive marker in identifying ACS patients who are at higher risk, even amongst the purportedly low-risk troponin-negative patients.

Over the recent years, clinical studies on head-to-head comparisons of the diagnostic and clinical utility of BNP and NT-proBNP abound. Both are promising marker in identifying left ventricular systolic dysfunction (LVSD). Both assays facilitate diagnosis of symptomatic as well as asymptomatic cardiac dysfunction and as an aid in the differential diagnosis of probable signs or symptoms of heart failure. More recent studies suggest NT-proBNP might be a more discerning marker for early cardiac dysfunction than BNP. Although both markers are reliable and have good diagnostic sensitivity of heart failure, the slightly wider detection range and the more stable structure of NT-proBNP compared with BNP suggest NT-proBNP could play an additional role in the evaluation of patients with LVSD.

Substantial evidence supports a pathogenic role of inflammation in ACS where local or systemic inflammatory processes play a role in determining arterial plaque stability. Biochemical markers of inflammation may thus help risk stratification and identify patient groups who would benefit from particular therapeutic interventions. C-reactive Protein (CRP) is amongst the most widely studied ones. Marked improvement in analytical sensitivity of CRP assay over the past decade enables detection of minute changes in CRP levels the high-sensitivity CRP (hs-CRP) assays. The landmark study by Liuzzo et al published in NEJM 1994 showed that patients presenting with unstable angina and raised CRP levels had a higher rate of death, MI and need for re-vascularization compared with those with normal CRP levels. The findings have been confirmed by more recent trials where hs-CRP was measured. It was shown in many of these recent trials that the predictive value of hs-CRP was independent of, and additive to, cardiac troponins. More importantly, hs-CRP was found to have prognostic value even among patients with negative cardiac troponins and no evidence of ongoing myocardial necrosis. However, the optimal cut-off for defining ACS remains to be determined and there is also no evidence that hs-CRP is helpful for identifying ACS patients who will benefit from a particular treatment. All in all, hs-CRP have a role in potential risk stratification, but it is not currently recommended for routine clinical use although more clinical trial data supporting this have been emerging.

Novel markers of myocardial ischaemia and oxidative stress are emerging; they are gradually moving from the bench to bedside, e.g., unbound free fatty acids, ischaemia-modified albumin (IMA), glutathione peroxidase 1, and myeloperoxidase. The question as to whether such novel biomarkers should be similarly introduced to the routine clinical laboratory remains to be answered.

A multi-marker approach for risk stratification has been the centre of debate of late. Although multiplex testing has gained much support over recent years, a consensus still lacking. According to the draft guideline of the National Academy of Clinical Biochemists, there is less evidence for measuring hs-CRP, BNP, and NT-proBNP in addition to cardiac troponins for assessing risk in patients with a clinical syndrome consistent with ACS than using cardiac troponin alone; but the document still gives the recommendation for multi-marker testing a class II nod.

ACHIEVING QUALITY THROUGH WORKFLOW SIMPLICITY IN A HOSPITAL OUTPATIENT LABORATORY

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While laboratory analysis of routine tests in biological fluids has improved considerably over the years due to advances in measurement technology and instrumentation, the quality of pre-analytical phase of testing such as sample collection, delivery, and checking of specimens prior to testing still poses a challenge as these steps are often verified visually by technologists in laboratories that are not fully automated. The choice of analytical platform to be placed in the laboratory can influence upstream processes, including the number of steps required to achieve a successful completion of specimen testing for each patient. In general, achieving workflow simplicity not only results in material savings and safety, but also reduces the likelihood for laboratory errors and improves the quality of work life for technologists. The technologists also assume the role of "quality assessors" verifying the quality of laboratory results throughout the day, rather than at specific critical time points in the day. We found that the availability of a "distributed" laboratory in the right setting, such as at outpatient clinics, can significantly contribute to the overall quality of test results obtained in a timely manner. More important, however, are the observed intangible benefits for patients and the satisfaction derived from more frequent direct face-to-face interaction between laboratory personnel and users of laboratory service.

APPLICATION OF ARRAY CGH IN MOLECULAR CHARACTERIZATION OF HUMAN DISEASES

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The development in comparative genome methods, principally driven by comparative genome hybridization (CGH) on high-density arrays, has enhanced our understanding of genetic diseases. In particular, the developments on identification of sequences mapped to specific regions through human genome project, arraying them on a slide, and using this array for comparing test and control DNA. This made high-resolution analysis feasible for the comprehensive analysis of detailed changes in genomic copy numbers. Furthermore, unlike classic CGH, array CGH is more amenable to automation and thus it offers significant cost cutting for detailed analysis of chromosomal alterations in reproductive medicine and prenatal/postnatal karyotyping in reproductive pathology. Rapidly accumulating evidence indicates that genomic array approaches allows high resolution characterization of the genomes from tumors and has led to the identification of genes that were not previously evaluated in cancer progression. It also uncovered an unexpectedly large extent of "structural variation" in the human genome. However, despite this progress there are still many challenges to be met before array CGH becomes a routinely ordered clinical test. We are currently exploring the application of array based CGH in clinical and molecular genetics in areas ranging from prenatal genetic diagnosis to fetal losses and cervical cancer.

MONITORING OF DRUG RESISTANCE IN HIV-1

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Human Immunodeficiency Virus Type 1 (HIV-1) is an emerging infectious disease which causes a global pandemic for more than two decades. The introduction of Highly Active Antiretroviral Therapy (HAART) to HIV treatment has significantly improved the treatment response of the HIV-1 infected patients. Therefore, HAART is still the most widely used treatment strategy for controlling the disease progression. As HIV-1 has an error prone reverse transcriptase and a high replication rate, drug resistance related mutations can be easily developed in the HIV genome under drug pressure. For close monitoring of the drug resistance, genotyping resistance test (GRT) has been widely used in the western developed countries as a routine diagnostic tool. However, due to the high cost of the commercial genotyping system, many developing countries in Africa and Asia cannot afford to set up GRT for longitudinal drug resistance monitoring. In Hong Kong, a fast and cost-effective in-house GRT was developed and optimized. It targets the protease and reverse transcriptase region of the HIV-1 *pol* gene. Viral RNA of HIV-1 is extracted and it is reverse transcribed and amplified by RT-PCR plus a nested PCR. The amplicons are sequenced and they are submitted to the Stanford HIV-1 Drug Resistance Database (http://hivdb.stanford.edu) for drug susceptibility interpretation and HIV-1 subtyping. For evaluating the in-house GRT, the performance of the in-house system was compared with the FDA-approved ViroSeq™ HIV-1 Genotyping System. In a panel of 232 plasma samples from 207 HIV-1 infected patients in Hong Kong, both the in-house and ViroSeq™ system could successfully amplify 97.50% of samples with viral load > 400 copies/ml. The in-house GRT showed a higher sensitivity and it provided longer sequences comparing to the ViroSeq™ system. Among 3016 drug resistance interpretations on 13 antiretroviral drugs, the two systems showed 98.54% concordance. Overall, our in-house GRT requires only basic molecular biology set-up and the turn-around time is about 3 days. It shows comparable performance as the commercial system and the cost can be cut down to 1/4. Therefore, it is highly recommended to include this in-house GRT as an essential part for HIV-1 drug resistance monitoring.

THERAPEUTIC POTENTIAL OF RNA INTERFERENCE FOR CANCER

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RNA interference (RNAi) is a powerful tool for gene functional study as well as a promising therapeutics for cancer. The emergence of RNAi has generated enthusiasm not only in scientific community but also in pharmaceutical industry. Many molecules contributed to tumorigenesis have been targeted by RNAi. Significant anti-tumor effects have been shown both in cell culture systems *in vitro* and in pre-clinical animal models *in vivo*. However, some challenges such as *in vivo* delivery, RNAi efficacy and non-specific immune response remain to be overcome before the clinical application of RNAi for cancer therapy can be fully realized.

RECENT ADVANCES IN DETECTION OF HUMAN PAPILLOMA VIRUS

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Human papilloma virus (HPV), a common DNA virus, has been recognized as a causative agent for cervical cancer, one of the commonest cancer in women. While cervical cancer prevention has been achieved through screening women with cervical cytology, advances in the development of molecular techniques to detect HPV has enabled the exploration of the application of HPV detection as an ancillary tool in cervical cancer prevention. HPV detection may be useful on (1) primary screening of cervical cancer, (2) triage of patients with atypical cervical cytology and (3) follow up of patients after treatment. Ideally, HPV test should be able to detect and identify multiple individual HPV types, assess viral load, easy to perform, automated, highly reproducible and demonstrate a high specificity and sensitivity. In actual practice, various approaches have been applied. Currently, HPV DNA tests that have been validated in large trials and epidemiological studies include the hybrid capture test (2^{nd} generation) and polymerase chain reaction (PCR)-based methods employing consensus primers. Thirteen most prevalent high-risk HPV types found in cervical cancer worldwide are included in the hybrid capture test. Other approaches are also being practiced, including DNA sequencing, in situ hybridization, HPV RNA assay, viral load and integration analysis, RMS Line Blot Assay and HPV chips. Distinct advantages and disadvantages exist in different detection techniques. With the increasing availability of various HPV molecular tests, one should bear in mind the importance of quality control and cost-effectiveness related to such HPV detection techniques. Sample quality as affected by storage condition and DNA preparation procedures can affect the performance of the test. Standardization of reference samples, reagents and protocols as well as inter-laboratory comparison is important to maintain the quality of the HPV detection systems. Correlation with clinical scenario and histo-cytological findings will enhance its application in prevention and management of cervical cancer and precursors.