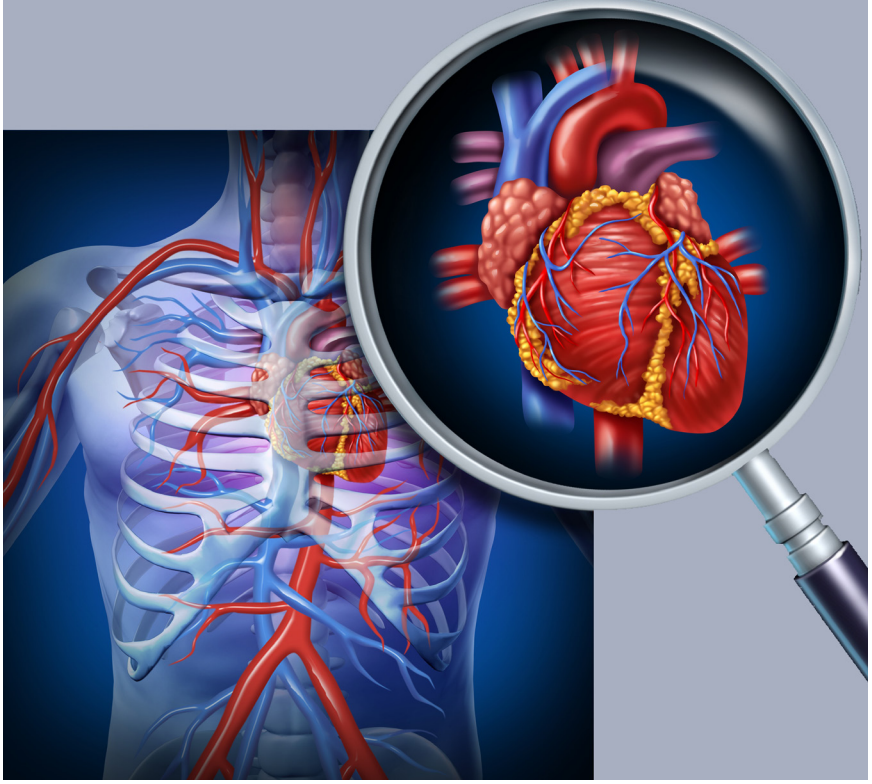

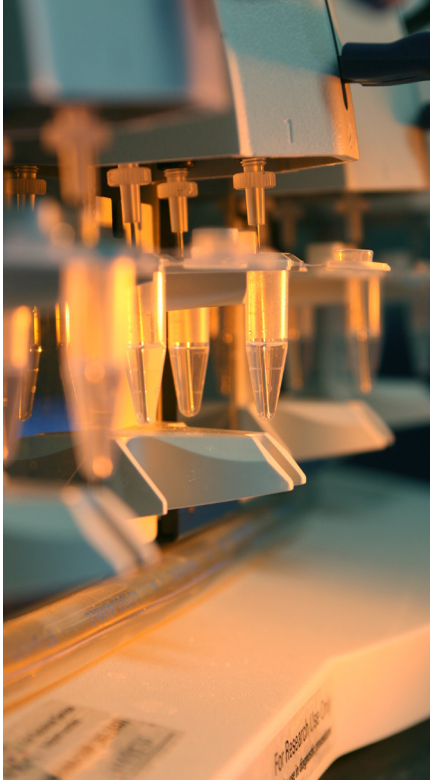

UNIVERSITY OF OTTAWA
HEART INSTITUTE
INSTITUT DE CARDIOLOGIE
DE L'UNIVERSITÉ D'OTTAWA

26th Annual Research Day
Abstract Booklet

May 14, 2013

Foustanellas Auditorium
University of Ottawa Heart Institute

www.ottawaheart.ca/research-day

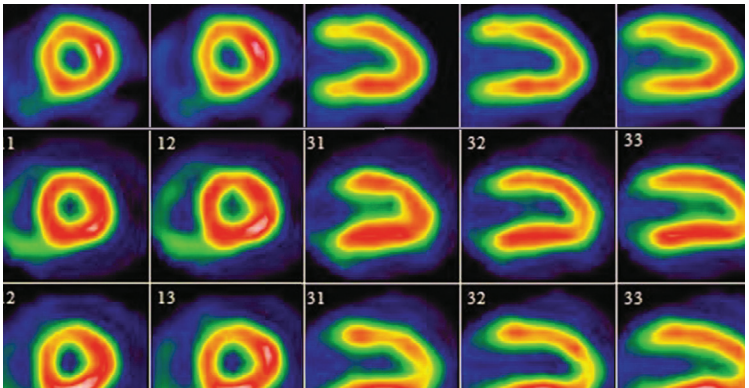

Table of Contents

ORAL PRESENTATION ABSTRACTS

O-1 Improved Diagnostic Accuracy When Combining Computed Tomography Angiography and Corrected Coronary Opacification in Patients with Coronary Stents

Taylor F. Dowsley, Brian Mc Ardle, Mohammed Alam, Girish Dwivedi, Benjamin J.W. Chow

Background: The evaluation of stented coronary segments can be challenging with coronary computed tomography angiography (CCTA) due to artifacts such as beam hardening and partial volume averaging ("blooming" artifact). The detrimental effects of these artifacts on stent evaluability are largely dependent on stent size and composition with the majority of stents </= 3 mm being non-evaluable. Methods that can increase the diagnostic accuracy of CCTA in percutaneous coronary intervention (PCI) patients are desirable. Preliminary studies have shown that corrected coronary opacification (CCO) gradients measured from CCTA can be predictive of abnormal resting coronary flow. In this proof of concept investigation, we determine if CCO differences across coronary stents can improve the accuracy of CCTA.

Methods/Results: PCI patients who underwent CCTA and invasive coronary angiography (ICA) within the subsequent 3 months were retrospectively analyzed. Coronary luminal attenuation values (normalized to the aorta) were measured proximal and distal to coronary stenoses and stents. CCO differences were calculated and compared to resting coronary flow as measured by the corrected TIMI frame count (cTFC). The ability of CCO to predict abnormal resting flow and functionally significant stenoses was assessed using receiver operator characteristics (ROC) analysis. The diagnostic accuracy of combined visual CCTA/CCO for detection of functionally significant stenosis was then compared with CCTA visual assessment alone. Seventy-five coronary arteries (n= 25, mean age = 61.4 years, men = 80.0 %) were assessed. In stented coronary vessels, CCO identified abnormal resting flow and functionally significant stenoses with an area under the curve of 0.867 and 0.929 respectively. Combined CCTA/CCO identified functionally significant stenoses in stented vessels with diagnostic accuracy of 88.9 % and sensitivity, specificity, positive predictive value and negative predictive value of: 66.7% (24.1-94.0%), 95.2% (74.1-99.5%), 80.0% (29.9-98.9%), and 90.9% (69.4-98.4%), respectively. In contrast, CCTA anatomic assessment in stented vessels had a lower overall diagnostic accuracy of 35.7%. This was driven by considerably lower specificity(30.8%, (10.3 to 61.1%), $p < 0.05$) and positive predictive value (10.0% (0.5 to 45.9%), ns) of CCTA anatomic assessment.

Conclusions: CCO differences are predictive of abnormal resting flow and of functionally significant stenoses in stented coronary vessels. Combining CCO analysis with visual anatomic assessment may improve the diagnostic accuracy of CCTA in PCI patients.

O-2 **A Novel Combined Approach Using Genetics and Clinical Variables to Predict High on-Clopidogrel Platelet Reactivity**

Garg N, Roberts JD, Wells GA, Labinaz M, Le May MR, Glover C, Froeschl M, Dick A, Marquis JF, McPherson R, Hibbert B, Bernick J, So DY

Background: High on-clopidogrel platelet reactivity (HPR) is associated with major adverse cardiovascular events following percutaneous coronary intervention (PCI). Several clinical variables and genetic polymorphisms influencing clopidogrel biotransformation have been linked to HPR. We aimed to determine the relative association of these variables to HPR and to derive and evaluate a novel combined genetic/demographic approach to identify patients at increased risk for HPR after PCI.

Methods: 185 patients who received a 600 mg loading dose of clopidogrel and underwent PCI for stable coronary artery disease or non-ST-elevation acute coronary syndrome (ACS) (derivation cohort) were genotyped for genetic variants influencing HPR: CYP2C19*2, CYP2C19*3, CYP2C19*17, and ABCB1 3435 C>T. A multivariate analysis of genetic and clinical variables was used to develop a weighted risk score for predicting HPR, based on the derivation cohort. The risk score was then applied to a validation cohort to evaluate its ability to predict HPR in patients who underwent PCI for ST-elevation ACS.

Results: Baseline demographics among patients in the derivation cohort include: mean age 60.1 ± 9.0, mean BMI 28.6 ± 5.6, 94.6% Caucasian, 22.7% with diabetes mellitus (DM), 19.4% on proton-pump inhibitors (PPI), 37.8% with ACS, and 31.4% current smokers. Baseline demographics among 72 patients in the validation cohort include: mean age 57.0 ± 9.2, mean BMI 28.7 ± 5.2, 91.7% Caucasian, 15.3% with DM, 6.9% on PPI, and 56.9% current smokers. CYP2C19*2 was present in 24.3% and 26.4% of patients in the derivation and validation cohorts, respectively. CYP2C19*2 ($p=0.002$), DM ($p<0.001$), age>60 ($p=0.015$), and BMI>28.1 ($p=0.032$) were independently associated with HPR and included in the prediction model for the risk score. Use of the risk score provided incremental predictive value for HPR compared to genetics alone. Sensitivity and specificity of the risk score in the validation cohort were 80% and 61.3%, respectively.

Conclusions: CYP2C19*2, DM, elderly age, and elevated BMI were independent predictors of HPR. A combined genetics and demographics approach using these variables provides incremental value in predicting platelet response to clopidogrel compared to genetics alone. Validation of this model in larger cohorts may lead to development of future strategies for personalized anti-platelet therapy.

O-3 **Clinical Impact of Mild Acute Kidney Injury Following Cardiac Surgery**

Elsayed Elmestekawy, Thierry Mesana, Bernard McDonald; Christopher Hudson, Marc Ruel, Vincent Chan, Munir Boodhwani

Objectives: Dialysis-dependent renal failure occurs infrequently following cardiac surgery but leads to substantial morbidity and mortality. In contrast, milder degrees of acute kidney injury (AKI), based on small increases in serum creatinine, occur frequently but the independent impact of mild AKI on outcome remains unclear.

Methods: Between January 2010 and December 2012, 4158 consecutive patients undergoing cardiac surgery comprised the study cohort. AKI was defined according the AKI Network Criteria as stage I, II, or III. A non-parsimonious multivariable logistic regression model including preoperative and intraoperative variables was constructed to determine a propensity score for the development of stage I AKI followed by a greedy matching algorithm to create 1:1 propensity-matched pairs.

Results: Incidence of stage I AKI in the entire cohort was 20%. Stage I AKI patients were more likely to be older, have diabetes, hypertension, preoperative renal dysfunction, and poorer LV function, require more urgent surgery and longer cardiopulmonary bypass. Following propensity matching, the 806 matched pairs were similar in terms of all of the above characteristics (all $p > 0.5$). Within the matched cohort, AKI patients had higher mortality (2.0% vs. 0.5%, $p = 0.006$), higher incidence of neurologic dysfunction (14% vs. 8%, $p<0.001$), and longer duration of mechanical ventilation (30±3 vs. 16±1 hours, $p<0.001$). Intensive care unit stay (4.2±0.2 vs. 2.4±0.1 days, $p<0.001$) and hospital length of stay (16±0.5 vs. 13±0.4 days, $p<0.001$) was significantly longer in matched AKI patients.

Conclusions: Patients with even mild degrees of AKI experience increased mortality and morbidity compared to their matched counterparts. Interventions that prevent or mitigate post-cardiac surgery AKI can yield substantial clinical benefit.

O-4 **Efficacy of Metoprolol in Preventing Cardiovascular Complications and All-cause Mortality in Emergent Surgeries**

Louise Sun[1], Homer Yang[1], George Wells[2], Philip J. Devereaux[3], Gordon Guyatt[3], Salim Yusuf[3], Kate Leslie[4]
[1]Department of Anesthesiology, University of Ottawa
[2]Department of Epidemiology and Community Medicine, University of Ottawa
[3]Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON
[4]Department of Anesthesiology, Royal Melbourne Hospital, Melbourne, VIC, Australia

Background: The Perioperative Ischemic Evaluation (POISE) (Devereaux et al Lancet 2008) randomized trial suggested a decrease in major cardiac complications (non-fatal myocardial infarction, non-fatal cardiac arrest, cardiovascular death), and a possible increase in all-cause mortality with administration of metoprolol to patients undergoing non-cardiac surgery. We addressed the question of whether these effects might differ in emergent versus elective surgery.

Methods: Following institutional REB approval, anonymized data from POISE was used for the analysis. Outcomes of interest were major cardiac complications (CVC) and all-cause mortality within 30 days. Time to event for each outcome was assessed using a multivariable Cox proportional hazards model with respect to treatment (metoprolol versus placebo) and urgency of surgery (emergent (i.e. non-elective surgery within 24 hours of acute hospital presentation) versus elective). Effect modification of treatment by urgency of surgery was tested using an interaction term of treatment by surgery. Measure of association was hazard ratio (HR, 95% CI) and Kaplan-Meier (KM) curves were used to describe the time-to-event patterns of the outcomes. All analyses were conducted using SAS 9.3.

Results: Of the 8351 patients, 5 were excluded since information on urgency of surgery was unknown; 10.5% (878/8346) were emergent patients. CVC occurred in 10.8% and 5.9% of emergent and elective patients respectively. For emergent patients we found a HR of 0.97 (95%CI 0.65-1.45) associated with metoprolol use and for elective patients HR 0.81 (95%CI 0.67-0.98; p=0.027) (interaction p=0.19, suggesting similar underlying effects of metoprolol in the two populations). Emergent surgery elevated cardiac risk in both the metoprolol (HR 2.09, 95%CI 1.52-2.87; p<0.0001) and placebo (HR 1.74, 95%CI 1.28-2.38; p=0.0004) treatment groups. Mortality occurred in 8.4% of emergent and 2.0% of elective cases. For mortality, in emergent patients we found a HR of 1.49 (95%CI 0.93-2.36) associated with metoprolol use and for elective a HR of 1.27 (95%CI 0.92-1.75) (interaction p=0.30, suggesting similar underlying effects of metoprolol). Emergent surgery elevated mortality in both the metoprolol (HR 4.61, 95%CI 3.20-6.64; p<0.0001) and placebo (HR 3.93, 95%CI 2.56-6.05; p<0.0001) treatment groups.

Conclusion: While risk of CVC and death were higher in patients undergoing emergent versus elective surgery, we found no evidence of a difference in the relative effect of metoprolol in the two populations.

O-5 **Caspase-dependent Pathways Governing Cardiac Hypertrophy**

Charis Putinski[a,b], Mohammad Abdul-Ghania[ab], Rebecca Stiles[a,b], Steve Brunette[a], Pasan Fernando[b,c,d] and Lynn A. Megeney[a,b,e]
[a]Ottawa Hospital Research Institute, Sprott Centre for Stem Cell Research, Regenerative Medicine Program, Ottawa Hospital, Ottawa, ON K1H 8L6, Canada
[b]Faculty of Medicine, Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada
[c]Division of Cardiology, Canadian Molecular Imaging Centre of Excellence (CMICE), University of Ottawa Heart Institute, Ottawa, ON K1Y 4W7, Canada
[d]Nordion Inc., Ottawa, ON, K2K 1X8, Canada
[e]Department of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada

BACKGROUND: Cardiac hypertrophy is the cellular response that mediates pathologic enlargement of the heart. This is initially an adaptive response; however, chronic stress on the heart is a maladaptive process that can progress to end-stage heart failure. This maladaptation is also characterized by cell behaviours that are typically associated with apoptosis, including cytoskeletal reorganization and disassembly, altered nuclear morphology and enhanced protein synthesis/translation. Here, we investigated the requirement of apoptotic caspase pathways in mediating cardiomyocyte hypertrophy.

METHODS/RESULTS: Primary ventricular cardiomyocytes were isolated from 2 day old rat pups followed by various treatments. Cardiomyocytes were treated with an adrenergic hypertrophy agonist phenylephrine (PE); caspase inhibitors (caspase 3 or caspase 9 peptide inhibitors; biologic caspase inhibitors p35 expressing adenovirus (AdV) or Mcl-1 expressing AdV) followed by PE treatment; and procaspase activating compound-1 (PAC-1), a small molecule activator of caspase 3. Cardiomyocytes treated with PE displayed rapid and transient activation of the intrinsic mediated cell death pathway, characterized by elevated levels of caspase 9, followed by caspase 3 protease activity. Disruption of the intrinsic cell death pathway at multiple junctures, led to a significant inhibition of cardiomyocyte hypertrophy during agonist stimulation, with a corresponding reduction in the expression of known hypertrophic markers and transcription factor activity. Caspase 3 mediated induction of cardiomyocyte hypertrophy was also examined in the intact myocardium. Osmotic mini-pumps were implanted in rats containing either saline or PE to induce pathologic hypertrophy. PE treated rat groups were then subject to ultra-sound guided micro-injection of the left ventricle wall with either control GFP or p35 expressing adenoviruses. Similar to the in vitro experimental observations, inhibition of effector caspase activity attenuated cardiomyocyte hypertrophy in the intact heart. Furthermore, treatment of cardiomyocytes with PAC-1 resulted in a robust induction of the hypertrophy response in the absence of any agonist stimulation. Finally, a temporal caspase-dependent decline in full length histone deacetylase 3 (HDAC3) protein levels suggests HDAC3 as a potential hypertrophic caspase cleavage target.

CONCLUSIONS: These results suggest that caspase-dependent signalling is both necessary and sufficient to promote cardiomyocyte hypertrophy. These results also confirm that cell death signal pathways behave as active remodelling agents in cardiomyocytes, independent of inducing an apoptosis response.

O-6 **Muscle Enriched A-Type Lamin Interacting Protein (MLIP) a Novel Regulator Cardiac Growth and Function**

Marie-Elodie Cattin, Esther Mak-Washburn, Patrick G. Burgon

The newly discovered muscle-enriched A-type lamin interacting protein (MLIP) is highly expressed in the heart. Its biological function remains elusive but it may be of high relevance for cardiac function as it has no paralogous homologue suggesting no functional redundancy.

To elucidate MLIP's function, we generated both hemizygous (MLIP+/-) and homozygous (MLIP-/-) null mice and investigated their cardiac phenotype. At 8 weeks of age, they both develop dilated cardiomyopathy characterized by enlarged hearts (heart to body weights MLIP+/+ 5.62mg/g vs MLIP+/- 10.73mg/g & MLIP -/- 11.03mg/g), functionally dilated (LVDD: +/+ 2.89mm; +/- 3.93mm; -/- 4.11mm) with reduced function (LVFS: +/+ 47%; +/- 31%; -/- 29%). Histological analysis shows no overt abnormalities of both MLIP+/- and MLIP-/- hearts. Preliminary data indicate a preserved cardiomyocyte size in MLIP+/- and MLIP-/- mice, suggesting that the increase in heart size might be due to hyperplasia rather than cellular hypertrophy. Moreover, cardiac gene expression of 12 week-old MLIP+/- and MLIP-/- mice reveals an upregulation of genes involved in cardiac development and control of cell cycle. Cardiomyocyte proliferation of post-natal and adult MLIP+/- and MLIP-/- hearts and cardiac response to pro-hypertrophic stimulus are currently under investigation.

In conclusion, MLIP is a newly discovered protein that impacts genes involved in cardiac development, growth and function.

O- 7 **Scaffold protein CARD11 Modulates Receptor Mediated Apoptosis in the Myocardium following Myocardial Infarction**

P. Wood[2,4], M. Moon[3,4], L. Zhang, F. Dawood[4], M. Nishi[4], K. Naito[4], P. Liu[2,3,4]
Department of Physiology[2], Institute of Medical Science[3], Faculty of Medicine, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada. University Health Network[4], Toronto, Ontario, Canada

Background: The CARD scaffold superfamily members, named for containing the common caspase-recruitment domain, are known to be important modulators of inflammatory and apoptotic signalling. CARD11, a membrane bound scaffold, is essential for the production of cytokines in the immune cellular response but is present in heart and lung tissue, where its effects are unknown, and described to associate with apoptotic players such as BCL10 and Caspase-8. Due to the complex nature of cardiac response to myocardial infarction (MI), we hypothesize that CARD11 can act as a regulator and integrator of receptor mediated apoptotic pathways following cardiac tissue injury.

Methods & Results: Experimental MI was created in CARD11-deficient mice (CARD11-/-) and wild-type littermate controls (CARD11+/+) by left coronary artery ligation. Survival over 28 days was significantly improved in CARD11-/- (95%) over littermates (47%) over 28 days. CARD11-/- mice exhibited lower rates of rupture between days 3 and 7 post-MI and reduced infarct size. TUNEL staining identified a lower percentage of apoptotic cells in the border zone of CARD11-/- hearts post-MI and western blot analysis displayed decreased levels of cleaved caspase-3, -8 and -9 as well as pro-apoptotic mitochondrial proteins Apaf1, cytochrome-C and Bid. CARD11 expression during this period is significantly increased in the myocardium and is paralleled by general stress induction by H2O2 (25µM) and death receptor activation by Fas agonist Jo2 (1µg/ml) and TNF-alpha (50ng/ml) in isolated neonatal cardiomyocytes. Annexin V (AV) and propidium iodide (PI) double staining demonstrated decreased levels of apoptotic cells (AV+/PI+) following H2O2, Jo2 and TNF-alpha treatments in CARD11-/- neonatal cardiomyocytes to that of placebo levels when compared to CARD11+/+ cells, as analyzed by flow cytometry. Pretreatment with caspase inhibitor zVAD (25µM) blocked this effect in CARD11+/+ and replacement of CARD11 through plasmid transfection restored the apoptotic phenotype in CARD11-/- cells.

Conclusion: From these results, the recruitment of CARD11 to death receptor signalling complexes propagates apoptotic signalling through caspase activation in the infarct and border zone and its removal can provide protection against cardiomyocyte death and cardiac rupture.

O-8 **The Redox-sensitive Transcription Factor NRF2 (NFE2L2) Promotes a Hyperfused Mitochondrial Network**

Timothy E. Shutt[1], Robert A. Screaton[2], Ross Milne[1], Heidi M. McBride[3]
[1]University of Ottawa Heart Institute
[2]Children's Hospital of Eastern Ontario
[3]McGill University, Montreal Neurological Institute

BACKGROUND: Mitochondria in the heart have been shown to fragment following ischemia. Blocking ischemia-induced mitochondrial fragmentation is protective, resulting in reduced infarct size. While it is known that mitochondrial morphology plays an important role in mediating both mitochondrial and cellular function, there is still much to be learned about the mechanisms regulating this process. In a process described as Stress Induced Mitochondrial Hyperfusion (SIHM), mitochondrial hyperfusion occurs rapidly following various types of cellular stress. This interconnected reticulum is refractive to apoptosis, suggesting that SIHM may play an important protective role during the early stages of cellular stress. Treatments that lead to a hyperfused mitochondrial network could prove a valuable therapeutic approach in the treatment and prevention of cardiac ischemic/reperfusion injury. We recently demonstrated a role for oxidized glutathione in the direct activation of the mitochondrial fusion machinery, providing a mechanistic link between oxidative stress and fusion. However, questions remain about the persistence of the fused mitochondrial state and whether fission may also be negatively regulated.

METHODS/RESULTS: As part of a genome-wide screen to find novel factors modulating mitochondrial morphology, we identified the redox sensor KEAP1, whose silencing led to a hyperfused phenotype. A key function of the redox sensitive protein KEAP1 is inhibition of the transcription factor NRF2 (NFE2L2) a regulator of the cell's antioxidant response. Using a combination of approaches, we demonstrate that NRF2 is the primary agent through which Keap1 exerts its effects on mitochondrial morphology. Although mitochondrial fusion was not activated in these cells, increased levels of NRF2 resulted in a significant decrease in the levels of the fission protein DRP1, explaining the hyperfused mitochondrial network.

CONCLUSIONS: This study suggests that the activation of NRF2 will prolong the hyperfused phenotype, thereby protecting the cell from apoptosis until the stress has been neutralized. NRF2 also provides additional protective effects as it upregulates many genes involved in antioxidant stress response and proteasome function. Notably, the NRF2 pathway is a drugable target. Several compounds currently in clinical trials, such as bardoxalone metyl and sulforaphane, have been identified that increase NRF2 activity. In summary, we have identified a novel role for the KEAP1/NRF2 redox pathway in maintaining a fused mitochondrial network following cellular stress.

O-9 **Integrin α2β1 is Required for Synergistic Effect of Cells and Matrix Therapy in Improving the Perfusion, Viability and Function of Infarcted Hearts**

A Ahmadi, B McNeill, B Vulesevic, M Kordos, L Mesana, S Thorn, JM Renaud, E Manthorp, D Kuraitis, H Toeg, TG Mesana, RS Beanlands, JN DaSilva, RA deKemp, M Ruel, EJ Suuronen

BACKGROUND: Injectable hydrogels are promising for improving cell engraftment and the efficacy of cardiac cell-based therapies; however, the mechanisms of action remain largely unknown. Here, we studied a combined therapy of collagen matrix and circulating angiogenic cells (CACs), which was applied to MI mice. We evaluated the role of integrins (Itg) and integrin-linked kinase (ILK) in CAC-matrix interaction.

METHODS/RESULTS: Seven days after LAD coronary artery ligation, female C57BL6/J mice received one of the following treatments: CACs, collagen matrix, CACs+collagen matrix, or PBS. CACs were green fluorescent protein (GFP)+ bone marrow cells from male C57BL/6-Tg(eGFP) mice. Echocardiography revealed that the ejection fraction (EF) of infarcted mouse hearts was ~36%, and improved to 56±2% 3 weeks after treatment of CACs+matrix; whereas no improvement occurred with other treatments (≤40±2%; p<0.001). PET analysis showed improved glucose metabolism (18F-FDG) and perfusion (13N-NH3) only with CACs+matrix by 35% and 29%, respectively (p ≤0.05). Histology showed that the anterior to posterior LV wall thickness ratio was greater for CACs+matrix (0.7±0.1) than for all other groups (≤0.3±0.0; p<0.001). More arterioles were detected in hearts injected with CACs+matrix (10.9±1.1/FOV) compared to other treatments (≤6.2±0.5; p<0.001). Moreover, Y chromosome q-PCR indicated increased intramyocardial retention of transplanted cells in CACs+Matrix group (by 8.6±1.4-fold; p=0.001) relative to CACs only. ILK expression was higher in hearts treated with CAC+matrix (1.4±0.1-fold) or matrix (1.6±0.1-fold) compared to CACs-only or PBS treatment (p ≤0.02). In vitro, collagen matrix culture increased ILK protein levels by ~2-fold in CACs compared to fibronectin (p=0.003). An integrin (Itg) expression screen revealed increases in the collagen-binding receptors Itgα2 in matrix-cultured CACs. Blocking Itg α2β1 resulted in ILK down-regulation, and reduced adhesion (82% less), proliferation (77% less) and secretion of SDF-1 (50% less) in matrix-cultured CACs, compared to non-blocked CACs (all p<0.05). Blocking Itg α2β1 in CACs also abrogated the in vivo therapeutic effects of CACs+matrix treatment in infarcted hearts: there was no improvement in EF (baseline=41±3%; follow-up EF=42±3%), vascular density was reduced and CAC engraftment was negligible, despite normal cell viability upon treatment delivery.

CONCLUSIONS: The collagen matrix does not simply provide passive delivery of CACs, but rather, it interacts with the cells through integrin α2β1 to activate intracellular signaling leading to superior proliferation, adhesion, and cytokine production. This was essential for the synergistic therapeutic benefit of CACs+matrix treatment.

O-10 **Intra-myocardial Delivery of Human Cardiac Stem Cells Genetically Engineered to Over-express Stromal Cell-derived Factor 1α Enhances Post-infarct Cardiac Repair**

Everad L Tilokee, Nicholas Latham, Robyn Jackson, Bin Ye, Buu-Khanh Lam, Marc Ruel, Erik J Suuronen, Duncan J Stewart, Darryl R Davis

Background: Phase 1 clinical trials have demonstrated that transplantation of cardiac stem cells (CSCs) into damaged myocardium is safe with clear hints of superiority above standard therapy. The mechanism underlying the benefit of first generation CSC products is unclear but appears to be largely cytokine mediated. Recent studies have shown that somatic gene transfer of the cytokine stromal cell-derived factor 1α (SDF1α) to animal models of cardiac injury enhances angiogenesis and resident stem cell recruitment with minor improvements in cardiac function. Given that the paracrine repertoire of CSCs lacks robust production of SDF1α, we investigated the impact of genetically engineering CSCs to over-express SDF1α on post-infarct function in an immunodeficient mouse model of myocardial ischemia.

Methods and Results: Human CSCs were cultured from atrial appendage biopsies donated by patients undergoing clinically-indicated surgery. The mesenchymal sub-population (CD90+) within CSCs underwent lentivirus-mediated gene transfer to over-express SDF1α. Following recombination with the non-transduced fraction, SDF1α expression in conditioned media increased threefold above non-treated CSCs (608±183 vs. 1727±80, respectively; $p<0.05$). Interestingly, cytokine array profiling of conditioned media demonstrated that SDF1α over-expression enhanced the paracrine signature of CSCs with 19 cytokines increased as compared to non-treated CSCs ($p<0.05$). Over-expression of SDF1α enhanced the capacity of conditioned media to stimulate angiogenesis in a matrigel-based tube formation assay and the recruitment of circulating angiogenic cells while demonstrating negligible effects on the potential of CSCs to undergo cardiogenic differentiation. NOD SCID mice injected with CSCs over-expressing SDF1α one week after LAD ligation had a greater echocardiographic ejection fraction at 4 weeks post-MI (39±2%) than animals injected with unmodified CSCs or saline (33±1% and 25±1%, respectively; $p<0.05$). SDF1α-mediated enhancement of myocardial salvage and endogenous progenitor recruitment underlie these benefits as injection of SDF1α over-expressing CSCs provided a 2-fold reduction in scar burden as compared to saline ($p<0.05$) with negligible benefits towards long-term persistence as demonstrated by equivalent qPCR detection of retained human alu sequences ($p=0.73$).

Conclusions: Over-expression of SDF1α in human CSCs boosts their ability to stimulate angiogenesis and to recruit endogenous stem cells. Genetic enhancement improved cardiac repair over current CSC products under clinical investigation.

O-11 **Characterizing Coronary Endothelial Function with Micro-PET Blood Flow Imaging**

Croteau Etienne, Archer Christine, Renaud Jennifer M, DaSilva Jean, Beanlands Rob, deKemp Robert

Objectives: Atherosclerosis, hypertension and diabetes are risk-factors associated with early endothelial dysfunction and progression to coronary artery disease. The main function of the endothelium in coronary arteries is to release nitric oxide (NO) to vasodilate the vessel in response to flow-mediated shear stress and neuro-hormonal stimuli. The cold-pressor test activates the sympathetic nervous system to release norepinephrine (NE), and has been used to measure coronary endothelial function with PET myocardial blood flow (MBF) imaging. The aim of this study was to characterize endothelial function in mice using [^{11}C]acetate micro-PET measurements of coronary endothelial flow reserve (EFR = adrenergic stress/rest MBF).

Methods: [^{11}C]acetate PET EFR was measured using i.v. infusion of NE (3.2 μg/kg/min) or salbutamol (SAL 1.0 and 0.2 μg/kg/min). NE is an α- and β-adrenergic receptor agonist and SAL is a selective β-agonist. The peripheral blood pressure (BP) and heart rate (HR) were measured in the carotid artery to assess efficacy of the dosage and treatment response in rate pressure product (RPP: systolic BP x HR). Transgenic endothelial nitric oxide synthase knockout mice (eNOS KO) and L-NAME pre-treated mice (eNOS inhibited) were used to characterize the NO-mediated flow response. β-adrenergic specificity was evaluated by NE and pre-treatment with α-antagonist phentolamine (PHE). β_2-selectivity was assessed using low-dose vs. high-dose SAL.

Results: The NE-stress/rest RPP ratio in PHE pre-treated mice (0.89 ± 0.15) was decreased vs CTRL (1.19 ± 0.10) ($p < 0.01$). The RPP response to NE was also decreased with eNOS inhibition (L-NAME) (1.07 ± 0.02) and eNOS KO (1.13 ± 0.04), whereas SAL-stress response was absent. Despite the variable RPP response, we observed similar EFR in normal mice with NE 1.7 ± 0.4, NE+PHE 1.6 ± 0.5 and high-dose SAL 1.7 ± 0.3 (ANOVA $p=0.87$). Low-dose SAL, more specific to β_2 adrenergic receptor showed a lower EFR with 1.4 ± 0.3. NE-stress in eNOS inhibition and KO groups showed significantly reduced EFR with 1.0 ± 0.2 and 1.1 ± 03 respectively ($p < 0.05$). Similarly, EFR was reduced of 20% using high-dose and absent using low-dose SAL-stress, in eNOS inhibited mice ($p < 0.05$).

Conclusion: NE and low-dose SAL stress can be used to evaluate NO-mediated coronary endothelial function in mice non-invasively using [^{11}C]acetate and micro-PET. Low-dose SAL may be an optimal adrenergic stress agent for application in human subjects.

O-12 **Clinical Evaluation of Functional Mitral Stenosis after Mitral Valve Repair for Degenerative Disease: Potential Impact on Surgical Strategy**

Al-Atassi T, Lam BK, Chan V, Mesana T

Background: The use of either a mitral annuloplasty band or ring is an integral part of all mitral valve (MV) repair procedures for degenerative MV disease. The impact of each type of annuloplasty device on outcomes is not well described. The objective of this study was to contrast the echocardiographic and functional characteristics of the two most commonly used annular remodeling strategies (band versus ring).

Methods/Results: We systematically evaluated 107 consecutive patients who had undergone mitral valve repair for degenerative mitral disease at our institution by stress echocardiography, 6-minute walk exercise testing and SF-36 questionnaire; 65 (61%) patients received a band and 42 (39%) a ring. Functional mitral stenosis (FMS) was characterized by RVSP > 60 mmHg and/or mean mitral gradient > 15 mmHg during peak exercise.

Mean age was 60+/-11 years and included 76 (71%) men. The mean band and ring size used for repair was respectively 30.7+/-2.8 mm and 30.4+/-2.1 mm (P=0.59). Resting mean mitral gradient was 3.7+/-1.9 mmHg and 5.8+/-2.6 mmHg for band and ring patients, respectively (P<0.0001). Mean mitral valve area at rest was 2.3+/-0.6 cm2 and 1.8+/-0.5 cm2 for band and ring patients, respectively (P<0.0001). Exercise duration was 12.9+/-5.7 min for band and 12.6+/-5.7 min for ring (P=0.78); distance covered was 471+/-77 m for band and 443+/-107 m for ring (P=0.12). At peak exercise, mean mitral gradient was 10.6+/-4.8 mmHg for band versus 15.3+/-8.2 mmHg for ring (P=0.0002) while RVSP was 45.8+/-9.5 mmHg for band and 52.6+/-14.2 mmHg for ring (P=0.004). FMS was present in 17% of band patients versus 55% of ring patients (P<0.0001). Congestive heart failure (P=0.05) and atrial fibrillation (P=0.006) were associated with the utilization of a ring annuloplasty; in addition, ring patients reported lower levels of energy (P=0.02) and general health (P=0.007) on their SF-36. Predictors of FMS included ring usage (OR 3.8, 95% CI1.3-11.8), smaller resting mitral valve area (OR 9.2, 95% CI 2.5-33.3) and younger patients (OR 6.9, 95% CI 2.3-21.5).

Conclusions: Patients with functional mitral stenosis following mitral valve repair for degenerative mitral valve disease were identified by using complete echocardiographic and functional assessments. Annuloplasty remodeling, using complete rings, was associated with a risk of functional mitral stenosis, recurrence of CHF and markers of poorer quality of life. Consideration should be given to the selection of an annuloplasty device type and size at the time of mitral valve repair.

O-13 **Improving Cardiovascular Health in Family Members of Patients with Heart Disease: A Randomized Trial**

Robert D. Reid[a], Lisa A. McDonnell[a], Dana L. Riley[a], Amy Mark[a], Lori Mosca[b], Louise Beaton[c], Sophia Papadakis[a], Chris M. Blanchard[d], Heidi Mochari[b], Patricia O'Farrell[a], George A. Wells[e], Monika E. Slovenic D'Angelo[a], Andrew L. Pipe[a]

[a]Division of Prevention and Rehabilitation, University of Ottawa Heart Institute, Ottawa, Ontario
[b]Preventive Cardiology, Columbia University Medical Center, New York, New York, USA
[c]North Bay Parry Sound District Health Unit, North Bay, Ontario
[d]Dalhousie University, Department of Medicine, Halifax, Nova Scotia
[e]Cardiovascular Research Methods Centre, University of Ottawa Heart Institute, Ottawa

Background: We conducted a randomized trial to determine whether a family heart health intervention would improve coronary risk factors in family members of patients with coronary heart disease.

Methods: Four hundred twenty-six participants completed a coronary risk factor assessment and were randomized to family heart health intervention (FHH; n = 211) or usual care (UC; n = 215). FHH participants received feedback on baseline risk factor levels, goal setting assistance, and ongoing cognitive-behavioural counseling for 12 months. Reports and recommendations were forwarded to their primary care physician. UC participants received brief advice and print materials. Primary outcomes were changes in: total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) ratio; levels of physical activity; and intake of fruits and vegetables. Secondary outcomes were changes in: other lipids; fasting glucose; blood pressure; smoking status; waist circumference; and body mass index.

Results: TC/HDL improved during follow-up but there was no difference between groups. FHH participants made greater improvements in physical activity (mean difference 36.7 minutes/week, 95% confidence interval 10.6 to 62.8 minutes/week), and consumption of fruits and vegetables (mean difference 1.2 servings/day, 95% CI 0.6 to 1.8 servings/day). Among smokers, verified abstinence was higher in FHH compared to UC (30.0% vs. 5.3%; P=0.04). FHH participants also showed greater improvements in fasting glucose, body mass index, and waist circumference.

Conclusion: Physical activity levels, fruit and vegetable intake, rates of smoking cessation, fasting glucose levels, and measures of adiposity improved with a family heart health intervention. The intervention is an important adjunct to primary care and should be extended to family members with diabetes and existing CVD.

O-14 **Metabolic Syndrome and Prediction of Fatal and Non-Fatal Cardiovascular Events in a Newfoundland Population: Preliminary Findings from the NFLD MetSyn Follow-Up Study**

Turton P, Fodor JG, Sussex B, McKay D, Gadag V

Background: Large waist circumference (central obesity) is related to an increased risk of developing cardiovascular disease (CVD) and diabetes. Central obesity is frequently associated with hypertension, dyslipidemia and dysglycemia, a constellation of risk factors commonly referred to as metabolic syndrome (MetSyn). We re-examined a random and representative sample of 791 middle-aged Newfoundland men and women to determine if MetSyn independently predicts the incidence of CVD events.

Methods/Results: In 2008-09, an observational follow-up study of this pre-established cohort was conducted to determine how many clinical events occurred over a mean follow-up period of 17.3 years. A composite endpoint of fatal CVD events (confirmed with primary healthcare records) and non-fatal CVD events (self-reported) was the study outcome. An individual was diagnosed as having MetSyn if they had any 3 or more of the following 5 criteria: obesity (determined by an increased waist circumference with ethnic-specific cut-points), high triglycerides, low high-density lipoproteins, high blood pressure (or treatment with antihypertensive medication) and elevated fasting glucose levels. All these criteria were measured at baseline. Of the original sample, 426 respondents were re-assessed and 99 deaths were recorded, representing a 66% follow-up rate (525 out of 791). The baseline prevalence of MetSyn in the follow-up cohort was 44.3%. In total, 64 major CVD events occurred (28 fatal and 36 non-fatal). After removing those with a history of diabetes at baseline, individuals with MetSyn had a 2.6-fold increased risk of having a CVD event in unadjusted logistic regression. After adjusting for sex and age, MetSyn remained an independent predictor of CVD incidence (RR, 2.157; 95% CI, 1.144-4.069; p=0.018). On the other hand, MetSyn was not independently linked to CVD outcomes in a regression model with MetSyn diagnosis and Framingham risk score (FRS) as explanatory variables. Interestingly, in a multivariate regression analysis that included the co-variables sex, age, MetSyn diagnosis and FRS, MetSyn – and not FRS – significantly predicted future CVD events (RR, 2.019; 95% CI, 1.012-4.028; p=0.046).

Conclusion: Baseline presence of MetSyn was associated with a 2.2-fold increased risk of having a fatal or non-fatal CVD event after adjusting for sex and age. Further analyses are ongoing to determine if MetSyn is a better predictor of CVD risk than the Framingham risk score.

POSTER ABSTRACTS

BASIC SCIENCE

P-1 In Vivo and Ex Vivo Imaging Indicate Effective Injection and Retention of a Collagen Matrix in a Mouse Model of Myocardial Infarction

A. Ahmadi, S. Thorn, M. Kordos, D.T. Padavan, T. Hadizad, G.O. Cron, R.S. Beanlands, J.N. DaSilva, M. Ruel, E.J. Suuronen, R.A. deKemp

BACKGROUND: Injectable biomaterials have been shown to improve the regenerative effects of endogenous and exogenous progenitor cells. However, much remains unknown concerning the basic injection and integration properties of the various injectable delivery systems. Our objective was to label a collagen matrix with hexadedecyl-4-[18F]fluorobenzoate (18F-HFB) PET tracer or Qdot® 525 ITK™ carboxyl quantum dots (q-dots) and assess its retention and distribution after ultrasound-guided injection in a mouse MI model applying PET and bioluminescence imaging.

METHODS/RESULTS: Ice cold glutaraldehyde cross-linked rat tail collagen type 1 matrix was labeled with 18F-HFB tracer and incubated at 37oC for 30 minutes. The labeling efficiency for 18F-HFB in the matrix 2 hours after solidification was 81.6±1.9% (n=4). In vivo retention of 18F-HFB-labeled collagen matrix at the site of injection was validated by PET scans. Seven days after ligation of LAD in 10-week old C57BL6/J female mice, the animals were injected with 18F-NaF (7.5±1.4 MBq) to demarcate the skeleton (as fiducial marker for image co-registration) and with 13N-NH3 (42.5±4.8 MBq) to delineate the infarct during a single scan. 18F-HFB (3.0±0.9 MBq) labeled matrix (total volume: 50 uL) was injected to the infarct and PET scans were performed after 10 minutes and 2 hours. PET images showed that the collagen matrix re-distributed evenly within the infarct. Signal intensity quantification was performed using Inveon Research Workplace software and revealed that the activity detected 2 hours after injection was 87.6±4.3% of initial activity detected at 10 minutes post-injection. After animal sacrifice, different tissues were collected for biodistribution assessment, which indicated significantly higher matrix retention in the myocardium compared to all other tissues (66.2±1.5% of injected dose; n=14, $p<0.001$). Collagen matrix covalently tagged with q-dots (labeling efficiency >96%), was also injected to MI hearts, which were then harvested 10 minutes and 2 hours later. IVIS® Spectrum bioluminescence imaging of the heart was then performed ex vivo. This confirmed the redistribution of the collagen matrix within the infarcted myocardium. Q-dot fluorescence signal quantification indicated that 84.1±7.4% (n=4) of the injected matrix was retained in the myocardium.

CONCLUSIONS: This study established the effectiveness of the collagen matrix to be retained and redistributed within the infarcted myocardium, making it a highly suitable therapeutic delivery vehicle for application in the heart.

P-2 Resistance to Weight Loss is Associated with Altered Skeletal Muscle Mitochondrial Energetics in Low Birth Weight Mice

Brittany Beauchamp[1], Michael Dysart[1], Sujoy Ghosh[2], Alphonse Chu[1], Alexandre Blais[1], Karunanithi Rajamanickam[3], Eve Tsai[3], Mary-Elizabeth Patti[4], Mary-Ellen Harper[1]
[1]Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, ON, Canada
[2]Biomedical/Biotechnology Research Institute, North Carolina Central University, Durham, NC, USA
[3]Ottawa Hospital Research Institute, Ottawa, ON, Canada
[4]Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA

Introduction: Suboptimal nutrition in utero is associated with increased risk for type 2 diabetes and obesity during adult life. Given the central role of skeletal muscle in whole body metabolism, we hypothesize that predisposition to metabolic disease is, in part, due to low oxidative capacity and dysfunctional mitochondrial energetics in muscle.

Methods/Results: We used an experimental mouse model system of maternal under nutrition during the last term of pregnancy to examine female offspring from undernourished dams (U) and control offspring from ad libitum fed dams (C). U had increased adiposity and impaired glucose tolerance compared to C, confirming previous studies. Weight gain and food intake post-weaning were similar for both groups. There was decreased expression in muscle of genes involved in PPAR-alpha/RXR-alpha activation; RAR activation; fatty acid metabolism; and NRF2-mediated oxidative stress response in U. Muscle mitochondria from U had decreased coupled and uncoupled respiration but increased maximal respiration compared to C. At 10 weeks of age, mice were 40% calorie restricted for 4 weeks. U lost half as much weight (15%) as controls, mirroring previously reported weight loss variation in diet-adherent obese women in an intensively supervised behavioral weight loss program at the Ottawa Hospital. Muscle from calorie restricted U had decreased oxidative fibers and mitochondrial content as compared to calorie restricted C.

Conclusion: Our data suggest that developmental alterations in skeletal muscle metabolism are associated with weight loss resistance. Findings are expected to further our understanding of developmental influences on obesity and its treatment.

P-3 **MicroRNA Expression Profiles in the Generation of Endothelial Progenitor Cell Phenotypes from Circulating Mononuclear Cells**

Behbahani, J; Stewart DJ

Background: Endothelial Progenitor Cells (EPCs) are believed to circulate in the peripheral blood mononuclear cell (PBMC) fraction and can be selected under endothelial-specific culture conditions. Early outgrowth EPCs appear after the first 3 to 5 days of selective culture, and are non-proliferative cells that still express mononuclear MNC markers (i.e. CD14 and CD45), as well as some endothelial cell (EC) markers including CD31 and VEGFR2. Late outgrowth EPCs appear after 2 weeks, and exhibit high proliferation potential and a strong EC phenotype. MicroRNAs (miRNAs) are small non-coding RNAs that have emerged as important regulators of gene expression.

Hypothesis: MNCs, early and late outgrowth EPCs express distinct miRNA profiles and manipulation of specific miRNA expression will influence the differentiation of EPCs in culture, either promoting or inhibiting the emergence of different EPC populations.

Methods and Results: Mononuclear cells were isolated from healthy participants by leukaphesis and cultured for 1, 3, 5, 7 and 9 days under conditions promoting endothelial differentiation (n=8). Late outgrowth EPCs were derived from the same cultures and appeared after 14-21 days (n=5). MiRNA expression was assessed by qRT-PCR (Taqman), using a panel of 13 miRNAs previously identified in PBMCs, EPCs or mature ECs. MiRNA expression profiles were grouped based on similarity in expression profiles and cell morphology (1-3 days, cultured MNCs); and (5-9 days, early outgrowth EPCs). In early outgrowth EPCs, the expression of miR-146a increased 2.9±0.04 fold, ($p<0.05$). In contrast, late outgrowth EPCs exhibited marked increases in expression of miRs-126, -222 and -221 compared to early EPCs (by 488-, 15.0- and 8.41-folds respectively; $p<0.05$). The ratio of miR-126 to miR-146 provided the strongest indicator of endothelial differentiation. MiR-146a knock down (KD) was performed using fluorescently-labeled oligonucleotides and uptake of KD miRs was confirmed using confocal microscopy. As well, a miR-126 mimic (OE) using lipofectamine transfection and a >4 log increase was confirmed using qRT-PCR compared to control ($p<0.01$). Transfected miR-126 cells were viable and exhibited a change in morphology compared to normal controls after 3 days. Further characterization of changes in phenotype and gene expression in EPCs after modulation of miR-146a and 126 is ongoing.

Conclusion: MNCs exhibit specific patterns of miRNA expression tightly associated with their progression from early to late outgrowth EPCs. MiRNAs may play a critical role in regulating the maturation of MNCs from early to late outgrowth EPCs, and enhancing miR-126 or suppressing miR-146 could enhance their differentiation to an endothelial phenotype.

P-4 **Attenuating Methylglyoxal Production Reduces Ischemic Injury and Improves Cardiac Function Post-MI**

Nick JR Blackburn, Branka Vulesevic, Ali Ahmadi, Brian McNeill, Ross Milne and Erik J Suuronen

Background: Myocardial infarction (MI) stimulates the rapid production of methylglyoxal (MG), but the resulting structural and functional consequences from the accumulation of glycation adducts have yet to be fully investigated. This study aimed to explore the potential benefit of attenuating MG production in a mouse model of MI. Since circulating angiogenic cells (CACs) are important mediators of post-MI neovascularization and collagen is particularly susceptible to glycation, we also explored the effect of collagen glycation on CAC properties in vitro.

Methods/Results: MI was induced in mice bred to over-express glyoxalase 1 (GLO1: MG detoxifying enzyme) and their wild-type (WT) littermates. Cardiac echocardiography was performed at baseline, 1 and 4 weeks post-MI. GLO1 mice had significantly higher left ventricular ejection fraction 4 weeks post-MI compared to WT (46.0±3.3% vs. 33.9±1.8%, $p=0.008$). Sections of hearts collected at 4 weeks were used to assess vascular density and apoptosis. In the infarct zone, GLO1 mice had a higher arteriole count (4.2±0.2 vs. 3.3±0.2 per FOV, $p=0.03$) and number of CD31+ endothelial cells (93.7±5.0 vs. 72.6±6.2 per FOV, $p=0.006$). The percentage of cardiomyocytes that were caspase-3+ (apoptotic) was less in GLO1 mice compared to WT (20±2% vs. 28±3% per FOV, $p=0.05$). For our in vitro work, CACs were cultured on collagen gels that were unmodified or modified by overnight incubation with MG at 37oC. We show that MG modification of collagen (glycation) results in: 1) reduced CAC adhesion (53% reduction vs. control, $p=0.005$); 2) increased susceptibility of CACs to hypoxia-induced apoptosis (43% increase vs. control, $p=0.05$); 3) a reduction in the number of pro-angiogenic CD34+ and CD133+ progenitors (75% and 44% reduction, respectively, vs. control, $p=0.005$); and 4) a decreased capacity to mediate neovascularization (1310±202, and 2150±212, for total network (μm) $p=0.005$).

Conclusion: We demonstrate that MG may contribute significantly to the pathogenesis of MI. Particularly, MG-mediated damage to the ECM, notably type I collagen, may play a role in the impaired endogenous repair response of CACs. This axis may also have important implications on the efficacy of regenerative therapy for treating MI hearts. Therefore, intervening and potentially restoring ECM signalling in the infarcted tissue may have the dual benefit of improving the heart's natural ability to repair while making the hostile cardiac environment more amenable to respond to therapy.

P-5 **Fibrillatory and Oxidative Stress Induce Mitochondrial Hyperfusion and Criticality Associated with Altered Calcium Handling in Cultured Atrial Myocytes**

M Bou-Khalil, G Drozdzal and CJ Redpath

BACKGROUND: Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. The high frequency electrical activity in the fibrillating human atrium *in vivo* is associated *in vitro* with calcium overload and apoptotic cell death. In surviving myocytes aberrant calcium handling promotes perpetuation of AF. We have previously demonstrated that stress induced mitochondrial hyperfusion is associated with increased formation of mitochondria-associated membrane and altered calcium handling. We sought to investigate if rapid stimulation alters mitochondrial function and calcium handling in cultured atrial myocytes.

METHODS: HL-1 cardiomyocytes were cultured at 37°C under a 5% CO_2 atmosphere in modified Claycomb medium. HL-1 cardiomyocytes were either (i) field stimulated at 1Hz (control) or 5 Hz for 24 h ("fibrillatory stress"); (ii) treated with BSO (200 μM, 24 h) to simulate oxidative stress; or (iii) infected with $DRP1_{K38E}$ or empty vector (500 MOI) to induce "forced fusion". Mitochondrial morphology was studied using electron and confocal microscopy. Laser scanning confocal imaging was used to monitor spontaneous calcium events and fluctuations in mitochondrial potential (Ψ_m). Subsequently, calcium release from intracellular stores was studied by fluo-4 video microscopy after administration of caffeine (4 mM).

RESULTS: The majority of HL-1 cells studied, regardless of prior stress, demonstrated spontaneous calcium activity at rest with the temperospatial pattern of detected signals invariably synchronous and homogeneous throughout. Fibrillatory stress was associated with a fivefold increase in mean mitochondrial 2D size and an almost 25-fold increase in SR: mitochondrial contact sites ($p< 0.0001$ for both comparisons, Students *t* test, n= 50 cells). Similar results following oxidative stress and forced fusion were associated with a sensitised response to caffeine; an approximate two to four fold increase in amplitude and velocity of caffeine-induced calcium release ($p< 0.0001$ for all comparisons, ANOVA, n= 40 cells per group). Fibrillatory stress was associated with a field stimulation-induced synchronization of Ψ_m previously described as mitochondrial "criticality" capable of rendering cardiac tissue refractory ($p< 0.01$, χ^2 test, n= 40 cells).

CONCLUSIONS: Our results reveal, for the first time, that "fibrillatory stress" induces mitochondrial hyperfusion, increased MAM formation and criticality in cultured atrial myocytes. This data provides direct evidence for the existence of a novel potential mechanism of functional refractoriness. Further work is required to elucidate the intracellular mediators involved and to demonstrate whether this novel mechanism promotes arrhythmogenesis in vivo.

P-6 **MR or AT1R Gene Silencing in the PVN Markedly Attenuates Ang II-induced Hypertension in Rats**

Aidong Chen, Bing S. Huang , Hongwei Wang, Frans H.H. Leenen

Functional studies suggest that circulating Ang II activates an aldosterone-MR-endogenous ouabain (EO)- AT1R pathway in the hypothalamus and thereby causes hypertension. The present study examined whether intra-PVN infusion of AAV-MR-siRNA or AAV-AT1aR-siRNA induces a knockdown of AT1R and MR specifically in the PVN, and whether this knockdown prevents the increase in AT1R and MR expression and in BP induced by chronic sc infusion of Ang II. Two wks after bilateral PVN infusion of AAV-MR-siRNA (1 μl of 5 x 1010 genomic particles / ml), MR mRNA and protein expression in the PVN were reduced by 56% and 31%, compared with rats with AAV-SCM-siRNA as a control. In a 2nd set of rats, pressor response to acute intra-PVN infusion of Ang II at 600ng/min was abolished in rats treated with PVN infusion of AAV-AT1aR-siRNA (1 μl of 2.5 and 25 x 1010 genomic particles / ml) a wk earlier. In a 3rd set of rats, sc infusion of Ang II at 500ng/kg/min for 2 weeks increased BP by ~80 mmHg, and MR and AT1aR expression in the PVN, SON and SFO by 50-90 % in rats with AAV-SCM-siRNA. In rats treated with AAV-MR-siRNA or AAV-AT1aR-siRNA 1 wk earlier, Ang II induced hypertension was largely prevented, and the increase in MR or AT1aR expression in the PVN was suppressed to levels lower than control without Ang II. Both AAVs had no effects on increases in gene expression by Ang II in the SON and SFO. These results indicate that MR and AT1R activation in the PVN plays a major role in Ang II-induced hypertension, and genetic intervention of MR or AT1R production by AAV-siRNA in specific brain nuclei provides new insights into the actual pathways of MR-EO neuromodulation. (Supported by grant # FRN:MOP-74432 from the Canadian Institutes of Health Research)

P-7 **The Role of miR-21 in Biomaterial–Endothelial Cell Interaction in a Post-MI Environment**

Helene Chiarella-Redfern, Drew Kuraitis, Tanja Sofrenovic, Brian McNeill, Ali Ahmadi, Marc Ruel, Katey Rayner, Erik J. Suuronen

BACKGROUND: The presence of hostile conditions after myocardial infarction (MI) such as ischemia, inflammation, and fibrosis/scarring contributes to the heart's inability to regenerate. This study sought to characterize the potential of an injectable collagen matrix to protect the environment post-MI, and to elucidate some of the cell-biomaterial interactions involved.

METHODS/RESULTS: MI was induced in C57BL/6J mice. One week later, mice received intramyocardial injections of: 1) PBS or 2) collagen matrix. Four weeks later, matrix treatment improved arteriole density (by 45%) and decreased inflammation, which was determined by the quantification of CD68+ cells and the levels of pro-inflammatory cytokines in the infarct tissue. The anti-inflammatory effect of the matrix was confirmed in vitro, whereby matrix-cultured macrophages produced significantly less pro-inflammatory cytokines than control cultures. The matrix also up-regulated the expression of pro-survival/proangiogenic miR-21 by 50% in MI hearts 1 week after treatment. To further explore this mechanism, miR-21 expression was assessed in human umbilical vein endothelial cells (HUVECs) grown on collagen matrix in vitro and compared to TCPS cultures. By qPCR analysis, the level of miR-21 was reduced in HUVECs and their supernatant (secreted miR-21) after 1 and 3 days of collagen culture compared to TCPS (by ≥60%), suggesting that endothelial cells may not be the source of miR-21 observed in matrix-treated hearts. One target gene of miR-21 is Spry1, which is a negative regulator of angiogenesis. Neovascularization involves a significant contribution from progenitor cells, so we examined Spry1 expression in CD34+ cells grown on collagen matrix for 1h, 2h, and 1day compared to fibronectin. Spry1 expression was reduced in collagen-cultured CD34+ cells at 2h (by 25%) and at 1 day (by 80%) vs. fibronectin. Therefore, it appears that the angiogenic potential of matrix-cultured CD34+ cells may involve the regulation of Spry1 activity.

CONCLUSIONS: Our results suggest that the matrix can mediate the MI environment through increasing vascularization and reducing inflammation, and this may involve control at the post-transcriptional level through microRNAs. Our collagen-based matrix treatment presents itself as a novel method for reducing hostile conditions that limit regeneration in the post-MI environment.

P-8 **Cellular Stress Induces Mitochondrial Hyperfusion, Increased MAM Formation and Altered Calcium Handling in Cultured C2C12 Myotubes**

G Drozdzal, M Bou-Khalil, CJ Redpath

BACKGROUND: Atrial Fibrillation is the most common sustained cardiac arrhythmia, abruptly inducing ultra-rapid conduction between atrial myocytes. This fibrillatory conduction is associated with oxidative stress and abnormally remodelled calcium handling which promote perpetuation of AF. Recently, in addition to being the major source of oxidative stress within cells, mitochondria have been observed to fuse, forming mitochondrial networks and attaching to sarcoplasmic reticulum (SR) in response to cellular stress. We sought to identify a potential role for rapid stimulation, oxidative stress and mitochondrial hyperfusion in remodelled myocyte calcium handling and increased formation of mitochondria associated membrane (MAM). We selected the C2C12 myotube model as it has previously been successfully used to investigate mitochondrial dynamics and has a myofibrillar system similar to atrial myocytes.

METHODS: Differentiated C2C12 myotubes were obtained by culturing C2C12 myoblasts in low-serum medium for 24-96 h at 37°C in an atmosphere of 10% CO2. C2C12 myotubes were either (i) field stimulated at 1Hz (control) or 5 Hz for 24 h ("fibrillatory stress"); (ii) treated with BSO (200 mM, 24 h) to simulate oxidative stress; or (iii) infected with DRP1K38E or empty vector (500 MOI) to induce "forced fusion". Myotube morphology was studied using electron and confocal microscopy. Confocal imaging was used to monitor spontaneous calcium events and caffeine induced calcium release. Mitochondrial pellets were obtained from C2C12 myotubes via centrifugation and analysed via immunoflourescence.

RESULTS: Cellular stress, whether from rapid stimulation or increased glutathione oxidation, resulted in mitochondrial hyperfusion, increased mitochondrial co-localisation with SR and increased formation of MAM in C2C12 myotubes ($p < 0.01$ for all comparisons, ANOVA, n≥ 40 cells per group). Oxidative stress reversibly increased and accelerated caffeine-induced calcium release threefold over control ($p < 0.0001$ for both comparisons, Students t test, n= 60 cells). "Forced fusion" also resulted in increased mitochondrial 2-D size, SR co-localisation and altered calcium handling ($p < 0.0001$ for all comparisons, Students t test, n≥ 40 cells per group). Intriguingly, the effects of "forced fusion" were reversed by co-incubation with the reducing agent N-Acetyl cysteine (NAC) in the absence of artificially induced oxidative stress ($p < 0.01$ for all comparisons, ANOVA, n≥ 40 cells per group).

CONCLUSIONS: Based on these results we conclude that the coordinate regulation of mitochondrial fusion, MAM formation and calcium handling may form a novel mechanism of stress-induced AF remodelling. We believe the MAM merits further investigation as a therapeutic target in AF arrhythmogenesis.

P-9 **A Novel Role of GATA6 Transcription Factor in Proper Development of Aortic Valve**

Lara Gharibeh, Mona Nemer

Heart morphogenesis, a revolutionary conserved pathway governed by many transcription factors, is disrupted in many congenital heart defects (CHD). Bicuspid aortic valve (BAV), affecting 1-2% of the population, was shown to be an autosomal-dominant disease with incomplete penetrance. Transcription factors GATA4 and 6, crucial to heart development, are known to be associated with cardiac outflow tract and septal defects. For instance, GATA6 KO mice fail to form a heart and die at E5.5-6.5 whereas *Gata4*$^{+/-}$*Gata6*$^{+/-}$ mice die at E13.5 developing vascular defects, thinning of the myocardium and Persistent Truncus Arteriosus. This study aims to characterize *Gata6* $^{+/-}$ mice while trying to understand GATA6 role in CHDs. Examination of the aortic valves of *Gata6* $^{+/-}$ hearts revealed the presence of BAV in 30% of the mice. BAV is a heart defect that is also known to cause aortic aneurism and regurgitation. Therefore, *Gata6* $^{+/-}$ mice were treated with hypertensive Angiotensin II and cardiac function was assessed by echocardiography and electrocardiograms. Results show that these mice have increased aortic valves pressure gradient, and some even developed aortic aneurisms. Treated *Gata*$^{+/-}$ mice have smaller hearts compared to their Wt littermates, and show signs of myocardial ischemia as revealed by ECG. In conclusion, this study reveals an important role for GATA6 in valvulogenesis and shows that haploinsufficiency of GATA6 leads to more pronounced cardiovascular complications in stressed hearts.

P-10 **Characterization of the LDL-PCSK9 Interaction: Effect of the R46L PCSK9 Loss-of-Function Mutation and Differential Binding of PCSK9 to Subclasses of LDL**

Mia Golder, Tanja Kosenko, Thomas Lagace

Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted protein that mediates the degradation of low density lipoprotein (LDL) receptors in the liver. PCSK9 is abundant in human plasma, and its levels are positively correlated with LDL cholesterol. Loss of function mutations in PCSK9, including the R46L mutation, are associated with decreased circulating LDL levels. PCSK9 interacts with LDL in a specific and saturable manner and this interaction fits a one-site binding model. LDL density ranges from 1.019 to 1.063 g/ml and can be divided into a number of subspecies based on density and particle size. To further characterize the interaction of PCSK9 and LDL, we investigated the interaction of PCSK9 with two subspecies of LDL – large, buoyant LDL (LBLDL; d=1.019-1.044 g/ml) and small, dense LDL (SDLDL; d=1.044-1.063 g/ml). Additionally, we investigated the effect of the R46L PCSK9 mutation on the LDL binding affinity of PCSK9.

Results: The interaction between purified PCSK9 and isolated LDL, LBLDL, and SDLDL was examined using in vitro binding assays. While the LDL-PCSK9 interaction had a Kd of 231.4 nM, the LBLDL-PCSK9 interaction had a Kd of 263.9 nM, and the SDLDL-PCSK9 interaction had a Kd of 361.1 nM. The binding affinity of WT and R46L PCSK9 in concentrated conditioned media to LDL was also examined using the in vitro binding assay. The LDL-R46L PCSK9 interaction had a Kd of 70.4 nM, significantly lower than that of the LDL-WT PCSK9 interaction (Kd=227.1 nM).

Conclusions: The ability of PCSK9 to bind to LDL is dependent on the subspecies of LDL and has a lower binding affinity for the more atherogenic form of LDL – SDLDL. The PCSK9 loss-of-function mutation R46L shows increased affinity for LDL. This increased affinity may partially explain the decreased circulating LDL levels.

P-11 **Strategies to Quantify Cardiac Stem Cell Retention after Intra-myocardial Injection**

Glenn Hay, Rick Seymour, Maryam Kamkar, Nicholas Latham, Robyn Jackson, Pasan Fernando, Glenn Wells, Darryl R. Davis

Recent clinical trials have shown that cardiac stem cell (CSC) therapy provides a novel means of reversing the progression of heart failure. Given the modest retention of injected cells, the next generation of CSC therapies will inevitably focus upon boosting CSC retention- thus mandating the development of simple non-toxic means of tracking CSCs. Therefore the purpose of this study was to assess the capacity of nano-dendrimers, mini-circle DNA and viral mediated gene transfer (adeno-associated virus (AAV) and lentivirus) to track transplanted CSCs tagged with bioluminescent or fluorescent tracers after injection into a murine model of experimental infarction.

*Methods and Result*s: All methods provided a robust non-toxic means of identifying transplanted CSCs (>95% labeling efficiency, p=ns). Progressive loss of the fluorescent tags through CSC proliferation and/or normal cell protein clearance limited the prospects for long-term cell-labeling using nano-dendrimer (fluorescent half-life 5.2±0.1 days) as compared to irreversible genetic labeling using AAV or lentivirus. Interestingly, non-integrating mini-circle DNA labeling resisted gene-silencing and delayed cell clearance to provide intermediate expression of the fluorescent tag. Lentiviral tagging of male CSCs to overexpress firefly luciferase permitted non-invasive in vivo cell tracking upon exposure to the bioluminescent substrate luciferin. Direct injection of male CSCs into the peri-infarct zone of female mice immediately after left anterior descending artery ligation demonstrated that injected CSCs persisted 3h after injection with non-invasive cell tracking correlating directly with cell counts obtained through qPCR detection of the Y chromosome from whole ventricular lysate.

Conclusion: Dendrimer cell labeling provides simple effective means of tagging CSCs but these techniques labels are not suitable for long-term tracking due to progressive signal attenuation to daughter cells and normal cellular clearance. While mini-circle DNA treatment permitted intermediate cell labeling, long term expression of cell labels was best accomplished using direct viral labeling.

P-12 **Characterization of Mice Deficient for IRF2BP2, a Transcriptional Co-repressor of IRF2**

Tiffany Ho, Nida Yap, Allen Teng, Naif Al-montashiri, Alex Stewart

Background: Coronary artery disease is the leading cause of death in North America today, caused by the hardening of the arteries due to atherosclerosis, resulting in reduced blood flow to the heart and tissues. In 2010, our lab found that IRF2BP2 (interferon regulatory factor-2 binding protein-2) coactivates expression of VEGF, a key protein in angiogenesis during ischemia. IRF2BP2 itself is an ischemia-inducible protein and a binding partner of IRF2 (interferon regulatory factor-2) for repression of IFN-α genes. IRF2BP2 mRNA is also a target of the RNA-binding protein Stau1, which may play a role in mRNA decay in skeletal muscle. Furthermore, genome-wide association studies have identified SNPs near IRF2BP2 that associate with elevated cholesterol in European and African individuals. Deletion of the IRF2BP2 gene in mice presents an excellent in vivo model to characterize the various roles of the IRF2BP2 protein.

Methods/Results: The 3'UTR of IRF2BP2 mRNA carries a common 9 nucleotide deletion polymorphism (rs3045215) which is associated with CAD risk in the Ottawa Heart Genomics study and the Duke Cathgen study. The RNA binding protein Stau1 was shown to interact with IRF2BP2 mRNA. Stau1 represses protein synthesis by causing mRNA decay when bound to 3'UTRs. Stau1 inhibits IRF2BP2 protein expression in differentiating skeletal myoblasts, possibly through the Staufen-mediated decay pathway. To characterize IRF2BP2 in vivo, we obtained transgenic mice with targeted disruption of the IRF2BP2 gene and maintained a breeding colony. Matings between IRF2BP2+/- hemizygous mice were initiated and genotypic analysis revealed <9% of IRF2BP2-/- knockout mice survive to adulthood (p=0.0001), indicating that knockout mice die during embryogenesis. The knockout mice that survive display low body weights, at less than 60% of their littermates upon weaning. The IRF2BP2-/- mice display elevated IgG and IFN-α in muscle, suggesting inflammation of the skeletal muscle. Furthermore, at least one IRF2BP2-/- mouse displayed an enlarged liver and spleen, and a complete blood cell count revealed polychromasia in the knockout mouse.

Conclusion: IRF2BP2 is an ischemia-inducible protein that is regulated by Stau1 in vitro. Knocking out the IRF2BP2 gene results in postnatal growth retardation and affects embryonic development in mice. These mice also display an upregulation of inflammatory proteins, with a phenotype of hepatosplenomegaly and elevated RBC counts. Deleting the IRF2BP2 gene in mice presents an excellent model for investigating its role in vivo.

P-13 **Paracrine Engineering of Human Cardiac Stem Cells with Insulin-like Growth Factor-1 Prevents Cell Death and Enhances Ischemic Myocardial Repair**

R Jackson, EL Tilokee, N Latham, B Ye, BK Lam, M Ruel, M Boodhwani, FD Rubens, V Chan, TG Mesana, EJ Suuronen, DJ Stewart, DR Davis

Insulin-like growth factor (IGF-1) is a potent activator of the AKT, MAPK and ERK pathways that stimulate cell growth and inhibit programmed cell death. While application of IGF-1 is known to improve post ischemic cardiac repair, this key cytokine is not robustly expressed by ex vivo proliferated human cardiac stem cells (CSCs). Here we explore the capacity of enhancing the CSC paracrine signature with IGF-1 to promote CSC survival and post ischemic cardiac repair.

Methods/Results: CSCs were cultured from human atrial appendages that were donated by patients undergoing clinically-indicated surgery. The mesenchymal subpopulation (CD90+) within CSCs underwent lentiviral mediated somatic gene transfer to overexpress human IGF-1 with a GFP reporter (CSCIGF-1) or a GFP reporter alone (CSCGFP). Analysis of the CD90-recombined CSC product using flow cytometry after transduction was reflective of pre-sorting levels (20.35±0.15%) and increased the IGF-1 content within conditioned media by 6.6 fold (149±39 vs. 1206±290 pg/ml*mg, respectively; p≤0.05) as compared to non-transduced CSCs (CSCNT). The impact of IGF-1 over-expression on CSC proliferation and apoptosis was examined using hypoxic (0.1% oxygen) and low serum (1%) culture conditions to mimic the post-infarct ischemic environment. Under these harsh conditions, CSCIGF-1 proliferation increased by 44±15% while CSCGFP and CSCNT proliferation decreased by 14±18% and 19±13%, respectively (p≤ 0.05). Over-expression of IGF-1 within CSCs reduced expression of the early (Annexin V) and late (7-AAD) apoptosis markers in response to stress conditions by 21±3% (p≤ 0.05) and 5±2% (p=0.06) as compared to CSCGFP. This resistance to hypoxia was mediated through downstream activation of the AKT, ERK and JNK pro-survival pathways as demonstrated through enhanced up regulation of BCL-2, FOS and JUN transcripts within CSCIGF-1 under stress conditions. Intra-myocardial injection of human CSCIGF-1 into immunodeficient mice one week after LAD ligation enhanced cardiac repair as compared to CSCGFP and PBS controls (ejection fraction 21 days post LAD ligation: 42±2% vs. 38±1% and 25±1%, respectively; p≤0.05).

Conclusions: Paracrine engineering of CSCs to over-express IGF-1 boosts CSC resistance to hypoxia and improves cell-mediated cardiac repair.

P-14 **Inducible Pluripotent Stem Cells as a Patient Specific in vitro Model of Atrial Rhythm Disorders**

MAJED JAMBI, DARRYL DAVIS

Recent advances in cellular reprogramming have now made it possible to generate embryonic-like cells from virtually any cell of the body. These inducible pluripotent stem (iPS) cells are capable of indefinite self-renewal while maintaining the ability to differentiate into all cell types. Nowhere will this technology have a greater impact than in the ability to generate disease and patient-specific cell lines. Here we explore the capacity of human iPS cells reprogrammed from peripheral blood endothelial progenitor cells lines to differentiate into atrial myocytes for the study of patient specific atrial electrophysiology.

Methods/Results: Late outgrowth endothelial progenitor cells (late-EPCs) were cultured from blood samples donated by volunteers free of cardiac rhythm disorders. Late-EPCs provided a highly proliferative (at least 73 PDTs) homogeneous population that exhibited a typical cobblestone morphology. In support of the notion that late-EPCs may readily undergo genetic reprogramming, quantitative PCR demonstrated 2 and 3 fold greater expression of the pluripotent markers Sox2 and Oct4 as compared to normal human dermal fibroblasts with no detectable differences in Klf4 or Nanog expression. To demonstrate the ease and reliability of late-EPCs for genetic reprogramming, cell lines maintained their phenotype and proliferative capacity after cryogenic storage. After 6 passages, late-EPCs were transduced with a doxycycline inducible lentivirus system encoding the human transcription factors Klf4, Nanog Oct4 and Sox2. After transduction, iPS colonies were maintained in feeder-free and defined media conditions. Following removal of doxycycline, genetically reprogrammed EPC-derived iPS colonies displayed phenotypical characteristics identical to human embryonic stem cells and expressed high levels of the pluripotent markers ALP, TRA1-60 and TRA1-81. After exposure to custom conditions known to favor cardiac identity, EPC-derived iPS cells were capable of differentiating into sheets of beating cardiomyocytes that expressing high levels of cardiac Troponin T (cTnT) and atrial specific connexin 40.

Conclusions: Late EPCs provide a stable platform for genetic reprogramming into a pluripotent state using a doxycycline conditional expression system that avoids re-expression of oncogenic/pluripotent factors as observed with constitutive expression systems. Human EPC-derived iPS cells can be differentiated into functional cardiomyocytes that express characteristic markers of atrial identity. This promising data demonstrates the suitability of iPS cells in modeling atrial physiology to better understand atrial rhythm disorders and enable the development of novel therapies based upon fundamental mechanistic insights.

P-15 **A Molecular Approach to Investigate the Autonomic Triggers of Atrial Fibrillation**

Maryam Kamkar, Richard J Seymour Michael H Gollob and Darryl R Davis

Atrial fibrillation (AF) is the most prevalent and persistent cardiac rhythm disorder worldwide. Unfortunately, current AF management strategies are not optimal. Drug therapy targets non-specific ion channels with limited efficacy, side effects and limited efficacy over time. Catheter ablation has received much attention but success rates are variable and the procedure carries significant risks. These limitations provide the impetus to identify novel, specific therapies. The target of this study is to visualize autonomic ganglia cells that trigger AF using a promoter driven construct directed towards parasympathetic ganglia located in the retro-cardiac fat pad that envelopes the left atrium.

Methods/Results: The neuron-specific enolase (NSE) and the nicotinic acetylcholine receptor sub-unit alpha 5 (CHRNA5) promoters was cloned upstream of GFP in a plasmid construct for somatic gene transfer using lentivirus and adeno-associated virus (AAV) vectors. When human embryonic kidney 293 cells or human neuroblastoma cell line (SK-N-SH) were transfected, GFP expression was reliably detected. However, direct injection of lentivirus conditional (CHRNA5 or NSE) or constitutive (cytomegalovirus) directed fluorescence tags into left atrial fat pad or intercostal muscle of Wistar Kyoto rats did not promote reliable gene expression due to presumed immune clearance. To overcome this limitation, the capacity of three different AAV serotypes (AAV1, AAV2 and AAV5) to reliably label neural cells was directly compared using qPCR screening within on-target and off-target tissues after in vivo injection. Simultaneous injection of AAV into the cardiac fat pad and highly vascular intercostal muscle resulted in broad off-target expression. In contrast, injection of AAV solely into the fat pad restricted expression of viral gene products to the local site. AAV5 provided the highest degree of expression but its migrant nature resulted in low level off-target expression in the liver, skeletal muscle and spinal cord. In contrast, treatment of the retro-cardiac fat pad with AAV1 or AAV2 confined transgene expression to the injection site, demonstrating restricted local expression essential for reliable in vivo labeling of a utonomic ganglia.

Conclusions: Conditional expression of GFP by promoter targeted transgenes reliably identifies cells of neural origin. While lentivirus-mediated gene transfer did not provide consistent in vivo gene transfer, injection of AAV resulted in high gene expression in target tissues with limited off-target spread. This approach will likely be a helpful tool to pinpoint critical cells that trigger this common disease for the development of new therapies based on fundamental insights.

P-16 **PGC1α, a Specific Target of microRNA-33, Regulates Immuno-metabolic Pathways in Atherosclerosis and Obesity**

Karunakaran D[1], Thrush B[1], Harper ME[2], Moore KJ[3], Rayner KJ[1]
[1]University of Ottawa Heart Institute
[2]University of Ottawa
[3]New York Unisity Langone Medical Centre

Background: microRNA 33 (miR33) has recently been identified as a novel and important regulator of lipid metabolism and atherosclerosis). Inhibition of miR33 using anti-sense oligonucleotides increased the expression of the cholesterol transporters (e.g. ABCA1, ABCG1), increased circulating HDL levels and decreased VLDL levels in animal models. In mice with pre-existing atherosclerosis, anti-miR33 reduced atherosclerotic plaque inflammation and promoted the regression of atherosclerosis. Recently, we performed a comprehensive microarray profiling of mRNA isolated from atherosclerotic plaque macrophages and found that PPARγ coactivator 1α (PGC1α), a novel miR33 target gene that was significantly de-repressed. PGC1α is a central regulator of energy metabolism and fatty acid oxidation and is highly expressed in brown adipose tissue, muscle and heart. Data from animal experiments and cell culture studies supports the notion that promoting the activity of PGC1α has positive outcomes on metrics of the metabolic syndrome, including insulin resistance and obesity. In humans, the levels of PGC1α are inversely correlated with insulin resistance and obesity. Here, we determine whether anti-miR33 treatment upregulates PGC1α and mitochondrial function in adipocytes and peritoneal macrophages, key cells in atherosclerosis.

Methods/Results: Inhibition of miR33 *in vivo* in Ldlr-/- mice upregulates PGC1□ mRNA expression in adipose tissue and atherosclerotic plaque macrophages. Using 3'UTR luciferase assays we showed that PGC1 □ was a direct and specific target of miR33. Transfection of macrophages with either miR33 mimics (overexpression) or anti-miR33 (inhibition) *in vitro* resulted in a decrease or increase of PGC1α mRNA and protein respectively. Interestingly, adipose tissue from anti-miR33 treated mice also showed upregulation of so-called "browning" genes (e.g. uncoupling protein-1) that contributes to increased energy expenditure. As PGC1□ is known to influence mitochondrial respiration, we utilized the Seahorse XF Flux Analyzer to investigate whether anti-miR33 directly affected mitochondrial respiration in macrophages. Preliminary data indicates that anti-miR33 increased mitochondrial respiration and induces changes in the oxidative phosphorylation patterns, confirming that anti-miR33 impacts positively on mitochondrial respiration. Further studies will examine the impact of miR33 modulation on metrics of energy metabolism *in vivo*, such as atherosclerosis progression and the development of diet-induced obesity.

Conclusion: PGC1α is a direct target of miR33 and is upregulated with anti-miR33 treatment in macrophages and adipose tissue, suggesting that it plays a critical role in regulating immunometabolic processes that contribute to atherosclerosis and obesity.

P-17 **Preventing Doxorubicin-induced Cardiotoxicity by a Novel GATA4 Partner: PEX1 Transcription Factor**

Hiba Komati, Wael Maharsy, Mona Nemer

Background: Doxorubicin (DOX) is an anthracycline drug that has been proved to be one of the most active antitumor drugs developed to date for the treatment of malignant neoplasias, such as solid tumors, leukemia, and lymphoma. However, its clinical usefulness is restricted to its cardiotoxicity, which might be either acute or chronic and eventually lead to cardiomyopathy following severe heart failure. Identification of transcriptional mechanisms underlying DOX action in the heart may provide new insight into the regulatory pathways of myocyte survival.

We previously showed that GATA4 transcription factor is an essential survival factor of postnatal cardiomyocytes and required for prevention of DOX-induced toxicity. More recently, we identified a novel transcription factor, phenylephrine-induced complex-1 (PEX1) also known as ZFP260, as a GATA4 partner in the adaptive hypertrophic response of the heart. In this work we aimed to assess the potential cardioprotective role of PEX1 transcription factor through gain- and loss-of-function studies.

Methods/Results: Transgenic mice lines with inducible and cardiac-specific overexpression of PEX1 were treated with a single i.p. injection of DOX at a dosage of 15 mg/kg body weight. After one week, two-dimensional guided M-mode echocardiography (Vevo® 2100 System) was performed on these mice under conscious sedation. Importantly, transgenic mice showed significantly improved cardiac function and reduced apoptotic nuclei comparing to control mice. Using cultured primary cardiac myocytes, we confirmed the direct cardioprotective role of PEX1. Interestingly, overexpression of PEX1 significantly attenuates DOX-induced myocyte apoptosis as evidenced by the absence of TUNEL-positive nuclei. In contrast, downregulation of PEX1 expression in cardiomyocytes showed decreased survival in the absence of treatment and an exaggerated response to DOX-induced apoptosis. We found that the mechanisms underlying the cardioprotective effects of PEX1 involve mitochondrial dependent apoptosis via upregulation of anti-apoptotic genes Bcl2 and BclXL and downregulation of pro-apoptotic genes BAX and PUMA. Most importantly, we identified PEX1 as a transcriptional regulator of sirtuin1, an NAD+-dependent protein deacetylase that has been recognized as a critical regulator of metabolism, aging and cardioprotection.

Conclusion: The work revealed an essential role of PEX1 in myocyte survival against DOX-induced cardiotoxicity. Identification of the transcriptional networks that regulate cardiomyocyte survival and adaptive stress response of the heart could lead to exciting new avenues for cardioprotection and prevention of end-stage heart failure.

P-18 **Blood and Cardiac Stem Cells are Stem Cells Sources with Differing Cardiomyogenic Potential**

Latham N, Ye B, Tilokee EL, Jackson R, Kuraitis D, Lam BK, Ruel M, Suuronen EJ, Stewart DJ, Davis DR

Transplantation of circulating angiogenic cells (CACs) or cardiac stem cells (CSCs) into areas of damaged myocardium enhances post-infarct cardiac repair with on-going clinical trials. Despite these benefits, cell engraftment and persistence is very modest with the majority of CAC and CSC gains mediated by paracrine effects on angiogenesis, recruitment of endogenous stem cells and rescue of reversibly damaged myocardium. Although CACs and CSCs possess the capacity to replace damaged cells, the magnitude following transplantation is not known. Therefore, we describe the long-term fate of transplanted human stem cells after injection into an immunodeficient mouse model of myocardial infarction.

Methods/Results: Human stem cells were cultured from samples donated by patients undergoing clinically-indicated cardiac surgery. Transplantation of CACs or CSCs alone provided equivalent improvements in angiogenesis, cardiac function and scar size as compared to controls. Simultaneous transplantation of CACs and CSCs enhanced cardiac function, attenuated scar formation and promoted revascularization when compared to single cell therapies. Quantitative PCR screening of the whole ventricular lysate for retained human alu sequences demonstrated greater retention of CACs 21 days after transplantation (16±5 vs. 2±1 cells/mg tissue, respectively; $p<0.05$) with equivalent retention 112 days after cell injection (1±1 vs. 2±1 cells/mg tissue, respectively; $p=0.40$). Despite favorable effects on cardiac remodeling, transplantation of the combination cell product did not enhance cells retention 21 (5±2 cells/mg tissue) and 112 (5±1 cells/mg tissue) days after cell transplantation. In all treatment groups small clusters of retained human cells (human nuclear antigen+, HNA+) were found within the infarct and peri-infarct regions. Random field analysis revealed that retained CACs differentiated into cells of a vascular origin (93±3% HNA+/vWF+) with no evidence for myocyte (HNA+/TNI+) or smooth muscle (HNA+/α-SMA+) differentiation. In contrast, CSC monotherapy and combination therapy with CACs demonstrated equivalent commitment towards a myogenic (51±9 vs. 36±7% HNA+/TNI+, $p=0.27$), smooth muscle (22±8 vs. 29%±1 1 HNA+/α-SMA+, $p=0.68$) and vascular (26±2 vs. 35±7% HNA+/vWF+, $p=0.42$) fate.

Conclusion: While direct replacement of injured myocardium is not the primary mechanism by which CACs and CSCs treatment enhance post-infarct cardiac function, transplanted cells persist and differentiate at low levels to fates reflective of their different ontogeny. Future work aimed at enhancing engraftment has the potential to build upon these findings to boost true cardiac repair by promoting CAC-mediated vascular remodeling and CSC replacement of working myocardium.

P-19 **Identification of a Novel Pathway for Protective Effect of Resveratrol against Atherosclerosis and Obesity in LDLR-/- Mice**

Yu Shi*, Haytham Ibrahim *, Sara Arab*, Eric Chun-wei Wang*, Youan Liu*, Guohua Li^,*, Mark Moon*, Robert F. Casper‡, Howard C. Tenenbaum‡, Ai-Hsien Li^, Liyong Zhang^,*, and Peter P. Liu^,*
^Cardiac Function Laboratory, Ottawa Heart Institute Research Corporation, University of Ottawa, Ottawa, Ontario, Canada
*Division of Cardiology, Heart and Stroke/Richard Lewar Centre of Excellence, University Health Network, University of Toronto, Toronto, Ontario, Canada. ‡ Faculty of Dentistry; University of Toronto, Department of Dentistry, Mount Sinai Hospital, Toronto, Ontario, Canada

Background: Atherosclerosis, one of the important contribu-tors to cardiovascular diseases, is a progressive process characterized by lipid accumulation combined with inflammatory and immune responses in the vascular wall. Besides, resveratrol is a natural phytopolyphenol compound that reduces cardiovascular diseases' risks by anti-obesity and anti-atherosclerotic effects with its underlying mechanisms remaining unclear. We investigated resveratrol on its preventive effects from developing obesity and atherosclero-sis in LDLR-/- mice fed on high calorie diet (HCD) as well as cultured adipocytes.

Methods/Results: In LDLR-/- mice, resveratrol treatment group significantly has less body weight, fat tissue accumulation, and atherosclerotic lesions compared with the control animals. In the mean while, through microarray analysis of the adipose 3T3-L1 cells treated with resveratrol, totally 151 genes were shown to be modulated : 108 were down-regulated and 43 up-regulated in resveratrol-treated cells vs. control ($P < 0.05$). Among the down-regulated genes detected above, we chose adipsin and adipsin-Acylation-stimulating protein (ASP) genes for further investigation because the related mechanisms of those were not reported previously and potentially involved in some innovative pathway regulating lipid synthesis in adipose tissues. And we demonstrated that resveratrol reduced the ASP expression in both adipose tissue of experimental animals (associated with less subcutaneous and visceral fat mass) and 3T3-L1 cells (with differentiation inhibition of 3T3-L1 cells into adipocytes). In LDLR-/- mice, plasma levels of adiponectin increased whereas leptin, inflammatory cytokines including GM-CSF, CCL2, and CCL5 decreased after resveratrol treatment. Resveratrol also inhibited foam cell transformation in U937 macrophage cell line exposed to lipopolysaccharide associated with decreased inflammatory markers as well as attenuated adipsin expression.

Conclusions: From our findings in vivo and in vitro, resveratrol down-regulated adipsin-ASP pathway in adipose tissues and cells. It was also associated with inhibition of adipose cell differentiation, foam cell formation, inflammatory modulation and adipokine regulation, and could consequently contribute to anti-atherosclerotic and anti-obesity effects.

P-20 **Mitochondrial Thiol Oxidoreductase Glutaredoxin-2 is Required to Regulate Mitochondrial Energy Metabolism in the Contracting Heart**

Jian Ying Xuan[1], Stephanie Thorn[2], Wael Maharsy[1], Rob deKemp[2], Jean DaSilva[2], Skye McBride[1], Mona Nemer[1], Marjorie Lou[3] and Mary-Ellen Harper[1]
[1]University of Ottawa, Faculty of Medicine, Department of Biochemistry, Microbiology, and Immunology, 451 Smyth Road, Ottawa, Ontario, Canada, K1H 8M5
[2]University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Ontario, Canada, K1Y 4W7
[3]Center of Redox Biology and School of Veterinary Medicine and Biomedical Sciences, University of Nebraska at Lincoln, Lincoln, USA

Background: Glutathionylation is a redox sensitive protein modification involving formation of a disulfide bridge between glutathione and a cysteine thiol. In the mitochondrial matrix of cardiomyocytes, glutaredoxin-2 (Grx2) catalyzes the reversible glutathionylation of proteins in response to changes in surrounding redox environment. Targets for Grx2-mediated glutathionylation include respiratory chain enzyme complex I. Changes in the reductive potential of the matrix alter the activity of complex I, which can have a profound impact on mitochondrial ATP production. Although glutathionylation has emerged as a key covalent modification required for control of protein function in cardiac mitochondria, our understanding of how it regulates mitochondrial biology and physiology in response to redox fluctuations is still in its infancy and remains unexplored.

Methods/Results: In the present study, we examined if loss of Grx2 altered cardiac energy metabolism and physiology using a novel Grx2 knockout (Grx2-/-) mouse model. Grx2-/- significantly increased heart weight and induced left ventricular hypertrophy. However, no changes in % fractional shortening or ejection volume were recorded. MicroPET imaging of glucose uptake by the heart revealed significantly higher rates of [18F]FDG uptake by the left ventricle indicative of changes in heart energetics. Cardiac mitochondria from Grx2-/- mice displayed a significant increase in mitochondria redox potential and an increase in the total amount of glutathionylated proteins. Examination of mitochondrial energetics using a novel high throughput multi-well technique revealed that Grx2-/- significantly decreased mitochondrial ATP output. Oxidative phosphorylation supported by complex I, complex II, and fatty acid oxidation substrates was substantially decreased in Grx2-/- cardiac mitochondria. Intriguingly, these effects could be reversed using DTT, a reductant that deglutathionylates proteins. These negative effects were partially attributed to the inhibition of complex I, which was more glutathionylated in Grx2-/- mitochondria. The activity of complex I could be recovered by restoring mitochondrial Grx levels or the reductive potential of the matrix.

Conclusions: We have identified Grx2 as a crucial component for the maintenance of proper mitochondrial ATP output in the contracting heart. Loss of Grx2 led to massive changes in heart energetics that were associated with left ventricular hypertrophy and possible hypoxia. In addition, mitochondrial ATP output by Grx2-/- mitochondria could be recovered by restoration of the reductive potential of mitochondria. Further, mitochondrial dysfunction in the diseased heart can be ameliorated by restoration of mitochondrial redox balance. (Funded by CIHR: INMD)

P-21 **Degradable Polar Hydrophobic Ionic Polyurethane Promotes Endothelial and Wound Healing Phenotype of Circulating Angiogenic Cells when Co-cultured with Monocytes**

Eva Mathieu, Kyle Battiston, Joanne McBane, Lien Davidson, Erik Suuronen, Paul Santerre, Rosalind Labow

Background: Strategies to yield vascular graft materials which could promote the endothelialization of the biomaterials along with a wound healing cellular phenotype are paramount to the goal of tissue engineering new blood vessels. This study investigated the interactive co-cellular response of human circulating angiogenic cells (CACs) and autologous monocytes (MN) with a degradable polar hydrophobic ionic polyurethane (D-PHI) and compared these findings to fibronectin-coated tissue culture polystyrene (TCPS), a tissue culture standard for CACs and endothelial cells.

*Methods / Result*s: It was found that D-PHI yielded good initial CAC attachment, and increased CD31 expression after 7 days suggesting that D-PHI favoured CACs to adopt an endothelial cell-like phenotype, compared to the fibronectin-coated TCPS surface. Moreover, cytokine expression by ELISA showed that the level of TNF-α was significantly lower than the level of IL-10 at day 7 when CACs were cultured on D-PHI (12.63±3.23 pg/mg DNA for IL-10 level vs. 1.58±0.46 pg/mg DNA for TNF-α level; $p<0.001$), which is indicative of a wound healing phenotype. The co-culture of MN with CACs did not appear to enhance the behavior of the latter cells in terms of cell attachment, growth and NO production. The co-culture confirmed that D-PHI favoured CACs to adopt an endothelial cell-like phenotype and the wound healing phenotype, while decreasing the activation of MN.

Conclusion: These data suggest that D-PHI selects for increase endothelial-like cells from CACs over time, and it favors the promotion of a wound healing phenotype, rather than an inflammatory response by the CACs. Moreover, D-PHI was less activating than TCPS for adherent MN. Furthermore, the presence of MN in co-culture with CACs did not appear to be necessary to enhance their activation on D-PHI. In summary, this study suggests that D-PHI seeded with CACs has the potential to promote functional endothelial-like cells required to tissue engineer a vascular graft.

P-22 **Encapsulation of Cardiac Stem cells within Matrix-enriched Hydrogel Capsules Promotes Cell Survival by Preventing Detachment Induced Apoptosis**

Audrey Mayfield, Everad Tilokee, Bu-Khanh Lam, Marc Ruel, Erik Suuronen, David Courtman, Duncan J Stewart, Darryl R Davis

Background: The use of ex vivo proliferated cardiac stem cells (CSCs) is an emerging treatment for patients suffering from heart disease. Despite advances in cell therapy, tissue regeneration is limited due to poor cell engraftment and survival. Biomaterials may provide a means to increase acute retention and survival post-transplantation by maintaining cell-ECM attachments. We explored the effect of encapsulating CSCs to prevent detachment induced apoptosis with the ultimate goal of boosting engraftment after myocardial injection.

Methods/Results: Human CSCs were cultured from atrial appendage biopsies donated by patients undergoing clinically-indicated surgery. CSCs were encapsulated in hydrogel supplemented with fibronectin and fibrinogen to enhance cell survival pathways. Encapsulation increased CSC viability while cells plated in suspension culture demonstrated decreased viability (1.4±0.1 vs. -0.9±0.1 fold change in cell number over 24 hours; $p>0.05$). Expression of downstream transcripts (Jun, Fos and Bcl-2) within the AKT, ERK, and JNK survival pathways were examined. Encapsulation maintained the expression of these transcripts compared to adherent conditions (p=ns). In contrast, CSCs in suspension uniformly increased the expression of Jun, Fos and Bcl-2, reflecting the apoptotic stresses applied to the cells (p≤0.05 vs. encapsulated conditions). Conditioned media (CM) was used to examine the paracrine abilities of encapsulated CSCs in culture. The tubule lengths of HUVECs exposed to CM from encapsulated CSCs were equivalent to tubule lengths from adherent conditions (286±26% vs. 220±31% of VEGF control, p=ns). In contrast, HUVEC tubule lengths of encapsulated CM was significantly greater than CM from suspended cells (93±12%, $p<0.05$). The ability to attract circulating angiogenic cells (CACs) was equivalent in CM from adherent and encapsulated cells (100±11% vs 108±17% of VEGF control CAC migration, p=ns). In contrast, CM from suspended condition attracted significantly less CACs (45±6%, $p<0.05$).

Conclusions: Encapsulation of CSCs in supplemented hydrogel enhances survival, proliferation, angiogenic capabilities, cytokine release and CAC attraction compared to suspended cells by providing vital attachment cues that prevent detachment induced apoptosis.

P-23 **Integration of Cysteine-rich Angiogenic Inducer 61 into Collagen Matrix Promotes the Therapeutic Potential of Circulating Angiogenic Cells**

Brian McNeill, Branka Vulesevic, Marc Ruel, Erik J. Suuronen

Background: Results from animal models and clinical trials have demonstrated the potential for CAC therapy to treat myocardial infarction (MI) but the benefits remain modest due, in part, to low cellular retention and engraftment. To overcome this hurdle, a collagen-based matrix has been developed to deliver and promote cell retention within the myocardium. The aim of this study was to modify the matrix to improve cellular function of the CACs and increase their therapeutic potential.

Results: From an mRNA expression profile, we have identified several integrins that become upregulated within the angiogenic CD34+ subpopulation of CACs when cultured on a collagen matrix that could be targeted to enhance cellular function. ItgαV and ItgB3, upregulated 55 and 67 fold respectively, were of interest as these are targets for Cysteine-rich angiogenic inducer 61 (CYR61), a secreted protein that associates with the ECM and promotes angiogenesis. By incorporating CYR61 into the collagen matrix, we were able to detect a 3.8 fold increase in total number of adherent cells on the collagen-CYR61 compared to collagen- only matrices. Furthermore, within these adherent cells there was a 3.3 and 2.7 fold increase in CD34+ and CD133+ cells, respectively. In addition to an increase in adhesive potential, CYR61 treated CACs incorporated more readily into tube-like structures during an angiogenesis assay (4.1 fold), 5 times more cells migrated towards CYR61 when used as a chemoattractant *in vivo* transplantation of matrix-cultured CACs pretreated with CYR61 resulted in a greater perfusion recovery in a hindlimb ischemia mouse model over injections with either matrix-cultured CACs or PBS.

Conclusion: These data suggest that incorporation of CYR61 into our collagen biomaterial may improve the therapeutic potential of CACs. Moreover, a matrix incorporating CYR61 may also recruit and stimulate host CACs to the site of injection further promoting reperfusion of the ischemic tissue.

P-24 **High Glucose Conditions Impair the Pro-angiogenic Capacity and Post-transplant Cardiac Repair of ex vivo Proliferated Cardiac Stem cells**

André Molgat, Nicholas Latham, Everad L Tilokee, Glenn Hay, Rick Seymour, Branka Vulesevic, Khanh Lam, Marc Ruel, Ross Milne, Eric Suuronen, Darryl R Davis

Diabetes is known to inhibit wound healing bynon-cardiac stem cells (CSCs). The impact of diabetes on first generation ex vivo proliferated CSC products is unknown but critical given that these are the very same individuals who will likely require CSC therapy in the future. Here, we tested the impact of high glucose conditions on CSC culture outcomes and the influence of diabetes on CSC-mediated cardiac repair.

Methods/Results: CSCs cultured from non-diabetic mice in high (25mM) or physiological (5mM) glucose conditions provided similar overall numbers of cells (0.7E6±0.1E6 vs. 1.0E6±0.4E6, p=0.32) and progenitor sub-populations (CD117+ 5±1 vs. 5±2%, p=0.7; CD90+ 23±4 vs. 27±6%,p=0.15). However despite equivalent production of pro-angiogenic cytokines, conditioned media (CM) from CSCs cultured under high glucose conditions displayed a reduced capacity to promote in vitro blood vessel formation as compared to CM from CSCs cultured under physiological glucose conditions (22±10vs. 45±6 mm cumulative tube length, $p<0.05$). Similarly, CM from CSCs cultured from the cardiac biopsies of streptozotocin-treated diabetic mice displayed a reduced capacity to promote in vitro blood vessel formation, compared to vehicle treated controls (37±3vs. 59±5 mm cumulative tube length, $p<0.001$). These effects were abrogated when CSCs were derived from cardiac biopsies of glyoxalase-1 transgenic animals (55±4 vs. 53±2 mm cumulative tubule length, p=0.8). CSCs cultured from the cardiac biopsies of diabetic patients demonstrated a reduction in overall cell numbers (0.5E6±0.2E6 vs. 1.2E6±0.2E6 cells, $p<0.05$). CM from diabetic patient CSCs displayed a reduced capacity to promote in vitro blood vessel formation (30±5 vs. 45±7 mm cumulative tubule length, $p \leq 0.05$),as compared to nondiabetic patient CSCs. Finally, transplantation of CSCs cultured from myocardial biopsies donated by diabetic patients demonstrated a reduced capacity to promote cardiac repair when injected into immunodeficient mice one week after LAD ligation as compared to transplantation of CSCs cultured from the myocardial biopsies of non-diabetic patients (change in ejection fraction28 days post LAD ligation 4±2 vs. 9±1%; $p \leq 0.05$).

Conclusions: Diabetes reduces the ability of CSCs to repair injured myocardium in an experimental model of myocardial infarction. Both diabetes and preconditioning CSCs in high glucose conditions attenuated thepro-angiogenic capacity of CSCs - which may in part explain the observed reduction in diabetic CSC mediated cardiac repair. Increased expression ofglyoxalase-1 enhanced the pro-angiogenic capacity of diabetic CSCs; suggesting a means of reversing diabetic CSC dysfunction by interfering with the accumulation of reactive glucose intermediates.

P-25 **Apelin Negatively Regulates Cardiac Fibrosis through Friend Leukemia Integration-1 and Vimentin post Myocardial Infarction**

Mark Moon[1,2], Xiaoxiao Wang[1], Jennifer Yang[1,2], Keiji Kuba[3],; Alan Valapeti[1], Fayez Dawood[1], Liyong Zhang[4], Yaacov Ben-David[2], Josef M Penninger[5],; Peter P Liu[1,2,4]
[1]University Health Network, Toronto, ON, Canada
[2]University of Toronto, Toronto, ON, Canada
[3]Akita University Graduate School of Medicine, Akita, Japan
[4] Ottawa Heart Institute, ON, Canada
[5]Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria

Background: Cardiac fibrosis is key component of cardiac remodeling. Apelin, a small regulatory peptide with multiple active fragments, exerts its functions via autocrine and paracrine pathways by binding to Apelin Receptor (APJ) receptors. It has both vasodilator effects on smooth muscle cells and inotropic effects on cardiomyocytes. We have previously found markedly increased cardiac fibrosis in apelin deficient (Apelin-/-) murine model of aortic binding. However, how endogenous apelin may regulate fibrosis remains unknown.

Methods and Results: Myocardial infarction (MI) in mice was generated by permanent left coronary artery ligation. Apelin and APJ mRNA levels were significantly increased at day 3 and 7, but returned to the baseline by day 28 post MI. Histology confirmed more interstitial fibrosis in Apelin-/- mice post MI; especially in the remote-infarct zones compare to its wild type littermates (Apelin+/+). The Apelin-/- mice also showed adverse remodeling and worse survival. Fibrosis-related proteins, including Collagen I and TGF-β, were up-regulated in Apelin-/- mice 28 days post MI. Importantly, apelin infusion rescued mice from excessive fibrosis and adverse remodeling post MI. Interestingly, vimentin (a pro-fibrotic factor) was found to be up-regulated in Apelin-/- mice post MI. This was also confirmed in Apelin-/- neonatal cardiomyocytes post stress such as hypoxia. Furthermore, we confirmed that viemntin over-expression enhanced fibroblast cell proliferation post hypoxia.

Conclusions: Absense of apelin aggravates cardiac fibrosis through up-regulation of vimentin and TGF pathway. This can be revealed in vivo with apelin infusion. APELIN may be a novel therapeutic target against cardiac fibrosis.

P-26 **PCSK9 Degrades Low-Density Lipoprotein Receptor (LDLR) in a Cell-type Specific Manner**

My-Anh Nguyen, Thomas A. Lagace

Background: The discovery that proprotein convertase subtilisin/kexin type 9 (PCSK9) mediates degradation of LDLRs suggested a critical role in LDL metabolism. PCSK9 is a secreted protein that binds to the epidermal growth factor-like (EGF)-A domain of LDLR and directs the LDLR for degradation in lysosomes by an unknown mechanism. In vivo studies in mice showed that physically relevant concentrations of PCSK9 in plasma were capable of decreasing hepatic LDLRs. However, no change in LDLR levels was observed in the adrenal glands, proposing a cell-type specific manner of PCSK9 activity. Herein, we assessed the responses of several cell lines to PCSK9–induced LDLR degradation, and investigated the mechanisms underlying these different responses in hepatic and non-hepatic cells. Because PCSK9 is a validated therapeutic target for cholesterol lowering in patients, this mechanism could be targeted to inhibit PCSK9 activity and preserve LDLR function in the liver.

Methods/Results: Indicated in biotinylation and flow cytometry studies, although PCSK9 was capable of inducing LDLR degradation in liver-derived cell lines, minimal effects on LDLR levels were observed in several lines of non-hepatic cells. Incubation of HepG2 human hepatoma cells for 6 hours with 5 µg/ml of wild-type PCSK9 reduced cell surface LDLR levels by >70%; in contrast, this dose of PCSK9 had no effect on LDLRs in SV589 human skin fibroblasts despite equal or greater levels of cell surface LDLR binding and cellular uptake of PCSK9. Similar results were obtained when comparing PCSK9 activity in HuH7 human hepatoma cells and 917 human foreskin fibroblasts. Especially, PCSK9 degradation assay showed that 125I-labelled WT-PCSK9 was internalized and degraded equally in both of SV589 and HepG2 cells, indicating dissociation of WT-PCSK9 from recycling LDLRs in PCSK9-resistant SV589 cells. Notably, PCSK9 recycling assay demonstrated that no recycling of WT-PCSK9 to the cell surface could be detected in either cell line. In contrast, more than 50% of internalized DY-PCSK9 recycled to the cell surface in SV589 cells and thus did not direct the LDLR for degradation despite persistent binding. Live cell imaging analysis confirmed that Alexa488-labelled DY-PCSK9 trafficked to both lysosomes and the endocytic recycling compartments in both hepatic and non-hepatic cells, whereas WT-PCSK9 exclusively trafficked to lysosomes.

Conclusions: We conclude that two factors diminish PCSK9 activity in non-hepatic cells: i) an increased dissociation from the LDLR in early endosomal compartments, and ii) a decreased ability of bound PCSK9 to direct the LDLR to lysosomes for degradation.

P-27 **Functional Analysis of GWAS Data based on Biological Properties of Complex Trait Genes**

Majid Nikpay, Paulina Lau, Thet Naing, Heather Doelle, Robert W Davies, George A Wells, Robert Dent, Ruth McPherson

Genetic factors behind a complex trait share particular functional features, namely, they tend to interact and mainly express in the tissues from which the trait originates. In this study, we used these functional features of complex trait genes to investigate the biological source of Obesity, a trait with known CNS origins and Coronary Artery Disease (CAD), a closely correlated trait to obesity and random gene-sets.

SNP Genotyping was done using Affymetrix Genome-Wide Arrays and followed with imputation using the 1000 Genomes Reference panel. In each study, nominally associated loci ($\beta \geq 0.1$ with $P < 0.01$) were searched in STRINGdb and the interacting genes with the highest confidence score (Score > 0.9) that also happened to display high modularity ($P < 0.0001$), were selected. We further reduced the number of the genes by testing for statistical-epistasis and keeping those with significant interactions ($P < 0.01$). Next, we searched our candidate genes in TiSGeDdb and selected genes that display tissue-specific pattern (SPM > 0.9). Finally, we did overrepresentation analysis and reassessed the results with gene expression analysis to find organ/systems that are the sources of obesity and CAD.

We did not find overrepresented organ/systems or a trend toward particular organ/system among the random genes; however, in both obesity samples, Central Nervous System (CNS) was the only overrepresented organ/system ($P = 2.3 \times 10^{-5}$ and $P = 4.2 \times 10^{-5}$). The same pattern was also observed in the CAD cohorts ($7.5 \times 10^{-4} \leq P \leq 0.002$ for CNS), with CNS being the only overrepresented organ/system.

We showed that functional properties of complex trait genes can be used to investigate the biological source of a complex phenotype. This approach can compensate for the limitations of the current GWAS single-marker-association tests and GWAS Pathway analysis approaches.

P-28 **Physically Cross-linked Injectable Chitosan and Collagen Derived Hydrogels for Cell Delivery and Regenerative Therapy**

Donna Padavan, Anna Badner, Kimberly McEwan, Julia Ranieri, Marc Ruel, and Erik J. Suuronen

BACKGROUND: Current cell-based therapies applied to CVD patients have yielded modest results, partly due to a low rate of engraftment and low persistence and function of cells in the target tissue. Tissue engineering offers the possibility of using biomaterials to address these limitations. Injectable hydrogels in particular have become popular scaffold platform materials for supporting cells since they mimic the highly hydrated tissue-like environment, have the ability to form *in vivo* and have previously demonstrated an ability to improve cell-based therapies in regenerative medicine. Many hydrogels, although biocompatible, typically require chemical cross-linkers, limiting their therapeutic potential for transplanting cells. Thus, physically cross-linked hydrogels used as injectables are attractive since they are capable of cell encapsulation and therapeutic cell delivery in a minimally invasive and non-toxic manner.

METHODS/RESULTS: Three injectable materials were generated via ionic cross-linking: a) chitosan derivative (HTCC) with sodium tripolyphosphate (TPP); b) pure chitosan (PC) with beta-glycerophosphate disodium salt (βGP); and c) collagen with βGP. Gels were evaluated for their chemical (functional groups and elemental analysis), physical (morphology and porosity) and mechanical (elastic moduli and degradation) properties. Cell compatibility (adhesion, viability) was assessed using circulating angiogenic cells (CACs) and human umbilical vascular endothelial cells (HUVECs) for up to 7d. For chitosan derivatives, PC-βGP gels were far less viscous (5.5±0.5Pa.s) and more elastic (6.33±0.87kPa) at 37°C compared to HTCC-TPP (11±4Pa.s and 0.05±0.01kPa, respectively). Morphology revealed homogenous pore distribution with HTCC-TPP having higher (14%) porosity. Gels in amylase (pH 7.2, 37°C, 250IU/ml) degraded 1.5× faster than gels in PBS at 7d. CACs and HUVECs adhered less on HTCC-TPP ($p \leq 0.001$) compared to PC-βGP and live/dead assays revealed comparable cell viability (>70%) between gels. The physically cross-linked collagen hydrogels showed increasing viscosity with higher βGP concentrations. There were no significant differences between fiber diameters with increasing βGP content (21.1±1 to 22.3±3μm); however, a greater number of cross-links per field of view were observed ($p<0.05$). Collagenase at 1unit/ml completely degraded the hydrogel within 24h whereas collagenase at 0.05units/ml degraded the hydrogel at a similar rate to PC-βGP. These collagen hydrogels promote high CAC viability after 24 and 48h of culture with increasing βGP content and support greater viability than their controls.

CONCLUSION: Physically cross-linked chitosan and collagen derived hydrogels are promising as injectable biomaterials for tissue regenerative therapies.

P-29 **Activity Measured as a Function of MLEM Iterations for a Dedicated Cardiac SPECT Camera**

Amir Pourmoghaddas, R. Glenn Wells

Background: Dedicated cardiac cameras may be able to provide dynamic images allowing quantitative blood flow measurement. Quantitative accuracy of regions in an iteratively reconstructed image can depend on the number of iterations used. In this study, we evaluate the consistency of activity as a function of MLEM iterations for a dedicated cardiac SPECT camera.

Methods/Results: A point source of 99mTc was displaced on an 8x6x6 grid in the field of view (FOV) of a multi-pinhole SPECT camera and data were acquired at each position for 60 seconds. Projection data were corrected for physical decay, reconstructed independently and then summed for reconstruction as a single image volume. 10 – 1000 iterations of the manufacturer MLEM-based software were used. The activity at each position and selected iterations was measured as the sum of activity in a 2-voxel radius. A cardiac-torso phantom was imaged with 99mTc in the myocardium and air in the other compartments to reduce the effects of attenuation. Activity in myocardial-wall volumes of interest (VOI) were measured and polar-map uniformity assessed. Reconstructed independently, the point sources had activity consistent to within 6% and converged to >95% of their final value in <= 100 iterations. The summed reconstruction reduced the accuracy of the sources by up to 15% and greatly increased the number of iterations required for convergence. The activity in the whole heart changed by less than 1% over the full range of iterations. The activity in the apical and mid-septal VOIs converged to >95% of their final value in <= 100 iterations at rates similar to those of the individual point sources. The uniformity of the polar map improved over the first 100 iterations and changed slowly thereafter.

Conclusions: Point-source experiment results suggest that extra-cardiac activity may decrease the rate of convergence and alter the quantitative accuracy of myocardial images from dedicated cardiac cameras.

P-30 **Stimulation of the Wnt Pathway Enhances the Proliferation of Cardiac Stem Cells**

Ola Qassim, Maryam Kamkar, Bin Ye, Majed Jambi, Nicholas Latham and Darryl R Davis

Cardiac stem cell (CSC) therapy holds promise for patients suffering from heart failure. We have developed techniques to extract and grow cells directly from a patient's own heart biopsy with a view towards transplanting these cells back into damaged myocardium. Within the heterogeneous population of cells that spontaneous emigrates from plated tissue in culture, a modest subpopulation (<10%) of true cardiac progenitor cells exists that express the surface marker c-Kit, indicative of cardiomyogenic potential. Given wnt/beta-catenin signalling promotes the *ex vivo* expansion of purified c-Kit^{+} cells, we explored the effect of targeted pathway stimulation on CSC potency and yield.

Methods/Results: Experiments were performed using adult c-KitBAC-EGFP transgenic mice (8-9 weeks old) with enhanced green fluorescent protein (eGFP) expression under the control of the *kit* locus; permitting the live cell detection of c-Kit+ cells. Quantitative morphometry of adult ventricle sections demonstrated that c-Kit+/GFP+ cells represented 0.2±0.1% of all cells. When adult murine hearts underwent enzymatic digestion for isolation of resident *in situ* c-Kit+/GFP+ cells using flow cytometry, only a modest number were isolated (2797±903 cells/isolation). In contrast, e*x vivo* culture of tissue from old c-KitBAC-EGFP mice permitted robust collection of the outgrowth cells surrounding plated tissue biopsies (1.3e6±1.7e5 cells/harvest; n=9) with predictable c-Kit+, CD34+, CD90+ and CD105+ sub-populations. Serial measures of GFP fluorescent from single plated c-KitBAC-EGFP tissue biopsies demonstrated a progressive increase in GFP fluorescence to 77±15% above the 12±18% baseline auto-fluorescence after 2 weeks in culture ($p<0.05$). Inspection of c-KitBAC-EGFP cultures demonstrated that the c-Kit+/GFP+ cells represented a larger proportion of the cells in areas distal to the plated tissue suggesting these c-Kit+/GFP+ cells possess a greater migratory capacity to colonize the periphery of the explant system (4±1% vs. 16±2%, respectively; $p<0.05$) with gradual infiltration by supportive endothelial and mesenchymal progenitors. Targeted stimulation of the wnt pathway using the small-molecule 6-bromoindirubin-3 (BIO) markedly increased the fraction of c-Kit+ cells as compared to the inactive methylated control (26±3 vs. 6±1%, respectively; $p<0.05$). Direct involvement of beta-catenin on c-Kit+ cell proliferation was confirmed through selective enhancement of beta-catenin degradation by XAV939 and iCRT14.

Conclusions: Ex vivo culture of myocardial biopsies markedly increases the number of c-Kit+ cells above that found in the native heart. Stimulation of the wnt pathway enhanced the c-Kit+ subpopulation within CSCs grown directly from cardiac biopsies. These results are consistent with the notion that wnt pathway stimulation plays an integral role regulating stem cell cycling.

P-31 **Advanced Donor Age Alters the Paracrine Profile of Cardiac Stem Cells**

Ghazaleh Rafatian, Nicholas Latham, Maryam Kamkar, Everad L. Tilokee, Catherine V. Cheng, Richard Seymour, Erik J. Suuronen, Darryl R. Davis

Background: Aging reduces the ability of organs to maintain homeostasis and respond to stressors. Despite mounting evidence that direct impairment of resident tissue stem cells underlies this phenomenon, cardiac stem cell (CSC) studies using tissue sourced from aged donors have largely focused on extraneous culture outcomes rather than the true intrinsic changes within CSCs. In this study, we examined the effect of advanced donor age on the fundamental mechanisms that provide CSC-mediated cardiac repair while quantifying intrinsic changes within CSCs that may account for this decline.

Methods and Results: Myocardial biopsies from healthy old (OM; 52 weeks) and young (YM; 8 weeks) C57/BL6 mice were minced, digested and placed in standard explant culture. Advanced donor age did not significantly reduce the overall yield of explant derived CSCs as compared to CSCs cultured from YM biopsies (4.3e5±6e4 vs. 4.0e5±4e4, p=0.72). Similarly, exposure of YM and OM CSCs to in vitro differentiation culture conditions known to favour cardiac identity did not demonstrate significant differences in the capacity of CSCs to differentiate into cardiac (troponin T, p=0.99), endothelial (von Willebrand Factor, p=0.16) and smooth muscle (Alpha-actin-2, p=0.20) lineages. Quantification of growth factors and cytokines secreted under hypoxic culture conditions demonstrated that OM CSCs expressed a greater amount of the pro-inflammatory cytokine IL-6 (828±99 vs. 497±98ng/ml, p<0.05) than YM CSCs with negligible effects on the production of the cardioprotective/pro-angiogenic growth factors VEGF, HGF, Angiogenin and Angiopoietin. CSCs cultured from OM tended to have greater amounts of reactive oxygen species (27±3 vs. 24±3 a.u. respectively, p=0.28) and lower glutathione peroxidase activity (0.15±0.02 vs. 0.23±0.05nmol/minute/ml, p=0.24) than CSCs cultured from YM.

Conclusions: Aging increases the production of the pro-inflammatory cytokine IL-6 in CSCs cultured from biopsies of normal donors with a tendency towards increased production of reactive oxygen species and reduced peroxide detoxification. Given the importance of paracrine factors in CSC-mediated cardiac repair, more work is needed to define the contribution of advanced age towards CSC function.

P-32 **Physically Cross-linked Collagen Hydrogel for Cardiovascular Tissue Regeneration**

Julia Ranieri, Donna T. Padavan, Duncan J. Stewart[*], Marc Ruel, Erik J. Suuronen
[+]Ottawa Hospital Research Institute

BACKGROUND: Cell-based therapies are being intensely investigated for improving blood flow and heart function in coronary artery disease. Tissue engineered hydrogels are also being developed to enhance cell therapy through increased survival, engraftment and function of cells at the target tissue. However, many of these hydrogels employ chemical cross-linkers, which may limit cell viability. Therefore, physically cross-linked hydrogels are an attractive biomaterial option, as they may promote greater cell compatibility than chemically cross-linked ones. This study aimed to characterize a type I collagen physically cross-linked hydrogel and determine its ability to support a therapeutic population of angiogenic cells.

METHODS and RESULTS: Type I collagen was derived and purified from the tails of 8-week old Sprague Dawley rats. A physically cross-linked injectable hydrogel was then developed using the rat-tail collagen via ionic cross-linking with beta-glycerophosphate disodium salt (β GP). A concentration study was conducted to determine which concentration of β GP was optimal for use as an injectable hydrogel. A picrosirius red collagen assay was conducted to determine the concentration of collagen in the gel. Cell-material interactions were evaluated with circulating angiogenic cells (CACs) cultured on the hydrogel, and compared to CACs on a chemically cross-linked collagen hydrogel (glutaraldehyde cross-linked). Adhesion was evaluated after 1, 4, and 24 hours. Six increasing concentrations of the βGP were tested to determine suitability in designing a hydrogel; however the least concentrated βGP (0.2M) in collagen did not form a gel and 1.8M βGP was not sufficiently soluble. The remaining four β GP concentrations underwent scanning electron microscopy and it was determined that 1.2M βGP was the optimal concentration to use based on porosity and lack of unused reaction products. The picrosirius red collagen assay on the samples of isolated rat-tail collagen revealed the concentrations to be in a range of 5.0-7.5mg/ml. Adhesion of CACs to the βGP physically cross-linked gel was superior to adhesion on the glutaraldehyde cross-linked gel at 1hr, 4hr, and 24hr (p=0.02, p=0.01 and p=0.01, respectively; n=5).

CONCLUSION: A physically cross-linked hydrogel was developed that promotes greater CAC adhesion than chemically cross-linked hydrogels. This presents the β GP-collagen hydrogel as a suitable injectable biomaterial for cardiovascular tissue regeneration.

P-33 **A Lymphocyte-Dependent Mode of Action for Imatinib Mesylate in Experimental Pulmonary Hypertension**

Mark L. Ormiston[1], YuPu Deng[2], NATALIE RUNDLE[2], Farid Bendjelloul[2], James N. Tsoporis[1], Thomas G. Parker[1], Duncan J. Stewart[2,3], David W. Courtman[2,3]
[1]Department of Medicine, Cambridge Institute for Medical Research, United Kingdom
[2]Regenerative Medicine Program, Ottawa Hospital Research Institute
[3]University of Ottawa

BACKGROUND: Pulmonary arterial hypertension (PAH) is a significant clinical challenge with few viable treatment options. PAH is marked by muscularization of the pulmonary arterioles, increased right ventricular systolic pressure (RVSP) and right ventricular hypertrophy (RV/LV+S). Smooth muscle hyperplasia is recognized as a causative factor in PAH; however, the importance of immune dysfunction is also gaining acceptance. In recent clinical trials, imatinib mesylate (Gleevec®) was effective in PAH treatment and this was attributed to its antiproliferative effects on vascular smooth muscle. However, in other diseases, e.g. gastrointestinal tumours and collagen-induced arthritis, imatinib's influence on the immune system contributes to its efficacy. Our objective was to test whether lymphocytes play a role in imatinib's effects in PAH. To address this question, we induced PAH in two immunocompromised rat models.

METHODS/RESULTS: Two immunocompromised models were used: nude rats, and immunodepleted F344 rats. The severity of PAH induced by monocrotaline (MCT) injection was evaluated by measuring cardiac hypertrophy (RV/LV+S) and right ventricular systolic pressure (RVSP). Muscularization of arterioles was scored by immunohistochemical analysis. Pulmonary leukocytes were detected by immunohistochemical analysis of lung cryosections immunostained for CD3 (Ts), CD68 (macrophages) and CD161 (NKs). Cytokine concentrations in lung lysates were measured using a multiplex bead immunoassay. Our main results were:

1. F344 rats treated with MCT demonstrated increased RVSP, RV/LV+S, and pulmonary arteriolar muscularization at 28 d, signifying PAH. NK and T leukocytes in the lung decreased in PAH while macrophages increased. All effects were blocked by imatinib. Increases in IFNγ, TNFα and IL-10 in the lung were observed after imatinib treatment, associated with activation of NK and T cells.
2. In nude rats, imatinib decreased RV/LV+S and pulmonary arteriolar muscularization but not RVSP. Notwithstanding the absence of T cells, cytokine levels and NK populations increased in the lung.
3. In F344 rats, NK and T cells were ablated by injecting ASGM-1 antiserum. The beneficial effects of imatinib were blocked; it did not decrease RVSP, RV/LV+S and muscularization of arterioles.

CONCLUSIONS: Our results provide insight into imatinib's mode of action in PAH. We demonstrate that activation of NK and T cells is an important counterpart to imatinib's inhibition of smooth muscle cell proliferation. PAH patients often co-present with diseases that compromise the immune system, e.g. HIV. Our work indicates that the immune status of patients will influence the efficacy of imatinib in PAH.

P-34 **Circulating Extracellular MicroRNA-26a as a Biomarker of Disease Activity in Clinical and Experimental Pulmonary Arterial Hypertension**

Kenny Schlosser[1], Yupu Deng[1], R. James White[2], Duncan J. Stewart[1]
[1]Ottawa Hospital Research Institute & University of Ottawa
[2]Division of Pulmonary & Critical Care Medicine, University of Rochester School of Medicine, Rochester, New York, USA

Rationale: Pulmonary arterial hypertension (PAH) is a progressive and fatal disease characterized by arteriolar remodeling and obliteration of the lung vasculature, ultimately causing right ventricular failure and death. Although the prognosis for PAH is poor, early diagnosis and treatment can increase patient quality of life. Unfortunately, prompt diagnosis remains a challenge as the early stages of PAH are asymptomatic, and there is no specific marker for this disease. MicroRNAs (miRNAs) are key regulators of gene expression that have recently been shown to circulate in blood in a stable extracellular format. Changes in specific circulating miRNAs have been associated with disease, and hold promise as non-invasive biomarkers.

Objective: To identify circulating miRNAs with utility as biomarkers of disease activity in PAH.

Methods and Results: RT-qPCR arrays were used to measure 1066 different miRNAs in plasma from 4 treatment-naive patients with idiopathic (I) PAH and 3 healthy controls. Twenty-five miRNAs differed between the IPAH and control groups within this discovery cohort (-2.8 to 3.4 fold change, $p<0.05$). The down-regulation of 4 miRNAs with novel links to PAH was confirmed in a separate validation cohort of 14 PAH patients (6 IPAH and 8 associated PAH) and 13 healthy controls. Among these, miR-26a exhibited significant diagnostic potential with an area under the receiver-operator characteristic curve of 0.85 ($p<0.05$), and also correlated with a functional index of PAH severity (i.e., 6 min walk distance, $r = 0.64$, $p<0.01$). MiR-26a was similarly found to be reduced (-1.3 fold, $p<0.01$, $n=18$) in plasma from rats with monocrotaline (MCT)-induced PAH, which exhibited characteristic elevations in right ventricular systolic pressure (61 ± 5 mm Hg, MCT; 28 ± 1 mm Hg, Con; $p<0.0005$) and right ventricular hypertrophy (RV/(LV+S) weight ratio, 0.31 ± 0.01, MCT; 0.22 ± 0.01, Con; $p<0.0001$) compared to controls after 3 weeks. Moreover, a reduction in miR-26a was observed in MCT-rat lung tissue (-1.6 fold, $p<0.01$), and specifically in the right (-1.9 fold, $p<0.05$), but not left ventricle of the heart. In vitro degradation assays demonstrate that miR-26a circulates in blood in a nuclease-resistant form, which becomes susceptible to endogenous plasma RNase activity following treatment with Proteinase K but not detergent (Triton X-100), indicative of a mechanism of transport and stabilization mediated primarily by protein complexation rather than vesicular encapsulation.

Conclusion: This study establishes novel links between circulating miRNAs and PAH, and supports their potential utility as biomarkers of underlying disease activity in affected tissues.

P-35 **Deficiency of Tie2 Predisposes to Severe Pulmonary Arterial Hypertension in a Model of VEGFR2 Inhibition and Chronic Hypoxia**

Mohamad Taha[1,2], Yupu Deng[2], and Duncan J Stewart[1,2]
[1]Department of Cellular & Molecular Medicine, Faculty of Medicine, University of Ottawa, ON, Canada
[2]Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada

Background: Pulmonary arterial hypertension (PAH) is characterized by degeneration and remodeling of the pulmonary microvasculature leading to right ventricle hypertrophy and ultimately heart failure and death. Endothelial cell (EC) apoptosis in the lung is believed to trigger PAH by inducing reactive vascular inflammation and cell proliferation, leading to obliterative distal arteriolar remodeling. Induction of lung EC apoptosis by SU5416 (SU), an inhibitor of VEGFR2, combined with chronic hypoxia (CH) leads to severe, irreversible PAH in rats, characterized by occlusive arteriopathy, similar to plexiform lesions in human PAH. In contrast, mice are relatively resistant to SU+CH induced PAH, even with multiple injections. Tie2 represents another major EC survival signaling pathway and Tie2 heterozygous knockout mice have been reported to be susceptible to lung EC apoptosis and PAH.

Hypothesis: Tie2-deficiency in mice will provide a “second hit” and lead to greater EC apoptosis and arteriolar remodeling in response to SU+CH, and thus a more marked PAH phenotype.

Methods/Results: Wild type or Tie2 heterozygous mice (Tie2+/-) were exposed to normoxia or 3 weeks of CH (9-10% O2), combined with a single subcutaneous injection SU (50mg/kg). Right ventricle systolic pressure (RVSP) and the ratio of RV over left ventricular plus septal weight (RV/LV+Septum) were determined at 3 weeks. Both WT and Tie2+/- mice demonstrated identical increases in (RVSP) and right ventricular (RV) remodeling when exposed to CH for 3 weeks compared to normoxia controls. There was no further increase in RVSP in WT mice exposed to SU+CH compared to CH alone. However, SU+CU significantly increased RVSP in Tie2+/- mice compared to WT littermates exposed to the same conditions (41±1.2 mmHg vs. 34±1.4 mmHg, respectively; n=13-17/group; $p< 0.001$) or Tie2+/- mice exposed to CH alone (41±1.2 mmHg vs. 35±1.5 mmHg, respectively; n=15-17/group; $p< 0.01$). This was combined with significantly greater RV remodeling (RV/LV+S: 0.48±0.01 SU+CH Tie2+/- vs. 0.42±0.01 SU+CH WT, 0.45±0.01 CH Tie2+/- n=14-17; $p< 0.001$, $p<0.05$ respectively). Histological assessment of lung sections of treated animals did not reveal any plexiform like lesions at 3 weeks, however, there was an increase in apoptosis ($p=0.05$) in the lungs of the Tie2+/- compared to CH only treated controls at three weeks.

Conclusion: Tie2-deficiency provides a “second hit” when combined with VEGFR2 inhibition and predisposes to severe PAH together with CH. SU+CH in a Tie2-deficient background may provide a robust murine model of severe PAH.

P-36 **Investigating the Metabolic Basis for Weight loss Variability in Obese Humans**

A. Brianne Thrush[1], Ghadi Antoun[1], Robert Boushel[2], Éric Doucet[3], Pascal Imbeault[3], Jean-Francois Mauger[3], Ruth McPherson[4], Robert Dent[5], Mary-Ellen Harper[1]
[1]Dept. of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Canada
[2]Heart & Circulatory Unit, Department of Biomedical Sciences, University of Copenhagen, Denmark
[3]Dept of Kinesiology, Faculty of Health Science, University of Ottawa, Canada
[4]University of Ottawa Heart Institute, Ottawa, Canada
[5]Ottawa Hospital Weight Management Clinic, Ottawa Hospital, Ottawa, Canada

Background: Response to weight loss treatments is highly variable and this may be due to biological factors such as altered skeletal muscle oxidative capacity and mitochondrial function. Diet adherent patients who completed the Ottawa Hospital Weight Management Program (OHWMP) and demonstrated the highest (Obese Diet Sensitive; ODS) and the lowest (Obese Diet Resistant; ODR) rates of weight loss have been recruited for studies of whole body and skeletal muscle energy metabolism. Our previous research demonstrated that ODS, compared to ODR have a higher proportion of oxidative fibres and mitochondrial proton leak in quadriceps muscle. We and others also demonstrated that proton leak through uncoupling protein 3 mitigates oxidative stress. It is proposed that a higher rate of mitochondrial proton leak contributes to higher rates of weight loss and may protect against oxidative stress.

Hypothesis: Skeletal muscle from ODS individuals will demonstrate higher rates of mitochondrial respiration and proton leak under basal conditions and in response to a high fat meal, which in turn will minimize oxidative stress in muscle.

Methods: Highly diet adherent, obese, weight stable women who have previously completed the OHWP and classified as ODS or ODR have been recruited to participate in this study. Prior to and 6 hrs following a high fat meal (60% dietary fat, 35% of total daily caloric requirements), vastus lateralis muscle biopsies are obtained for assessment of mitochondrial respiration using high resolution respirometry and determinants of mitochondrial function and oxidative stress. Resting metabolic rate and post prandial response to the high fat meal are also measured.

Results: Initial results suggest that mitochondrial respiration rates are higher in ODS compared to ODR both prior to and following a high fat meal.

Conclusions: This research will provide valuable insight into the biological basis for weight loss variability in humans.

P-37 **Effects and Mechanistic Correlates of using a Liquefied, Injectable Porcine Submucosa Extracellular Matrix, with or without Angiogenic Cells, in a Myocardial Scar Model**

Rashmi Tiwari-Pandey, Hadi Toeg, Richard Seymour, Ali Ahmadi, Suzanne Crowe, Branka Vulesevic, Erik J. Suuronen, Marc Ruel

Background: We examined some of the mechanisms involved in cardiac remodeling and vasculomyogenesis after injection of a clinically applicable, porcine derived small intestinal submucosa-extracellular matrix (SIS-ECM), with and without circulating angiogenic cells (CACs), in a mouse model of post-infarction myocardial scar.

Methods: 9-10 week old female C57BL/6J mice had their left anterior descending coronary artery ligated. Seven days post-ligation, 38 randomly allocated animals received echocardiographic-guided intramyocardial injections of phosphate buffered saline (PBS), CACs, SIS-ECM, or SIS-ECM + CACs. Repeat echocardiography and immunohistochemistry were performed 28 days post-ligation.

Results: Baseline, post-ligation left ventricular ejection fraction was equivalent in all groups. The matrix was identified 3 and 7 days after injection in the peri-infarction sites. After 21 days post-treatment, ejection fraction improved in SIS-ECM + CACs (by 38 ± 2.12 %) and SIS-ECM (by 36 ± 3.71 %), compared to CACs alone and PBS treatment groups ($p < 0.1$). Masson's trichrome staining showed reduced infarct size in SIS-ECM + CACs (34.2 ± 3.1 %) and SIS-ECM alone (34.5 ± 4.7 %), compared to CACs alone (47.3 ± 6.0 %) and PBS (61.9 ± 5.5%) ($p < 0.002$). There was also reduced deposition of collagen rich scar tissue and increased viable myocardial tissue in SIS-ECM alone (66.70 ± 4.19; $p<0.002$) and SIS-ECM + CACs (63.6 ± 2.78; $p<0.001$) groups as compared to PBS (38.07 ±5.57) and CACs only group (52.68 ± 4.77; $p<0.05$). Arteriolar density in peri-infarct regions was enhanced in both SIS-ECM treated groups (by ≥78 ± 7 %; $p = 0.03$). More GATA-4 and β-catenin positive cardiac cells were found in the myocardium of SIS-ECM treated animals.

Conclusions: Intramyocardial delivery of SIS-ECM 7 days after myocardial infarction in a mouse model reduces infarct size, improves myocardial vessel density and function, and when combined with CACs, helps restore myocardial cellularity. These findings suggest a potential therapeutic role for SIS-ECM in cardiac regeneration.

P-38 **Controlling Methylglyoxal to Prevent the Development of Diabetic Cardiomyopathy**

Branka Vulesevic, Ali Ahmadi, Dr Ross Milne, Dr Erik Suuronen

BACKGROUND: Hyperglycemia caused by diabetes leads to increased glucose oxidation and mitochondrial generation of superoxide. One of the most abundant by-products of glucose oxidation is methylglyoxal (MG). MG has been implicated in the development of diabetic complications. Diabetic cardiomyopathy, characterized by systolic and diastolic dysfunction, has a complex etiology, including the accumulation of MG-formed advanced glycation end products (AGEs). Methylglyoxal is detoxified by the activities of the enzymes glyoxalase 1 (GLO1) and glyoxalase 2.

METHODS/RESULTS: We have developed a mouse line that carries a transgene encoding human GLO1 (hGlo1) under the control of the preproendothelin promoter. The hGlo1 transgene has been shown to reduce MG-derived AGEs in tissues of streptozotocin (STZ)-induced hyperglycemic mice. We have also shown that over-expression of hGlo1, solely in the bone marrow, can rescue defective post-ischemic revascularization in STZ-treated mice. In this present study, the hGlo1 mice have been used to test the hypothesis that reduced MG levels can offset the development of diabetic cardiomyopathy. Diabetes was induced by intraperitoneal injection of STZ; 4 and 8 weeks post-STZ treatment, echocardiography of the wild type (WT) diabetic group showed systolic dysfunction. At 4 weeks of hyperglycemia, the ejection fraction (EF) was 54% and fraction shortening (FS) 37%. In contrast, cardiac function in the GLO1 diabetic mice as well as non-diabetic controls remained normal over time. Both the EF and FS were significantly lower in WT diabetic mice at 4 weeks post STZ-treatment compared to the control mice ($p=0.02$ and $p=0.04$, respectively). Western blots of heart tissue collected after 8 weeks of diabetes showed a significant increase in the expression of RAGE (receptor for AGE) in WT diabetic mice compared to other groups ($p=0.038$). Heart tissue collected from GLO1 diabetic and non-diabetic mice had increased GLO1 activity by 1.7-fold compared to the non transgenic mice.

CONCLUSION: These results suggest that MG contributes to the development of diabetic cardiomyopathy and that reducing methylglyoxal levels could be a potential therapeutic target for prevention.

P-39 **Identification of GATA4 Regulatory Mechanisms Associated with Heart Development and Disease**

Whitcomb, Jamie & Nemer, Mona

Introduction: GATA4, a member of the GATA family of zinc-finger transcription factors, has wide-ranging roles in cardiac development and hypertrophy. Point mutations of GATA4 can lead to congenital heart disease due to impaired DNA binding and/or protein-protein interactions. These impairments lead to cardiac abnormalities such as septal and valvular defects. Given the importance of GATA4 in the cardiac gene program, it is therefore important to gain insight into its positive and negative regulators, including post-translational modifications and protein-protein interactions. In this study, we aim to determine sites on the GATA4 protein that are post-translationally modified as well as novel interacting proteins that regulate GATA4 protein function.

Methodology/ Results: TripleFlag-GATA4 extracted from the TC13 endocardial precursor cell line was analyzed via HPLC-ESI-MS/MS and has yielded 3 phosphorylation sites: S67, S374 and S385. Mutational analysis was used to determine the functional relevance of the S67 residue. As well, multiple potential protein partners were determined. Additionally, 2 N-terminal GATA4 serine mutations implicated in human cases of congenital heart disease, S52 and S160, were determined to affect GATA4 transcriptional activation without changes to protein expression, DNA binding or cellular localization.

Conclusions: Specific sites on the N-terminal of the GATA4 protein were identified as potentially clinically relevant residues by mass spectrometry analysis and mutagenesis studies. From here, we aim to further characterize GATA4 post-translational modification in an endocardial model and extend this study to a myocardial model using HL1 atrial cardiomyocyte cell line. This study will elucidate the processes by which GATA4 is modified to help determine the molecular mechanisms leading to congenital heart disease.

P-40 **KLF13, a Novel Congenital Heart Disease Candidate Gene**

A.Yamak, S. Hayek[1], W. Maharsy, R. Darwich, G. Andelfinger[2], H. Komati, M. Nemer
[1]Emory University, School of Medicine, Atlanta, Georgia 30322, USA
[2]Cardiovascular Genetics, Department of Paediatrics, Sainte-Justine University Hospital Center, Université de Montréal, Quebec, Canada

BACKGROUND: KLF13 is a member of the Krϋppel-like transcription factors that are important regulators of cell proliferation and differentiation. Several KLF members are expressed in the heart in a spatial and temporal specific manner. KLF13 is highly enriched in the developing heart where it is found in both myocardial and endocardial cells.

METHODS/RESULTS: In xenopus, knock down of Klf13 causes developmental heart defects suggesting its essential role in heart morphogenesis. To test whether this role is evolutionary conserved in the mammalian heart, we deleted the Klf13 gene in transgenic mice using homologous recombination and analyzed the knockout mice at variable embryonic stages. Mice lacking both Klf13 alleles are born at reduced frequency; variable cardiac phenotypes are observed in these knockouts ranging between myocardial and endocardial defects. Epithelial-mesenchymal transformation (EMT) is affected in these mice and they have reduced proliferation in the AV cushion. Surviving Klf13 null mice have several structural cardiac anomalies and altered cardiac gene expression profile.

CONCLUSION: Our data uncover a role for a new class of transcription factors in heart formation and point to KLF13 as a potential congenital heart disease causing gene.

P-41 **Derivation and Characterization of Human Peripheral Blood Derived Endothelial Progenitor Cells: Osteopontin Surface Modification and eNOS Transfection Enhances Functional Activity**

Yifan, Yuan, Wafa A, Altalhi, Jeannette, Chan, David W, Courtman

Background: Complete endothelial coverage of the blood contacting surfaces of cardiovascular biomaterials has been difficult to achieve. A readily available autologous source of endothelium would improve the chances of developing functional and biocompatible surfaces.

Methods and Results: Here we describe methods to derive high quantities of Endothelial Progenitor Cells (EPCs) from Peripheral Blood Monocytes (PBMCs) obtained by leukapheresis. These cells are morphologically and phenotypically indistinguishable from Human Umbilical Endothelial Cells; however, their expression of the key vascular factor, endothelial nitric oxide synthase (eNOS), is markedly lower than that observed in human umbilical vein derived endothelial cells. We demonstrate here that eNOS levels can be elevated with transient plasmid based transfection. To further enhance EPC adherence and function we examined Osteopontin (OPN), as a substrate showing dose and time dependent responses of OPN in EPC adhesion and spreading. OPN also promoted haptotactic migration of EPCs in Boyden chamber assays. In addition, the OPN coating successfully enhanced the adhesion of eNOS overexpressing EPCs (39.6±1.7 and 49.4±2.4 cells/field for 0 and 1 nM OPN) and spreading (84.7±3.5% and 92.1±3.9% for 0 nM and 1 nM OPN).

Conclusions: These data confirm the direct effects of OPN on EPCs adhesion, suggesting that OPN works by mediating cell adhesion during vascular injury and the combination of autologous EPCs and OPN coatings could be a promising method of developing endothelialized biomaterials.

P-42 **Genetic Enhancement of Mesenchymal Stem Cells with Heme Oxygenase-1 for the Therapy of Pulmonary Arterial Hypertension**

Alexander Zhai, Xiaoxue Wen, Dr. Duncan Stewart

Background: Pulmonary arterial hypertension (PAH) is a disease associated with a significant burden of morbidity and mortality, in spite of our current standard of care. Numerous studies have shown that gene-enhance, cell-based approach to the treatment of PAH has the potential to further advance the therapy of PAH. Heme oxygenase-1 (HMOX-1) is an interesting candidate for the enhancement of stem cells in the development of PAH therapy. It is an enzyme that catalyzes the rate-limiting step in the breakdown of heme into carbon monoxide and bilirubin, both of which has pleiotropic effect on cellular function. HMOX-1 is induced by a variety of stressors, and has been shown to exert anti-inflammatory, anti-apoptotic, or anti-proliferative effects on different cell types.

The aim of our current study is to evaluate the therapeutic potential of mesenchymal stem cells (MSCs) that have been genetically enhanced to express heme oxygenase-1.

Methods/Results: Using the minicircle-DNA expression system, which has minimal bacterial backbone, we have transfected both rat and human MSCs with human gene HMOX-1, using JetPEI reagent. Preliminary studies show that the expression of HMOX-1 in MSCs follows a predictable time course. Functional analysis of heme oxygenase will be done to confirm the enzymatic activities of HMOX-1.

Conclusion: Our preliminary studies show that heme oxygenase-1 (HMOX-1) can be expressed transiently in human and rat MSCs. The therapeutic efficacy of MSCs genetically enhanced with HMOX-1 will be evaluated in rat models of PAH.

P-43 Comparison of Survival and Neurologic Recovery Following Initiation of Therapeutic Hypothermia in Patients With STEMI and Patients Without STEMI

Ifrah Abdirahman MD, Michel LeMay MD

Background: Therapeutic hypothermia (TH) has proved to be neuroprotective and increase survival in comatose pts presenting after out-of-hospital cardiac arrest (OHCA). However, there is limited data on pts with STEMI treated with primary PCI and therapeutic hypothermia. We sought to compare clinical outcomes in pts presenting with STEMI and pts without STEMI who survived cardiac arrest and were treated with TH.

Methods: Using the University of Ottawa Heart Institute return of spontaneous circulation (ROSC) database, we retrospectively identified, consecutive comatose pts presenting with OHCA between August 2010 and April 2012 who required TH. We compared pts with STEMI to pts without STEMI. The primary endpoint was defined as favorable neurological outcome at discharge, using discharge home from hospital as a surrogate; and secondary endpoint being hospital survival.

Results: We identified 73 OHCA pts treated with TH: 37 were STEMI and 36 were without STEMI. Of the 73 pts, 34 (92%) with STEMI and 26 (72%) without STEMI presented with ventricular fibrillation/ventricular tachycardia (VF/VT) on entry ECG, p= 0.03. All the STEMI pts underwent cardiac catheterization. The procedure was done within 24 hours in 35 of these pts (95%): primary PCI was performed in 31 (84%) and a pharmacoinvasive strategy was used in 4 (11%). In the without STEMI cohort, 18 (50%) underwent cardiac catheterization and 9 (25%) received successful PCI within 12hrs. The decision to perform cardiac catheterization in the without STEMI cohort was at the discretion of the attending cardiologist. The primary endpoint, the proportion of pts surviving to home discharge in the STEMI group was 49% and 50% in the group without STEMI, p=0.91. Hospital survival was 60% in the STEMI group as compared to 56% in the group without STEMI, p=0.74. The median length of hospitalization for STEMI pts was 10 (IQR 4-17) days versus 15 (IQR 7-28) days for pts without STEMI, p= 0.002.

Conclusions: In comparison to pts without STEMI, pts with STEMI treated with an invasive reperfusion strategy, appear to equally benefit from TH.

P-44 The Assessment of Mechanical RV Dyssynchrony using Phase Analysis of RNV Imaging in Subjects with Normal and Severely Reduced LV Function

R Abo-Shasha, T Haddad, G Dwivedi, RG Wells, TD Ruddy, RS Beanlands, MS Green, B Chow, H Haddad

Background: Phase analysis during radionuclide ventriculography (RNV) is an evolving and promising technique for measuring left ventricular (LV) mechanical dys-synchrony. However, the feasibility of phase analysis to assess right ventricle (RV) synchrony in normal population and RV dys-synchrony pre and post cardiac resynchronization therapy (CRT) have not been previously investigated. We evaluated a normal value of RV phase standard deviation (SD) and also assess the effect of CRT on RV phase SD in severe LV dysfunction patients.

Methods: We retrospectively identified 50 normal subjects (LV ejection fraction >50%) with diagnostic RNV images. 50 patients who underwent CRT implantation and diagnostic pre and post CRT RNV were also identified. Electrocardiographic gated equilibrium planar acquisitions in the left anterior oblique projection (40-50°) were used for the calculation of RV phase SD.

Results: Compared to those who underwent CRT, normal subjects were younger (62 vs. 53 years respectively; P< 0.0001) and demonstrated higher RV ejection fraction (20% vs 54% respectively; P< 0.0001). No difference in the RV phase SD (44.57±16.29 degree vs. 46.80±24.0 degree; P=0.58 respectively] was observed between the normal and at baseline in the LV dysfunction group. Post CRT, no change in the RV phase SD (46.8±24.0 degree vs. 48.30±21.20 degree; P=0.74 respectively) was observed.

Conclusions: Phase analysis during RNV for measuring RV mechanical dys-synchrony is feasible. No evidence of RV dys-synchrony on phase analysis is seen in patients with severe LV dysfunction. Following CRT therapy, the RV phase SD remains unchanged.

P-45 **Mapping and Ablation of Autonomic Ganglia in Prevention of Postoperative Atrial Fibrillation in Coronary Surgery: MAAPPS AF Randomized Control Trial**

Al-Atassi T, Mesana T, Lam BK

BACKGROUND: Despite pharmacologic prophylaxis, the incidence of postoperative atrial fibrillation (PAF) remains high after coronary artery bypass grafting (CABG). There have been limited efforts to intervene on cardiac autonomic ganglionic plexi (AGP) during surgery with mixed results. This randomized trial looks at the efficacy and safety of map-guided ablation of AGPs during CABG in preventing PAF.

METHODS/RESULTS: This single-center, partially blinded pilot trial randomized patients undergoing isolated CABG into an intervention group who underwent mapping and ablation of AGP (AGP+ group), and a control group who did not undergo mapping and ablation (AGP- group). Using a bipolar high frequency stimulation pen, active AGPs were identified and ablated. Cardiac rhythm monitoring was started on all patients immediately following surgery until discharge. Postoperative atrial fibrillation was defined as atrial fibrillation lasting > 5 min or requiring intervention. Daily ECG's, serum electrolytes, and postoperative medication administration was recorded.

A total of 47 patients were enrolled (24 AGP+ and 23 AGP-). There was no significant difference between the two groups in terms of baseline characteristics, past medical history, and current use of medications. Mapping and ablation added a median of 15 minutes to the operative time. The incidence of PAF and mean time in PAF was not statistically different between the two groups (AGP+ 21% vs. AGP- 30%, p=0.450; AGP+ 298 minutes vs. AGP- 514 minutes, p=0.566). The median length of hospital stay was similar in both groups (AGP+ 5 days vs. AGP- 6 days, p = 0.485). There were no postoperative arrests, strokes, myocardial infarctions or deaths until discharge in either group. The incidence of postoperative acute kidney injury and surgical site infections was similar in both groups (AKI: AGP+ 17% vs. AGP- 9%, p=0.345; SSI: AGP+ 8% vs. AGP- 4%, p=0.999). Postoperative medication use and serum electrolytes were similar in both groups.

CONCLUSIONS: Ablation of autonomic ganglionic plexi following CABG does not significantly reduce the incidence of PAF, total time in PAF, postoperative morbidity, or hospital LOS. Ablation of AGPs during CABG is safe and does not confer added risk or operative time. A larger multi-center trial is warranted.

P-46 **Can Body Mass Index and Body Surface Area as Measures of Obesity Predict Coronary Artery Calcification?**

Ahmed Aljizeeri, Benjamin Chow

Introduction: Obesity is a major health problem in the developed countries. Although it is considered as a risk factor for many cardiovascular diseases, many have reported that increased body weight is associated with better cardiac prognosis in patients "The Obesity Paradox". Whether or not the obesity paradox exist, remains to be determined. Since coronary artery calcification (CAC) is a predictor of future cardiovascular events, the correlation between obesity and CAC may assist in determining whether or not the obesity paradox exists.

Objective: To determine the potential relationship between measures of obesity and cardiovascular outcome, the agreement between body mass index (BMI) and body surface area (BSA) and CAC were calculated.

Methods: We retrospectively analyzed patients from the Cardiac CT database. Patients BMI and BSA were calculated and the cohort was categorized according to the BMI into 6 groups according to WHO BMI classification (underweight (BMI<18), normal (18<BMI< 25), overweight (25<BMI<30), mildly obese (30<BMI<35), moderately obese (35<BMI<40) and severely obese (BMI>40)). The cohort was also grouped into two groups depending on BSA (normal <1.71m2 and large >1.71m2). CAC was measures (Agatston score). We employed a multiple logistic regression model to study the relationship between BMI and CAC as well as BSA and CAC.

Results: 7133 patients (3791(53%) male and 3342 (47%) female) with a mean age of 57.2 + 11.2 were studied. 23 (0.3%) were underweight, 1595 (22.4%) were normal weight, 2778 (38.9%) were overweight, 1612 (22.6%) were mildly obese, 674 (9.5%) were moderately obese and 451 (6.3%) were severely obese. Mean BSA was 1.96 + 0.26 and mean Agatston score was 156.8 + 344.2. There was no association between MBI and Agatston score (P = 0.547). However there was a statistical association between BSA and Agatston score (P = 0.001).

Conclusion: BSA appears to be a predictor of CAC whereas BMI is not. BSA maybe a better predictor of cardiovascular outcomes and may explain the obesity paradox observed when only BMI as a measure of obesity is utilized.

P-47 **A 'Reduce-to-Quit' Pilot Program for Smokers with Cerebrovascular Disease: Transitioning Smokers to Set a Quit Date**

A Armstrong, R Reid, M Sharma*, DA Aitken, AL Pipe
*McMaster University/Population Health Research Institute

Background: Smoking cessation is a life-saving intervention for patients with cerebrovascular disease, however, only 35% of smokers with TIA or stroke are ready to make a quit attempt after their event. Little is known about smoking cessation in smokers with cerebrovascular disease who are not ready to quit. The aim of this analysis is to assess whether reducing daily cigarette consumption leads to a quit attempt in those smokers who are not ready to quit.

Methods/Results: The Stroke Prevention Clinic (SPC) 'reduce-to-quit' (RTQ) pilot program offers smokers who have experienced a stroke or TIA, and who are not ready to quit, a 4-week supply of nicotine replacement therapy (NRT) patch. Patients are encouraged to use the NRT patch each day while the attempting to reduce their cigarette consumption. Since December 2009, 181 smokers with stroke or TIA attending the SPC were not willing to set a quit date; 49 (27%) were willing to participate in the RTQ program and are included in the current review. Males constituted 48% male of the sample with a mean age of 59.5 +/- 10.6. The average number of cigarettes smoked per day was 23.5 +/-11.9. Of the 49 individuals participating in the RTQ program, 82% of the patients reduced the number of cigarettes they were smoking per day, the average smoker in the program reduced daily consumption by 13.2 cigarettes (SD = 9.7). Overall, 15 smokers (30%) in the RTQ program set a quit date at one month follow up, with those who had reduced cigarette consumption becoming "ready-to-quit" at a higher rate (33%) then those smokers who did not reduce their cigarette consumption (22%). Of those RTQ patients who set a quit date, the abstinence rates (bio-chemically confirmed) for those reached at follow-up were 25% and 38% at 6 and 12 months respectively. Males were more likely to set a quit date at one month follow-up: 44% of males set a quit date compared to 21% of females, ($p = .09$).

Conclusions: The results from the RTQ pilot program suggest that offering treatment to smokers who are not ready to quit can lead to an increase in quit attempts as well as 6 and 12 month cessation success in this population. A randomized control trial is needed to test the effectiveness of an RTQ intervention in those who are not ready to quit.

P-48 **Contemporary Referral Patterns for Transthoracic Echocardiography: Barriers to Determining Appropriateness in the Echocardiography Laboratory**

Behnam Banihashemi, Kasra Maftoon, Jordan Bernick, George Wells, Ian Burwash

Background: Healthcare costs have significantly grown in the last several years. In particular, echocardiography volumes have markedly increased at a rate that exceeds the increase in disease prevalence. Concerns exist that the increase in echocardiography volumes may not be appropriate. There is general consensus that echocardiography procedures should be remunerated based on appropriateness. However, there is no data on the utility of the echocardiography requisition to determine the appropriateness of an echocardiography referral.

Methods: We prospectively evaluated the requisitions for all transthoracic echocardiography (TTE) referrals to the UOHI echocardiography laboratory over a one-month period to determine the appropriateness of the referrals, based on the ACC 2011 appropriate use criteria for echocardiography. Patient demographic information, inpatient/outpatient status, previous echocardiography study, referring physician specialty, and primary indication for the study were identified. When the study indication was not clear based on the information provided on the requisition, relevant electronic medical records were reviewed and referring physicians contacted.

Results: Between July-August 2012, 1303 TTE examinations were performed (median age 65 years, 56% male). Inpatient examinations accounted for 24% of studies. Cardiologists were the largest physician referral group, accounting for 49% of referrals. Internists and family physicians accounted for 10% and 19% of referrals, respectively. Of the referral requisitions, 26% (n=339) provided inadequate information to determine the appropriateness of the referral. The most common reasons for requisition inadequacy were 1) failure to report a change in patient symptoms (n=326) or 2) failure to provide information about a previous echocardiogram (n=300). Cardiologists were more likely than other physicians to request a TTE with inadequate information on the requisition (41%; $p<0.001$). After adjusting for potential confounders, referring physician specialty ($p<0.001$), outpatient status (OR 0.20[0.12-0.32]), presence of a previous study (OR 0.39[0.27-0.56]) and the reason for the study were independent predictors of an inadequate requisition. Age and gender were not associated with an inadequate requisition.

Conclusion: In a contemporary large population of patients referred to an academic echocardiography laboratory, one quarter of TTE requisitions do not provide adequate information to determine the appropriateness of the examination. However, standardized requisitions that request specific information, along with education directed at physician groups, have the potential to improve the adequacy of the requisition as a tool to evaluate the appropriateness of echocardiography referrals.

P-49 **Usefulness of Estimated Left Ventricular End Diastolic Volume (calculated from 75% phase parameters) for the Prediction of All Cause Mortality**

Kevin Boczar, Girish Dwivedi, Mohammed Alam, Benjamin J Chow

Introduction: Computed tomographic (CT) coronary angiography (CTA) is a key diagnostic modality for the non-invasive detection of coronary artery disease (CAD). To minimize radiation exposure prospective- electrocardiographic (ECG) gated image acquisition algorithms are preferred whereby image acquisition is restricted to ventricular diastasis. This, however, leads to the loss of left ventricular (LV) functional information. Previous studies have demonstrated that LV end diastolic volume (LVEDV) has prognostic value. We have previously shown that LVEDV can be reliable estimated using CTA data obtained during ventricular diastasis. In the present study, we evaluated prognostic value of such estimates of LVEDV prospective ECG-gated CTA.

Methods: 101 patients with adverse events (all-cause mortality) on follow up were identified on our CT database. A matched (matched according to the Morise score) control list of 101 patients (control population) with no adverse events on follow up was also generated.
Images were reconstructed at the 75% phase (mid diastasis). Left atrial and LV volumes were measured at the 75% phase. Using our previously developed model [(LVEDV= (1.021 × 75% phase LV volume) + (0.259 × 75% phase left atrial volume)] by our group, predicted LVEDV was calculated.

Results: Baseline characteristics were not different between the test population and control group. The majority of patients were male, smokers with history of hypertension and hyperlipidemia. Mean follow up duration was 20 ± 12 months. Predicted LVEDV index was significantly larger (p=0.006) in the test population (167.50±83.09) compared to the control population (138.90 ± 58.14).

Conclusion: Significantly larger predicted LVEDV are seen with patients who experienced mortality on follow-up. On the basis of our results, assessment of the LVEDV may be considered from the data obtained during mid ventricular diastasis images.

P-50 **[18F]-fluorodeoxyglucose, but not C-reactive Protein, is Related to Intraplaque Inflammatory Burden in Human Carotid Plaque: A Sub-study of the Canadian Atherosclerosis Imaging Network (CAIN)**

M.Cocker, B. Mc Ardle, R Hammond, R deKemp, C. Lum, G Youssef, Y Yerofeyeva, T Karavardanyan, A Adeeko, A Hill, G Stotts, M. Sharma, JM Renaud, Cathy Kelly, J Brennan, Linda Garrard, M Alturkustani, L Hammond, J DaSilva, JC Tardif, R Beanlands, J David Spence

Background: Inflammation underlies the development and progression of atherosclerotic plaque. An actively inflamed plaque is considered to be a 'vulnerable' high-risk, rupture-prone lesion. It is imperative to develop surrogate biomarkers that enable for the early detection of disease. C-reactive protein (CRP) is a marker of systemic inflammation and a risk factor associated with cardiovascular events. Alternatively, radiolabelled glucose or [18F]-fluorodeoxyglucose (18FDG) imaged with hybrid positron emission tomography (PET) and computed tomography (CT) may serve as an imaging-derived biomarker of inflammatory burden within plaque. In this investigation, we directly compared systemic CRP and 18FDG carotid uptake to intraplaque inflammatory burden using CD68 immunohistology.

Methods: Thirty-four patients (67±10 years, 27 male) scheduled for carotid endarterectomy were prospectively recruited. Patients underwent FDG-PET and CT angiography of carotids. Prior to imaging, blood was collected for the assessment of CRP. Maximum 18FDG uptake at the left and right internal carotids was quantified and normalized to blood, resulting in a tissue to blood ratio (TBR). Following endarterectomy, excised plaque was fixed, sectioned and immunostained for CD68. CD68 expression was quantified semi-automatically.

Results: Carotid endarterectomy was performed in 34 patients; one patient received a 2nd carotid endarterectomy due to bilateral disease. Immunohistology was performed in 23 excised plaques. The extent of inflammation, as quantified with CD68 immunohistology, was related to maximum 18FDG uptake ($r=0.636$, $p=0.001$). However, there was no association between CD68 expression and CRP ($r=0.190$, $p=0.42$). Furthermore, CRP was not associated with maximum 18FDG uptake of the endarterectomy lesions ($r=0.179$, $p=0.34$).

Conclusion: As opposed to systemic CRP, 18FDG uptake is strongly related to the extent of inflammatory burden within high-risk carotid plaque. However, CRP may not be useful for detecting vulnerable carotid plaque (based on histopathology nor 18FDG uptake). Large prospective outcomes-based studies are required. 18FDG may serve as a robust and direct biomarker of carotid plaque vulnerability.

P-51 **Evidence for Actively Inflamed Bilateral Carotid Plaque in Patients with Advanced Atherosclerosis, Insight from [18F]-fluorodeoxyglucose Imaging: A Sub-study of the Canadian Atherosclerosis Imaging Network (CAIN)**

M.Cocker, J David Spence, R Hammond, B. Mc Ardle, R deKemp, C. Lum, G Youssef, Y Yerofeyeva, T Karavardanyan, A Adeeko, A Hill, S Nagpal, G Stotts, JM Renaud, Ran Klein, Cathy Kelly, J Brennan, Linda Garrard, M Alturkustani, L Hammond, J DaSilva, JC Tardif, R Beanlands

Background: Atherosclerosis is the most common underlying pathology responsible for myocardial infarction and stroke. It is a significant source of mortality and reduced quality of life. Inflammation of vasculature is associated with the development and progression of atherosclerotic plaque. macrophages are highly expressed in actively inflamed 'vulnerable' plaque. Relative to other plaque components, these cells have high metabolic rates. Therefore, the uptake of radiolabelled glucose or [18F]-fluorodeoxyglucose (18FDG) imaged with hybrid positron emission tomography (PET) and computed tomography (CT) can be utilized to identify inflamed plaque. the presence of bilateral carotid artery disease has been associated with an increased propensity towards developing adverse cerebrovascular events. The incidence of bilateral carotid plaque inflammation in patients presenting with high-risk carotid disease is unclear. Therefore, in patients with high-risk carotid disease, we examined 18FDG uptake in both carotids to determine if there is evidence for bilateral inflammation.

Methods: Thirty-four patients (mean age: 67±10 years, 27 male) scheduled for carotid endarterectomy within two weeks were recruited prospectively. Seven patients were referred for carotid endarterectomy due to the presence of high-grade severely stenotic internal carotid artery. The remaining 27 patients presented with symptomatic carotid vessel disease including stroke or transient ischemic events. Patients underwent PET-FDG and CT angiography of carotid vasculature. Maximum 18FDG uptake at the left and right internal carotid arteries was quantified and normalized to blood, resulting in a tissue to blood ratio (TBR).

Results: All patients had evidence for the presence of bilateral atherosclerotic plaque. Although bilaterally increased, maximum 18FDG uptake at the side of carotid endarterectomy and the contralateral carotid artery did not differ (3.2±1.2 TBR at the side of endarterectomy vs. 3.0±1.2, p=0.48). However, patients referred for carotid endarterectomy due to a symptomatic presentation (27 patients, 54 plaques), had greater 18FDG uptake than patients referred for a severe high-grade stenosis (7 patients, 14 plaques) (symptomatic plaque: 3.3±1.3 TBR vs. stenotic plaque: 2.7±0.6 TBR, p=0.03).

Conclusion: In patients with advanced atherosclerotic disease, there is evidence for actively inflamed bilateral carotid plaque, as demonstrated by 18FDG uptake. Symptomatic and stenotic plaque may reflect different stages of disease progression and inflammatory burden, as captured by 18FDG PET/CT imaging. These findings support the concept of a 'vulnerable patient'.

P-52 **The Influence of Initial Thrombolysis in Myocardial Infarction Flow Grades on Outcomes of Patients with ST-Elevation Myocardial Infarction**

Esam Elbarasi, Melissa Blondeau, Chris A. Glover, Derek Y. So, Michael P.V. Froeschl, Alexander Dick, Jean-Francios Marquis, Marino Labinaz, Michel Le May

BACKGROUND: Initial and post-procedural Thrombolysis In Myocardial Infarction (TIMI) flow are known to have significant prognostic implication among patients undergoing primary PCI for ST-segment elevation myocardial infarction (STEMI). We sought to determine factors associated with initial TIMI-flow 3 in STEMI patients in a large Canadian primary PCI program.

METHODS/RESULTS: We analyzed data collected between July 2004 to June 2010 on a total of 2062 consecutive STEMI patients entered in the Ottawa Heart Institute STEMI database. The study cohort was divided into two groups based on initial TIMI-flow in the infarct-related artery at the time of the first coronary injection: 1577 (76.5%) patients with initial TIMI-flow 0-2, and 485 (23.5%) patients with initial TIMI-flow 3. All patients received chewable ASA 162mg, clopidogrel 600mg orally, and intravenous unfractionated heparin 60units/kg (max 4000 units) before cardiac cathterization.

The two groups were well matched for baseline characteristics with the following exceptions: initial TIMI-flow 0-2 patients were more likely to present with a lower systolic blood pressure (134±29 mmHgvs139±28mmHg, p= 0.001), however, no differences observed in Killip class compared to patients with initial TIMI-flow 3(4.31%vs1.89%, p=0.09).

We observed no difference in the median door-to-balloon time of TIMI-flow 0-2 group compared to TIMI-flow 3 group: [98 (71-131) min vs. 103(79-136) min, p=0.07]. Similarly there was no difference in the median clopidogrel-to-balloon time between groups: 76 (IQR=50-101) min in the TIMI-flow 0-2 group vs. 82 (IQR=58-102) min in the TIMI-flow 3 group, p=0.07.

Higher rates of Glycoprotein IIb/IIIa inhibitors, and intra-aortic balloon pump use were noted in patients with initial TIMI-flow 0-2 compared to initial TIMI-flow 3. Patients with initial TIMI-flow 3 were more likely to achieve TIMI-flow 3 post revascularization compared to patients with initial TIMI-flow 0-2 (99%vs87.3%, p<0.001).

Compared to patients with initial TIMI-flow 3, those with TIMI-flow 0-2 had higher rates of stent thrombosis (1.65% vs. 0.41%, p=0.04), reinfarction (1.84% vs. 0.41%, p=0.02), cardiogenic shock (9.32% vs. 3.71%, p<0.0001). Moreover, higher mortality was noted in the TIMI-flow 0-2 at all time points measured: in-hospital mortality was 5.26% vs. 2.68%, p=0.02; 30-day mortality was 5.7% vs. 2.9%, p=0.02; and 180-day mortality was 8% vs. 4.4%, p=0.009.

CONCLUSIONS: This study highlights the importance to achieve TIMI-flow 3 in STEMI patients prior to arrival to the catheterization lab to improve clinical outcomes. Novel antiplatelet agents such as ticagrelor or cangrelor may play an important role to achieve this goal in the future.

P-53 **The Effect of CRT Therapy on RV Function in Patients with Severe LV Dysfunction: A Matched Control Study**

Tony Haddad, G Dwivedi, Rami Abo-Shasha, BA McArdle, RG Wells, T Dowsley, B Marvin, LM Mielniczuk, TD Ruddy, RS Beanlands, MS Green, B Chow, H Haddad

Background: Whilst numerous studies have established the prognostic and symptomatic benefits of cardiac resynchronization therapy (CRT) on left ventricle (LV), its effect on right ventricular (RV) function has not been well described. Radionuclide ventriculography (RNV) is a well established technique for RV function assessment. We evaluated the effect of CRT on RV function using RNV in patients with severe LV dysfunction.

Methods: 76 patients who underwent CRT implantation and diagnostic pre and post CRT RNV were retrospectively identified. The control group (n=76) included patients who underwent implantable cardioverter defibrillator (ICD) implantation alone and were matched for age, gender, etiology of heart failure and LV function. Electrocardiographic gated equilibrium planar acquisitions (24 frames/RR interval) in the left anterior oblique projection (40-50º) were used for the analysis of RV function. RV ejection fraction, RV volumes and RV peak filling rate were calculated.

Results: Mean age (65 vs. 66 years), gender ratio (83% vs. 82% males), ischemic etiology (76% vs. 76%) and LV function (21% vs. 23%) were similar in the test and control groups respectively. Significant improvements in RV systolic [(RV ejection fraction (9.05 vs. 1.54; P<0.00001 respectively) and end systolic volume 17ml vs 10ml; P=0.005 respectively)] and diastolic [RV peak filling rate (0.24 mls-1 vs. 0.04 mls-1; P=0.0003), respectively] parameters were observed in the CRT compared to the ICD group.

Conclusions: In patients with LV dysfunction, CRT improves RV systolic and diastolic function. A multicenter study is needed to confirm these findings.

P-54 **Clinical Outcomes of Patients Classified as False Activation of Catheterization Laboratory in a Regional Primary Angioplasty Program**

Nabeel Ismaeil, Chris A. Glover, Derek So, Michael P.V. Froeschl, Alexander Dick, Jean-Francois Marquis, Marino Labinaz, and Michel R Le May

BACKGROUND: Primary PCI has emerged as the dominant strategy for reperfusion in patients presenting with STEMI, if it can be performed in a timely fashion. However, false activation of the STEMI team may reduce the efficiency of the system. We sought to determine the frequency, etiology, and the clinical outcomes of patients identified as “false activation” (FA) of the Code STEMI.

METHODS/RESULTS: We defined FA as the absence of any one of the following: 1) ECG criteria (i.e. ST segment elevation of ≥ 1mm in at least 2 contiguous leads) or 2) symptoms of ischemia ≥ 30 minutes and ≤ 12 hours, or 3) angiographic findings to support STEMI. Between June 1, 2005 and May 31, 2010, a total of 2213 patients were referred for primary PCI. Amongst these, 369 patients (15%) were labeled as FA. Baseline characteristics of the FA cohort indicate that the mean age was 61±7 yr; 73% were male; 21% were diabetics; 34% had prior MI; and 5% had cardiogenic shock on presentation. Among patients with FA, 226 (61%) underwent immediate coronary angiography. The remaining patients were referred to the local ED for further evaluation. Eighty patients (35.4% of those who had cardiac catheterization were found to have significant coronary disease needing PCI in 67 and urgent CABG in 13 patients; the remaining 146 patients did not have obstructive coronary disease. Among the entire cohort of FA, 30 patients (8%) died during the acute hospitalization. As compared to survivor patients, the non-survivors were more likely to be older (71±7 versus 60±7; p=0.001), diabetic (40% versus 19.9%; p=0.02), have a history of previous MI (52% versus 32%; p= 0.03), have shock on presentation (48% versus 1.8%; p<0.001), or elevated cardiac biomarkers (84% versus 44%; p<0.001).

Major reasons for FA of the PCI team were: early repolarization (23.8%); LVH (20.6%); pericarditis (16.8%); persistent ST elevation from old MI (12.6%); LBBB (10.1%); RBBB (7.7%); Takotsubo cardiomyopathy (3.8%); coronary spasm (2.4%); and a paced rhythm (1%)

CONCLUSIONS: FA is relatively frequent occurrence in regional STEMI systems. Some of the patients referred to a PCI-capable hospital with FA may have poor outcomes. Awareness of these findings is important for health care workers involved in STEMI systems, especially when repatriation to the sending hospital is considered. Triage protocols are needed to identify FA patients at higher risk.

P-55 **Should We Routinely Replace Implantable Cardioverter-defibrillator Generators? Patients' Perspectives**

Lewis, K.; Birnie, D.H.

Background: ICD pulse generator replacement should be a time for shared decision-making. There should be careful review of the patient's health status, lived experience with the ICD, and health care goals. However, it has been suggested that current practice is often a relatively automated process with little discussion. Our objectives for this study were to assess whether patients and the health care team are engaging in a shared decision-making process when ICD replacement is indicated, and whether patients are aware that electing to not have their battery replaced is an option.

Methods: We evaluated current practices regarding ICD battery replacement in our tertiary care institution. We included all consecutive patients who have undergone battery replacement in the previous 3 years, and were 18 years of age or older. Pacemaker dependent patient and patients with cardiac resynchronization therapy were excluded. A questionnaire was sent out to eligible patients to gain insight on how the process unfolded, and to capture patients' preferences regarding a shared decision-making process.

Results: There were a total of 106 (72.1% response rate) responses. Fifty-five (51.9%) patients were unaware that replacement is nonobligatory. If given the option, 15/55 (27.2%) respondents stated they would have considered not replacing the ICD at battery change. For 88/106 (83.0%) of the respondents, it was "important" or "very important" to discuss all pros and cons of continued device therapy before making a decision about future replacement.

Conclusion: We found that more than half of our patients were not aware that they had the option of not replacing their ICD. Furthermore, 27% of these patients would have considered not replacing their ICD if they had known. Together these findings suggest that we have to improve on current practices. The next phase of our project is to design and implement a patient decision tool.

P-56 **Aortic Valve Repair in Acute Type-A Aortic Dissection**

T. Malas, R. Saczkowski, G. El Khoury[*], T. Mesana, MB M. Boodhwani
[*]Department of Cardiovascular Surgery, St-Luc Hospital. Brussels, Belgium

Objective: Repair and preservation of the aortic valve in type-A aortic dissection remains controversial. We performed a meta-analysis of outcomes for AV repair and preservation in acute type-A aortic dissection focusing on long-term valve-related events.

Methods: Structured searches were performed in Embase (1980-2012) and Pubmed (1966-2012) for studies reporting AV repair or preservation in acute type-A aortic dissection. Early mortality and linearized rates for late mortality and valve-related events were derived. Outcome data were pooled with an inverse-variance weighted random-effects model.

Results: Of 5325 screened articles, 19 observational studies met eligibility criteria consisting of 2402 patients with a median follow-up of 4.1 years (range: 3.1 – 12.6, total 13733 pt-yrs). The cohort was principally male (median = 68.1%, 39 – 89%) with a median age of 59 years (range: 55 – 68) and Marfan's syndrome was present in 2.5%. AV resuspension was performed in 85% and the remainder underwent valve-sparing root replacement. Pooled early and late mortality were 18.7 % (CI: 12.2 – 26.2), and 4.7 %/pt-yr (CI: 3.3 – 6.4), respectively. Linearized rates for AV reintervention was 2.8 %/pt-yr (CI: 1.5 – 4.4), recurrent AI (>2+) was 4.6 %/pt-yr (CI: 3.1 – 6.2), and endocarditis was 0.5 %/pt-yr (CI: 0.08 – 1.3). The composite rate of thromboembolism and bleeding was 1.4 %/pt-yr (CI: 0.7 – 2.3).

Conclusion: Repair of acute type-A aortic dissection is associated with poor long-term survival. Preservation and repair of the aortic valve is associated with a moderate risk of reoperation and recurrent AI and a low risk of thromboembolism, bleeding, and endocarditis.

P-57 **Is Aortic Valve Repair Reproducible? Analysis of the Learning Curve for Aortic Valve Repair**

Tarek Malas, Sophia Chaudry, Benjamin Sohmer, Marc Ruel, Thierry Mesana, Munir Boodhwani

Objectives: Aortic valve (AV) repair, while effective, is performed in a limited number of centers. Wider application is due, in large part, to challenges in dissemination of tacit surgical knowledge. We examined the learning curve in a single-center initiating a dedicated program in AV repair.

Methods: Detailed intra-operative, post-operative, and echocardiographic data on the first 100 consecutive patients undergoing AV repair was analyzed focusing on safety, efficiency, and efficacy. Safety parameters included mortality, perioperative myocardial infarction or stroke, need for AV re-exploration, reopening for bleeding, or pacemaker implantation. Efficiency was assessed through aortic crossclamp (ACC), cardiopulmonary bypass (CPB), and operating time. Efficacy was assessed through systematic echocardiographic follow-up. The cohorts were divided into three equal terciles (T1,T2,T3) to define the learning curve. Median clinical follow-up was 18 months.

Results: Overall early mortality was 1%. No patients developed severe AI or required AV reoperation during the follow-up period. A total of 16 safety events occurred in 14 patients with an incidence of 8(24%), 6(13%), and 2(6%), in T1, T2, and T3 respectively ($p=0.04$). ACC and CPB times were similar between T1 and T2 but decreased significantly during T3 (Figure 1, $p<0.01$). At follow-up, efficacy endpoints were achieved in 27(82%), 30(91%), and 31(91%) in T1, T2, and T3 patients respectively ($p=0.3$).

Conclusions: Significant improvements in safety and efficiency of AV repair were observed only after the second tercile with similar efficacy across terciles. Aortic valve repair is reproducible but appears to have a learning curve of approximately 60 cases.

P-58 **Triaxial Accelerometer Cutpoints to Assess Physical Activity in Individuals with Coronary Artery Disease**

Amy E Mark, Robert D Reid

Background: Accelerometers provide an objective measure of physical activity, however to date the latest generation triaxial accelerometers have not been validated in a coronary artery disease population. The primary purpose of this pilot investigation was to establish accelerometer thresholds denoting moderate and vigorous intensity physical activity (PA) among individuals with coronary artery disease (CAD); the secondary purpose was to determine inter-monitor reliability.

Methods/Results: Patients (n=11) were recruited from the cardiac rehabilitation program at the University of Ottawa Heart Institute, and had a cardiac stress test ordered as part of their routine care. Demographic (e.g., age, sex) and descriptive (e.g., height, weight) characteristics were extracted from participants' cardiac rehabilitation file. A ramp protocol was used for the stress test and exercise intensity was measured at each stage. The ActiGraph GT3X accelerometer was worn during the stress test; a 15-second sampling epoch was used. Mixed models and receiver operator curves were used select appropriate thresholds to denote moderate and vigorous intensity PA. Patients ranged in age from 45 to 80 years; all participants had diagnosed CAD. Based on receiver operator characteristic curve results, thresholds of 1700 and 3700 were found to best identify moderate and vigorous intensity PA, respectively. Inter-monitor reliability was found to be high ($r= 0.779$-0.992).

Conclusion: Researchers should consider using the thresholds established by this investigation when analyzing objectively measured PA by accelerometers in individuals with CAD. Further research is needed to confirm these results in a larger sample of CAD patients, preferably with varying functional capacities.

P-59 **Prognostic Value of Rubidium-82 Positron Emission Tomography for Evaluation of Patients following Cardiac Transplantation**

Mc Ardle B., Small G.S., Haddad H., Mielniczuk L.M., Stadnick E., Guo A., Etele J., Beanlands R.S. , deKemp R.A., Davies R.A., Chow B.J.

Background: Cardiac Allograft Vasculopathy (CAV) is a limiting factor in long term survival for patients following cardiac transplantation. There is widespread variability between transplant centers in how patients are screened for CAV. Positron Emission Tomography (PET) using Rubidium-82 (Rb-82) may be a valuable modality for risk stratification in this population but it's prognostic value has not yet been delineated.

Methods: Patients with a history of previous transplantation undergoing Rb-82 PET at our center were prospectively enrolled in the Rubidium ARMI registry. Informed consent was obtained and this registry has the approval of the Research Ethics Board of this institution. Details on baseline characteristics and subsequent clinical events were obtained from the patient's clinical records. Values for Summed Stress Score (SSS) as well as LVEF at rest and stress were recorded by the interpreting physician based on visual analysis. Automated absolute flow quantification both at rest and following dipyrimadole stress was performed for each patient and the ratio of mean global flow at stress and at rest, termed the Myocardial Flow Reserve (MFR), was recorded. The primary end-point was a composite of all cause death, acute coronary syndrome and cardiac hospitalization. The secondary end-point was all cause death. RV biopsy results were also compared with MFR results.

Results: A total of 140 patients (Males 80%, Mean Age 59+/-14, Mean Follow-up 459+/-271 days) underwent Rb-82 PET scanning from July 2009- February 2013. Eight patients died during follow-up with 1 ACS and 2 admissions for symptomatic heart failure. Global MFR was significantly lower in patients who died (1.64+/-0.5 vs. 2.44+/-0.8, p=0.008) or experienced an outcome (1.67+/-0.5 vs. 2.45+/-0.8, p=0.002), Values for SSS, Rest LVEF and Stress LVEF did not differ significantly however. MFR also differed significantly in patients with a history of more than ISHLT Grade 1 rejection on RV biopsy(1.91+/-0.6 vs. 2.46+/-0.85, p=0.024), and correlated significantly with baseline serum creatinine (r2= -0.33, p<0.001) and the time since transplantation (r2=-0.18, p=0.01). On univariate analysis an MFR<2.0 conferred an OR of 4.86 (1.23-19.25, p=0.02) for an adverse outcome and OR of 5.31 (1.03-27.36, p=0.04) for death.

Conclusions: Global MFR as measured by Rb-82 PET may have superior prognostic value to relative perfusion and LV function in patients following cardiac transplantation. MFR is also significantly lower in patients with histological evidence of rejection. However larger studies with longer follow up are required to confirm this finding.

P-60 **Sex-related Differences in Quality of Life Following Transcatheter Aortic Valve Implantation**

Matthew McDonald, Tarek Malas, Buu-Khanh Lam, Aneil Bhalla, Bernard McDonald, Donna Nicholson, Mark Hynes, Christopher Glover, Thierry G. Mesana, Marino Labinaz, Marc Ruel

Background and Study Aim: Transcatheter Aortic Valve Implantation (TAVI) constitutes an emerging modality to treat severe aortic stenosis. Despite its growing use and dissemination, quality of life (QoL) in patients having undergone TAVI remains to be fully elucidated, specifically sex-related differences. The aim of this study is to explore the dissimilarities in QoL between genders among TAVI recipients.

Methods: 90 patients (27 women and 63 men) having undergone TAVI at the University of Ottawa Heart Institute between 2007 and 2012 were administered a comprehensive questionnaire through telephone follow-up at a mean of 2.3±1.3 years postoperatively. The questionnaire probed post-surgical complications and included the Canadian Cardiovascular Society (CCS) Angina Grading Scale, New York Heart Association (NYHA) Functional Classification, Duke Activity Status Index (DASI), as well as the Short Form 12 Health Survey Questionnaire.

Results: No differences were observed in either mean NYHA class (women 1.7+/-0.2 vs men 1.5+/-0.1, P = 0.2261) or mean CCS class (women 0.1+/-0.1 vs men 0.3+/-0.1, P=0.2746) however, men outscored women with regards to mean DASI (women 18.4+/-2.2 vs men 29.6+/-1.9, P= 0.0004). Additionally, 29.6% of women reported having experienced atrial fibrillation postoperatively, compared to only 19.1% of men. Women were also more likely to indicate having accomplished less of their work or regular activities in the 4 weeks prior to interview due to physical health (70.4% vs men 45.2%) or emotional problems (22.2% vs men 13.1%).

Conclusion: After TAVI, men demonstrate a greater functional capacity than do women, as evaluated by the DASI. Men are also less hindered in their regular activities by physical health or emotional problems, and experience less atrial fibrillation. Both sexes are equally symptomatic of heart failure and angina, as per NYHA and CCS classifications respectively.

P-61 **Cost-utility Analysis of a Hospital-initiated Intervention for Smokers with Chronic Disease in Ontario, Canada**

Kerri-Anne A. Mullen, Douglas Coyle, Douglas Manuel, Hai V Nguyen, Ba' Pham, Andrew L. Pipe, Robert D. Reid

Introduction: Cigarette smoking causes many chronic diseases that are costly and result in frequent hospitalization. Smoking cessation interventions commencing in hospital and continued for at least 1 month after hospitalization increase the likelihood that patients will become smoke-free. We modelled the cost-effectiveness of a hospital-initiated smoking cessation intervention, the Ottawa Model for Smoking Cessation (OMSC), compared to usual care among smokers hospitalized in Ontario, Canada with acute myocardial infarction (AMI), unstable angina (UA), heart failure (HF), and chronic obstructive pulmonary disease (COPD).

Methods: We completed a cost-utility analysis based on a decision-analytic model to assess smokers hospitalized in Ontario for AMI, UA, HF, and COPD, their risk of continuing to smoke, and the effects of quitting on re-hospitalization and mortality over a one year period. We included intervention costs and costs associated with re-hospitalization.

Results: From the hospital payer perspective, the OMSC is dominant (both cost saving and effective) for HF and COPD patients. Delivery of the OMSC to UA and AMI patients can be considered cost effective with incremental costs per QALY gained of $127 and $108, respectively. In the first year, we calculated that provision of the OMSC to 15,326 smokers would generate 4689 quitters, and would prevent 107 re-hospitalizations, 849 hospital-days, and 125 deaths. Results were robust within numerous sensitivity analyses.

Discussion: Smoking cessation interventions initiated during hospitalization are cost-effective from the hospital system perspective. Important consideration is the relatively low cost of the intervention compared to the reduction in costs related to readmissions for illnesses associated with continued smoking.

P-62 **Usefulness of 18F-Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) and Cardiac Magnetic Resonance (CMR) in Patients with Conduction Disease due to Cardiac Sarcoidosis**

Hiroshi Ohira[1], David Birnie[1], Brian Mc Ardle[1], Keiichiro Yoshinaga[2], Ichizo Tsujino[3], Takahiro Sato[3], Osamu Manabe[4], Masaharu Nishimura[3], Nagara Tamaki[4], Jordan Bernick[5], George Wells[5], Myra Cocker[1], Eugen Leung[6], Rob S. B. Beanlands[1], Pablo Nery[1]
[1]Molecular Function and Imaging Program, the National Cardiac PET Centre, Division of Cardiology, and the Cardiac Research Methods Centre, University of Ottawa Heart Institute, Ottawa, Canada
[2]Department of Photobiology, Division of Molecular Cellular Imaging, Hokkaido University School of Medicine
[3]First Department of Medicine, Hokkaido University school of Medicine
[4]Department of Nuclear Medicine Hokkaido University school of Medicine
[5]Cardiovascular Research Methods Center, University of Ottawa Heart Institute, Ottawa, Canada
[6]Division of Nuclear Medicine, Department of Medicine, the Ottawa Hospital, Ottawa, Ontario, Canada

Background: 18F-fluorodeoxyglucose-positron emission tomography (FDG-PET) and cardiac magnetic resonance (CMR) are accurate modalities for detection of cardiac sarcoidosis (CS) a condition that should be considered in young patients with advanced atrioventricular block (AVB). In order to facilitate the decision regarding device insertion a prompt diagnosis is essential. However, the diagnostic accuracy of each modality in this population has not been well evaluated.

Methods: We included 25 patients from 2 centers who presented with conduction disease and were subsequently diagnosed with CS, and were underwent both FDG-PET and CMR. They were classified into 2 groups based on degree of conduction disease (Table 1). We also included 13 patients with idiopathic advanced AVB as controls (FDG-PET n = 7; FDG-PET&MRI n = 6).

Results: FDG-PET and CMR showed good agreement with concordance in mild conduction disease (prevalence adjusted bias adjusted kappa <PABAK> = 0.6). However, in patients with advanced AVB, the 2 modalities did not show significant concordance (PABAK = 0). The sensitivity of FDG-PET (90%) was higher than that of CMR (60%) and specificity was comparable in both modalities.

Conclusions: There was good agreement FDG-PET and CMR in CS patients with mild conduction disease with CMR appearing to be more sensitive. However FDG-PET appeared to have higher sensitivity than CMR in patients with advanced AVB. FDG-PET may be useful in patients with advanced AVB due to cardiac sarcoidosis.

P-63 **How Physically Active are Patients with Coronary Artery Disease who recently Completed Cardiac Rehabilitation?**

Jennifer L. Reed, Amy E. Mark, Andrew L. Pipe, and Robert D. Reid

Background: Cardiac rehabilitation (CR) programs are successful in ensuring patients initiate regular exercise, but long-term maintenance of exercise behaviour remains a challenge. The purpose of this study was to assess the number of minutes per day CR graduates with coronary artery disease (CAD) engage in sedentary, light, moderate and vigorous physical activity.

Methods/Results: Twenty-seven patients (25 males, 2 females; mean ± SD = age: 60 ± 10 yrs; height: 173.0 ± 8.0 cm; weight: 87.3 ± 15.9 kg; waist circumference: 101.7 ± 13.1 cm; BMI: 29.1 ± 4.7 kg/m2; resting blood pressure: 118/72 ± 16/8 mm Hg; resting heart rate: 66 ± 10 beats per minute) from the University of Ottawa Heart Institute (UOHI) with diagnosed CAD participated in this study. All patients previously attended ≥ 75% of scheduled CR sessions. Participants wore an Actigraph GT3X accelerometer (Actigraph, Pensacola, Florida) over the right hip for at least 600 minutes per day for 4.9 ± 1.8 days. According to published cut points developed in healthy adults to classify sedentary (0 – 100 counts/min), light (101 – 2689 counts/min), moderate (2690 – 6166 counts/min) and vigorous (≥ 6167 counts/min) physical activity, participants in the present study engaged in 520.9 ± 78.6, 195.7 ± 41.1, 63.5 ± 22.8 and 4.9 ± 7.2 minutes per day of sedentary, light, moderate and vigorous physical activity, respectively. Based on cut points developed from a pilot study conducted at the UOHI to classify sedentary (0 – 100 counts/min), light (101 – 1699 counts/min), moderate (1700 – 3699 counts/min) and vigorous (≥ 3700 counts/min) physical activity in a sample of CAD patients, participants in the present study engaged in 520.9 ± 78.6, 147.9 ± 31.2, 81.4 ± 25.2 and 35.1 ± 19.0 minutes per day of sedentary, light, moderate and vigorous physical activity, respectively.

Conclusion: Patients with CAD who recently completed a CR program appear to be meeting the ACSM and CSEP guidelines for patients with cardiac disease of at least 20-60 minutes of moderate intensity physical activity (40-80% VO2peak) on most days (4-7) of the week. Specifically, 96% (26/27) of CR graduates met PA guidelines for moderate intensity physical activity according to published cut points developed in healthy adults, with this value increasing to 100% (27/27) when using population specific cut points. Future studies are needed to assess whether patients continue to meet these guidelines 6 and 12 months following CR.

P-64 **Effect of Transthoracic Impedance on the Cardioversion Success Rates Using Biphasic Defibrillators**

Mouhannad Sadek, Varsha Chaugai, David Birnie, Timothy Zakutney, Mark Cleland, Adrian Chan, Andy Adler

Introduction: The advent of impedance compensating biphasic defibrillators (ICBD) in clinical use has resulted in higher cardioversion success rates at lower energies. However, the biphasic technology only partially compensates for the variation in transthoracic impedance (TTI). Therefore, we sought to examine the influence of TTI on cardioversion success rates.

Methods: We collected cardioversion data from 698 consecutive patients with atrial fibrillation (AF) or ventricular tachycardia (VT) at our institution. Shocks were defined as “successful” if it resulted in the resumption of normal sinus rhythm for a single beat or more. Shocks were adjudicated by two electrophysiologists. Based on the first shock impedance, the data were stratified into two groups of high (> 70 Ω) and low TTI (≤ 70 Ω) followed by categories of arrhythmias and shock energy levels. The comparison in the success rate was performed using Chi-square testing at a significance level of α = 0.05.

Results: In patients with AF, there was a significant difference in outcome between the two levels of impedance. Shocks with a high TTI had a lower chance of success at energy levels of <150 J (71.04% vs 55.9%, p<0.01) and 200J (69.93% vs 58.03%, p<0.01). When 200 J was used, patients with a successful last shock had a lower TTI (78.84 Ω vs 71.84 Ω, p<0.01).

The average number of shocks delivered for patients with AF were calculated to be 1.39 ± 0.75 for low TTI and 1.63 ± 0.86 for high TTI (p<0.01), while for VT, the average shocks for low and high TTI patients were 1.31 ±0.85 and 2.41 ± 2.24 respectively (p<0.05).

Conclusion: Patients with lower TTI had significantly higher cardioversion success rates and required fewer shocks than patients with higher TTI which suggest that TTI is still clinically relevant even with ICBD. Perhaps real time display of TTI might improve success rate by guiding clinicians to reduce impedance in high TTI patients (e.g., by changing pad position, etc.).

P-65 **Surgical Ablation of Atrial Fibrillation Associated with Aortic Valve and Coronary Artery Disease: Is It Worth It?**

Hadi Toeg M.D., Talal Al-Atassi M.D., Buu-Khanh Lam M.D., Paul Hendry M.D., Fraser Rubens M.D. M.Sc., Thierry Mesana M.D. Ph.D.

Purpose: Atrial fibrillation (AF) is often associated with mitral valve (MV) disease and is usually treated by surgical ablation at the time of MV surgery, with variable results. AF that is Non-MV (NoMV) related, like aortic valve and/or coronary artery disease, is often left untreated. The objective of this study was to determine the efficacy and outcomes of surgical ablation in NoMV AF patients.

Methods and Results: Between 2002 and 2008, 177 patients had MV repair or replacement +/- other procedures while 100 patients underwent coronary bypass surgery and/or aortic valve replacement with concomitant AF ablation by radiofrequency ablation. The mean age was 68 +/- 11 years and 60% were male. Overall hospital mortality was 3% (P=0.07); 1 and 3-year survival was, respectively 97% and 93% (P=0.65). Predictors of AF recurrence for the entire group included older age (OR 1.03, CI 1.01-1.63) and longstanding persistent AF (OR 2.7, CI 1.1-7.2). At follow-up, 68% (MV 64%, NoMV 75%, P=0.12) were in NSR with 69% (MV 62%, NoMV 83%, P=0.002) off anti-arrhythmic drugs and 45% (MV 32%, NoMV 70%, P<0.0001) off anticoagulation.

Conclusion: Concomitant surgical ablation of AF associated with aortic valve and coronary artery disease is an effective approach to reducing the burden of AF without increased risk to patients. Longstanding persistent AF patients were associated with increased risk of AF recurrence long-term. Postoperative prevalence of AF is similar between MV and NoMV AF patients while the latter group was more likely to be weaned from anti-arrhythmic drugs and anticoagulation.

P-66 **Finding the Ideal Biomaterial for Aortic Valve Cusp Repair**

Hadi Toeg[1], Ovais Abessi[2], Talal Al-Atassi[1], Laurent de Kerchove[3], Gebrine El-Khoury[3], Michel Labrosse[2], Munir Boodhwani[1]
[1]Division of Cardiac Surgery, University of Ottawa Heart Institute, Ottawa, Ontario, Canada
[2]Department of Mechanical Engineering, University of Ottawa, Ottawa, Ontario, Canada
[3]Department of Cardiovascular and Thoracic Surgery Cliniques Universitaires Saint-Luc, Brussels, Belgium

Objective: Cusp replacement in aortic valve repair (AVr) is associated with increased long-term repair failure. We measured hemodynamic and biomaterial properties after porcine AVr with 4 types of clinically relevant biomaterials to ascertain which material(s) would be ideal.

Methods: Porcine aortic roots with intact aortic valves were placed in a left heart simulator for baseline and post-repair valve assessment including: flow, pressure, LV work, valve opening (VO) and closing velocities (VC), and maximum geometric orifice area (GOA) evaluated over a range of cardiac outputs. The non-coronary cusp was excised and replaced with autologous porcine pericardium (APP), glutaraldehyde-fixed bovine pericardium patch (BPP; Synovis®), extracelluar matrix scaffold (CorMatrix ®), or collagen-impregnated Dacron (HEMASHIELD®). Biomaterial properties, along with St. Jude Medical® Pericardial Patch (SJM), were determined and finite element models (FEM) were constructed to evaluate leaflet stresses.

Results: Post-repair GOAs were 10+/-2% smaller in all groups compared to native valves ($p<0.05$) with no significant changes in LV work between groups. VOs and VCs were indistinguishable between original valves and bovine pericardial patch (p=NS), while other biomaterials demonstrated 30% reductions in these velocities ($p < 0.05$). FEM displayed differences in percent changes in leaflet stress for both repaired and non-repaired cusps with SJM® (+4%,+24%) and APP (+5,+26%) having lower values than CorMatrix® (+13%,+32%), BPP (+54%,+18%), and HEMASHIELD® (+63%,+9%), respectively.

Conclusions: All biomaterials provide reasonable hemodynamic result following cusp replacement. APP and SJM® have the closest profile to normal aortic valves. Increased stresses found with BPP may be associated with late repair failure.

P-67 **Treatment of In-stent Restenosis Using Everolimus Eluting Stents - A "Real World" Analysis**

Alana McEvoy, Luan Tran, Derek So

Background: The treatment of in-stent restenosis (ISR) after bare metal stent (BMS) or drug eluting stent (DES) implantation remains a challenge. The use of novel second generation "limus"-based DES for ISR may be ideal based on drug potency and thinner struts. Effectiveness of these stents for ISR in a real-world setting has been understudied.

Objective: To evaluate the effectiveness of Everolimus-eluting stents (EES) in the treatment of ISR.

*Methods***:** Retrospective cohort study of patients treated with EES for ISR. The primary endpoint was clinical target lesion revascularization (TLR) at one year. Secondary endpoints included: mortality, non-fatal myocardial infarction (MI), MACE (cardiac death, MI, TVR), target vessel revascularization (TVR), and stent thrombosis (ARC definite or probable).

Results: Among 81 consecutive patients with ISR enrolled between January 2009 and May 2012, there were 107 ISR lesions. Baseline demographics include: mean age 64.5 ± 11.8, 71.6% males, 41.7% diabetics, 74.1% hypertension, 88.9% dyslipidemia, and 16.0% smokers. Of lesions treated, 56.1% were for BMS ISR. At one year follow up; the overall rate of TLR was 2.5% (0 pts of DES and 2 of 40 pts of BMS). One-year cardiac mortality was low at 1.2%(0 pts of DES, 1 of 40 pts of BMS), and incidence of stent thrombosis was 1.2% (0 pts of DES, 1 of 40 pts of BMS). The overall MACE rate at 1-year was 4.9% (0 pts of DES, 4 of 40 pts of BMS) with a non-fatal MI rate of 2.5% (0 pts of DES, 2 of 40 pts of BMS).

Conclusion: In this "real-world" analysis, the utilization of the EES in the treatment of ISR was safe and demonstrated a low rate of adverse clinical outcomes at one year. Further prospective and larger studies are warranted to determine the effectiveness of EES in the treatment of ISR.

P-68 **Can Minimally Invasive Coronary Artery Bypass Grafting Be Initiated and Practiced Safely? Learning Curve of Small Thoracotomy CABG**

Dai Une, Harry Lapierre, Benjamin Sohmer, Vaneet Rai, Marc Ruel

Background: This study examined the effects of learning curve on clinical outcomes and total operative time in minimally invasive coronary artery bypass grafting (MICS CABG).

Methods: Logistic regression analysis was performed to investigate the relationship between 1-month clinical events (bleeding, myocardial infarction, revascularization, mortality) and the number of MICS CABG performed by a surgeon. Curve regression analysis and a cumulative summation technique were performed to assess the correlation between the duration of operation and the number of MICS CABG performed.

Results: In the first 200 consecutive MICS CABG operations performed by the same surgeon, there were 3 cardiopulmonary bypass (CPB)- assisted single vessel small thoracotomy (SVST), 87 off-pump SVST, 51 CPB-assisted multivessel small thoracotomy (MVST), and 59 off-pump MVST. The median number of grafts was 2 (range 1-5). There was no perioperative mortality, and 5 patients (2.5%) underwent reopening for perioperative bleeding. No complications occurred as a result of CPB assistance. Experience was not associated with perioperative events; however, in off-pump SVST and off-pump MVST, experience numbers correlated with operative time (mean duration 122±30 and 241±80 minutes, respectively; $R2 = 0.16$ and $R2=0.38$; $p<0.001$ and $p<0.001$), but not in pump-assisted MVST (mean duration 258±44 minutes; $R2 < 0.001$; $P=0.9$). The learning period for off-pump MVST to reach optimization was calculated to be 45 cases.

Conclusions: MICS CABG can be safely initiated, with a very low perioperative risk. Pump assistance is safe and may be a good strategy to alleviate some of the learning curve when initiating a multi-vessel MICS CABG program.

P-69 **Twenty-Year Durability of the Aortic Hancock II Bioprosthesis in Young Patients: Is it Durable Enough?**

Dai Une[1], Susan Armstrong[2], Marc Ruel[1], Tirone E. David[2]
[1]Division of Cardiac Surgery, University of Ottawa Heart Institute, Ottawa ON, Canada
[2]Division of Cardiovascular Surgery of Peter Munk Cardiac Centre, Toronto General Hospital and University of Toronto, Toronto, ON, Canada

Background: There is a current trend toward the use of bioprosthetic aortic valves in the aortic position in young patients but there is limited information on durability beyond the first decade. The Hancock II bioprosthesis has been reported to have excellent durability in patients ≥60 years of age. This study examined the long-term durability of the Hancock II bioprosthesis in the aortic position, in patients <60 years of age.

Methods: From 1982 to 2008, 304 patients aged 59 years or less underwent aortic valve replacement (AVR) with a Hancock II bioprosthesis at 2 centers. Valve function was longitudinally assessed by echocardiography. Median follow-up was 14.6 years (maximum 27.5 years). Survival and freedom from adverse events were calculated by using a Kaplan-Meier method. Independent predictors of structural valve deterioration (SVD) were assessed with a Cox-Hazard regression method.

Results: Survival and freedom from repeat AVR at 20 years were 57.0±6.1% and 25.4±4.7%, respectively. Over the follow-up duration, 100 patients (32.9%) underwent repeat AVR: 78 for SVD, 11 for endocarditis, 4 for nonstructural valve dysfunction, and 7 for other reasons. The 10- and 20-year freedom from SVD were 90.9±2.1% and 25.2±5.0%, respectively. The independent predictors of SVD were age (hazard ratio 0.97/yr; 95%CI: 0.95, 0.99; p<0.01) and prosthesis-patient mismatch (effective orifice area index≤ 0.80 cm2/m2) (hazard ratio 1.60; 95%CI: 1.02, 2.49; p=0.039].

Conclusions: This study showed that the Hancock II bioprosthesis in AVR patients less than 60 years of age is associated with excellent durability during the first decade of follow-up. However, SVD increases dramatically during the second decade and by 20 years, especially in younger patients with prosthesis-patient mismatch. These findings may assist with prosthesis selection between patients and their surgeons.