TRAINEE SECTION

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Scientific overview: CSCI-CITAC annual general meeting and young investigator's forum 2011

Abstract

In 2011, members of the Clinician Investigator Trainee Association of Canada - Association des cliniciens-chercheurs en formation du Canada (CITAC-ACCFC) and the Canadian Society for Clinician Investigators (CSCI) held a joint Annual General Meeting (AGM) and Young Investigator Forum (YIF) September 12-14 in Ottawa, ON, Canada. The theme of the meeting was "The Role of Government and Regulatory Organizations in Shaping the Environment of the Clinician Scientist". The meeting was well attended by established clinician scientists and clinician investigator trainees from across Canada. The aim of this scientific overview is to highlight the research presented by trainees at both the oral plenary session as well as the poster presentation sessions of this meeting. This work covers a wide variety of medical disciplines, focusing on translational medicine, from the basic sciences to clinical application.

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Background

The purpose of the joint Annual General Meeting (AGM) hosted by the Clinician Investigator Trainee Association of Canada - Association des cliniciens-chercheurs en formation du Canada (CITAC-ACCFC) and the Canadian Society for Clinician Investigators (CSCI) is to provide trainees from across Canada with the opportunity to network with established clinician scientists, and to showcase their research during the Young Investigator Forum (YIF). Similar to previous years, research areas covered a wide variety of topics from the basic sciences to clinical studies across a number of medical disciplines [1, 2]. The research presented at the meeting is summarized below.

Retrospective Studies and Database Reviews

Several abstracts presented information from comprehensive databases, or retrospectively-collected patient data to provide valuable information about health care delivery, outcomes, programs and patient management.

A study evaluating patient data from the APPROACH database found that individuals with schizophrenia, despite having a high burden of cardiac disease and reduced cardiac function, were less likely to receive coronary revascularization. This was, in part, explained by their relatively healthier coronary anatomy as compared with matched controls, although the issue remains that the rate of mortality remains increased in this population.

The BC organ transplant program was reviewed to examine the efficacy and issues involved with liver transplantation for HIV positive patients. They reported positive outcomes with regards to transplant success rates, and provided an important reference to support the successful implementation and feasibility of transplant centers throughout Canada to accept HIV-positive candidates. In a separate study, data concerning the incidence of Retinopathy of Prematurity (ROP) in the Neonatal Intensive Care Unit (NICU) was retrospectively acquired from the Canadian Neonatal Network for 423 premature neonates, and found that gestational age and birth weight were the biggest predictors of ROP.

Another group conducted a population-based case-control study of male youth between the ages of 16-19 years hospitalized for road trauma (treatment) or appendicitis (control) in Ontario, Canada looking for prior psychiatric diagnoses in the decade before admission. The conclusion was that disruptive behavior disorders explain a significant number of road traumas in adolescent males, recommending programs to address this issue.

With the goal of quantifying oppression in epidemiological research in aboriginal women's health, systematic database searches of peer-reviewed literature were conducted. The factors that have been used include perceived racism, racist attitudes, domestic and sexual violence, individual-level and area-level economic disadvantage as measures of social exclusion, and parental residential school attendance and historical loss trauma as measures of multigenerational colonization impact. There have been limited measurements of gender oppression beyond domestic and sexual violence and also little focus on intersection of sources of violence. Improvements in evaluation of these measurements will assist in designing more effective interventions.

Surgery and Patient Management

With regards to surgical procedures and patient management, considerable research was conducted to address outcomes and current practice standards; for example, the relationship between surgical wait times and outcomes in patients with ureteral stones was examined. Overall, the success of the procedure was high despite considerable wait times; wait times that were still within government defined targets.

A retrospective cohort study compared the management of pleural effusions with either an Indwelling Pleural Catheter (IPC) or Chemical Pleurodesis (CP). By evaluating median survival times from time of catheter insertion as compared with duration of CP treatment, they found a possible survival benefit through management with IPC.

Use of NSAIDs during elective colorectal surgery was not shown to have any significant effect on frequency of anastomotic leaks in a case control study conducted between 2006 and 2010.

Another group evaluated the morphological changes in ovarian adenocarcinomas before and after chemotherapy treatment. Other than tumor grading, which remained the same pre and post therapy, there was an observed morphological change in the carcinoma following chemotherapy, likely as a result of the treatment.

A recent development in robotic-assisted surgery at the University of Calgary created an integrated surgical platform combining contact technology to provide tactile feedback to surgeons with haptic feed-back to transmit forces of surgical dissection. This was successfully tested in rodent models and manufacturing of clinically usable versions are being pursued.

A group from the Ottawa Hospital Research Institute suggested that the hyper-coagulable state that develops after surgical procedures, promotes formation of tumor cell emboli (TCE), which leads to increased metastasis.

Natural Killer (NK) cells were also shown, by knockout mouse models, to be indispensable in this process - reducing post-surgical metastasis when absent. Thus, administration of anticoagulants could reduce the chances of post-operation associated metastasis.

Microbiology and Immunology

Many interesting and novel studies to approach various disease mechanisms and pathways were presented during the YIF. Pulmonary failure due to chronic respiratory infections is prevalent amongst patients diagnosed with Cystic Fibrosis (CF). Resistance of streptococcal isolates from CF patients' sputum to common antibiotics was tested, as was the mechanism of resistance to the antibiotics. Researchers were able to determine that over 60% of the isolates were resistant to erythromycin and azithromycin, with even higher rates of resistance for some of the more novel streptococci strains. Researchers also demonstrated that mutation, rather than horizontal gene transfer, was the primary mechanism of antibiotic resistance in the CF population. During a systemic inflammatory response, neutrophil recruitment to the liver is highly pronounced, but poorly understood. One abstract discussed that the role of these neutrophils was to create Neutrophil Extracellular Traps (NETs) to prevent against bacterial dissemination during a septic infection. The production of vascular NETs by neutrophils was observed during infection, and was dependent upon platelet-neutrophil interactions. NETs did indeed significantly reduce the bacteria disseminated to other organs by ensnaring them in the liver vasculature.

A group from Calgary also characterized the response of smooth muscle cells from the lower and upper segments of the human uterus to prostaglandins. Researchers determined that prostaglandin E2 was able to reduce IL-1 β -mediated upregulation of IL-8 in cells isolated from the lower segment of the uterus, but did not repress IL-8 output in cells from the upper segment.

Cutaneous wound closure involves transition of dermal fibroblasts to myofibroblasts through TGF- β signaling. Using Cre-lox recombination knock out of the ILK gene, a group from the University of Toronto showed that ILK is necessary for dermal fibroblast to myofibroblast transition by regulation of TGF- β type II receptor turnover and signaling.

Regulatory T-cells (TregS), important in inflammatory response mediation in peripheral organ-centered disease processes, were also found to be important in the subsequent/comorbid development of "sickness behaviours." This was studied in a mouse model of hepatic inflammation and determined to

be mediated via TregS inhibition of IL-6 release, and the subsequently reduced liver-brain signaling, via circulating IL-6.

The potential mechanism of immune system evasion by *Neisseria gonorrhoeae* was further examined through the interaction of the pathogen with the Carcinoembryonic Antigen-related Cellular Adhesion Molecule-1 (CEACAM1), revealing that a 5'-inositol phosphatase, SHIP-2, associates with CEACAM1 when bound to a pathogen and reduces phosphoinositide signaling with variable effects on pro-inflammatory secretions. Thus, SHIP2 recruitment by this bacterium may contribute to inhibition of immune cells.

The chaperone Heat Shock Protein 90 was found to play a critical role in both late anaphase and cytokinesis of *Candida albicans* during mitotic exit. Thus, Hsp90 inhibition was proposed as a possible treatment option for candidiasis.

The high morbidity, mortality, and antibiotic resistance seen in human extraintestinal *Escherichia coli* (ExPEC) initiated a study into the possibility of horizontal transfer of virulence genes from Avian Pathogenic *Escherichia Coli* (APEC), found in retail meat. Microarray analysis disproved this possibility but suggested pork and chicken as potential reservoirs for ExPEC virulence factors.

Another study focused on the culturing of bacteria from the airways of cystic fibrosis patients and challenged the idea that the complexity of such a microbiome becomes apparent when non-culture-based molecular profiling is used. The researchers showed that by using a diverse set of growth media, and various anaerobic/aerobic conditions, the majority of the organisms can be grown on plates and that this is particularly useful since an experimental model can be established for most of these organisms as well.

Furthermore, apolipoprotein III from *Locusta migratoria*, was shown to have antimicrobial properties as it had stronger interactions with phosphatidylglycerol of unilamellar vesicles, representing bacterial membranes, than phosphatidylcholine and cholesterol-based vesicles, representing mammalian membranes, as measured via calorimetry and circular dichroism.

Ophthalmology

Identification of the structural and functional changes that occur in the retina with age allows for identification of potential biomarkers of age-associated retinal degeneration. Histological evaluation of the *harlequin* disease mouse model, a model of accelerated aging, demonstrates that nuclear kinesis to the segment layer occurs prior to retinal thinning, and may serve as an early structural marker of age-associated retinal degeneration.

Another study looked into eye-care services across the Canadian provinces for individuals under 18 years of age. Researchers showed, in locations where such services are not covered by the provincial healthcare, that individuals were 24% less likely to use available services. Additionally, due to a shortage of specialists in the territories of Yukon, North West and Nunavut, children and adolescents were 40% less likely to use eye-care services. The importance of addressing these issues was also pointed out.

Neurology and Psychiatry

Using rat cerebellar slices, one group recorded potassium and calcium currents and found that fluctuations in extracellular calcium (Ca^{2+}) are important regulators of neuronal excitability via the Cav3-Kv4 signaling complex. The reduction in intracellular Ca^{2+} decreased neurotransmitter release and, therefore, negatively influenced synaptic transmission. In addition to modulating synaptic transmission, when homeostatic control over intracellular Ca^{2+} is lost, neuronal cells are more susceptible to cell death. The group then examined the role of mitochondria in regulating Ca^{2+} transients. They used GCaMP4, a novel genetically encoded Ca^{2+} indicator, and were able to show that inhibition of mitochondrial respiration produced a delayed decay in Ca^{2+} transients and regional elevations in intracellular Ca^{2+} . The authors conclude that mitochondrial respiration is important to maintain proper Ca^{2+} dynamics in neuronal cells. Another group identified that the Ca^{2+} -permeable cation channel, transient receptor potential cation channel, subfamily M, member 2, TRPM2, is inhibited by glutathione, a naturally occurring antioxidant that diminishes with age. The group further demonstrates that TRPM2 currents are enhanced over time in cultured hippocampal neurons. Since the TRPM2 channel is activated following exposure to oxidative stress, and prolonged activation promotes cell death, the authors suggest that the TRPM2 channel may contribute to the age-related susceptibility of neurons in a variety of neurological disorders.

In an effort to track the efficacy of nerve injury treatments for nerve regeneration, an advanced imaging modality to simultaneously image multiple nerve branches was developed. Multi-labeling of motor neurons was successful in a rat model, which will potentially be useful in helping developing nerve therapies.

One group established the hypothesis that schizophrenia is associated with demyelination in oligodendrocytes by creating hyperactivity and impaired spatial working memory in mice upon treatment with cuprizone which leads to demyelination. Enhanced remyelination of oligodendrocytes was achieved by

administration of the atypical antipsychotic quetiapine and this led to improved spatial working memory.

Oncology

In cancer research, use of novel sequencing technologies and microarray-based methods stood out. Next generation sequencing technology led to the discovery of novel translocations in epithelioid sarcoma, a disease of both mesenchymal and epithelial morphology. Such fusions would not have been identified via traditional cytogenetic analyses. Additionally, microarray analysis of the microRNA profile in cutaneous squamous cell carcinoma led to further studies that preliminarily found miR-125b to have a significant role in dedifferentiation and aggressive nature of this cancer. In terms of therapeutics, use of oncolytic Reo virus in renal cell carcinoma was evaluated and was found to be effective in both *in vitro* and *in vivo* models; however, it was shown to have antagonistic effects when combined with Sunitinib, a first line treatment currently used for renal cell carcinoma.

Previous research identified Y-Box-Protein-1 (YB1) as a potential contributor to the genesis of pediatric glioblastoma multiforme (GBM), a highly aggressive astrocytoma. Investigators built upon these findings by overexpressing or silencing YB1 in various astrocyte cell lines that were then assessed *in vitro* and, with mice xenografts, *in vivo*. Results showed that nuclear YB1 enhances cell proliferation, while cytoplasmic YB1 decreased proliferation and enhanced cell migration and metastasis. Analysis of pediatric GBM associated nuclear YB1 with poor prognosis, suggesting that targeting YB1 in the treatment of GBM may have questionable therapeutic efficacy. A second group examined critical regulatory molecules in human medulloblastoma brain tumors. This study assessed whether the sonic hedgehog (Shh) pathway regulates expression of Bmi1, a protein previously shown to enhance proliferation and tumorigenesis. Results indicate that a positive feedback loop may exist to maintain medulloblastoma brain tumor-initiating cells (BTICs); whereby Shh drives Bmi1 expression and Bmi1 may activate Shh pathway genes.

Another group developed a lentiviral-based cellular barcoding method as a means of tracking stem cells and their clonally-generated progeny. This barcoding method allowed researchers to evaluate the currently accepted model of mammary epithelial cell differentiation.

Chronic Lymphocytic Leukemia (CLL) is often diagnosed in elderly populations with has a relatively poor prognosis. Whether the prognosis for these elderly patients was associated with increases in age-related cytokine levels (IL-6 and IL-8) was investigated. Researchers assessed mortality as caused by

CLL, as opposed to co-morbid conditions associated with advanced age, and found that IL-6 and IL-8 may be useful prognostic indicators, and subsequently, important targets to improve outcomes in these patients.

Endocrinology

An amyloid mediated-inflammatory pathway, determined by increased macrophage release of IL-1, was identified in pancreatic islet cell transplant mouse models. Researches were able to add to the current literature supporting innate immune activation with amyloid diseases, and also provide support for the use of anti-IL-1 therapy to improve islet graft function.

In ApoE deficient mice, glucosamine supplementation was tested as a potential contributor to the accelerated atherogenic processes observed in diabetes. Quantitative measures of atherosclerotic lesion size in the aortic root found that lesion size was increased in these mice.

Urology

The use of “traction therapy” in Peyronie’s disease, in which fibrous plaque build up occurs in the connective tissue of the penis to cause both histological and functional changes, was evaluated. The therapy involved a microsurgical method of inducing longitudinal stress on the plaques, and examination of the tissue showed that this may be a novel method to remodel the plaques, and therefore act as an important treatment option.

Radiology, Molecular Imaging and Clinical Measurements

One study in this category found that three-dimensional visualization, as compared with two-dimensional visualization, provided increased efficiency and reduced operating-times in robotics-assisted cardiac surgery. Another group utilized Cardiac MRI in a type 2 diabetes group without previous ischemic heart disease; qualitative stress-perfusion imaging data was used to show coronary microvascular disease was present even in a well-controlled patient population.

Noticing the large variation in lung density in the adult population and between patients, one group from the University of Western Ontario examined whether the incorporation of lung density information into attenuation correction algorithms of whole-body PET/MRI could improve quantitative accuracy. Indeed, improvement was significant for the lungs and its surrounding tissue, including the myocardium.

The muscle innervations and architecture pattern of the superior lateral pterygoid (SLP) were studied using 3D modeling which showed that the superior part of SLP can be divided into quadrants based on innervation patterns. This indicates that the movement of the disc capsule complex at the temporomandibular joint (TMJ) is finely controlled, and improper activation of the quadrants may result in TMJ disorder progression. Similarly, another group constructed 3-D models of the piriformis muscle, establishing that its musculotendinous architecture provides support to the anatomic impingement of the sciatic nerve as it passes through the sciatic foramen. This architectural support mechanism may be affected in the piriformis syndrome.

One group visualized skeletal muscle fiber bundles three dimensionally using computerized script and were able to apply a mathematical model to determine the fiber bundle distribution and an architectural map of the fiber bundles. Another group investigated pulse-oximetry measurements on the ear, a standard clinical method to detect hypoxemia. This group demonstrated that pulse-oximetry was less accurate than finger clip oximetry readings, as validated to arterial blood oxygen saturation levels.

Orthopedics

MRI using 3D multi-echo imaging and a multi-point fat-water separation method was used to measure patellar cartilage volume and quadriceps muscle volume upon voluntary knee contractions in 23 osteoarthritis patients. The results showed that weakness of the distal vastus medialis allows for maltracking of the patella during knee flexion and may contribute to patellofemoral knee arthritis.

Using sandwich enzyme-linked immunosorbent assay (ELISA) levels of Proteoglycan 4 (PGL4) and hyaluronan (HA) were measured in clinical samples of synovial fluid from osteoarthritis (OA) patients and compared with cartilage-on-cartilage friction test results. Results showed that PGL4 levels may be reduced in some OA patients and its supplementation could be of benefit.

Cardiology

Low-level inflammation has been indicated to contribute to atherosclerosis and cardiac disease progression. Recruitment of monocytes contributes to the inflammatory process and attenuating monocyte migration may diminish inflammation. Treatment with Slit2 was able to inhibit monocyte migration in an *in vitro* transwell assay and an *in vivo* model of murine irritant-induced peritonitis. Interestingly, inflammation also

plays a role in post-myocardial infarction patients. In response to molecules such as reactive oxygen species, ATP and urate, a multi-protein complex named inflammasome is formed. Inflammasome activates IL-1b, causing inflammation. Mice with cardiac-specific over-expression of calcineurin (CN/Tg) develop hypertrophic hearts with inflammation capturing the aforementioned inflammatory conditions. It was shown that inflammasomes are active and significant in myocardial dysfunction and arrhythmogenesis in CN/Tg mice in a Protein Kinase C-dependant manner. Thus, inflammasomes present a potential new therapeutic target for treatment of heart failure.

One study investigated the use of statins in young patients, between 20 and 40 years, of who have secondary dyslipidemia and are considered to exhibit mild to moderate risk for cardiovascular disease (CVD). Review of the literature shows a general lack of knowledge on benefits of long-term use of statins in young adults with secondary dyslipidemia. The authors suggest that patients should be provided with the option of short-term statin use after being informed of the benefits and risks. Improved risk assessment and management of CVD among young adults is recommended.

Genetics and Cell Signaling

A thorough review of copy number changes seen in congenital renal dysplasia was carried out by comparative genomic hybridization techniques. The results showed novel deletions in 1p36.2 (87 genes) and in 16q24 (32 genes) in individual patients. Additionally, copy number changes in genes such as SIK1, LACTB2, and PTPRD were also identified. These genes are specifically expressed in mouse ureteric buds, the source of nephrogenic signals in the kidney, and, thus, they may regulate renal development.

Another condition that was studied using genetic techniques was chronic pain, a significantly prevalent and costly healthcare problem. The researchers found a correlation between expression of the dorsal root ganglion *Chrna6* (alpha6 subunit of nicotinic cholinergic receptor gene) and neuropathic mechanical allodynia following spared nerve injury. Also, *Chrna6* null mice and gain of function mice were examined for allodynia following injury and the results indicate a protective role for *Chrna6* in chronic pain, thus identifying a new target for future therapeutics.

A genetic epistasis analysis and immunoprecipitation followed by Western blotting was used to assess whether PI3K signaling regulates the serine/threonine kinase Akt following exposure to DNA damaging agents. The investigators employed a *C. elegans* model and were able to determine that DAF-2 and PDK-1, downstream kinases in the canonical PI3K

signaling pathway, functioned independently of one another. Furthermore, Akt phosphorylation was increased at Ser517, and not at the PDK-1 phosphorylation site Thr350, suggesting that a pathway independent of PI3K regulates Akt activity in response to DNA damage.

Characterization of signals required for the generation of stem cells is currently an area of active investigation with significant potential clinical implications. One study assessed the role of high-mobility group A2 (Hmga2) in the generation of hematopoietic stem cells (HSCs). Hmga2 expression is significantly enhanced in fetal HSC when compared with adult HSCs. Hmga2 knockout mice were employed. The absolute number of HSCs was reduced in Hmga2^{-/-} animals and the expression of Hmga2 increased the number of daughter cells produced following transplantation. The authors conclude that Hmga2 is critical for the high self-renewal of fetal HSCs.

Clinical and Public Health Practices

The practice of using blue lights in public bathrooms to discourage intravenous drug users was examined through qualitative research on a cohort of 18 individuals from the cities of Vancouver and Abbotsford. Under the blue lights, the task of finding veins for injection becomes more difficult. The results outstandingly indicated that use of these lights does not have any decision making impact on the drug users choice of the location for their injection but that it has negative effects due to increased injuries and more drug seeking behavior caused by poor injections.

Surveys on education in the Canadian healthcare system at the six medical schools of Ontario showed a general sense of a lack of enough time spent on clinical and public health practices in the curriculum. As future Canadian physicians, students desire to know more about the healthcare system to which their profession is dedicated and this is therefore an important subject for medical schools to cover.

Further surveys on the use of computerized clinical decision support systems (CCDSS) and Electronic Medical Records (EMR) in clinical practice showed improved processing of care but not necessarily better patient outcomes with these more modern clinical tools.

Furthermore, epidemiologic efforts pointed to potential factors that can aid in categorization of bipolar disorder. Diagnosis of bipolar disorder I vs. II is currently based on differentiation of manic/mixed and hypomanic episodes and is a challenging task. But researchers found differences between the two conditions based on demographics, clinical features, depressive symptomatology and co-morbidities, which can help clinicians when making a diagnosis. In terms of diagnosis of

metabolic syndrome, use of different definitions by the National Cholesterol Education Program Adult Treatment Panel and the International Diabetes Federation were shown to lead to different results. The later system led to the finding of increased prevalence of the condition and better prediction of cardiovascular risk in men while the former predicted cardiovascular risk better in women.

Lastly, adherence to guidelines in the care of heart failure, community-acquired pneumonia and acute exacerbations of chronic obstructive pulmonary disease in two Canadian tertiary centers showed that the majority of physicians were appropriately following the guidelines. A different study looking at management of heart failure patients and screening/treating for thyroid function as part of the clinical approach, a requirement by the international heart failure guidelines, in three Calgary clinics found strong adherence to the guidelines as well; however, both studies found variation in practices.

Conclusions

The high quality and breadth of translational research presented by clinician investigator trainees at the 2011 CSCI-

CITAC annual general meeting demonstrates the strength of training received by our next generation of clinician scientists, and highlights the benefits of mentorship from established investigators. The 2012 meeting is expected to further expand upon these exciting discoveries and allow trainees to network further with other trainees and mentors.

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