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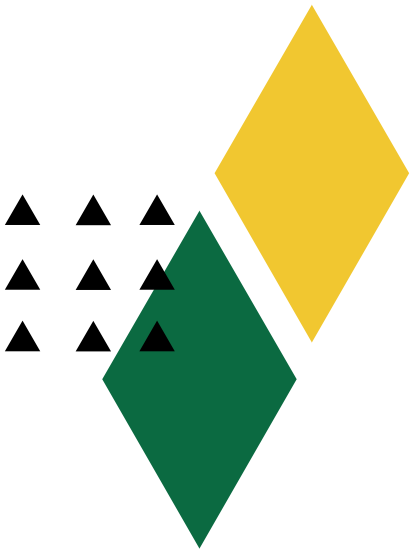

Contents

A MESSAGE FROM THE VICE-DEAN OF RESEARCH

Dear Colleagues,

Welcome to the 2020 College of Medicine Research Award (CoMRAD) symposium, that features results stemming from 2018 CoMRAD competition awardees.

The CoMRAD competition was designed to provide seed funding for researchers in the college to fund novel pilot or feasibility studies, with the intention that preliminary funding and data would facilitate future applications to external funding sources.

I am delighted to acknowledge an exceptional dedication and quality of work conducted by these investigators, It is with great joy that we celebrate our 2018 winners, and allow them to share the fruits of their labor.

With kind regards,

DR. MAREK RADOMSKI
Vice-Dean of Research

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Biomedical

New Therapies for Triple Negative Breast Cancer (TNBC)

PLETT, R., MELLOR, P., AND ANDERSON, D.

INTRODUCTION

DR. DEBORAH ANDERSON

Breast cancer is the 2nd leading cause of death from cancer, affecting approximately 1 in 8 Canadian women [1]. There are 4 major subtypes of breast cancer: luminal A (Estrogen (ER)+, Progesterone (PR)±, human epidermal growth factor receptor (HER2)-; 40-60%), luminal B (ER+, PR±, HER2+; 10-20%), HER2 amplified (HER2+,10-15%) and triple negative breast cancer (TNBC; 15-20%) [2]. TNBCs lack the 3 receptors (ER, PR and HER2) that define the other subtypes and which allow them to benefit from targeted treatments. As a result, TNBCs rely on several combinations of cytotoxic chemotherapy agents for treatment [4]. The lack of targeted therapies for TNBC contributes to their high mortality rates and high risk of relapse compared to other subytpes of breast cancer. Developing targeted therapies for TNBC is an unmet clinical need. Our lab has described the metastasis suppressor cAMP-responsive element-binding protein 3-like protein 1 (CREB3L1) as a novel molecular feature of TNBCs. CREB3L1 suppresses the metastatic properties of breast cancer cells, including in vitro cell migration and invasion. Additionally, re-expression of CREB3L1 in metastatic breast cancer cells has been shown to block tumor progression, angiogenesis and metastasis in rat models of breast cancer [3, 5]. In low-grade human breast tumors CREB3L1 expression is initially upregulated [5]. In contrast, CREB3L1 expression is low in advanced breast tumors [5]. Approximately 30% of breast cancers have decreased CREB3L1-expression (including ~90% of TNBCs) and these patients usually have a poor prognosis [5]. These results show that loss of CREB3L1 contributes to the metastatic phenotype and that targeting CREB3L1-deficient breast cancer cells should reduce metastases. In this project, inhibitors that selectively block the growth and/or survival of metastatic CREB3L1-deficient breast cancer cells have been identified and validated as new treatments for CREB3L1-deficient breast cancers.

MATERIALS AND METHODS

FDA Approved Compounds - High Throughput Drug Screen:

A high-throughput screen was carried out using an FDA-approved drug library (1,818 compounds; 1 μM) using the CREB3L1-deficient TNBC cell line HCC1806 +/- re-expressed CREB3L1. Cells were engineered to express red-fluorescent protein (RFP), allowing live cells still adhered to the plate to be imaged and counted by an automated imaging fluorescence microscope system (ImageXpress Micro XLS Widefield). Cells were seeded at 1300 and 2600 cells/well for HCC1806 and HCC1806 +HACREB3L1 Cl3, respectively, in a final volume of 50 μL in 384-well plates and allowed to adhere overnight. After 24 hours, each of the 1,818 drugs/inhibitors was added (final 1 μM) using a Biomek FX liquid handling system (A31843). Cells were imaged once per day over a five-day period and quantified (ImageXpress Micro XLS Widefield software).

Validation Assays and EC50 Determinations:

From the initial screen, 47 promising drugs were identified and purchased (CEDARLANE). A 1 μM validation assay was performed in triplicate to validate the compounds and data from the initial 1 μM drug screen for these 47 compounds. Using an Integra ASSIST PLUS liquid handling system, HCC1806 and HCC1806 + HACREB3L1 Cl3 cells were seeded at 500 and 600 cells/well, respectively, in a final volume of 50 mL in 384-well black-walled plates (4505 INTEGRA Biosciences AG). After 24 hours, each of the 47 compounds were added, in triplicate, to the plates (final 1 μM). After 4-days, Hoechst and ImageIT Dead Green dye were added to the plate to stain cell nuclei of live and the cytoplasm of dead cells, respectively. The plates were imaged, and viable cells were quantified (ThermoFisher Scientific CX7 imager software). This identified 27 drugs that were validated as killing both cell lines. Drug titration experiments were carried out on these to determine the EC50 values of each and to identify drugs that selectively killed the CREB3L1-deficient cells, at the lowest possible drug concentration. Cells were plated as before and drugs were serially diluted in a deep 96-well plate, using the ASSIST PLUS. Each drug, in a range of concentrations, was then added to the 384-well plate, in triplicate. After 4-days the plates were imaged and quantified as above.

RESULTS AND DISCUSSION

FDA Approved Compounds - High Throughput Drug Screen:

A total of 47 drugs were identified from the initial screen that warranted further analyses. Of these drugs, 21 showed 40% or greater more cytotoxicity to the TNBC cell line, HCC1806 than to the same cell line re-expressing CREB3L1, HCC1806 +HACREB3L1 Cl3 at 1 mM. These were not pursued further. The remaining 26 drugs were similarly cytotoxic to both cell lines.

EC50 Determinations:

From the initial validation assays, 26 drugs were cytotoxic towards one or both cell lines at 1 mM. When assessed over a range of concentrations, the majority of these drugs did not selectively kill either the HCC1806 or HCC1806 +HACREB3L1 Cl3 cells below 1 mM (example, Fig 1A). However, there were 4 drugs that selectively killed the CREB3L1-deficient HCC1806 cells at concentrations lower than 1 mM (example, Fig. 1B). The EC50 determinations for these drugs revealed that a lower concentration of drug was sufficient to achieve half of the maximal response (lowest % Viability) in the CREB3L1-deficient TNBC cells as compared to the CREB3L1-expressing TNBC cells.

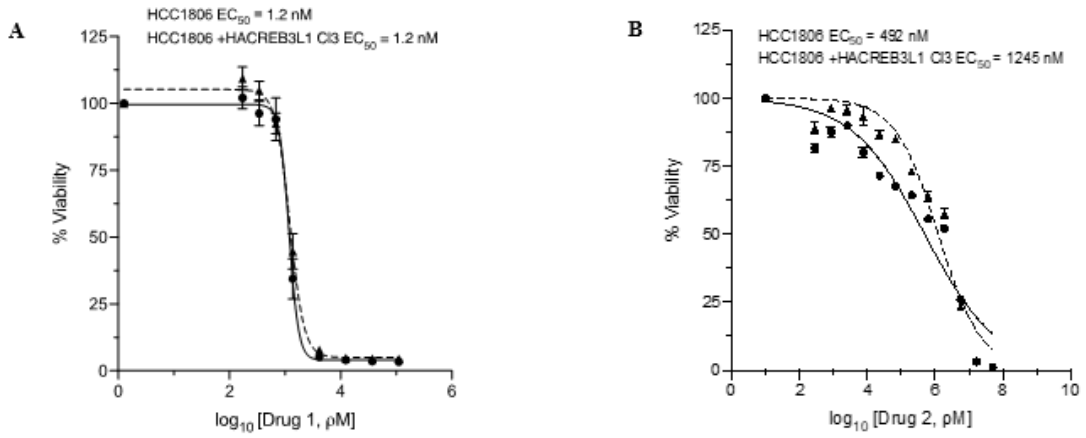

Figure 1. Examples of EC50 determination for drugs +/- CREB3L1 expression. % Viability values are reported with standard error of the mean (SE; n=3). (A) Drug 1 that killed both HCC1806 (circle) and HCC1806 +HACREB3L1 Cl3 (triangle) cells with similar efficacy. (B) Drug 2 preferentially killed CREB3L1-deficient HCC1806 cells.

CONCLUSION

Four promising drugs were identified from these in vitro studies that show increased cytoxicity towards CREB3L1-deficient metastatic TNBC cells. These drugs will be assessed in additional TNBC cell lines. They will also be tested in combination with doxorubicin and paclitaxel, two chemotherapy agents commonly used to treat TNBCs, to determine if they can increase the efficacy of these treatments. The results of this work should lead to new more effective treatment options for TNBC and better outcomes for these patients.

ACKNOWLEDGMENT

The authors would like to express appreciation for the support of the sponsors CoMRAD (421028) and scholarship support to RP from: Frederick Banting and Charles Best Canada Graduate (CIHR), College of Graduate and Postdoctoral Studies and the Department of Anatomy, Physiology and Pharmacology.

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Dysfunction in Protein Degradation in Autonomic Neurons and NGF-Transformed PC12

MUSTAFA, W., MOMENI, Z., BAUTISTA, M., FALZONE, T., AND CAMPANUCCI, V.

INTRODUCTION

DR. VERONICA CAMPANUCCI

Approximately 50% of diabetic patients develop peripheral neuropathy, which impacts quality of life and life expectancy (Vinik & Erbas, 2013). Peripheral neuropathy results from damage to peripheral nerves, including those involved in the control of autonomic functions. Diabetic autonomic neuropathy (DAN) is the most under-recognized complication of diabetes; it can affect multiple organ systems leading to widespread clinical manifestations (Vinik & Erbas, 2013; Vinik et al.; 2003; Donaghue et al., 2005: Kuehl & Stevens, 2012; Melendez-Ramirez et al. , 2010; Pop-Busui R, 2010; Marcovecchio et al. , 2010), and is a major contributor to cardiovascular dysfunction in diabetes (Trotta et al., 2004; Clarke et al. , 1999). The oxidative stress hypothesis is one of the most explored in the development of diabetic neuropathy. Small free radicals generated during hyperglycemia lead to the oxidative damage of proteins, and compromise neuronal function. To retain a fully functional proteome under oxidative stress conditions, neurons invest significant energetic effort in a dynamic equilibrium between de novo protein synthesis and degradation of damaged proteins (Rousseau & Bertolotti, 2018). Protein degradation is achieved by the combined actions of the autophagy-lysosome system, which degrades larger and integral proteins, and the ubiquitin-proteasome system (UPS), which degrades smaller and soluble peptides. Dysfunction in the proteolytic machinery has been previously linked to the accumulation of misfolded and aberrant proteins characteristic of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases. In diabetes, lysosome proteolytic dysfunction has been reported in renal (Medina-Navarro et al 2019) and in pancreatic β cell failure (Pasquier et al 2019). However, the potential role of proteolytic dysfunction in diabetic neuropathy has not been considered thus far. Work from the Campanucci' s laboratory revealed that hyperglycemia-mediated oxidative stress contributes to the development of DAN. The nicotinic acetylcholine receptors (nAChRs), which are responsible for driving autonomic transmission at sympathetic ganglia, are irreversibly targeted by oxidation leading to symptoms of DAN (Campanucci et al., 2010; Chandna et al., 2015, Lam et al., 2018). The irreversible changes in nAChRs, which persist even after normal glycemic conditions have been restored, suggest that turnover and/or degradation of aberrant receptors may be impaired in diabetes. nAChRs are mostly degraded by the lysosome-system, however, their level and stability at the plasma membrane is positively regulated by the UPS (Rezvani et al., 2009; Teng et al., 2015). Therefore, in the current project we aim at understanding the potential role proteolytic pathways in autonomic function during diabetes.

MATERIALS AND METHODS

2.1. PC12 Cell Line Transformation Treatment, and Collection: PC12 cell line (ATCC® CRL-1721.1™) were cultured in F-12K growth media (15% horse serum ,2.5% fetal bovine serum). PC12 were transformed by 50ng/mL NGF (Alamone Labs) for 10 days. NGF-transformed PC12 cells (PC12NGF) were maintained in either control (5mM) or a high glucose (25mM) for 10 days.

2.2 Diabetes induction, sample collection, and Western blotting: The diabetic state (blood glucose > 15 mM) was induced by streptozotocin (STZ) (50mg/kg; i.p.) as previously described (Campanuci et al., 2010), and controls were injected with citrate buffer. After 4 weeks from the induction of diabetes, whole SCGs were harvested from control and diabetic mice. Samples were flash frozen and maintained at -80 °C. We used SCG whole extracts from control and STZ-induced diabetic mice were homogenized in ice-cold CelLytic™ MT Cell Lysis Reagent (Sigma-Aldrich) containing a protease and phosphatase inhibitor cocktail. At least 3 replicas were used per condition (CNT or STZ) for both WT and RAGE-KO mice. Equal amounts of protein were loaded per group, separated on 12% SDS polyacrylamide gels and then electrotransferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were incubated overnight at 4°C with the following primary antibodies against: (mouse from Sigma) α-Tubulin, β-actin, (rabbit from Abcam) LC3b, LAMP1, ubiquitin, mTOR, MAP2, NF-L, and nicotinic α3 (rabbit from Alomone Labs). Afterwards, the membranes were washed with the blocker solution followed by the treatment of the appropriate, either anti mouse or anti rabbit, HRP conjugated secondary antibodies (1:5000, Abcam) in room temperature for 2 hours. The membranes were washed for 2x5min with blocker solution, 2x5min PBST, and 2x5min PBS and 1x 1min ECL. Lastly, Densitometry analysis was carried out on these membranes using ImageJ software.

RESULTS AND DISCUSSION

Short-term STZ-induced diabetes caused a significant increase in protein oxidation by Oxyblot analysis. Oxidation-damaged proteins can undergo fragmentation or form large aggregates (Davies, 1987; 2001; Bence et al., 2001; Tyedmers et al., 2010; Aiken et al., 2011). Consistently, our findings monitoring the level of proteins marked for degradation revealed a significant increase in protein ubiquitination in PC12NGF cells exposed to either control or high glucose. Normally, ubiquitinated proteins will follow the lysosome or UPS degradation pathway, however, their accumulation indicates these proteins are not entering degradation and that downstream proteolysis may be dysfunctional. Considering that most soluble and regulatory proteins are degraded by the UPS, we concentrated on monitoring the activity of the 20S proteasome in PCF12NGF cells under control or high glucose conditions. The formation of the 20S proteasome complex is induced by oxidative stress, which drives the expression of subunits more efficient in protein degradation during oxidative conditions (Aiken et al., 2011). We detected no significant changes in the 20S activity level, the 26S proteasome subunit expression levels, and the accumulation of nicotinic subunits in the presence of the proteasome inhibitor (MG-132) during high glucose. Next, to study lysosomal function we monitored accumulation of nicotinic subunits in the presence of the lysosome inhibitor hydroxychloroquine (HCQ) in PC12NGF cells maintained in control or high glucose conditions. We observed a significant accumulation of α3 nicotinic subunit during high glucose, suggesting lysosomal function is required for its degradation. In addition to degradation of the α3 nicotinic subunit, we found that in the SCG of STZ-diabetic mice there was a significant reduction in the expression levels of the α3 subunit. Taken together, our findings indicate that during hyperglycemia the synthesis-degradation equilibrium of the α3 nicotinic subunit is altered. Furthermore, the active (phosphorylated) mTOR inhibitor (rapamycin, which induces autophagy) (Jung et al., 2010), was significantly increased in the STZ of diabetic mice.

CONCLUSION

Taken together our data suggest that proteasome expression and function is not affected by hyperglycemia. However, lysosomal function not only is required to degrade a3 nicotinic subunits but also the lysosomal function seems to be impaired in short term diabetes. Our findings support the hypothesis that diabetes alters proteolytic pathways, which may contribute to reduced autonomic function in DAN.

ACKNOWLEDGMENT

The authors would like to express appreciation for the support of the sponsors.

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Molecular Function of the Breast Cancer Metastasis Modulator MEMO1

UHLEMANN, E., BONIECKI, M., DOLGOVA, N., VIZEACOUMAR, F.S., TONELLI, M., VIZEACOUMAR, F.J., AND DMITRIEV, O.

INTRODUCTION

DR. OLEG DMITRIEV

MEMO1 is a highly conserved protein found in the cytosol of eukaryotic cells from yeast to humans. MEMO1 has been linked to several biological processes, but its primary function is unknown. MEMO1 appears to play a crucial role in cell motility. In particular, it supports the ability of breast cancer cells to invade surrounding tissues, leading to metastasis (1,2). The ultimate goal of our research is to determine the primary function of MEMO1, to understand the common denominator of its involvement in various intracellular processes, in particular, in cancer metastasis, and to identify new targets for the therapy of metastatic breast cancer. Several lines of evidence establish the crucial role of MEMO1 in cancer metastasis. MEMO1 knockdown reduces breast cancer cell migration, and decreases the number of lung metastases in mice receiving breast cancer xenografts. Retrospective analysis of the resected tumors showed strong correlation between increased expression of MEMO1, and poor outcomes and reduced patient survival time (1). These effects have been linked to the interaction between ERBB2 receptor and MEMO1, which, in turn, was proposed to relay the activation of ERBB2 heterodimers by the extracellular growth factors to the microtubule cytoskeleton, inducing growth of lamellipodia, cell protrusions that enable cancer cell migration (2). The interaction with ERBB2 gave MEMO1 its name (Mediator of ERBB2-driven Cell Motility 1). MEMO1 was shown to bind copper and catalyze redox reactions, such as superoxide production (1), suggesting that MEMO1 is required for sustained reactive oxygen species (ROS) production, likely in conjunction with NADPH-oxidase 1 (NOX1) activation. However, some data suggest that modulation of ROS generation by NOX1 is not the primary activity of MEMO1, and the physiological metal cofactor of MEMO1 has remained uncertain. To determine the molecular function of MEMO1 and use it as a target for metastasis suppression in breast cancer, we set out to: (i) identify the type and oxidation state of the metal cofactor in MEMO1, (ii) generate MEMO1 knockouts in a breast cancer cell line, and (iii) conduct synthetic lethality screening in silico to identify genetic interactions involving MEMO1.

MATERIALS AND METHODS

To analyze MEMO1 activity in vitro, we have expressed the protein in E. coli as a fusion with chitin-binding domain and intein, and purified the protein by chitin affinity chromatography and size-exclusion chromatography. MEMO1 variants with mutations in the metal-binding site were generated by site-directed mutagenesis. NMR experiments were conducted on a 900 MHz spectrometer with a CryoProbe.

The X-ray diffraction and X-ray fluorescence data on the crystals of the metal-bound MEMO1 were collected at the Canadian Light Source and the structure was solved by molecular replacement. Synthetic lethality and dosage lethality analysis was performed by screening the Marcotte, Achilles Project, CERES, ATARiS and RSA databases for significant ($P<0.05$) differences in the gene essentiality scores between the high- and low- MEMO1 expressing cell lines. MEMO1 knockouts were created in MDA-MB-231 breast cancer cell line using TrueGuide crRNAs, TrueCutTM Cas9 protein, and Lipofectamine CRISPRMAX Reagent (Invitrogen).

RESULTS AND DISCUSSION

We have solved the X-ray structure of metal-bound MEMO1 and identified the amino acid residues involved in metal binding. To understand the function of the metal cofactor in MEMO1 biological activity, we have generated mutant variants of MEMO1, replacing each of the metal-coordinating residues at a time, and demonstrated by NMR that all the mutants are properly folded. To enable substrate binding studies and drug design by structure-activity relationship (SAR) method, we optimized the sample conditions and obtained high quality NMR spectra for the backbone chemical shift assignment by NMR, which is currently in progress. We have generated CRISPR/Cas9 knockouts of MEMO1 in an aggressive breast cancer cell line (Fig. 1) and validated a wound closure assay for MEMO1 biological function. By genome wide synthetic lethality and synthetic dosage lethality (SDL) screening in silico, we have identified genetic interactions partners of MEMO1, which, in turn provided leads to its molecular function. Based on SDL data, we chose the top 30 candidate targets for metastasis suppression in breast cancer, including 12 that are targets of the existing approved or experimental drugs.

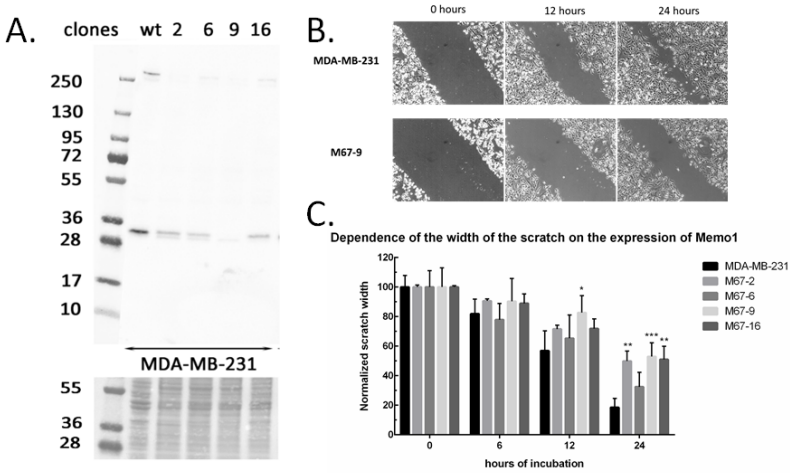

Fig. 1 Cell motility as a function of MEMO1 expression level. (A) MEMO1 Western blot in the parental cell line (wt) and several independent MEMO1 knockout clones. Loading control is below. (B) Wound closure in the parental cell line (*top*) and clone 9 (no detectable MEMO1, *bottom*) (C) Wound closure as a function of time in the parental cell line, and each MEMO1 knockout clone. Significant difference between each of the clones (M67) and the parental cell line is indicated (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$)

CONCLUSION

We have identified the physiological metal cofactor and determined metal binding mode in the cancer metastasis modulator MEMO1. We have completed the groundwork and generated a set of tools and genomic data for analyzing molecular function of MEMO1, and for discovering and validating novel targets for metastasis suppression in breast cancer.

ACKNOWLEDGMENT

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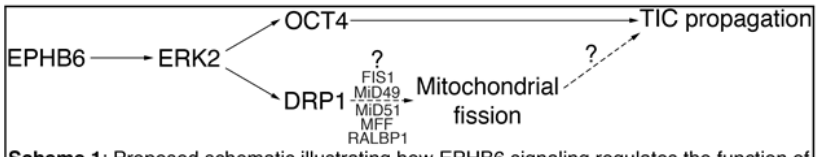
Targeting DRP1 Receptors in Triple-Negative Breast Cancer Tumor-Initiating Cells

KATRII, T., LEARY, S., AND FREYWALD, A.

DR. ANDREW FREYWALD

INTRODUCTION

Breast cancer (BC) is the most frequent malignancy in women, with an average of 500 new cases diagnosed in Canada per day. Despite advances in BC therapy, it remains the second leading cause of cancer-related deaths in the female population and thus represents a significant challenge with respect to finding new, targeted approaches for BC treatment. Expression profiling of key molecules including the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) allowed clinical classification of BC into three major subtypes. They are represented by HER2-amplified (HER2+), ER-positive (ER+) and triple negative BC (TNBC; ER-, PR-, HER2-) tumors(Mohamed et al., 2013). TNBC patient outcomes are the worst among BC subtypes due to the lack of clinically relevant targeted therapies and the aggressive nature of this malignancy, which exhibits elevated metastatic activity and a higher incidence of tumor recurrence following initial treatment. Therefore, there is an urgent need to discover effective therapeutic targets for TNBC(Chang-Qing et al., 2020; Nakhjavani et al., 2019). The so-called cancer stem cells or tumor initiating cells (TICs) are thought to be at the heart of tumor aggressiveness and recurrence (Dick, 2008; Harris and Kerr, 2017; Wei and Lewis, 2015).Therefore, identifying and characterizing the molecular mechanisms that control TIC populations in TNBC tumors is a crucial step towards the development of targeted therapeutics for this devastating disease(Bao and Prasad, 2019). While an understanding of these molecular mechanisms is far from complete, our previous work shows that the same signaling pathway that supports the propagation of TIC populations in TNBC tumors also triggers mitochondrial fission in TNBC cells(El Zawily et al., 2016; Toosi et al., 2018) (Scheme 1). Although a detailed understanding of how mitochondrial dynamics is controlled in TNBC TICs is currently lacking, our findings collectively suggest that mitochondrial fragmentation is an adaptive response



Scheme 1: Proposed schematic illustrating how EPHB6 signaling regulates the function of OCT4 and DRP1 and leads to the activation of two pathways integral to TIC proliferation.

required for the efficient propagation of TNBC TICs, and that mechanisms controlling mitochondrial morphology are likely to represent good therapeutic targets for the elimination of TICs in TNBC tumors. Consistent with this idea, previous findings emphasize that pharmacological targeting of some components of the machinery that governs mitochondrial fragmentation selectively kills malignant cells(Seo et al., 2019), although the effect of this intervention on TNBC TICs remains unknown. We therefore, attempted to determine if proteins that induce mitochondrial fission in TNBC cells are crucial for TNBC TICs propagation and maintenance.

MATERIALS AND METHODS

Cell culture:
MDA-MB-231 TNBC cells were obtained from ATCC and passaged for less than three months following resuscitations. Therefore, no additional authentication was performed. Mycoplasma testing was performed. The cells were grown in DMEM cell culture medium supplemented with 1% penicillin/streptomycin, 1 mM sodium pyruvate and 10% FBS (all from Gibco, Life Technologies, Burlington, ON, Canada).

Experimental methods:
Gene silencing, Western blotting, tumorsphere cell propagation (by direct cell counting) and ALDEFLUOR assays were performed as described in our previous publications(Baker et al., 2017; El Zawily et al., 2016; Toosi et al., 2018).

RESULTS

Mitochondrial organization is determined by the balance between signaling events that control organelle fission and fusion. Of these two opposing processes, fission is better understood, with dynamin related protein 1 (DRP1) being a central player. DRP1 localizes to the cytosol and is recruited to the outer mitochondrial membrane to promote fission(Pernas and Scorrano, 2016). DRP1 recruitment to mitochondria also requires the involvement of at least one of four outer membrane proteins, MFF, MiD49, MiD51 or FIS1, that are referred to as DRP1 receptors (DRP1Rs). Each DRP1R may act alone in DRP1-induced fission(Koirala et al., 2013) and recruited DRP1 molecules act as mechano-enzymes, oligomerizing into a helical structure and constricting the mitochondrial tubule to achieve membrane scission(Sesaki et al., 2014). To assess the effect of suppressing individual DRP1Rs on TNBC TICs, we individually silenced expression of two DRP1Rs expressed in MDA-MB-231 cells. Knockdowns were done with two independent shRNA molecules for each DRP1R and confirmed by Western blotting. Excitingly, our analyses indicate that individual silencing of these DRP1 receptors strongly inhibits propagation of MDA-MB-231 cells in tumorsphere cultures, which are mostly composed of TICs(Morrison et al., 2012; Pece et al., 2010; Stankic et al., 2013; Stratford et al., 2010). Moreover, these knockdowns also preferentially suppressed TICs in MDA-MB-231 cell monolayers by reducing the frequency of ALDH+ cells, which represent TIC subpopulation(Ginestier et al., 2007).

CONCLUSION

Overall, our observations strongly suggest that at least some DRP1Rs are crucial for the propagation and maintananance of TNBC TICs. Based on the role of TICs in multiple aspects of tumor behaviour that determine cancer aggressiveness, elimination of these cells is commonly accepted as an important new approach in cancer therapy. Therfore, we anticipate that our work will identify new targets for TNBC therapy and trigger the development of conceptually novel treatment approaches that will improve tumor eradication, while also preventing tumor metastasis and relapse. Future investigations in animal models of human TNBC and analyses of the remaining untapped DRP1Rs will be performed for selecting optimal therapeutic targets.

ACKNOWLEDGMENT

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IFNα Subtype-Specific Gene Expression

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INTRODUCTION

DR. KERRY LAVENDER

Induction of the type I interferon (IFN) response is the primary innate immune defense against viral infection. Type I IFNs comprise a family of cytokines that includes 12 human IFNα subtypes. IFNα subtypes share significant protein sequence similarity. They also have significant functional homology and signal through the same interferon alpha receptor (IFNAR) to induce expression of a host of interferon-regulated genes (IRGs) that encode anti-viral proteins and moderators of the immune response. Much of what we know about the IFNα response to HIV-1 and other viruses assumes that the different IFNα subtypes are functionally redundant. Recent advances have indicated that IFNα subtypes are biologically distinct and elicit unique signaling cascades. This appears to result in differential downstream responses and ultimately differences in their biological effects and control of distinct viral infections1-3. Despite growing recognition of the variable biological activities of different IFNα subtypes, very little is known regarding the differential gene expression that mediates their observed disparate activities. Controversy remains regarding whether IFNα is beneficial or detrimental in HIV-1 infection4. Much of the confusion regarding the role of IFNα in driving HIV-1-mediated pathology may be due to the plethora of studies that do not distinguish between IFNα subtypes. The vast majority of studies have used the IFNα2 subtype, which is produced in excess during HIV-1 infection5 and may be driving IFNα-associated pathology in HIV-1 infection. My work and the work of others has shown that different IFNα subtypes do not mediate equivalent effects in suppressing HIV-1 replication and identified IFNα2 as a relatively ineffective anti-HIV-1 subtype in vitro2,6 . Further work in vivo using humanized mice, including our triple knockout-bone marrow-liver-thymus humanized (TKO-BLT) mice highlighted IFNα14 as a significantly more potent anti-HIV-1 subtype in vivo than IFNα2. Additionally, IFNα14 did not induce the pathogenic indicators associated with IFNα-mediated HIV- 1 pathology2,7 . Thus, we set out to determine what IRGs are differentially expressed upon treatment of TKO-BLT mice with either IFNα14 or IFNα2 that might explain the differential effects mediated by each of these subtypes during HIV-1 infection. We assessed the IRG profiles of total leukocytes as well as the CD4+ subset to determine what genes may be differentially mediating protection both overall within the immune system and directly within the cell type targeted by HIV-1 infection.

MATERIALS AND METHODS

TKO-BLT mice were produced as previously described8,9 from six different human donor tissues. At 12 weeks post-humanization, mice were assessed for successful reconstitution with human immune cells (^{3}2x105 hCD45+ cells/ml blood). Five days later, three mice from each donor received a single intravenous injection of 1.5x105 Units of either IFNα2 or IFNα14 or a saline control injection. Six hours later, magnetic beads conjugated to anti-Ter119 and anti-mouse CD45 antibodies (Miltenyi, San Diego, CA) were used to isolate total human leukocytes from spleens of TKO-BLT mice produced from three of the human donors (2 male, 1 female). Additional purification using a human CD4+ T cell isolation kit (Miltenyi) was performed on the splenocytes of TKO-BLT mice produced from the remaining three human donors (2 male, 1 female). RNA was isolated from each of the cell samples using Zymo quick RNA kits (Zymo Research, Irivine, CA) and then underwent RNA-seq analysis using a NextSeq 500/550 v2.5 kit (Illumina, San Diego, CA).

RESULTS AND DISCUSSION

mRNA transcriptome analysis (RNA-seq) was performed on total human leukocytes and enriched human CD4+ T cells isolated from TKO-BLT mice treated with saline, IFNα2 or IFNα14. Gene counts were normalized using DESeq2. On average, 21±5 million quality-controlled reads were obtained per sample. Differences in gene expression were defined using a 2-fold cut-off between treatment conditions and a minimum base mean count of 100. Principle component analysis (PCA) revealed that the greatest variance in gene expression was observed between leukocytes and CD4+ cells, suggesting both background gene expression and genes induced by IFNα differ dependent on the cell population(s) being examined. The second greatest variance in gene expression occurred between cells from animals treated with saline, IFNα2 or IFNα14 (Figure 1) and suggested that the distinct effects of IFNα subtypes may be

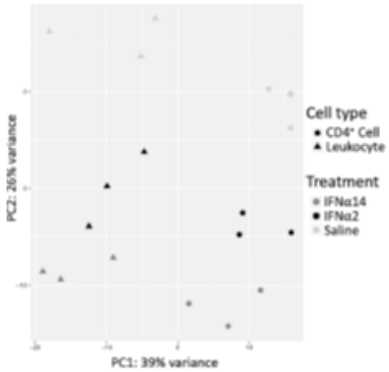

Figure 1. PCA analysis demonstrated segregation of gene expression dependent on both the cell types(s) analysed and the IFNα treatment administered.

understood through analysis of their transcriptional profiles. More genes exhibited altered expression in total leukocytes compared to the CD4+ subset. In both cell sample types, IFNα14 altered the expression of more genes from baseline compared to IFNα2. Most significantly, 182 genes were differentially regulated by IFNα14 compared to IFNα2 in total leukocytes and 134 genes in the CD4+ cell subset (Table 1). Thus, IFNα2 and IFNα14 induced distinct gene expression profiles in individual cell types and globally within the leukocyte population that may result in the engagement of distinct biological pathways leading to differential HIV-1 control.

Table 1. Number of genes differentially upregulated between treatment groups.

Cell type	Treatment comparison	Number of genes padj<0.05
Leukocytes	Saline vs. IFNα2	450
	Saline vs. IFNα14	2113
	IFNα2 vs. IFNα14	182
CD4^{+} Cells	Saline vs. IFNα2	237
	Saline vs. IFNα14	1598
	IFNα2 vs. IFNα14	134

CONCLUSION

These results reveal that gene expression varies depending on the cell type(s) analysed and the IFNα subtype administered in vivo. Both IFNα subtypes modified gene expression compared to saline. However, IFNα14 and IFNα2 induced different overall transcription profiles thereby identifying gene targets for follow-up. Ingenuity pathway analysis of differentially expressed genes will be employed to identify biological pathways responsible for differential control of HIV-1 by IFNα14 and IFNα2.

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New Biochemical Markers of Acute Kidney Injury Following Cardiac Surgery

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INTRODUCTION

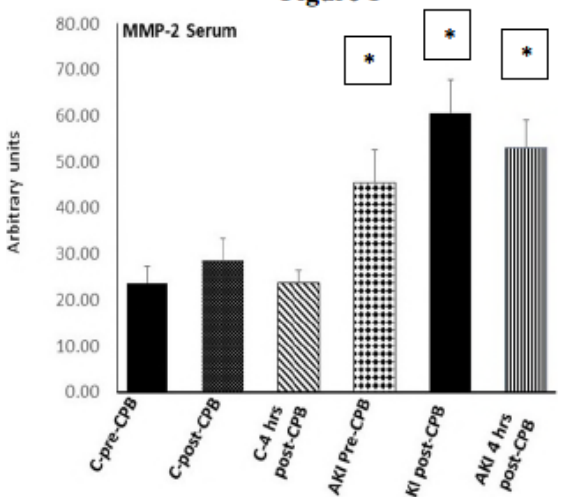
DR. ERICK MCNAIR

The majority of cardiac surgical procedures depend upon an arrested heart with circulatory support by cardiopulmonary bypass (CPB)1. Providing blood flow to other organs while the heart is stopped is not without risk, and has been shown to cause injury, particularly to the kidneys2 . Up to 43% of patients undergoing heart surgery develop cardiac surgery associated-acute kidney injury (CSA-AKI)3. CSA-AKI can be defined as a sudden and continuous decrease in kidney function possibly leading to fluid overload and dialysis while costing healthcare millions of dollars each year4 . CSA-AKI, is diagnosed by an slow abnormal rise in blood creatinine levels. However, creatinine levels do not become elevated from several hours to days after surgery, thereby prolonging the time to diagnosis and treatment of CSA-AKI5 . Having an earlier rising biomarker for CSA-AKI immediately following cardiac surgery may help clinicians move past the use of blood creatinine levels for prediction, diagnosis or monitoring CSA-AKI. Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein covalently bound to MMP-9 by neutrophils6 . Normally expressed at low concentrations, NGAL is significantly increased in injured epithelial cells including the kidney, thus making it a promising biomarker for human CSA-AKI7. In adults who underwent elective cardiac surgery and subsequently developed AKI, NGAL measurements in urine and plasma significantly increased within 1-3 hours after surgery8 . Moreover, adults undergoing CPB and later requiring dialysis were found to have had the highest NGAL levels directly following surgery9 . However, limitations of this biomarker remain because of its low to moderate sensitivity10 and specificity11 for diagnosing CSA-AKI. In animal12 and human13 studies my colleagues have shown that there is a massive release of matrix metalloproteins (MMPs) from the heart during aortic cross clamping (ischemic period). Recently, in a small-scale human study, (Figure 1) we have also shown that MMP levels in blood are higher in patients that develop CSA-AKI as compared to those patients that do not develop CSA-AKI. The data suggests that MMPs may be closely related to the development and diagnosis of AKI. The present feasability study was conducted to discover whether matrix metalloproteins (MMP-2 and -9) released from the heart and, or, the kidney are linked with CSA-AKI.

Hypothesis:
We hypothesized that elevated levels of serum MMPs released from the heart contribute to the development of AKI in patients undergoing CPB-supported cardiac surgery.

Specific Aims:
The specific aims to fulfill the long-term objectives are the following: 1) Collect blood and urine at designated time intervals; 2) Group patients as AKI and non-AKI; 3) Analyze blood and urine for levels of MMP-2, -9, and NGAL; 4) Gather clinical outcome data; and 5) Determine the clinical sensitivity and specificity of biomarkers. The relationship of the specific aims to each other is through the correlation of the measured biomarkers with accepted criteria for AKI.

Figure 1



Long-Term Objectives:
Support for our hypothesis will drive research towards the long-term goal of clinical trials to study MMP-inhibitors such as doxycycline. Doxycycline has the potential to protect the patient's kidneys and heart from MMP-induced injury. Specifically, the findings of this research may benefit patients by leading to further research towards pre-treatment of this population with an MMP inhibitor before CPB-supported cardiac surgery, which in turn may decrease the risk of the development of kidney injury and the need for costly long-term care.

MATERIALS AND METHODS

Patient Sample:
The University of Saskatchewan Biomedical Research Ethics Board approved the study protocol and the Provincial Health Authority granted operational approval. Patients undergoing CPB- supported cardiac surgery signed a written consent agreeing to participate in this research. Approached as a pilot study, we enrolled 40 non-CSA-AKI and 20 patient with CSA-AKI, between December 2018 and December 2019. The inclusion criteria consisted of: both sexes; 18-85 years of age; elective or urgent cardiac surgery and; a hemoglobin >100 g/L. The exclusion criteria were patients: with pre-existing chronic kidney disease (eGFR<30 mL/min); the need for dialysis, on nephrotoxic mediations; or serum hemoglobin < 100 g/L.

Cardiopulmonary Bypass:
Heparin dosing was determined by a hemostasis management system (Medtronic, Minneapolis, MN). CPB was initiated after attaining an activated clotting time (ACT) of ≥ 480 seconds. A Sorin heart-lung machine (Sorin, Munich, Germany) was used with flows of 2.4 - 2.6 L/min/m2 in the non-pulsatile mode. ACT's during CPB exceeded 480 seconds, and heparin concentrations ≥ 300 u/kg. Following decannulation, heparin was reversed by protamine and the return of the ACT to baseline was confirmed.

Data Collection and Patient Classification:
Because all cardiac surgical patients are at risk of developing AKI14 , we aimed to recruit all adult patients undergoing CPB-supported cardiac surgery. Following surgery, patients were separated into two groups (AKI vs. Non-AKI) based upon the KDIGO/AKIN criteria for acute kidney injury: an increase of SCr by ≥ 26.5 µmol/l within 48 hours; or an increase of SCr to ≥ 1.5 times baseline, which occurred within 7 days of surgery or; urine volume <0.5 ml/kg/h for 6 hour15 . Urine and serum samples were collected at designated time points (pre-CBP, post-CBP, and 4-hours post-CBP) for comparison of MMP-2, -9, and NGAL activity in AKI vs. non-AKI groups. Blood samples were drawn from the indwelling venous line, while urine samples were drawn from the indwelling Foley catheter. Serum creatinine was measured by the central hospital laboratory.

Biochemical Measurements of MMPs and NGAL:
MMPs in serum and urine were quantitated via Gelatin Zymography16 . The measurement of serum and urine for NGAL was performed using commercially available quantitative sandwich enzyme- linked immunosorbent assay (ELISA) kits. Measurements were performed in duplicate and the results were averaged.

RESULTS AND DISCUSSION

The results discussion and conclusion will be published in our upcoming manuscript.

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Impaired Relaxation of the Mesenteric Arterial Bed in an Animal Model of Primary Aldosteronism May Be Rescued by Constituents of the Endothelial Glycolax

SHEVCHENKO, B., BRANNEN, M., AND NDISANG, J.

DR. JOSEPH NDISANG

INTRODUCTION

Primary aldosteronism (PA) is characterized by hypertension, elevated aldosterone and low renin [1]. PA is associated with a high risk of cardiovascular complications independently of hypertension [2, 3] and many PA patients still exhibit severe cardiovascular complications even with treatment [4]. A common cause of many cardiovascular disease is endothelial dysfunction [5, 6]. A common problem associated with endothelial damage is the destruction of the glycocalyx, the semi-permeable layer that covers the endothelial surface layer of the vasculature [7]. However, there is very little information about the glycocalyx in PA. The glycocalyx is made up of different classes of glycosaminoglycans and proteoglycans including hyaluronan and heparan sulphate [7, 8]. The role of hyaluronan, heparan sulphate and chondroitin on vascular endothelium and vasorelaxation in PA is unknown. Similarly, the specific input of hyaluronan and heparan sulphate to vasorelaxation has not been reported. Therefore, this study is designed to address one of the most consistent gaps in knowledge in PA, particularly the effects of hyaluronan and heparan sulphate on the vascular contractility in an animal model of primary aldosteronism [uninephrectomized deoxycorticosterone-acetate (DOCA-salt) hypertensive rats]) [9].

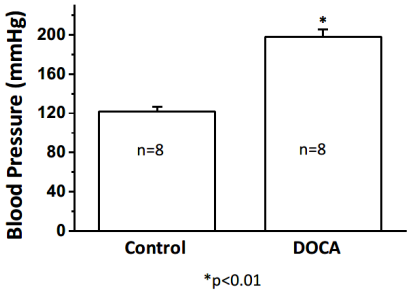
MATERIALS AND METHODS

C57BL mice of age 8 weeks were purchased from Charles River Laboratories, Inc. (Willington, MA), housed at 21°C with 12-h light/dark cycles, fed standard chow, and had access to drinking water and libitum. After a week of acclimatization, DOCA-salt hypertension was induced as described previously [9]. In brief, the right kidney was removed through a dorsal flank incision under isoflurane anesthesia, and DOCA pellets (50 mg, 5-week release, Innovative Research of America, Sarasota, Florida, USA) are implanted subcutaneously. The animals were salt solution of 0.9% NaCl plus 0.2% KCl for five weeks at which time the animals became hypertensive. Blood pressure was monitored weekly by tail-cuff method. Ex vivo relaxation studies were done using third order branches of mesenteric arteriole were isolated, cut into rings and mounted to a wire myograph perfused with Kreb's bicarbonate as we previously [10, 11]. The mesenteric arteriole rings were pre-contracted with submaximal dose of phenylephrine (0.3 μM), and later treated with several drugs including acetylcholine, L-NAME, hyaluronan and heparan.

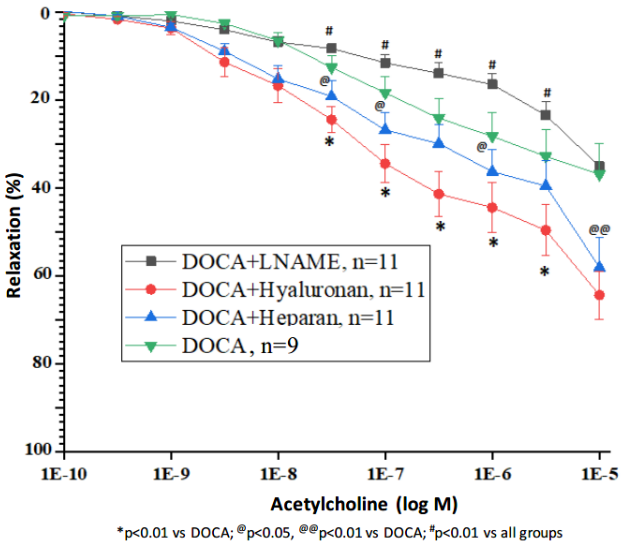
RESULTS AND DISCUSSION

As expected, DOCA treatment caused severe hypertension in the animals (Fig. 1), whereas control animals were normotensive. Acetylcholine (Ach) is generally used to evaluate endothelium-dependent relaxation [12], so it to assess nitric oxide (NO)-induced relaxation in the mesenteric arterioles. The relaxation of DOCA mesenteric arterioles is partly endothelium dependent and is less than 50%, and may be impaired. Treatment with the NO inhibitor, L-NAME, abrogated NO and caused a rightward shift of DOCA+L-NAME curve as compared to all other groups (Fig. 2). Interestingly, treatment with two different components of the glycocalyx such as hyaluronan and heparin shifted these two curves to the left of DOCA, suggesting improved vasorelaxation in DOCA+heparan and DOCA+hyaluronan respectively, although hyaluronan was a better relaxant than heparan. Our results suggest that relaxation of the mesenteric arterial bed of DOCA-mice is compromised and this may be due to defects of the glycocalyx given that components of the glycocalyx such as hyaluronan and heparin significantly improved vasorelaxation. Defective glycocalyx may underpin many vascular diseases [7, 13-15]. Consistently, the glycocalyx may also be impaired in DOCA-salt induced hypertension, a model of human primary aldosteronism.

i) **Figure-1: Effect of DOCA-salt on systolic blood pressure**



i) **Figure-2: The effects of hyaloran and heperan on relaxation of mesenteric arterioles**



CONCLUSION

The structure and function of the glycocalyx is critical for effective vascular physiology, and perturbations of components of the vasculature including the glycocalyx may cause vascular dysfunction and other related diseases. Our findings suggest an important role of heperan and hyaluronan in the restoration and gylcocalyx in a microscale, and on a macroscale of maintenance of vascular integrity and a wide range of physiological functions of the vasculature such as vasorelaxation, leukocyte adhesion, mechano-transduction, thrombogenesis, rheology and vascular permeability [7].

ACKNOWLEDGMENT

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Epitope Tagging of Endogenous Diacylglycerol Acyltransferase-2 Using Gene Editing with CRISPR/Cas9

MCFIE, P. AND STONE, S.

INTRODUCTION

DGAT2 catalyzes the final step of the triacylglycerol biosynthetic pathway and has been an attractive anti-obesity drug target [5]. However, information regarding how DGAT2 functions at the cellular level is lacking. A major limitation and criticism in studying the function of DGAT2 in cells has been the lack of useful antibodies to detect endogenous DGAT2 protein. We (and others) have unsuccessfully tested many commercial antibodies as well as our own "in-house" antibodies. There is currently no evidence that DGAT2 undergoes processing such that epitopes to these antibodies are removed. Consequently, our studies and others have been restricted to overexpressing tagged versions of DGAT2 in cells, which may or may not reflect the function of endogenous DGAT2. In this study, we used gene editing with CRISPR/Cas9 to add a FLAG epitope to the C-terminus of endogenous DGAT2 in HepG2 cells. HepG2 cells, derived from a human hepatocellular carcinoma, have been routinely used as a cell model to study human hepatocyte lipid and lipoprotein metabolism [2]. Using this model, we are now able to detect endogenous DGAT2 protein in a relevant cell line and study its function.

DR. SCOT STONE

MATERIALS AND METHODS

2.1. Construction of homology directed repair (HDR) template - Homology arms (LHA and RHA) for human DGAT2 were cloned into pFetch on either side of a neomycin resistance cassette (NeoR) (a generous gift from E. Mendenhall and R. M. Myers [4]).The final HDR construct contained DNA sequences encoding the 3XFLAG epitope, P2A sequence and NeoR, flanked by the two DGAT homology arms, LHA and RHA (Fig. 1).

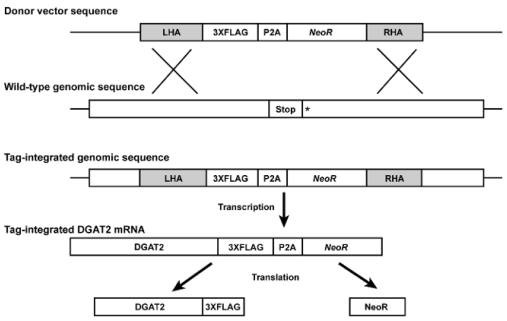

Figure 1. Strategy for adding a 3XFLAG tag to endogenous DGAT2 using sgRNA and homology directed repair. The asterisk after the stop codon of DGAT2 is the target site for sgRNA-mediated DNA cleavage.

2.2. Small guide RNAs (sgRNA) - Complimentary oligonucleotides for two sgRNAs (sgRNA11 and sgRNA19) were annealed and ligated into pSpCas9(BB)-2A-GFP (PX458) (a generous gift from Feng Zhang) downstream of the U6 promoter [3].

2.3. Cell Culture and Transfection - HepG2 cells were grown to 70% confluence and then co-transfected with 5 µg HDR plasmid (pFetch_Donor) and 2.5 µg sgRNA plasmid DNA (PX458) using 0.1% polyethylenimine [1]. Cells were selected with 0.6 mg/ml G418 48 h post-transfection. G418-resistant pools were expanded and used subsequent experiments. PCR fragments containing recombination sites were amplified from genomic DNA and Sanger sequenced.

RESULTS AND DISCUSSION

Gene editing via CRISPR/Cas9 was used to introduce a FLAG epitope to the C-terminus of endogenous DGAT2 in HepG2 cells. The strategy, outlined in Fig. 1, was to use a sgRNA to direct Cas9 to a region near the stop codon of DGAT2 where it will introduce a double stranded DNA break. Cellular machinery will repair the break using an HDR template that will replace the stop codon of DGAT2 with three FLAG epitopes, a P2A sequence and NeoR. This design will allow for the co-transcription of FLAG-tagged DGAT2 and NeoR, which will be translated as two separate polypeptides due to the presence of the P2A sequence promoting ribosomal skipping [6]. Due to the addition of the 3XFLAG tag and the P2A sequence, DGAT2FLAG will be ~50 kDa. Untagged DGAT2 is predicted to be ~42 kDa. HepG2 cells were co-transfected with PX458 containing either sgRNA11 or sgRNA19 and a plasmid containing the HDR template. Cells were incubated with G418 to select for transfected cells. Both stable pools of cells and individual colonies were picked and expanded in order to determine if genome editing was successful. PCR amplification of genomic fragments over key junctions was used to demonstrate the successful insertion of the 3XFLAG epitope at the C-terminus of DGAT2 (not shown). Sequencing of the junctions at the insertion site of the HDR template confirmed the correct in-frame integration of the 3XFLAG tag, P2A sequence and NeoR into the DGAT2 locus (not shown). Immunoblotting with a mouse monoclonal anti-FLAG antibody was used to determine if DGAT2FLAG was successfully expressed in HepG2 cells. A weak signal ~50 kDa in size could be detected when probing cellular lysates with anti-FLAG (Fig. 2A). Immunoprecipitation with anti-FLAG was used to concentrate DGAT2FLAG and confirmed that DGAT2FLAG was expressed in the two gene edited HepG2 cell lines. When immunoblotting the immunoprecipitates with anti-FLAG, we were able to detect an immunoreactive protein that was present in the gene edited cell Lines but was not present in wild-type cells or the buffer control (Fig. 2B).

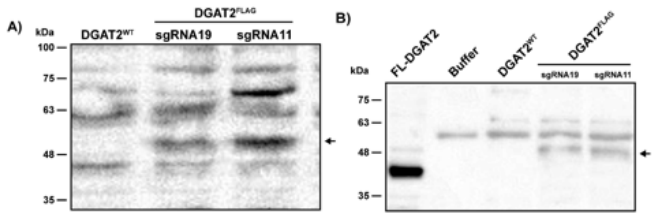

Figure 2. Detection of DGAT2^{FLAG} in HepG2 cells by A) immunoblotting or B) immunoprecipitation with anti-FLAG. DGAT2^{FLAG} is indicated by an arrow.

CONCLUSION

We successfully generated DGAT2 gene edited HepG2 cells and were able to detect endogenous DGAT2 protein.

ACKNOWLEDGMENT

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Establishment of an MS Model to Investigate a Novel Therapeutic Intervention

TOKARSKA, N., SALAPA, H., LEVIN, M., MUIR, G., AND VERGE, V.

DR. VALERIE VERGE

INTRODUCTION

Multiple Sclerosis (MS) results in segmental axonal demyelination that early on can be followed by efficient remyelination, supporting that endogenous repair mechanisms exist which can be targeted at later points when effective remyelination is not the case. Our lab focuses on therapies that enhance intrinsic repair mechanisms of the injured nervous system. We find acute intermittent hypoxia (AIH; intermittent periods of reduced oxygen), a novel non-invasive therapy, improves outcomes in spinal cord (Prosser-Loose et al., 2015; Hassan et al 2018) and peripheral nerve injured animals, the latter in a manner akin to that observed with electrical stimulation (ES; McLean et al., 2014; 2016), likely due to AIH's ability to increase neural activity. But its potential for repair in MS is unknown. Thus, our goals with our CoMRAD grant were to (i) establish the MOG33-35 experimental autoimmune encephalomyelitis (EAE) in female C57BL/6 mice and its clinical behavioral assessment in our lab and (ii) use this model to gain insights into the potential of AIH treatment to effect better remyelination, axon protection, immune cell polarization toward a pro-repair state, oligodendrocyte precursor cell recruitment, and delay EAE disease progression.

MATERIALS AND METHODS

All animal procedures were conducted in accordance with CCAC guidelines and as approved by the UofS AREB.

EAE:
EAE was induced and assessed as per Douglas et al., (2016) in 11 wk old female C57BL/6 mice by immunization with an emulsion of MOG35–55 (myelin oligodendrocyte glycoprotein) in complete Freund's adjuvant (CFA) + pertussis toxin (PTX), with a second PTX injection the next day(as per Hooke Labs EAE induction kit; Hooke Laboratories Inc., Lawrence, MA, USA). Each day, EAE mice were examined clinically for disease severity and progression using a scoring system composed of scoring values ranging from 0 to 5.0 increasing at increments of 0.5. A score of 1.0 indicates tail atony " limp tail" (an initial sign of clinical disease onset), whereas 5.0 is moribund. Scoring details can be found at https://hookelabs.com/services/cro/eae/RatEAEscoring.html Clinical scores (conducted daily by an individual blind to therapeutic intervention) were averaged from all experimental runs and differences between groups assessed. The score assigned to each mouse allows correlatation of disease state with histochemical analysis. Three runs of EAE mice were generated over the course of this past year for a total of 12-13 mice per experimental group; 5 naive mice served as controls.

Acute Intermittent Hypoxia (AIH):
Mice were acclimated to Plexiglas chambers under normoxia (21% inspired O2). On each treatment day, EAE mice are exposed to AIH, consisting of ten 5min hypoxic episodes (11% inspired O2) alternating with 5min normoxic intervals. Control EAE mice were in adjacent chambers at the same time under continuous normoxia. Chamber oxygen levels are continuously monitored. EAE mice had 7d daily AIH treatment begun at near peak of disease clinical score of 2.5 (limp tail and dragging of hind legs) to assess impact on repair.

Histochemical Assessment of the impact of AIH on EAE:
4% paraformaldehyde fixed lumbar/thoracic spinal cord regions (most affected in MOG35-55 EAE) were removed, cryoprotected and frozen. Ten micron longitudinal and transverse serial sections were cut from each experimental group (AIH EAE, normoxia EAE, naïve) and processed for immunofluorescence using antibodies to detect myelin basic protein (MBP), the marker of axonal protection phosphorylated neurofilament (NF; SMI-31), activated microglia/macrophage markers-CD68/ED-1, the paranodal protein Caspr, juxtaparanodal potassium channel Kv1.231to examine re-establishment of nodes of Ranvier, the oligodendrocyte precursor cell (OPC) marker Olig2 (or PDGFαR), activinA which transitions OPCs into oligodendrocytes(OLs) and the pro-myelinating molecule, BDNF and the hypoxia sensing/repair molecule, hypoxia-inducible factor (HIF)1a. With respect to macrophage/microglial polarization we examined the same markers as in our focally demyelinated peripheral nerve study9 pro-inflammatory-iNOS and TNFalpha; pro-repair- CD206, ActivinA and Arg-1. All alterations in protein expression of the markers within demyelinated regions or within macrophages were quantified in a blinded manner using Northern Eclipse or ImageJ analysis programs as previously described (McLean et al., 2014; 2016). Tissue sections from each group were also processed with classic histological stains to gain additional insights into the pathological state effected by the AIH.

RESULTS AND CONCLUSION

Preliminary data supports that 1 week of daily AIH treatment begun at near peak of disease score of 2.5 has a remarkable impact on significantly improving behavioural scores in the EAE mouse relative to normoxia controls. Qualitatively there were also marked impacts of AIH on markers of axon protection, oligodendrocyte precursor cell recruitment, remyelination and immune response modulation toward a pro-repair phenotype.

CONCLUSION

With the establishment of the EAE mouse model in our laboratory we were able to generate preliminary data supporting a significant impact on AIH on behavioural outcomes with improved clinical scores and associated histopathology. This provides a strong rationale for further investigation into defining the optimal AIH protocol to bolster nervous system repair after demyelinating lesions and elucidation of the underlying mechanisms of how AIH is effecting this enhanced repair response.

ACKNOWLEDGMENT

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MiR-122 Promotes HCV Replication by Modifying Viral Genomic RNA Structures

PALMER, M., MROZOWICH, T., PATEL, T., AND WILSON, J.

DR. JOYCE WILSON

INTRODUCTION

Chronic HCV causes liver failure and hepatocellular carcinoma [10] but with the recent development of direct acting antiviral therapy it can now be cured. However, major hurdles remain to treat the 80 million people who are infected worldwide and a vaccine and research on drug resistant viruses will be required [10]. Efficient HCV replication requires an RNA structural element formed by annealing of the liver specific miRNA, miR-122, to two sites on the HCV 5' untranslated region (UTR) (Figure 1A) and miR-122 antagonists are powerful HCV inhibitors and efficiently suppress HCV replication. However, recent evidence suggests the emergence of resistant viruses [9]. While the mechanism of action of miR-122 is not clearly understood, a major breakthrough was an hypothesis made by my lab that miR-122 promotes HCV by altering the 5' UTR RNA structure (Figure 1B) [2]. We also hypothesized that viruses escape the need for miR-122 by incorporating point mutations that induce the pro-viral RNA structure in the absence of miR-122 [2, 14]. This hypothesis is supported by i) RNA structure analyses that predict the 5' UTR RNA forms a non-canonical 5' UTR structure in the absence of miR-122 and the canonical structure in the presence of miR-122 annealing (Figures 1B and 2A and B) [2, 14], ii) that alternative small RNAs that promote HCV also induce the canonical structure (Figure 1B and 2D), and iii) that mutant 5' UTR sequences that support miR-122-independent HCV replication are predicted to form the canonical RNA structure even in the absence of miR-122 [2, 14] (Figures 1C and 2C). However, it has not been tested by direct biophysical analysis of HCV 5' UTR structures.

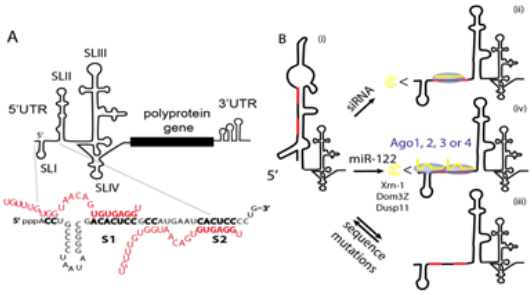

Figure 1. A) schematic diagram of the HCV genome highlighting the 5' terminal sequence, canonical RNA structure, and pattern of miR-122 annealing. B) Model of the predicted RNA structures (i) without miR-122 annealing, ii) with small RNA annealing, iii) of 5' UTR sequences that promote miR-122-independent replication and iv) with miR-122 and recruited Ago proteins

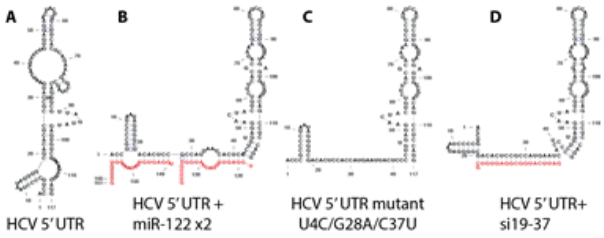

Figure 2. *In silico* RNA structure analysis of the 5' UTR region of the HCV genome A) without miR-122 annealing, B) with miR-122 annealing, C) of a mutant capable of miR-122-independent replication, and D) with annealing of si19-37, which promotes HCV replication as potently as miR-122.

MATERIALS AND METHODS

RNA structure analysis by Small Angle X-ray Scattering (SAXS): The underlying goal of my CoMRAD grant was to assess the molecular details of the RNA structural switch element formed by annealing of miR-122 to the HCV 5' UTR and how viruses escape a therapeutic strategy the exploits an RNA structure. HCV 5' UTR RNA structure was analyzed using size exclusion chromatography (SEC) and SAXS in collaboration with co-Investigator Trushar Patel (University of Lethbridge). SAXS generates information on the low-resolution structures or 'envelopes' (20-30 Å) of RNA or protein in solution as well as assesses complexes and conformational changes [3, 13, 16]. 5' UTR HCV RNA was prepared using in vitro transcription, followed by SEC to remove aggregated/degraded material and subsequently analyzed by SAXS. We employed SEC-SAXS to prepare and analyze the 5' UTR alone, in complex with miR-122 or spmRNAs, as well as 5' UTR having mutations that support miR-122-independent replication. The analyses were done at the DIAMOND synchrotron in the UK using the SEC-SAXS setup. The Patel lab processed the SAXS data to obtain low-resolution structures of 5' UTR and its complexes [5-7].

RESULTS AND DISCUSSION

Our preliminary SAXS results demonstrate that, indeed, the HCV RNA undergoes conformational changes after miR-122 annealing (Figure 3A). We synthesized and purified the HCV 5' UTR 117 nucleotides by SEC and analyzed the RNA by SAXS. The experimentally generated SAXS envelopes of the 5'UTR 1-117 with and without miR-122 annealing are shown in Figure 3. Also shown are high resolution molecular models of the RNA complexes generated by combining RNA structure predictions, SHAPE data, and SAXS envelopes [4, 14]. The $\chi 2$ value of less than 2.0 indicates good fit. However, these experiments have been done only once and need to be repeated for confirmation. We have also begun to analyze SAXS structures of the 5' UTR RNA annealed to spmRNA 19-37 (Figure 3B si19-37), and of an RNA having mutations that support miR-122-independent HCV replication (Figure 3B, U4C/G28A/C37U). Our preliminary findings suggest they form an RNA structure similar to that induced by miR-122 annealing. In the future we will use SAXS to analyze RNA structures formed by 5' UTRs annealed with other spmRNAs and 5' UTR mutations

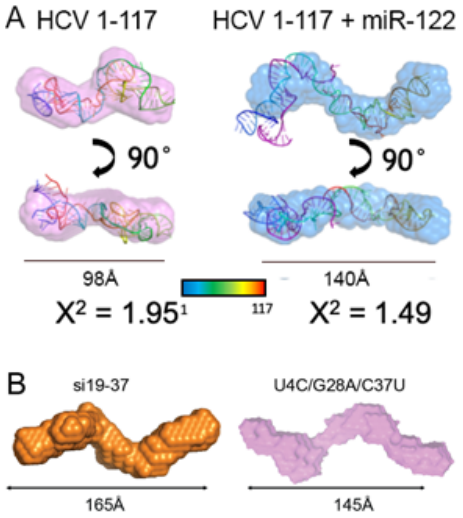

Figure 3. SAXS analysis and molecular modeling of the HCV 5' UTR 117 nucleotides with and without miR-122 annealing. (A) SAXS envelopes containing modeled HCV 5' UTR RNA (B) SAXS envelope of 5' UTR annealed with si19-37 and of miR-122-independent HCV mutant U4C/G28A/C37U

CONCLUSION

SAXS analysis of the HCV 5' UTR RNA structures support our hypothesis that miR-122 annealing modifies the structure of the viral genomic RNA. Combined with other data from our lab we have generated a model that miR-122 promotes HCV replication by modifying the 5' UTR RNA structure to induce the formation of the active HCV internal ribosome entry sequence and stimulate virus translation. Genome stabilization by miR-122 has a secondary role that alone cannot induce virus replication, but enhances replication induced by translation stimulation. Future experiments will test the model by assessing the impact of alternative small RNAs on the 5' UTR structure and resolve the mechanism by which HCV mutations permit the virus to escape its reliance on miR-122.

ACKNOWLEDGMENT

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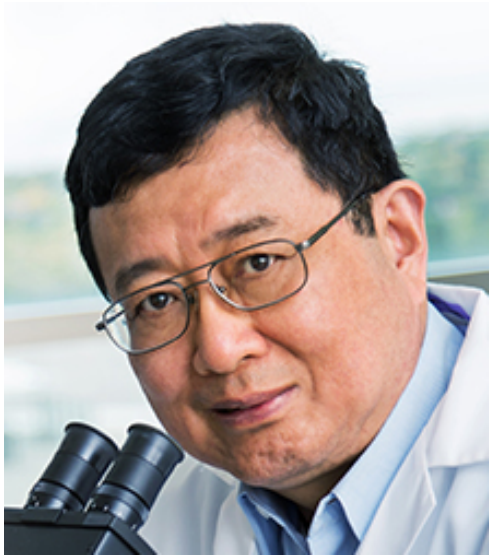
Critical Role of FAK/RhoA in Controlling mTORC1/PKM2-Regulated Triple-Negative Breast Cancer (TNBC) Cell Proliferation, Adhesion and Invasion

LI, R. AND XIANG, J.

INTRODUCTION



DR. JIM XIANG

Breast cancer represents a highly heterogeneous disease comprised by several subtypes with distinct histological features and clinical behaviors. Triple negative breast cancer (TNBC) with negative expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) and constituting 20% of all breast cancer cases is one of the most aggressive subtypes, oftena highly orchestrated transitional program for epithelial-to-mesenchymal transition (EMT) and associated with a poor patient outcome due to the development of metastasis in secondary organs [1]. Beside the well-known transforming growth factor-beta (TGF-β)-Smad pathway regulating EMT [2], the mTORC1 (mammalian target of rapamycin complex-1) pathway has recently been found to be another critical pathway for EMT [3] via regulation of pyruvate kinase-M2 (PKM2) [4], a crucial glycolytic enzyme with influence in aerobic glycolysis, EMT, tumor growth and metastasis. However, the up-stream signal controlling mTORC1/PKM2-regulated EMT is still elusive. We have recently applied a commonly used a three-dimensional (3D) clinostat (a random positional machine) to model SMG. We have found that SMG reduces FAK (focal adhesion kinase)/RhoA (ras homolog gene-A) activity, and revealed that SMG enhances B16 melanoma cell apoptosis and inhibits B16 melanoma cell proliferation and metastasis via suppressing FAK/RhoA-regulated mTORC1 pathway [5]. We then hypothesize that FAK/RhoA may be the upstream signal controlling the mTORC1/PKM2-regulated EMT.

MATERIALS AND METHODS

A human TNBC cell line MDA-MB-231 [6] obtained from American Type Culture Collection (ATCC), which has passed EMT. To examine the gravitational effect on EMT, MDA-MB-231 cells growing in DMEM medium plus 10% FCS in T25 culture flasks or Chamber Culture slides (Nunc Inc) under ground condition (1g) or on the 3D clinostat under SMG condition (μg) for 3 days [5] will be subjected to or prepared for cell lysates for cellular and molecular characterization by biochemistry, molecular biological, and histopathological analyses including confocal microscopy, Western blotting as well as cell proliferation and invasion assays.

RESULTS AND DISCUSSION

To assess cell morphology, we performed confocal microscopy analysis, and showed that SMD changes cell morphology from M to E and reduces focal adhesions (FAs) (Fig 1). To assess the MET phenotype, we assessed expression of the mesenchymal (fibronectin and vimentin) and epithelial keratin markers by Western blotting analysis using lysates from MDA-MB-231 cells cultured under 1g and μg, respectively, and showed that SMG increases keratin, but reduces fibronectin and vimentin expression (Fig 2A). To assess SMG-affected FAK/RhoA, mTORC1 and PKM2 signaling, we similarly performed Western blotting analysis, and demonstrated that SMG also reduces expression of mTORC1 (pS6K, Ser235) and PKM2 (Fig 2B), suggesting that SMG converts EMT to MET via controlling mTORC1-PKM2 axis. In addition, we also displayed that SMG reduces expression of pFAK (Tyr379) and RhoA (Fig 2B), suggesting that FAK/RhoA may be the upstream signal of mTORC1/PKM2. Furthermore, we demonstrated that SMG inhibits in vitro MDA-MB-231 cell's proliferation, adhesion and invasion using Cell Migration and Invasion kits (Fig 3).

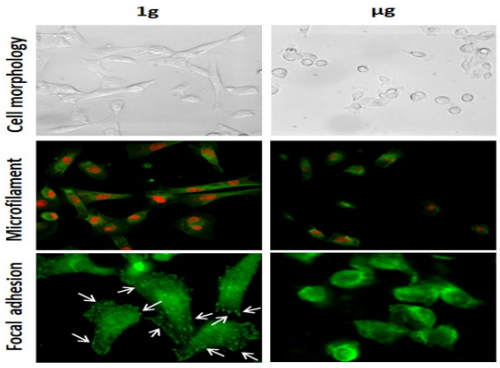


Figure 1. SMG (A) changes cell morphology from flat mesenchymal stellate shape (1g) to epithelial-like collestone shape (μg), (B) alters microfilaments (MF) and (C) abrogates focal adhesions (FAs) in MDA-MB-231 cells under μg, compared to control cells under 1g, which were stained by FITC-anti-F-actin (for MF) and FITC-anti-paxillin (for FAs) antibody, respectively, and examined by confocal microscopy. Arrows, FA green spots.

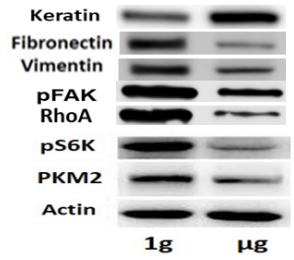


Figure 2. SMG down-regulates pFAK, RhoA, pS6K, PKM2 and mesencymal gene-encoding molecules fibronectin and vimentin and epithelial gene-encoded keratin in MDA-MB-231 cells under μg, compared to cells under 1g, by Western blotting analysis.

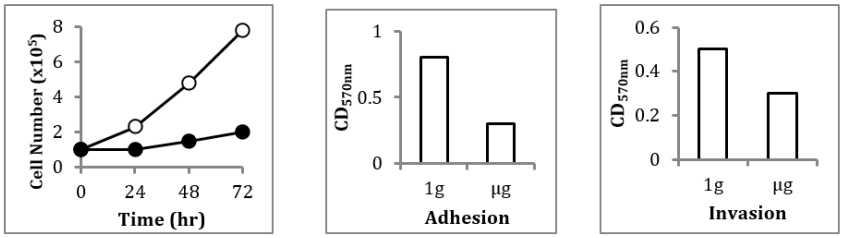


Figure 3. SMG inhibits MDA-MB-231 cell proliferation, adhesion and invasion. MDA-MB-231 cells grown under 1g (open circle) and μg (solid circle) were counted daily foe cell proliferation analysis or used for measurement of cell adhesion and invasion using Cell Adhesion and Invasion kits (Cell Biolabs).

CONCLUSION

We showed new evidence that the FAK/RhoA signal controls mTORC1/PKM2-regulated TNBC's MET, cell proliferation, adhesion and invasion, indicating that the upstream signal FAK/RhoA may become a new molecular target for novel therapeutics for TNBC patients.

ACKNOWLEDGMENT

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Clinical

Effectiveness of the Early Psychosis Intervention Program and the Examination of Risk Factors Interfering with its Use

ADAMS, C., WRATH, A., ADAMS, S., MITCHELL, A., CLARK, M., HINZ, T., AND SHEBELSKI, K.

DR. CAMELIA ADAMS

INTRODUCTION

Canada has the highest incidence rates (new diagnoses each year) of schizophrenia compared to other first-world nations (Dealberto, 2013). It has been suggested that patients with psychosis enrolled in specialized early intervention programs such as Saskatchewan's Early Psychosis Intervention Program (EPIP) can have a higher recovery rate at one-third the cost of non-specialized psychiatric outpatient services and remarkably positive impact on individuals and society (Aceituno, Vera, Prina, & McCrone, 2019; Mihalopoulos, Harris, Henry, Harrigan, & McGorry, 2009; Strålin, Skott, & Cullberg, 2019). While numerous risk factors for psychosis have been identified, most of them are unmodifiable by the time the first episode of psychosis develops (e.g., sex, genetic predisposition, trauma, etc.). Still, several aspects such as cannabis use and individual attachment are modifiable, representing possible areas for intervention. A wealth of research has shown the negative impact of cannabis use on the development of psychosis (for a review, see Radhakrishnan, Wilkinson, Cyril, & Hillemacher, 2014). Individuals with a history of cannabis use have an earlier onset of psychosis, are younger when first hospitalized for psychosis and have more severe symptoms than individuals with psychosis who never used cannabis (Hosseini & Oremus, 2018; Large, Sharma, Compton, Slade, & Nielssen, 2011). These data suggest that cannabis can be a risk factor for the development of psychosis, and contribute to its persistence. Attachment, an interpersonal descriptor developed early in life in relationship with caregivers, has been shown to persist over the life span and to impact the way individuals relate in relationships (Mikulincer & Shaver, 2016). Attachment is often considered to be comprised of two continuous dimensions: attachment anxiety and attachment avoidance. Individuals with anxious attachment have poorer self-view, tend to dwell in negative emotions and have higher needs for closeness and reassurance from others. Conversely, individuals with avoidant attachment tend to mistrust others and cope alone by isolating themselves during difficult times. These individuals inhibit their own emotions, avoid feeling distressed and rarely seek support when in need. There is significant evidence that higher attachment anxiety and avoidance are associated with substance use and more severe symptoms of psychosis (Mikulincer & Shaver, 2016). However, these bodies of literature are small, and more research in this area is needed. The current study improves on previously employed cross-sectional methodologies by collecting prospective data from EPIP patients throughout their treatment. We will assess changes in symptom severity as a function of changes in attachment and past and prospective cannabis use. This is the first study to examine this relationship in individuals with early psychosis.

MATERIALS AND METHODS

Participants include new as well as previously enrolled EPIP patients between 18 and 35 years old. Participants completed the Positive and Negative Syndrome Scale (PANSS) interview as a measure of symptom severity. The PANSS has multiple subscales, such as negative symptoms (e.g., blunted affect, emotional withdrawal, etc.), positive symptoms (e.g., hallucinations, delusions, etc.) and general psychopathology (e.g., anxiety, depression, etc.). Current and lifetime cannabis use was measured by the Daily Sessions, Frequency, Age of Onset and Quantity of Cannabis Use Questionnaire. Attachment anxiety and attachment avoidance were measured using the Psychosis Attachment Measure. Current results are cross-sectional in nature. Descriptive statistics were performed and correlations between variables of interest were conducted.

RESULTS AND DISCUSSION

3.1 Results: Twenty-one participants have completed baseline measures. The sample is predominately male (n=15) and in early adulthood (Mage=22.4). Participants' symptom severity ranged from the 4th to 82nd percentile (Mpercentile=18). Sixteen participants reported lifetime cannabis use. The mean age at first cannabis use was 14.5 years old. Cannabis began to be used regularly (2 or more times/month) by the age of 15.9 (n=13), and daily or nearly daily by age 16.5 (n=12). Five participants were currently using cannabis at the time of enrollment. The age of regular cannabis use onset was approaching a significant association with attachment anxiety ($r=-0.55$, $p=0.05$) and positive symptoms ($r=-0.55$, $p=0.05$). Attachment avoidance was significantly correlated with scores on the negative symptoms subscale of the PANSS ($r=0.46$, $p<0.05$)). Attachment anxiety was significantly correlated with the general psychopathology subscale of the PANSS ($r=0.45$, $p<0.05$). Both attachment dimensions were approaching significant correlations with the positive symptom subscale of the PANSS ($r=0.38$-0.43, both $p<0.09$). Attachment anxiety was approaching a significant correlation with thought disturbance ($r=0.43$, $p=0.06$).

3.2 Discussion: Our results show that individuals with early psychosis begin using cannabis at a very young age and begin using daily or nearly daily within two years. We found that the age at which cannabis begins to be used regularly is positively associated with positive symptoms of psychosis. Although only approaching significance, this finding may indicate that more frequent exposure of the developing brain to cannabis may be a risk factor for developing early psychosis. Another risk factor for early-onset regular use of cannabis may be attachment anxiety. Interestingly, attachment anxiety is also associated with the age of onset of regular use of cannabis and positive symptom severity. Specifically, individuals with greater attachment anxiety begin using cannabis at a younger age. This may be due to peer pressure and/or wanting to please others to maintain a sense of security in their social circle. Attachment avoidance was significantly and positively associated with negative symptom severity. Negative symptoms include emotional and social withdrawal, which compliments the cognitive and behavioural tendencies of individuals with higher attachment avoidance who down-regulate their negative emotions and avoid potentially uncomfortable social interactions, particularly if the situations are emotionally charged. Attachment anxiety was significantly and positively associated with more severe general psychopathology. Attachment anxiety has frequently been associated with more severe symptoms of multiple mental health and medical disorders and our findings indicate that this may also be true in psychosis.

CONCLUSION

From the current data, we see that early onset of regular cannabis use, as well as insecure attachment, are associated with the severity of symptoms of psychosis. As we move forward with longitudinal measurements of changes in these variables throughout treatment, we hope to discover how these variables influence each other and treatment outcome. If we find that the changes in the modifiable risk factors we have identified have significant impacts on the recovery of individuals with psychosis, this knowledge can be used to develop new interventions and ideally improve the efficiency of psychiatric care in this population.

ACKNOWLEDGMENT

We would like to thank all staff that provide support to EPIP patients on their journey to recovery and who support the need for this project.

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Heart Failure After Laboratory Confirmed Influenza Infection (FLU-HF)

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DR. PAYAM DEHGHANI

INTRODUCTION

Influenza has been shown to exacerbate heart failure (HF). This can further compound patient morbidity, mortality, and result in prolonged hospital admissions. Importantly, no study to date has examined the relationship between HF hospitalizations (HFH) with laboratory confirmed influenza infections. This study evaluated the association between laboratory-confirmed influenza infection and HFH in the two largest hospitals in Saskatchewan, Canada.

MATERIALS AND METHODS

We used a retrospective self-controlled case-series design to evaluate the association between laboratory-confirmed influenza infection and HFH. We compared the incidence ratio for HFH during the influenza risk interval with the control interval. We defined the influenza risk interval as the seven days after a laboratory confirmed influenza result and the control interval as one year before and after the risk interval.

RESULTS AND DISCUSSION

We identified 114 HFH that occurred within one year before and after a positive test result for influenza between April 1, 2010, and April 30, 2018. Of these, 28 (28 admissions per week) occurred during the risk interval and 86 (0.853 admissions per week) occurred during the control interval. The incidence ratio of a HF admission during the risk interval as compared with the control interval was 33.53 ((95% confidence interval (CI), 21.89 to 51.36). A decline in incidence was observed after day seven; between days eight to 14 and 14 to 28 incidence ratios was 0.91 (95% CI, 0.13 to 6.52) and 0.91 (95% CI, 0.22 to 3.68) respectively.

CONCLUSION

We have observed a significant association between acute influenza infection and HFH. However, further research with a larger sample size and involving a multicenter setting is warranted.

Cannabidiol in Inflammatory Bowel Disease: Assessment of Current Use in Saskatchewan and Phase I Dose Escalation Study

CHAMI, J., ALCORN, J., AND FOWLER, S.

INTRODUCTION

DR. SHARYLE FOWLER

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract and comprises two major forms: Crohn's disease (CD) and ulcerative colitis (UC). The symptoms of IBD are variable and can include diarrhea, vomiting, rectal bleeding, abdominal pain, weight loss, and malnutrition. The therapeutic goals in managing IBD are to induce and maintain remission thereby eliminating symptoms, improve quality of life, and prevent complications of the disease. Conventional IBD therapies demonstrate only modest clinical efficacy and have the potential for adverse side effects.1 As a result, a significant proportion of IBD patients are using complementary and alternative medicine (CAM), including medicinal cannabis.2,3 Cannabis contains over 100 different constituents with Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) the best characterized.4,5 Cannabinoids act on the endocannabinoid system and have been shown to have anti-inflammatory, anti-diarrheal, and analgesic effects in animal models of gastrointestinal inflammation.6-11 Epidemiological studies have shown rates of active cannabis use among patients with IBD between 6.8-17.6%, with lifetime use up to 48% in one study.13-16 Patients reported using cannabis for abdominal pain, nausea, diarrhea, anorexia, and to improve mood and quality of life (QoL).12-15 However, two recent Cochrane database reviews have concluded that the effects of cannabis and cannabidiol on CD and UC are uncertain and no firm conclusions regarding the efficacy and safety can be drawn.16,17 This is also in line with a position statement from the Canadian Association of Gastroenterology in 2018.18 Significant gaps in knowledge exist regarding medicinal cannabis use for the treatment of IBD, making many healthcare providers unprepared or unwilling to counsel patients in this area or authorize the use of medicinal cannabis for IBD. Our study has 3 objectives: 1) To assess patient and physician knowledge on the use of cannabis in IBD and identify patterns and reasons for use of cannabis amongst patients with IBD in Saskatchewan; 2) To determine the safety and tolerability of CBD-enriched cannabis therapy in patients with IBD; 3) Gather preliminary data on the effect of CBD-enriched cannabis on patient's QoL, clinical disease activity and markers of inflammation, as well as the relationship between dose escalation and steady-state trough levels of bioactive cannabinoids.

MATERIALS AND METHODS

Part 1: Patient and Healthcare Practitioner Survey

This project involves a questionnaire distributed to patients with IBD and healthcare providers who treat IBD in Saskatchewan (gastroenterology and general surgery). This questionnaire was developed using the IBD Ontario Survey as a general framework as well as previously published surveys by the Canadian Consortium for the Investigations of Cannabinoids.12,19 The patient questionnaire includes demographics and psychosocial characteristics, disease characteristics, and questions on cannabis use. The physician questionnaire includes information on physician knowledge of the use of cannabis in IBD, experience authorizing cannabis in IBD, attitudes regarding the use of cannabis in IBD, and barriers to the use of cannabis in IBD. Statistical Analysis: Descriptive statistics will be used to summarize respondent's knowledge, experiences, attitudes, barriers, and demographic information. Means with corresponding standard deviations (SD) and proportions with their corresponding 95% confidence intervals (95% CI) will be reported.

Part 2: Pilot Study

This trial will be a single center, open-label, dose-escalation study with two phases: placebo and treatment.
Participants meeting inclusion and exclusion criteria will receive placebo for 2 weeks, CBD 12.5 mg BID for two weeks, 20 mg BID for two weeks, then 25 mg BID for two weeks. Placebo will be assessed to ensure patients tolerate the CBD-delivery agent with no side effects. Patients will be assessed every 2 weeks during treatment, and at week 10 for a follow up safety visit. Participants will be assessed for disease activity, adverse events, and QoL. Blood samples will be drawn to assess steady state trough concentrations of bioactive cannabinoids using liquid chromatograpy-mass spectrometry (LC-MS/MS). Inflammatory markers (C-reactive protein and fecal calprotectin) will be measured at week 8. As this is a dose escalation safety and tolerability study a sample size of 28 will be used, which is within usual guidelines for phase I clinical trials. Statistical Analysis: Data will be analyzed using descriptive statistics (mean and SD, frequencies and 95% CIs). Friedman's ANOVA Tests will be used to determine rank differences in clinical disease activityqw and QoL scores across the four assessments (week 2,4,6,8). Wilcoxon Matched-Pairs Signed Rank Tests will be used to identify differences in QoL and disease activity measures between placebo and CBD, as well as between doses. These non-parametric comparisons will test the effect of CBD on QoL and disease activity measures, as well as the effect of dose escalation.

RESULTS AND DISCUSSION

Part 1: Patient and Healthcare Practitioner Survey

Surveys have gone through patient feedback via think aloud sessions. Ethics approval has been obtained and data collection is currently underway. Results are pending.

Part 2: Pilot Study

Several barriers have impeded the progress of the pilot study. The Cannabis Act and Regulations provide the framework for legal access to cannabis and control and regulate its production, distribution, sale and research. The University of Saskatchewan has applied for an institutional research license and expects that this will finally be granted in March 2020. Additionally, in order to complete a Health Canada Clinical Trail Application, an Investigator's Brochure is required from a Licensed Producer. We continue to work on developing partnerships with various Licensed Producers in order to obtain this for our Pilot Study.

CONCLUSION

Use of cannabis in IBD is a recent and emerging area of study and, therefore, considerable knowledge gaps exist. Cannabis shows promise for its effect on inflammation but its safety and efficacy in the treatment in IBD requires investigation. Although we have faced several barriers to successful implementation of this pilot study, we will continue to persevere and work to help fill some of the gaps in knowledge relating to cannabis use in IBD.

ACKNOWLEDGMENT

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Focused Cardiac Ultrasound in Stroke: A Feasibility Study

LEIS, B., ANS, L., GRAHAM, B., VERITY, R., BRYCE, R., MONDAL, P., AKHTAR, J., AND HUNTER, G.

INTRODUCTION

Transthoracic echocardiography (TTE)[1] is a standard investigation in ischemic stroke.1-3 Coupled with a negative heart rhythm monitoring study, a negative TTE excludes most cardioembolic sources of stroke.2 However, multiple reviews report low diagnostic TTE yield between 5%-7%,4-6 which limits their cost-effectiveness.7 For expensive, low yield testing such as this, creative cost-reducing strategies are needed.8,9 Conversely, a positive cardiac study (e.g. clot identification) has major implications for patient management and prevention of stroke recurrence.5,10 The goal of this project was to evaluate the practicality of a revised, more cost-effective approach to TTE in ischemic stroke. We designed a "focused" sequence that directs sonographers to efficiently gather only the necessary images to inform the management of stroke, done with a handheld probe/tablet combination at bedside 11-14, as opposed to a large machine within the imaging department.

DR. GARY HUNTER

MATERIALS AND METHODS

This is a cross-sectional study evaluating patients undergoing echocardiography for evidence of possible cardioembolic stroke, examined with both standard and focused imaging approaches. Such that participants act as their own comparator. All patients greater than 17 years of age, admitted with ischemic stroke to RUH, and deemed by the neurology team to require a TTE were eligible for the study. Patients with known complex congenital heart disease and mechanical valves were excluded. In this feasibility study, we aimed to recruit 225 patients, which will be adequate for preliminary evaluation of non-inferiority with a 3% margin. Recruitment began in February 2019 and is still ongoing. Variables of interest for the respective focused and standard TTE's were the presence of any wall motion abnormality, cardiomyopathy, atrial size and atrial septal abnormalities, endocarditis, mitral or aortic valve abnormalities, aortic plaque, or cardiac masses.15,16 The reading cardiologist was blinded to the identity of the patient and to the results of the standard TTE when interpreting the focused TTE. Standard TTE results were interpreted and reported as per usual practice.

RESULTS AND DISCUSSION

Sixty-one patients have been recruited. Data is currently available for 15 patients who have completed full interpretation of both imaging studies, and is presented in Table 1. As expected, the mean time to finish image acquisition for the focused TTE was significantly shorter than the complete TTE. Focused TTE may show a tendency to reporting smaller atrial volumes than the complete TTE; this may be explained by a reduced ability of the handheld probe to image distant structures.

Table 1. Selected reporting of positive TTE findings (N=30 ultrasounds).

	Type of statistic	ALL	COMPLETE (n=15)	FOCUSED (n=15)	p-value
IMAGE ACQUISITION (MINUTES)	Mean, (SD)	20.7 (15.9)	34.9 (12.5)	8.4 (2.3)	<0.0001
ATRIAL SIZE (CM)	Mean, (SD)	3.3 (0.6)	3.6 (0.4)	3.1 (0.7)	0.03
LEFT ATRIAL VOLUME INDEX (ML/M^2)	Mean, (SD)	27.3 (9.6)	29.0 (9.8)	23.2 (8.6)	0.22
LEFT VENTRICULAR EJECTION FRACTION (%)	Mean, (SD)	59.7 (10.9)	62.0 (10.0)	55.6 (12.0)	0.19

As expected based on the low yield of cardiac echo in this population, no positive findings were found in either group to affect the course of stroke care, such that agreement on clinically meaningful findings between the two modalities is 100% at this stage. The focused TTE report was more likely to comment on pertinent negative findings, but also more likely to be ambiguous about a positive finding. Of note, the Canadian Society of Echocardiography15 does not stipulate the need to report the absence of these findings in a standard report, suggesting that a lack of reporting is equivalent to an assumption of absence. If this is the case, the focused and complete TTE results in our sample show some diagnostic agreement for the absence of abnormalities, but the focused TTE is more explicit about it in the report.

CONCLUSION

The performance of a focused TTE in ischemic stroke patients is cheaper and more efficient than a complete TTE, with estimated cost of $150 vs $500 respectively, faster image acquisition, and improved patient experience as it can be done at the bedside with a handheld tablet and probe. Clinically relevant findings have so far demonstrated 100% agreement between the two modalities, all with negative findings at this stage. A larger sample size will help to clarify whether there is a difference in positive findings between the two approaches. Long-term impacts may include improved access to rapid focused echo at the bedside with reduced cost, and avoiding delays to discharge while awaiting conventional TTE.

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Proteomics Studies in Parkinson's Disease

KATSELIS, G., CHUMALA, P., THOMPSON, B., EANES, S., BOCKING, S., RAJPUT, A., AND RAJPUT, A.

INTRODUCTION

Parkinson syndrome is characterized by two of the three clinical features – bradykinesia, rigidity and resting tremor1. There are several different pathological conditions, which can produce somewhat similar symptoms. The most common is known as Parkinson's disease (PD) which is pathologically characterized by marked loss of substantia nigra dopaminergic neurons and Lewy body inclusions in the neurons2. Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP) are two other neurodegenerative disorders often misdiagnosed as PD3. This study aims to address the gap that exists during clinical diagnosis in differentiating among various forms of Parkinson syndrome by using mass spectrometry-based brain and blood proteomics. It also aims to identify, characterize, and validate protein markers, which can be used in PD diagnosis.

DR. GEORGE KATSELIS

MATERIALS AND METHODS

Brain samples from diseased patients and blood (plasma and serum) samples from current patients as well as from healthy controls were collected at the Saskatchewan Movement Disorders Clinic at Royal University Hospital, University of Saskatchewan. Proteins from brain samples were obtained after homogenization in extraction buffer and centrifugation. Blood samples were depleted of high-abundant proteins using an Agilent MARS-2 spin cartridge. All protein samples were digested in-solution and tryptic peptides were analyzed on an Agilent 6550 Chip iFunnel QTOF in data dependent acquisition mode. Data were analyzed by MassHunter Bioconfirm software and protein identification and characterization was done on Spectrum Mill, searching against the UniProt Human database, and Mass Profiler Professional / Pathways Architect software, respectively.

RESULTS AND DISCUSSION

Fifteen brain samples (5 each from clinically diagnosed PD, MSA, and PSP cases, respectively), collected after autopsy, were studied. Spectrum Mill database search identified 7,098 proteins in all samples. Mass Profiler Professional statistical analysis using FC > 2 and $p < 0.05$ produced 117 differentially expressed proteins. Principal component analysis on the differentially expressed proteins showed that individual samples from the three groups cluster well within each group and that there is clear separation between the three clinically diagnosed cases (63% separation on x-axis; Figure 1). Of these significant proteins, 17 were found to be upregulated and 96 downregulated in MSA samples when compared to PD samples.

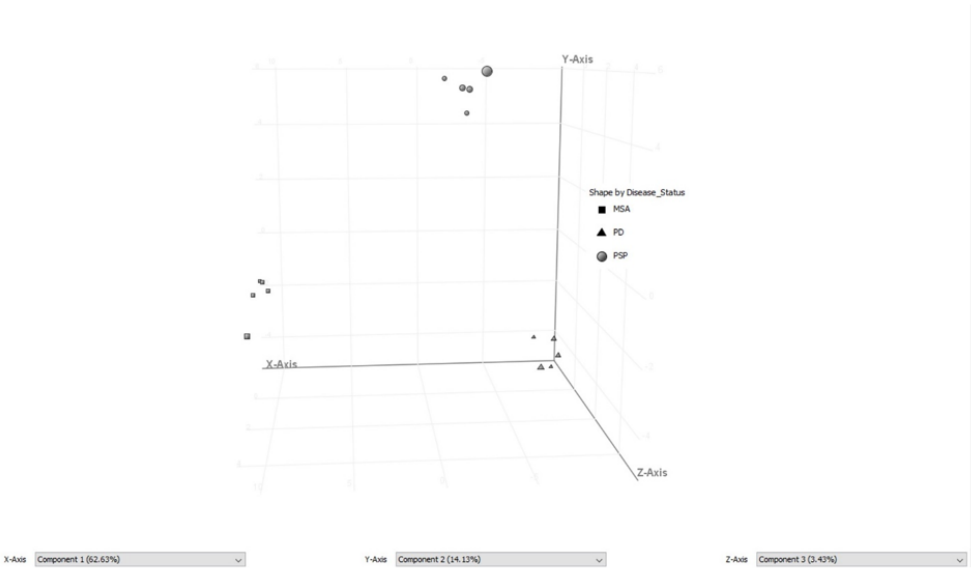

Figure 1. Principal Component Analysis plot showing the individual brain samples from the 3 groups cluster well within each group and that there is clear separation between the 3 clinically diagnosed cases. Plot is based on the 117 differentially expressed proteins; fold change (FC)>2; $p<0.05$.

On the other hand, 13 proteins were found to be upregulated and 31 downregulated in PSP samples when compared to PD samples. Further comparison of the proteins revealed that 7 proteins upregulated were unique to MSA and 10 upregulated were common between MSA and PSP samples. Similarly, 66 proteins downregulated were unique to MSA and 30 downregulated were common between MSA and PSP samples. In addition, 3 proteins upregulated and 1 downregulated were unique to PSP, respectively. Pathway analysis of the 117 differentially expressed proteins identified 168 different pathways that these proteins belong. Most prominent pathways included Parkinson's disease, signaling, ion channel transport, axon guidance and transmembrane transport of small molecules pathways. The list of proteins we have identified and can potentially be considered as markers for diagnosing Parkinson's disease include septins, STE20/SPS1-related proline-alanine-rich protein kinase, synapsin-2, which regulates alpha-synuclein functions, brain acid soluble protein 1, sodium/potassium/calcium exchanger 2, MARCKS-related protein, synaptophysin, complexin-2, neuroplastin and others. Analysis of blood samples and validation of the marker compounds are currently underway.

CONCLUSION

Mass spectrometry-based proteomics can differentiate among various forms of Parkinson syndrome and identify potential marker compounds for diagnosing Parkinson's disease

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Characterizing Iron Dysregulation in the Brain After Thrombolytic Treatment for Ischemic Stroke

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DR. MICHAEL KELLY

INTRODUCTION

Iron dysregulation leading to iron accumulation has been linked with significantly increased risk of ischemic stroke. (Ellervik, et al. 2007; Gill, et al. 2018) High iron status is associated with diabetes mellitus, hemochromatosis (the most common genetic disease in Canada, and common in Caucasians), and thalassemia (common in people of African descent). Administration of thrombolytics, such as tPA to dissolve blood clots in thromboembolic stroke, has been shown to be xa significant predictor of hemorrhagic transformation of ischemic stroke in an animal model with high iron status.(García-Yébenes, et al. 2018) Thrombolytics are the frontline of defence for ischemic stroke, however, a significant proportion of the Canadian stroke patient population may have high iron status and therefore be at increased risk of tPA-mediated hemorrhagic transformation. Reviews of admission records demonstrate that patients with poor stroke outcomes typically have high serum ferritin (a marker of elevated iron) at the time of admission,(Dávalos, et al. 1994, 2000; Erdemoglu, et al. 2002; Milan, et al. 2007; Chakraborty, et al. 2013) and that ferritin levels above 275 ng/mL, measured within 24h of stroke onset, increased the risk of subsequent stroke 33-fold.(Erdemoglu, et al. 2002) The underlying mechanism of how elevated iron status contributes to hemorrhagic transformation of ischemic stroke is unknown. We hypothesize that iron accumulation at the blood brain barrier may contribute to post-stroke damage, leading to increased risk of hemorrhage. We will characterize changes in iron distribution and chemical form in tissues before and after thrombolytic treatment in mouse models of ischemic stroke. This knowledge will help us develop a mechanistic model of how iron contributes to hemorrhagic transformation of ischemic stroke.

MATERIALS AND METHODS

Animal Models:

All animal work is conducted with approval from the University of Saskatchewan's Animal research Ethics Board and carried out in accordance with the Canadian Council on Animal Care guidelines for humane animal use. Our lab employs several mouse models of ischemic stroke,(Caine, et al. 2016; Pushie, et al. 2018) including the middle cerebral artery occlusion (MCAO) model and the photothrombotic (PT) model, both in C57BL/6 mice. The MCAO model employs a silicone-coated filament that is introduced into the left internal carotid artery until it occludes the middle cerebral artery.(Chiang, et al. 2011) After 30-minutes the filament is withdrawn, allowing reperfusion of the ischemic tissue. PT mice are administered intraperitoneal injection of the photoreactive dye, Rose Bengal (100mg/kg, Sigma, USA), which is allowed to circulate for 5 minutes. A sterile mask with a 1.5 x 1.5 mm opening is applied overlying the primary somatosensory cortex to minimize scatter of the incident laser light and the site is irradiated for 20 minutes with a green laser (532 nm, laser power 23-24.5 mW, confirmed prior to each use using a C-Series Optical Power Meter, Thorlabs, Inc.) to photoactivate the Rose Bengal, generating a focal thrombus.(Caine, et al. 2016) At 1h post-stroke, mice are administered saline or thrombolytic (either tPA or TNK).

We employ equal numbers of 11-week-old male and female C57BL/6 mice for all experiments. We have established a hemochromatosis (iron overload) mouse colony, where the gene for iron export is knocked-out in all tissues (strain B6.129P2-Hfetm1Gfn/J, backcrossed to C57BL/6 (from Jackson Laboratory, designed HFE0/0). Purchased wild type mice are fed either normal chow or a diet with elevated iron from 7 to 11 weeks old, following established protocols for rodents.(Castellanos, et al. 2002) Mice are euthanized at 24-72h post-stroke in accordance with allowed practices of the Canadian Council on Animal Care. Heads are immediately frozen in liquid N2 and the brains are removed from the skull at -20°C, followed by cryo-sectioning.

Tissue Sectioning:
Brain cryosectioning for X-ray fluorescence imaging (XFI) were cut at 30 um-thick onto metal-free Nunc Thermanox coverslips (Thermo Fisher Scientific Inc.) allowed to air-dry at ambient temperature before analysis, while tissue for Fourier transform infrared (FTIR) imaging was cut at 14 um-thickness and mounted onto CaF2 discs.(Caine, et al. 2016) Whole body tissue sections were prepared by freezing the whole body immediately post-mortem followed by embedding within a frozen cellulose gel matrix. Sagittal cryosections were collected using a Leica CM3600 XP Cryomacrotome (Leica Microsystems Inc.) at 30 um thickness,(Okutan, et al. 2016) mounted on Kapton tape (DuPont de Nemours, Inc.) for XFI, and freeze-dried.

XFI.:
Elemental maps were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamlines 10-2 and 2-3, with the SPEAR3 storage ring operating in top-up mode at 3GeV and 500 mA, and at the Canadian Light Source (CLS) on the BioXAS Imaging beamline, with the storage ring operating at 2.9 GeV and between 150-220 mA. SSRL Beamline 10-2 is equipped with a 30-pole 1.45-Tesla wiggler, using a Si(111) double-crystal monochromator (phi = 90o orientation). A 35 um x 35 um microfocussed beam and samples were raster scanned using Newport IMS Series stages (Irvine CA, USA) in 30 um steps with a dwell time of 200 ms per point. SSRL Beamline 2-3 is equipped with a 1.3 T bend magnet. Data was collecting using a Si(111) double-crystal monochromator and a Kirkpatrick-Baez mirror system to achieve a beam size of ~2 um x 2 um. Newport stages were used with a 2 um step size and a dwell time of 200 ms per point. CLS beamline BioXAS Imaging is equipped with a 164-pole in-vacuum undulator with a peak field of 1.011 T. Data was collected with a liquid N2-cooled Kohzu Si(111) monochromator and a aperture to achieve a 40 um (brains) or 100 um (whole body) spot size. Newport stages were used with a 30 um step size (oversampling with 40 um beamspot) and 100 um step size (100 um beamspot), using a dwell time of 100 ms and 60 ms, respectively. Fluorescence was acquired using a silicon-drift Vortex detector with an incident energy of 13,450 eV.

RESULTS AND DISCUSSION

We have established the HFE0/0 colony. Iron accumulation continues into adulthood. Due to the potential for pups to be born with elevated iron status, above the previous generation, we are tracking iron levels across successive generations (the colony is now in 4th-gen). We have begun characterizing the HFE0/0, HFE0/+ and wild type mice, as well as wild type mice fed a high iron diet for 1-month. Pilot ischemic stroke experiments demonstrate that high iron animals have more hemorrhage than wild type mice. Evidence from pilot experiments for increased hemorrhagic transformation in our post-stroke treatment groups using tPA or TNK has not reached statistical significance. Comparison of HFE0/0, HFE0/+ and wild type mice is currently underway at the CLS.

CONCLUSION

Experiments are on-going and will be published in peer-reviewed high impact journals.

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Worker Radiation Safety Practices During Mobile Equine Radiography and Associated Radiation Doses

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INTRODUCTION

Portable X-ray equipment is routinely used in equine clinical practice during radiographic procedures. Equine workers perform radiographic studies under different roles such as cassette holder, X-ray tube operator, and horse restrainer. These workers may be exposed to high levels of scattered radiation because of factors inherent to the nature of radiographic examination of the equine patient. Use of mechanical devices to hold the X-ray tube and the cassette as well as use of personal protective equipment (PPE) is recommended by the National Council on Radiation Protection and Measurements (NCRP) during the use of portable X-ray equipment. There is limited data describing radiation safety practices (Surjan et al., 2015) among equine workers and reporting accurate measurements of radiation doses received by them. (Šterc and Lepková, 2006; Hupe and Ankerhold, 2008; Tyson et al., 2011) Objectives of this study were to describe radiation safety practices among equine technicians and to report radiation doses to the hand for six radiographic studies.

DR. NIELS KOEHNCKE

MATERIALS AND METHODS

In the first part of the study, a questionnaire was distributed electronically to 884 members of the American Association of Equine Veterinary Technicians and Assistants. The survey contained questions regarding radiation safety practices during radiographic examinations. Wilcoxon signed-rank test was used to compare the percentage of time PPE was used between workers involved as cassette holders and as tube operators. Kruskal-Wallis and Wilcoxon Rank Sum were used to assess correlations between risk factors and outcomes. In the second part of the study, an equine cadaver in a standing position was used to simulate six radiographic studies for radiation dose measurements for workers holding the X-ray tube and the cassette by hand. An experienced veterinarian positioned a portable X-ray tube and a radiographic cassette. Scatter radiation doses to the hand was measured using a 180cc pancake flat ion chamber which was placed in the same orientation as the worker's hands with no shielding, with the ion chamber enclosed in a 0.5 mm lead equivalent glove, and with the ion chamber enclosed in a lead- free glove. Thirty exposures were performed for each study view and each condition (n = 1920). A solid-state ion chamber was used to measure doses in the primary beam. Mean doses were assessed with mixed linear regression accounting for repeated measures for role of worker and study type.

RESULTS AND DISCUSSION

One-hundred and fifty-eight respondents had been involved in an equine radiographic examination in the last year, comprising our study population. Eighty-four percent of these workers reported operating an X-ray tube, and 96% reported being involved as the cassette holder. Only three-quarters of our study population wore aprons, and only one-third wore thyroid shields. The reported use of eye shielding was very low, in part due to lack of availability; 90% of workers never wore lead eyeglasses, with 46% not having them available. More than half of X-ray tube operators and of cassette holders reported holding the tube and the cassette by hand, respectively. When holding equipment by hand, more than half of tube operators and one-quarter of cassette holders reported that they never wore gloves. Based on previous data (Ellis et al., 2019) and on the close proximity to the patient, it is generally assumed that cassette holders are exposed to higher levels of scattered radiation than tube operators. However, mean scattered radiation doses to the cassette holder's hand were higher than for the tube operator's hand for fetlock and hock studies ($P < .001$) only, whereas mean doses to the tube operator's hand were higher than for the cassette holder's hand for thoracic spine, lumbar spine, and hoof studies ($P < .01$). In addition, doses to tube operators for thoracic and lumbar spine were the highest among all studies, considering both roles of workers.

CONCLUSION

Our findings indicate that there is a lack of compliance of some of the technicians' practices with radiation safety regulations and, therefore, their exposure to ionizing radiation is at times not as low as reasonably achievable. Based on doses reported in the simulation study, both cassette holders and tube operators should wear protective gloves during radiographic examinations with portable equipment.

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The Utility of Radiation to Treat Vascular Prosthetic Graft Injections

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INTRODUCTION

DR. DAVID KOPRIVA

Prosthetic graft infections following vascular surgery are associated with a high risk of morbidity and mortality. The risk of major limb amputation is as high as 70%, and the mortality risk up to 75% with aortic graft infections (O'Hara et al., 1986; O'Connor et al., 2006; Legout et al., 2012; Erb et al., 2014). Prosthetic graft infections are often associated with biofilms that make antibiotic therapy ineffective in clearing the infection (Khardori and Yassien, 1995). It is therefore necessary to remove the infected graft surgically to afford the patient a cure. Removal of infected aortic prosthetic grafts, followed by reconstruction of lower extremity blood flow is complex, high risk and morbid. Our experience has shown that reconstruction of infected aortic grafts, using the neo-aorto iliac segment (NAIS) technique, was associated with a mean operative time of 439 minutes, 9.3 units of blood transfused and 27% mortality (Tse, Kopriva and McCarville, 2010). Less invasive approaches to the treatment of prosthetic aortic graft infections would be desirable. Ionizing radiation has been utilized as radiotherapy for cancer. However, it is also useful for non-malignant diseases, such as Dupuytren's Contracture and Acne Vulgaris (Sagher and Tas, 1960; Zirbs et al., 2015). Industrial applications have taken advantage of the ability of ionizing radiation to destroy bacteria in sterilization of pharmaceuticals, food and medical devices (Gopal et al., 1988; Hansen and Shaffer, 2001). The most common organisms to cause aortic prosthetic graft infection are Staphylococcus aureus, coagulase negative Staphylococcus species (Staphylococcus epidermidis) and Enterobacteriaceae (Escherichia coli and Enterobacter species) (Legout et al., 2012). Other common organisms include Enterococcus species, and anaerobic bacteria (Erb et al., 2014). D-values represent the radiation dose needed to inactivate 90% of bacterial burden within a sample (MOREIRA, EKPANYASKUN and BRABY, 2010). Staphylococcus aureus ATC 13565 in the stationary phase had a gamma radiation d-value of 360 Gy in deboned chicken meat (THAYER and BOYD, 1992). For E. coli O157:H7, the estimated d-value in meat isolates is 340 Gy (RAJKOWSKI and THAYER, 2000). To place this within context, radiotherapy for lung cancer utilizes fractionated doses with a total delivery of 50-60 Gy (Dillman et al., 1990).

Delivery of the total dose is limited by toxic effects of the radiation. The d-value for typical causative microorganisms in aortic prosthetic graft infection are a factor of six to seven times the radiation dose that can be delivered in advanced lung cancer, so it is unlikely that external beam radiation will be useful in treating infected vascular grafts. However, endovascular techniques have been revolutionary in the field of vascular surgery. Using endovascular systems to deliver a source of low penetration radiation, directly into the lumen of an infected aortic graft, might provide a means to eradicate an aortic prosthetic graft infection without inducing host toxicity. The purpose of the present research is to determine, in an ex-vivo model of prosthetic vascular graft infection, the response to radiation of common causative organisms. This will determine the feasibility of endovascular radiation delivery as a treatment of vascular prosthetic graft infection

MATERIALS AND METHODS

Portions of sterile knitted polyester (Dacron®) vascular graft (Vascutek Gelsoft Plus – Terumo; Refrewshire, Scotland) were cut with a punch into uniform 1 cm pieces, weighing 4 mg and incubated to stationary phase in Tryptic Soy Broth innoculated with one of the following bacterial organisms: Staphylococcus aureus ATCC#25923, Escherichia coli ATCC#25922, Staphylococcus epidermidis ATCC#12228. The experimentally infected portions of vascular graft were transferred to a sterile 4 x 6 well container and immediately transferred to the linear accelerator at the Western College of Veterinary Medicine where radiation dose from 0 – 500Gy was delivered with 6MV photons, using a CT-based computerized treatment plan, and bolus to ensure the planned dose was delivered. The samples were then sonicated and quantitatively plated at various dilution factors to quantitate bacterial presence. After 24-hour incubation, colony counts were used to calculate the number of colony forming units per gram (CFU/g) of Dacron. The radiation dose-response analysis was performed using R software version 3.5.0. The data were logarithmically transformed using a base of 10 and were modelled with a linear model adjusted for pre-irradiation concentration and fitted with restricted cubic spline functions and 4 knots.

RESULTS AND DISCUSSION

Dose response curves are shown below for each of the organisms studied. One logarithm reduction in bacterial counts on infected vascular graft is acheived at doses of 100-300 Gy, depending on the organism. S. epidermidis appeared most sensitive to radiation, followed by E. coli. S. aureus was the most resistant to killing by irradiation.

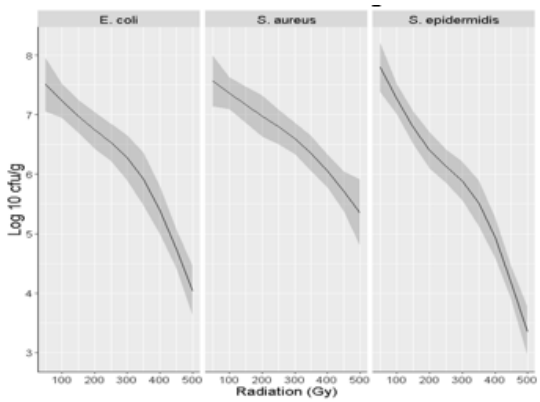

Figure 1: Plots of bacterial survival by species, as a function of radiation dose.

CONCLUSION

The feasibility of endovascular irradiation as a treatment for prosthetic vascular graft infection depends on the ability to deliver doses of 100-300 Gy of low penetrating radiation within an infected prosthetic vascular graft.

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An Investigation into the Relationship Between Childhood Asthma and Mental Health Conditions

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INTRODUCTION

DR. JOSHUA LAWSON

Multimorbidity has been widely researched in adults but much less so in children.1 It can create a set of complex needs along with increased social and health service utilization and significant impacts on clinical decision-making.2 Asthma and mental health conditions (e.g. anxiety and depression) are two of the most important sets of chronic conditions experienced by children. Among children, the prevalence is high for each (asthma: 14-20%3 ; mental health conditions: 1.1% to 31.9%4-6), they account for a huge amount of health care utilization and health care costs,7-9 and they are the two most common reasons for participation and activity limitations.10 An increased risk of having anxiety or depression among children with asthma has been observed.11-13 Despite the importance of multimorbidity and the potential impact on health care utilization, little work has been completed to look at the relationship between mental health conditions and asthma in this area.14 The overall aim of this analysis was to investigate the relationship between childhood asthma and mental health conditions. Our specific research questions were:

1. What are the temporal relationships between asthma and mental health conditions?
2. Are there personal characteristics (e.g. age, sex, location of dwelling) that predict mental health conditions among children with asthma?
3. Are there differences in medication use and health care utilization between those with and those without a mental health condition among those with asthma?

MATERIALS AND METHODS

We used data from Saskatchewan Health. Use if this data was approved by the Saskatchewan Health Data Access Review Committee for ethical and practical review as well as through the University of Saskatchewan (Bio 16-91). The Saskatchewan Health databases cover 99% of the Saskatchewan population with exceptions including RCMP, armed forces, inmates, etc. In addition to this, Saskatchewan Health does not have information on drug filling for First Nations persons as this is covered by the Federal government. Therefore, we excluded this group from the analyses. The person registry includes information on beneficiary status, birthdate, sex, and location of dwelling. It also has a common identifier which can be used to link the databases. Other databases used included the outpatient prescription drug (date, type, form, amount, strength, days' supply), hospitalization (dates, diagnoses, procedures), and physician visit (date, diagnoses) databases.

We used a retrospective birth cohort study design. Participants were born between January 1, 1995 and December 31, 2013. Every person born between these dates and who had either a physician visit or hospitalization where there was a diagnosis of asthma included as part of a health care visit were included in the cohort. The date of that first physician visit for asthma or the discharge date of that first hospitalization for asthma was considered the index date or date of diagnosis. Once a person was entered into the study, their entry date will be considered either the date of birth or Saskatchewan Health coverage initiation. Their exit date for the study was the earliest of their 18th birthday, coverage termination, death, or December 31, 2014 (end of study). All requested information from the health services history (i.e. the outpatient prescription drug, hospitalization, and physician visits databases) was compiled for each person included in the cohort from entry date until exit date. Condition presence was based on the physician services and hospitalization databases using ICD 9 and 10 coding. Among children with asthma, we determined if and when a mental health condition developed relative to asthma diagnosis. Predictors of a mental health condition, a specific type of health care utilization, or medication use was examined by Cox Regression survival analysis adjusted for sex, age, location of residence, cohort, moved, allergic disease.

RESULTS AND DISCUSSION

The mean age of asthma diagnosis was 3.8 years (SD=3.5) and 24.4% of children had a mental health condition. Specifically, 10.8% had anxiety and 5.0% had depression. Most of these were diagnosed after asthma diagnosis (99.5% and 92.2%, respectively). After adjustment for confounders, age and cohort of birth showed a higher likelihood of depression and anxiety. Rural dwelling and being male showed an inverse association with each mental health condition. Depression was associated with an increased risk of asthma hospitalization and filling a prescription for oral corticosteroids. The results are consistent with the literature where the likelihood of a mental health condition increases with age, appears to be increasing over time, and is more common in females. While, in general, mental health conditions are less likely in rural areas, this has rarely been studied in children and never as a comorbid condition with asthma. There are a number of potential explanations for this finding including personal, environmental, social, behavioural, and health care access-related but to date this has rarely been examined. Asthma hospitalization and filling an oral corticosteroid are considered indicators of poor control or higher severity. Depression, specifically, could lead to worse asthma outcomes with proposed mechanisms being through inflammation or depression leading to lower adherence to medications.15

CONCLUSION

A high proportion of children with asthma develop a mental health condition after their asthma diagnosis and, at least with depression, could impact poor asthma control or increased severity. Future research should further consider the presence of comorbidity, potential explanatory factors, and impact. Specifically, consideration of the role of location of dwelling should be considered as rural and urban areas are known to differ in a number of the determinants possibly related to asthma or mental health conditions16-20 and provide an opportunity to examine some of the explanations for presence of comorbidity and its impact on outcomes.

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Treatment of Weight Gain in Psychotic Patients on Clozapine Using Low Dose Psychostimulants

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DR. ROHIT LODHI

INTRODUCTION

Patients with psychosis have lower life expectancy largely due to a high prevalence of metabolic and cardiovascular diseases (1,2). Clozapine is a medication used for treating resistant psychosis but it has the worse metabolic profile among all antipsychotics. Weight loss or a reduction in the amount of weight gain is difficult to achieve with this medicaiton. Psychostimulants (methylphenidate, dextroamphetamine and lisdexamphetamine) have been reportedly used with clozapine to reduce sedation, cognitive symptoms and psychomotor retardation in patients with psychosis (4,5,6). There is no study on the long-term protective effects of psychostimulants on clozapine induced weight gain (7). In the Saskatoon Severe and Persistent Mental Illness Clinic (SPMIC), there are patients that were started on stimulant medications. We investigated if the addition of stimulants changes the weight and blood pressure trajectory.

MATERIALS AND METHODS

Retrospective clinical chart review was performed on patients (18-65 years old) with a clinical diagnosis of Schizophrenia, Schizoaffective disorder and Psychosis NOS who attended the SPMIC between (2013-2018). Eligibile patients at the SPIMC include those that had been on clozapine for more than six months. Case notes were used to extract information on age, gender, duration of clozapine exposure, clozapine dosage and type of stimulant medication over the next 18 mnths. Clozapine patients on stimulants considered as the 'exposed group' and those on clozapine only considered as the 'control group'. Multilevel Linear Mixed Model Analysis (MLMM) was conducted using STAT 15.1 to compare weight and blood pressure change in stimulant treated (ST) with no stimulant treated (NST) groups using a group-time interaction variable.

RESULTS AND DISCUSSION

200 case notes were examined but only 45 had enough data relevant to our study. Adequate inforation was available at approximately 0,6,12 and 15 months. Number of patients in the ST and NST groups were 26 and 19, respectively. NST was significantly older than the ST group (P=0.002), but otherwise the groups were comparable. MLMM analysis revealed that there was no difference between the ST and NST groups in terms of weight change over time (β=-0.03, P=0.98), systolic BP (β=-0.34, P=0.69), and diastolic BP (β=-0.87, P=0.27).

Table 1. Sample description

	ST	NST	*p*
N	26	19	
Mean Age	33.05	45.52	**0.002**
Sex (%)			0.926
Male	57.89	59.26	
Female	42.11	40.74	
Diagnosis (%)			0.884
Schizophrenia	56.52	58.82	
Schizoaffective/Bipolar	43.48	41.18	
Mean Clozapine dose	268.63	270.83	0.971
Mean follow up (years)	0.82	0.93	0.482

Table 2. MLMM results – Change in weight over time

	Coeff	SE	p	95% CI	
Age	0.072	0.28	0.80	-0.49	0.63
Sex	-7.06	8.41	0.40	-12.0	9.42
Diagnosis	6.53	9.20	0.47	-11.5	13.57
Clozapine dose	-0.039	0.02	0.11	-0.08	0.01
Time	1.355	1.15	0.24	-0.91	3.62
Group	7.48	8.20	0.36	-8.59	15.5
Group#time					
ST	-0.031	1.54	0.98	-3.05	2.99

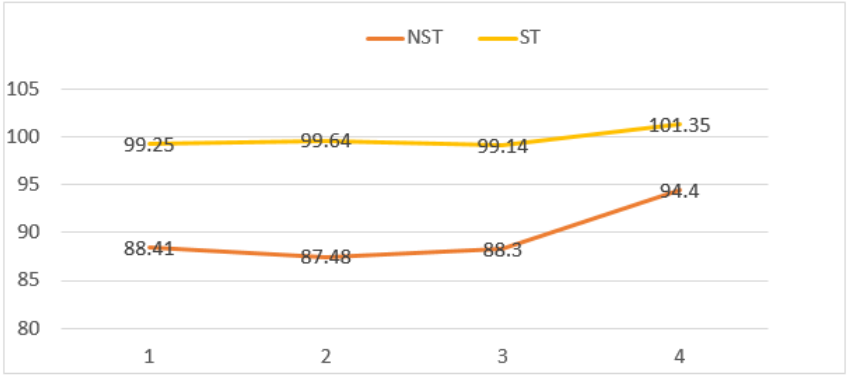
Table 3. MLMM results – Change in Systolic BP over time

	Coeff	SE	*p*	95% CI	
Age	0.42	0.12	0.001	0.17	0.66
Sex	-3.83	3.07	0.21	-9.85	2.18
Diagnosis	-0.34	2.53	0.88	-4.95	4.26
Clozapine dose	-0.022	0.01	0.03	-0.04	-0.02
Time	0.10	0.48	0.83	-0.84	1.04
Group	2.67	3.64	0.46	-4.45	9.81
Group#time					
ST	-0.37	0.94	0.69	-2.22	1.47

Table 3. MLMM results – Change in Diastolic BP over time

	Coeff	SE	*p*	95% CI	
Age	0.044	0.08	0.60	-0.12	0.21
Sex	-2.77	2.12	0.19	-6.94	1.40
Diagnosis	1.70	1.62	0.23	-1.47	4.88
Clozapine dose	-0.001	0.007	0.87	-0.03	0.01
Time	-0.55	0.43	0.19	-1.40	0.28
Group	-0.65	2.57	0.80	-5.69	4.39
Group#time					
ST	-0.87	0.79	0.27	-2.42	0.68

Fig. 1: Change in weight (in KG), over visits (at approximately 0,6,12 and 15 months) in the stimulant (ST) and non-stimulant (NST) clozapine groups



CONCLUSION

Our pilot study indicates that co-prescription of therapeutic stimulant medications does not change the trajectory of weight and blood pressure in clozapine treated patients. Clozapine related metabolic dysfunction should be monitored on a regular basis.

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Blood Markers and AVF Maturation in an End Stage Renal Disease

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INTRODUCTION

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Arteriovenous fistula (AVF) is associated with a lower incidence of patency-related procedures. Asif et al. [1] indicated that the percentage of AVFs that fail to develop sufficiently for successful cannulation and hemodialysis is 28-53%. Research studies conducted on predictive markers of an adequate and operative fistula have failed to reach a consensus on AVF predictive markers, due to the fact that they have focused on different aspects and have used different definitions of maturation, study design, clinical factors and patient samples [2]. Thus, surgeons are confronted with the challenge of classifying the different risk factors as well as deciding on the parameters to use in order to evaluate the possibility of successful fistula development. It may be possible to improve the end results of vascular access by gaining a more comprehensive picture about the various factors involved in the maturation of fistulae. This could then in turn provide important information during the pre-surgical evaluation that surgeons can base their decisions on. Independent predictive factors may be beneficial in anticipating successful fistula maturation without the use of invasive tests, and this could be cost-effective. The main purpose of this study was to examine the potential influence of patient factors, blood markers on the maturation of AVF on the maturation of AVF.

MATERIALS AND METHODS

This is a prospective cohort study conducted within the Regina General Hospital. This study has been reviewed and approved on ethical grounds by the research ethics board of the former Regina Qu'Appelle Health Region, Regina, SK, Canada (REB/18-77). After ethics review board approval, potential participants were assessed by the consultant vascular surgeon. The participants were purposively recruited by vascular surgeons on the basis of the inclusion and exclusion criteria; diagnosed 18 years and older ESRD patients with expected initiation of hemodialysis within 12 months of enrolment, or current dialysis dependence and those who will receive first time AVF surgery in Southern Saskatchewan. Patients who had undergone a repeat AVF creation (2nd and further fistula creations) and had previous history of kidney transplant were excluded. Statistical analysis was performed using SPSS (IBM SPSS Statistics 24.0). Analysis of categorical variables by Chi square test and Fisher's Exact Test was performed to assess difference between mature and immature fistulae outcome (mature to needle and those that failed to mature-inadequate for dialysis). The association between blood markers between immature and mature AVF cohort was assessed utilizing Independent sample t-test.

RESULTS AND DISCUSSION

A total of 30 study participants were recruited. Of those, about 16 participants who have had their 6 week follow up with the rest pending. Successful maturation of the AVF was achieved in 11 (68.8%) patients as assessed by vascular surgeons. No statistical significant association ($p > 0.05$) was observed between fistula outcomes (mature/immature) and patient's demographics (Table 1).

Table 1. Selected demographics of study participants

	Mature AVF	Immature AVF	Total	P Value
Age ≤ 65 yrs.	8 (72.7)	4 (80)	12 (75)	0.63
Male	9 (81.8)	5 (100)	14 (87.5)	0.46
Left arm fistula	7 (63.6)	5 (100)	12 (75)	0.18
Radiocepahilic	4 (36.4)	4 (80)	8 (50)	0.14
Brachiocephalic	7 (63.6)	1 (20)	8 (50)	0.14
No PVD	8 (72.7)	4 (80)	12 (75)	0.63
Diabetics	6 (54.5)	4 (80)	10 (62.5)	0.35
Smoker	5 (50)	3 (60)	8 (53.3)	0.57
Hypertensive	9 (81.8)	5 (100)	14 (87.5)	0.46
Obese	6 (54.5)	2 (40)	8 (50)	0.50
Previous use of CVC for HD	9 (81.8)	2 (40)	11 (68.8)	0.14

No significant difference was observed in vein diameter (p-0.15 95% CI: -1.5 to 1.5). However, artery diameter was significantly (p- 0.008; 95% CI: 0.5 to 2.8) wider among patients with mature AVF (4.32±1.60) compared with immature AVF (2.66±0.48). Similarly significant difference ($p>0.05$) was observed between mature and immature AVF patient's serum urea, parathyroid hormone, triglycerides and hemoglobin level (Table 2).

Table 2. Blood markers of study participants

	Mature AVF (mean±SD)	Immature AVF (mean±SD)	P Value
Vein Size (mm)	4.67±1.65	3.70±0.91	0.15 (-1.5 to 1.5)
Artery Diameter (mm)	4.32±1.60	2.66±0.48	0.008 (0.5 to 2.8)*
Serum Albumin	32.64±4.7	32.80±4.8	0.95 (-5.6 to 5.3)
eGFR (ml/min/1.73m^2)	9.54±3.7	12.20±2.2	0.16 (-6.5 to 1.2)
K^+ (mmol/L)	138.82±2.7	140.20±5.3	0.80 (-0.93 to 0.74)
Na^+ (mmol/L)	4.76±0.8	4.86±0.64	0.60 (-7.8 to 5.0)
Ca^{++} (mmol/L)	2.46±0.16	2.30±0.18	0.13 (-0.06 to 0.38)
Blood PH	24.50±3.7	23.00±5.9	0.63 (-5.7 to 8.7)
Creatinine (mmol/L)	578.09±221.1	457.80±74.9	0.13 (-40.26 to 280.8)
Urea (mmol/L)	16.12±3.9	25.44±6.1	0.003 (-14.9 to -3.7)*
Parathyroid Hormone (pmol/L)	30.75±15.0	52.56±26.6	0.052 (-43.8 to 0.26)*
SBP (mm of Hg)	152.45±16.5	157.20±34.63	0.78 (-46.9 to 37.5)
DBP (mm of Hg)	86.45±10.8	74.00±12.9	0.06 (-0.78 to 25.7)
HbA1c	6.88±1.9	6.90±0.69	0.98 (-2.0 to 1.9)
PTT (second)	32.00±3.0	32.75±3.3	0.71 (-5.6 to 4.1)
INR (ratio)	0.97±0.1	1.04±0.1	0.28 (-0.19 to 0.06)
TC (mmol/L)	4.25±0.9	3.25±0.9	0.08 (-0.14 to 2.13)
TG (mmol/L)	2.24±1.2	1.13±0.5	0.02 (0.19 to 2.03)*
HDL (mmol/L)	0.96±0.2	1.10±0.3	0.33 (-0.46 to 0.18)
LDL (mmol/L)	2.10±1.0	1.64±0.7	0.32 (-0.51 to 1.4)
Hemoglobin	115.60±10.7	103.40±7.3	0.02 (1.88 to 22.5)*
WBC level	7.5±2.7	8.00±1.9	0.68 (-3.1 to 2.1)
Platelet count	232.90±74.7	216.00±75.8	0.69 (-78.4 to 112.2)

CONCLUSION

The clinical utility of risk categories in the maturation of arteriovenous fistula requires further validation in a lager sample size, and multi-centre for longer follow-up.

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Exploration of End of Life Care in Lung Cancer and COPD

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INTRODUCTION

DR. ERIKA PENZ

Lung Cancer and Chronic Obstructive Pulmonary Disease (COPD) are two of the leading causes of death in Canada (lung cancer the leading cause of cancer deaths and COPD the 4th common cause of death)[1]. Death for these patients, often due to respiratory failure, is heralded by symptoms of breathlessness, anxiety and sometimes confusion and agitation, leading to distress for both patients and caregivers. Even more grim for lung cancer patients in our province is that 80% of those who die end up dying in hospital [2]. The costs associated with dying in hospital are arguably enormous, both from a personal/caregiver perspective but also from a health system point of view [3]. Hospice and out-of-hospital care models have the potential to improve the experience of patients at the end of life and potentially reduce the burden on the hospital system at a lower cost [4]. However, widespread adoption of a hospice/end of life program in the absence of a fulsome understanding of the costs and outcomes associated with our current paradigm compared with a new program creates uncertainty for many decision makers and contributes to a reluctance in adopting change. The primary objective of our study is to describe and compare healthcare utilization, including palliative care services, for Saskatchewan residents living with lung cancer and COPD in the last year of life using Saskatchewan Health Administrative and Cancer Registry Data. The Secondary Objective of our study is to develop a simulation model, using inputs obtained from Objective 1, to evaluate the impact of different care models for end of life care on healthcare costs and patient outcomes.

MATERIALS AND METHODS

We will access an existing cohort comprised of Saskatchewan residents age 35 years and older diagnosed with 1) lung cancer between January 1, 2000 and December 31, 2015 through the Saskatchewan Cancer Agency (SCA) Registry AND 2) COPD, identified using Saskatchewan Health administrative databases during this same time period, using nationally validated Canadian Chronic Disease Surveillance System definition[5]. Access to our cohort data will occur at the Saskatchewan Health Quality Council within a data sharing agreement. Descriptive analysis of demographic and clinical characteristics of the cohort will be performed. Within our defined cohort of lung cancer and COPD patients, we will identify a subset of this cohort whom have died between 2010 and 2015. For this subset, we will describe overall healthcare utilization in the year prior to death, stratified by diagnosis. We will then compare healthcare utilization and costs for those individuals who received palliative care services versus those who did not receive palliative care services. We will develop an agent-based model, incorporating data from our cohort, to describe the care path experience for those with COPD and lung cancer at the end of life.

The model will simulate observed patients' transitions between different states based on data obtained from our analysis described above. Each state depicts a care setting (e.g., hospital) with transition probabilities between states derived from the cohort data. The simulation model will start from the current provision of services to these patients in their last year of life to establish the baseline scenario. By altering the baseline scenario, we will then begin to develop and test different care pathways by increasing the uptake levels of the community, hospital & palliative care services (which will result in decreasing hospital admissions and/or acute length of stay). The model will estimate and compare the total healthcare costs for these patients under different modelling scenarios.

RESULTS AND DISCUSSION

A total of 117,952 patients are included in the cohort, including 3132 with lung cancer, 110,884 with COPD and 3936 with lung cancer and COPD combined. Mean age of those with lung cancer and COPD combined is older than those with COPD or lung cancer alone. Among those with lung cancer and COPD, a higher proportion of those with non-small cell lung cancer are early stage (24%) versus those with lung cancer alone (14%). Death among individuals in the cohort between 01/01 2010 and 31/12 2015 will be reported. Proportion of individuals with palliative care involvement, identified via physician billing claims will be identified including time from first consultation to death. Healthcare utilization will be compared amongst groups. See Table 1 for a visual display of results to be reported.

Table 1. Deaths and Healthcare Utilization last year of life

	Lung Cancer		COPD		Lung Cancer & COPD	
Deaths	N=		N=		N=	
Healthcare Utilization	Mean (STD)		Mean (STD)		Mean (STD)	
MD visits						
Hospitalizations						
Hospitalization (last 30 days life)						
Intensive Care Unit (last30 days life)						
Hospitalization at time of death						
Home care involvement %						
Opioids prescribed %						
Palliative Care Consultation	Yes	No	Yes	No	Yes	No
% received						
Mean time from 1st consult to death						
Healthcare Utilization	Mean (STD)		Mean (STD)		Mean (STD)	
MD visits						
Hospitalizations						
Hospitalization (last 30 days life)						
Intensive Care Unit (last30 days life)						
Hospitalization at time of death						
Home care involvement %						
Opioids prescribed %						

CONCLUSION

The purpose of this project is to build an understanding of end of life care experiences using administrative data for patients with lung cancer and COPD and use simulation modelling to help understand the impact of different care pathways on health outcomes and costs. Administrative data, although it has its own limitations, provides rich information detailing care patterns for large groups of patients; combined with sophisticated modeling of "what if" scenarios exploring different care pathways, can be a powerful tool for decision makers when making healthcare system change. How we design our health system matters. It determines where we spend our limited resources and by extension, what we forgo (the opportunity cost). Given our limited resources and that, choices must be made amongst programs and services, it is imperative that we understand value for money spent on healthcare at the end of life. We can only know this by asking ourselves what care is currently being delivered and whether it meets the needs and preferences of the people we serve. Key components of this project, funded through the College of Medicine Research Award (COMRAD) include completing data access requests with the SCA, a data sharing agreement (between SCA, Health Quality Council(HQC), and the Ministry of Health) and an ethics application; almost complete.

We have received Saskatchewan Centre Patient Oriented Research (SCPOR) support to complete the remainder of the analysis and use our results in the development of a simulation model to explore alternate delivery-of-care models at the end of life and study the impact on health system costs. We plan to share these results with stakeholders at the Saskatchewan Health Authority, Ministry of Health and Cancer Agency.

ACKNOWLEDGMENT

The authors would like to express appreciation for the support of Tracey Sherin, Tanya Verrall at the Health Quality Council and Serena Kozie at the Saskatchewan Cancer Agency. This study is based, in part, on de-identified data provided by the Saskatchewan Ministry of Health and eHealth Saskatchewan. The interpretation and conclusions contained herein do not necessarily represent those of the Government of Saskatchewan, the Saskatchewan Ministry of Health, or eHealth Saskatchewan.

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Pediatric Dentistry Under General Anesthesia: A Saskatchewan Profile

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DR. MATEEN RAAZI

INTRODUCTION

More than 19,000 Canadian children aged 0-6 years undergo dental treatment under general anesthesia (GA) annually.1 National data suggest that children of low socioeconomic status, from rural areas, and from neighbourhoods with a high proportion of Indigenous peoples are more likely to receive dental care under GA.2 In addition to the usual risks of GA, concerns remain about the potential long-term neurodevelopmental risks of early and repeated exposure to GA.3-7 The objectives of this study were to determine the prevalence and predictive factors for dental treatment under GA in Saskatoon, Saskatchewan, a tertiary care referral center with a catchment area of 500,000 people.

MATERIALS AND METHODS

Following approval from the Research Ethics Board at the University of Saskatchewan, we conducted a retrospective review of children aged 0-6 years who underwent dental treatment under GA in Saskatoon between the years 2015-2018. Demographic, dental diagnostic and treatment data, and number of previous exposures to GA were collected. Quantitative analysis included comparisons of proportions and measures of associations between demographic clinical variables.

RESULTS AND DISCUSSION

A total of 570 patient records were reviewed. The mean age of children in the sample was 4.57± 0.73 with 21.4% having required dental treatment under GA on more than 1 occasion. Fifty percent of the sample lived within a 1-hour driving distance from Saskatoon, compared to 22.7% who lived greater than 3 hours away. The dental treatment needs for the sample were complex, with children having a mean of 10.85 ± 3.56 teeth treated, at an average dental treatment cost of $3231.72 ± 898.95 per child (Table 1). Comparisons based on driving distance revealed that children who lived greater than 1-hour driving distance away from Saskatoon had a significantly higher severity of tooth decay, total number of teeth treated, total extractions, total crowns placed, and cost of dental treatment (Table 2). Additionally, children who lived greater than 1-hour driving distance away from Saskatoon were 30% more likely to have had previous dental treatment under GA (OR: 1.3, 95% CI: 1.03 – 1.65) when compared to those who lived closer.

Table 1. Summary of clinical findings for children requiring GA services for dental treatment

Outcome Measure	Mean ± SD
Total time under GA in minutes	104.46 ± 27.40
DMFT/dmft index [decayed, missing, filled teeth]	11.52 ± 3.84
Total teeth treated	10.85 ± 3.56
Total restorations placed	8.62 ± 3.06
Fillings	2.51 ± 2.93
Stainless steel crowns	6.11 ± 3.13
Total extractions	2.22 ± 2.53
Total sealants	0.31 ± 1.05
Total dental treatment cost per child	3231.72 ± 898.95
Total anesthesia cost per child ($)	784.33 ± 159.81

Table 2. Differences by driving distance from primary treatment site

Outcome measure	Within 1-hr	Greater than 1-hr	95% CI
Repeat treatment under GA (OR)	0.80	1.30**	0.70-0.95;1.03-1.65¥
Total time under GA in minutes (mean)	105.16	103.74	99.9 - 108.97 ₸
DMFT/dmft index [decayed, missing, filled teeth]	10.91	12.14***	10.85 - 12.04 ₸
Total teeth treated (mean)	10.32	11.59***	10.28 - 11.42 ₸
Total restorations placed (mean)	8.35	8.90*	8.12 - 8.78 ₸
Total extractions (mean)	1.77	2.68***	1.81 - 2.63 ₸
Total dental treatment cost per child ($)	3056.01	3408.99***	3025.60 – 3336.40 ₸
Total anesthesia cost per child ($)	788.46	780.16	757.74 – 810.26 ₸

₸ Independent T-Test; ¥ Chi-Squared Test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

CONCLUSION

Our results demonstrate that children who live greater than 1-hour away from Saskatoon have a higher burden of disease, are more likely to require repeated GA exposures for dental treatment, and have a higher associated treatment cost. Further analysis is pending to uncover the incidence of dental care under GA for First Nations children. Targeted prevention strategies to reduce the burden of oral disease for high-risk children must be considered to reduce the incidence of dental cases under GA. We further recommend that Health Authorities prioritize addressing geographic disparities that exist with access to timely dental care.

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Effectiveness of Pre-Operative Per-Protocol Selective Testing Versus Routine Testing in Elective Joint Replacement Surgery

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INTRODUCTION

Preoperative tests are done to determine a patient's fitness for anesthesia and to identify those who are at high risk for complications after surgery. Routine preoperative tests are defined as those done in the absence of any specific clinical indication or purpose and are conducted in all patients undergoing a given procedure, regardless of medical history or other patient features. Investigations and tests are unnecessary when they do not provide information that will alter the care or outcome of a patient before or after surgery. In per-protocol testing, preoperative testing is done based on patient medical conditions. Currently, several Canadian medical authorities discourage extensive non-selective routine testing for patients undergoing elective surgeries. Choosing Wisely Canada (CWC) recommends against any unnecessary testing before surgery and the Canadian Society of Anesthesiology (CSA) recommends performing tests based solely on an individual patient's history and medical conditions. Based on the Choosing Wisely Canada recommendations, a quality improvement initiative is currently underway at Saskatoon City Hospital (SCH) to reduce unnecessary testing before hip and knee joint replacement surgery. We hypothesize that by using per-protocol selective testing rather than routine testing, there will be a reduction in the unnecessary tests before planned (elective) hip and knee joint replacement surgery by 25% without increasing the number of complications following surgery, cancellations of surgery, and length of stay in hospital after surgery. Primary Objectives: 1) To determine the impact of per-protocol testing in the pre-admission clinic on the proportion of unnecessary tests as per Choosing Wisely Canada guideline in patients undergoing planned (elective) hip and knee joint replacement surgery in Saskatoon City Hospital. Secondary Objectives: 1) Compare the difference in the mean number of complications following surgery within 30 days between per-protocol and routine testing group. 2) Compare the difference in the mean number of cancellations of the surgery between per-protocol and routine testing group. 3) Compare the difference in the length of stay in hospital after surgery between per-protocol and routine testing group after adjusting for confounding variables.

MATERIALS AND METHODS

This is a quasi-experimental, pre-post intervention study design. The per-protocol group is the post-intervention group that will be compared to the routine test group before the intervention.

RESULTS AND DISCUSSION

Currently data collection is undergoing and results are not available.

Cultural Orientation of HIV Care to Reduce Stigma and Enhance Screening, Diagnosis, and Linkage to Care

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INTRODUCTION

In 2015, 81% of individuals with newly diagnosed HIV infections in Saskatchewan self-identified as Indigenous. Indigenous people living on-reserve in Saskatchewan have an HIV incidence roughly 7.3-times higher than the Canadian average (42.2/100,000) (Charlton, 2020) and is a significant issue in rural First Nations communities. As a result of colonization, Indigenous people are disproportionately affected by a myriad of socioeconomic issues and intergenerational trauma that put them at an increased risk of HIV infections (John, 2008). In addition, stigma, discrimination, and previous negative experiences with mainstream healthcare services are major barriers to accessing healthcare, which leads to underutilization of such services by Indigenous people and consequently, poor health outcomes (Browne et al., 2011). Healthcare professionals should be cognizant of this and ensure cultural safety for all to improve access and utilization of health services; particularly for those living with HIV (Kirmayer et al., 2003). HIV treatment and prevention in Indigenous communities requires innovative interventions that address socioeconomic determinants of health along with disease management (Negin et al., 2015). Recent consultation with members of the Big River First Nation revealed that stigma and lack of knowledge about HIV are barriers to accessing screening and treatment in community. The objective of this study was to explore the current level of HIV-related stigma in the community, and to explore strategies to address stigma and increase HIV-related knowledge.

DR. STUART SKINNER

MATERIALS AND METHODS

Employing community participatory research design (Ramsden et al., 2017); three community consultation meetings were hosted in the community of the Big River First Nation. The research team worked in collaboration with community representatives to complete the ethics application, identify pertinent discussion topics, and finalize research design. The team collectively agreed: a) a strengths-based approach would be adopted, with focus given on identifying strategies to address the current health issue rather than simply identify gaps; and b) participation in research will help build community's research capacity, enhance in-community services and help community access additional resources. There were no resources for a culturally responsive strengths-based questionnaire to measure HIV-related knowledge and stigma; therefore, focus group discussions were chosen to honour oral Indigenous traditions (Kovach, 2010). A story telling approach (Datta, 2018) to explore the current status of HIV-related knowledge and stigma in the community was employed. Based on the findings of focus groups, strategies were to be adopted to improve knowledge and address stigma in the community.

Community representatives assisted with participant recruitment. Focus group 1 consisted of six participants (2 female; 4 males) aged 40 years or older. Focus group 2 consisted of 18 participants (3 female; 15 male) aged between 18-39 years. As per community protocol, tobacco was offered to elders, beginning focus groups with prayer. The focus groups were audio recorded and all participants signed informed consent form. The facilitators kept field notes and identified salient points that emerged during the discussion. These points were then shared with the group at the end of the discussion ensuring that the perspectives of the participants were documented. Light refreshments and a $20 gift card to the community convenience store were provided for each participant. Focus group data was transcribed, coded, and analyzed according to qualitative methods (Miles et al., 2019) using QSR NVivo® 9 (QSR International Pty Ltd, 2010).

RESULTS AND DISCUSSION

Participants indicated lack of HIV related knowledge among all age groups in the community. Participants reported presence of considerable stigma attached to the HIV diagnosis. This often discouraged individuals from accessing screening and treatment available in community. Fast spreading rumors negatively influenced confidentiality and impacted individuals' decision to access HIV screening and treatment in-community. Older adults were very concerned about the impacts of addictions on youth. Currently clients are engaged in several cultural activities with elders such as moccasin making, ribbon skirt making and some land-based activities will be added weather permitting. However, participants mentioned that individuals seemed to be totally disconnected and alienated from life while under the influence of substances. Strategies supporting individuals using or abusing substances had minimal impact thereby further increasing their risk for HIV infections. Participants indicated that HIV education sessions should begin in schools. Education sessions should be offered consistently and ongoing. HIV-related information should be tailored towards specific age groups and offered to all in the community. The content should be simple and easy to remember. Social media was identified as an appropriate mode of knowledge translation among youth, in-person sessions or radio was suggested for older adults, and education through health curriculum can be employed for children. Additional sessions to educate and empower women were suggested. Posters and billboards should be displayed at various locations in the community, e.g., at community gatherings, to normalize HIV diagnosis within the community. The study indicated that in a geographically isolated First Nations community, lack of HIV-related knowledge and stigma can be major barriers to accessing screening and treatment even when such services are available in community. Geographic isolation, lack of access to modern communication mediums like internet, combined with other socioeconomic factors, can impede individuals' access to otherwise easily available health information. Participants identified the need for a systematic strategy targeting all age groups in the community. Participants recognized the need to customize HIV-related information to effectively reach respective age groups. Multiple mediums were suggested to ensure all age groups received reliable HIV-related information. Participants also indicated the need to address addictions, and the importance of starting HIV-related education early on in school. Further, connection with elders, traditional healers, cultural and traditional ceremonies is proposed to positively impact clients with HIV diagnosis. As a next step for this research, it would be of value to assess stigma intervention strategies that arose as a result of this study, as there are relatively few articles that focus on assessing stigma reduction interventions (Mahajan et al., 2008).

CONCLUSION

Lack of HIV-related knowledge can lead to stigma and impact individuals' ability to access screening and treatment in remote First Nations communities. Age appropriate education and information sessions delivered through appropriate knowledge translation mediums are required to address HIV-related stigma and enhance HIV-related knowledge.

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Early OA Detection in Women with Hypermobility of the First CMC Joint

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DR. REGINA TAYLOR-GJEVRE

INTRODUCTION

Osteoarthritis (OA) of the joint at the base of the thumb, the first carpometacarpal (CMC) joint, is a common finding in women that increases in prevalence with age and impacts quality of life and functional abilities [Zhang, 2002; Sodha, 2005; Haara, 2003]. The stability of this unique biconcave saddle joint is dependent on integrity of supporting soft tissue structures [Ladd, 2014]. Disruption or damage to these supporting structures may predispose to development of OA [Hakim, 2003; Wolf, 2011]. Joint hypermobility is a relatively prevalent finding, which has been associated with joint laxity and possibly OA [Castori, 2015; Flowers, 2018; Connelly, 2015; Jónsson, 1996]. OA has generally progressed beyond an early stage by the time it is clinically apparent. Plain radiographs will reveal changes in osseous structure and joint space narrowing, but lack capacity to identify soft tissue changes. MRI is advantaged in the capacity to examine these peri-articular and articular structures [Saltzherr, 2017]. As there is evidence of a dominant genetic inheritance pattern for OA of the hands in women [Irlenbusch, 2006], women whose mothers had OA of the hand were identified as at high risk of developing OA of the hand themselves. In this study we sought to focus on women at high risk of developing OA to explore associations between hypermobility and early changes of OA on MRI [Jónsson, 2009].

MATERIALS AND METHODS

Women between the ages of 30-60 with a self-reported family history of OA in the hand(s) of their mothers were recruited for the study. A pre-existing diagnosis of OA of the hands was an exclusion. Joint hypermobility was identified using a self-reporting, 5-point hypermobility questionnaire designed by Hakim (^{3} 2 positive responses indicate hypermobility) [Hakim, 2003]. Assessment of potential early hand [OA] symptoms on the activities of daily living in hypermobile women [N=28] and non-hypermobile women [N=30] was assessed using the validated Functional Index for Hand OA (FIHOA). The FIHOA questionnaire consists of a 10 question, 4-point scale, with score outcomes ranging from 0 (zero functional impairment) -30 (maximum impairment). [A score ^{3} 5 differentiates symptomatic from asymptomatic participants] (2). General demographic data was collected. Further assessment for symptoms of stiffness, pain and function was made using a 5-question, self-reporting, 100mm (10cm) Visual Analogue Scale questionnaire. Hand morphology tracings, with fingers extended and abducted, were collected. Using the tracings, the distance between the tips of the first and second digits was measured, in mm, using a ruler. Using the hand tracings, a line was drawn, from the middle of the first and second digits respectively, extending towards the hand. The point of intersection between these lines determined the angle between the first and second digits, as measured by protractor. Imaging studies focused on the carpometacarpal joint of the dominant hand. X-Ray: X-ray and MRI imaging of the CMC on the dominant hand was taken. Standard positioning was used for anterior/posterior, oblique and lateral x-ray views of the wrist. MRI: Patients placed in a prone position with the arm extended over the head, on a 3T MRI using a Body Array Coil.

Imaging protocol was run for four static and one kinematic MRI. A 4x6 inch, ¾ inch thick wooden block was used as a spacer between the camera and the table, so as to not restrict the patient's mobility during the kinematic sequences. The hand was prone with the anterior wrist closest to the table. The wooden block was placed flat on the table on the thumb side of the hand leaving enough room for thumb movement. MRI data was collected for cartilage changes, bone changes, soft tissue changes, subluxation and effusion. X-ray images were assessed for sclerosis, cysts, joint space narrowing, subluxation and osteophytes.

RESULTS AND DISCUSSION

[All questionnaire data and images have been collected for this study, however interpretation results from both X-ray and MRI images are not yet available for analysis.] In total 58 women were enrolled in this study. All were right hand dominant. Demographic, morphometric and questionnaire instrument scores are detailed below in Table 1. Non-parametric analysis revealed no significant differences for individual variables between groups based on hypermobility status. Examining this population based on functional status (FIHOA) scores and using a cutpoint of 4 to define groups, we observe significant differences between groups for some individual variables [VAS pain scores ($p<0.001$) and number of pregnancies ($p=0.008$)]. Further analysis is anticipated once imaging interpretation data is available.

Table I: Demographic, morphometric and questionnaire instrument scores for patient groups

Variable	Hypermobile (n=28)	Non-Hypermobile (n=30)
Mean age (years)	46.21 (SD: 8.16)	44.50 (SD: 9.53)
Mean height (m)	1.66 (SD: 0.06)	1.63 (SD: 0.06
Mean BMI (kg/m^2)	27.65 (SD: 5.13)	26.90 (SD: 6.54)
Mean # pregnancies	1.89 (SD: 1.50)	1.17 (SD: 1.41)
Hand morphology: Mean distance between index finger and thumb R (mm)	118 (SD: 17.87)	112.02 (SD: 15.00)
Hand morphology: Mean angle between index finger and thumb (degrees)	45.80 (SD: 12.08)	42.37 (SD: 10.75)
Pain with hand activities VAS mean score	27.58 (SD 23.59)	22.86 (SD: 24.45)
FIHOA score	4.04 (SD: 4.04)	2.92 (SD: 3.31)

CONCLUSION

(Preliminary from data available to date): In women at high risk of hand OA, a significant association between higher FIHOA scores and higher numbers of pregnancies was observed. Higher FIHOA scores were also associated with greater pain during hand activities.

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The Nursing Initiated Community HIV/Hepatitis Engagement (NICHE) Project

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INTRODUCTION

Saskatchewan remains in the midst of significant HIV and HCV epidemics driven by high rates of intravenous drug use in vulnerable populations. Unique aspects of these epidemics in Saskatchewan mirror the epidemiology of at-risk individuals in the province and include disproportionate representation of persons of Indigenous heritage, as well as large numbers of women and persons under the age of 40. HIV incidence rates in the province remain over twice that of the Canadian average with regional rates as high as 10 times greater than the national average. The World Health Organization (WHO) has set ambitious treatment targets to end the HIV/AIDS epidemic by 2020, and to largely eliminate HCV by 2030. Despite an increased investment in resources to diagnose and treat blood-borne infections in Saskatchewan, provincial HIV and HCV incidence rates continue to rise. The ID Clinic at Regina General Hospital serves as the primary tertiary referral clinic for persons living with HIV and/or HCV in southern Saskatchewan, and currently manages over 500 persons living with HIV and over 1,500 persons living with HCV. To address barriers to improving clinical outcomes for persons with HIV and HCV in southern Saskatchewan, the ID Clinic in collaboration with Public Health in Regina have established a series of outreach initiatives designed to improve the availability and consistency of care for persons living with HIV and HCV across Regina. We hypothesized that a well-trained public health nurse (the NICHE nurse) working under protocol and physician supervision to test and manage both HIV and HCV strategically placed in various community settings populated by at-risk persons could successfully improve clinical outcomes for persons living with HIV and HCV in Regina.

MATERIALS AND METHODS

The ID Clinic and Public Health in Regina together supported a 1.0 FTE public health nurse to fill the NICHE role. The NICHE nurse was situated in a variety of clinical settings, including various community outreach clinics as well as the local provincial correctional facility. Protocolized testing and management of HIV and HCV was developed, including the ability for the NICHE nurse to arrange initiation of HCV therapy after discussion with the supervising physician. The position was supported from December 1, 2017 to December 31, 2019. A clinical database was established in ID Clinic to capture demographics and outcome data, and is reported descriptively. Data is reported cumulatively from December 1, 2017 through September 30, 2019 inclusive.

RESULTS AND DISCUSSION

A total of 934 unique persons were seen by the NICHE nurse over a 22 month period. 389 of 934 (41.6%) were male, and 464 of 934 (49.7%) were under the age of 35. 209 of 464 (45%) of persons seen under the age of 35 were female, compared to 180 of 470 (38.3%) of persons over the age of 35. The majority of persons (774/934, 82.9%) were initially seen in one of the three primary community outreach settings across Regina (Parliament Medical Clinic [an opioid agonist therapy clinic), Street Workers Advocacy Project [a community-based organization for at-risk persons], and Harm Reduction Clinic [an opioid agonist therapy clinic]), the remainder were seen at Regina Provincial Correctional Center, a men's only prison. Of 794 persons with self-declared ethnicity data available, 602 (75.8%) were of Indigenous heritage. A total of 319 clinic days and 2,665 patient encounters were charted by the NICHE nurse over a 22 month period, an average of 8.4 persons per clinic, and 121 patient encounters per month. 101 persons seen were infected with HIV (with or without HCV coinfection), representing one-fifth of the entire ID Clinic cohort. 368 of 934 persons (39.4%) seen were negative for both HIV and HCV. 465 of 934 were mono-infected with HCV, represented by HCV antibody positivity (with or without HCV viremia). From June 1/18 onwards, vaccinations (Hepatitis A and B, Haemophilus influenzae type B, Pneumovax®, Tdap, Influenza) and HIV / HCV services provided by the NICHE nurse were recorded. Between June 1/18 and September 30/19, 584 vaccinations were provided, 463 persons consented to HIV testing, and the proportion of persons who were HIV positive receiving antiretroviral therapy and achieving viral suppression increased from 40/50 (80%) and 25/50 (50%) on June 1/18 to 34/40 (85%) and 33/40 (82.5%) by September 30/19. A total of 104 persons were initiated on HCV treatment by the NICHE nurse. As of February 23, 2020 91/104 (87.5%) had completed treatment, and of these 76/91 (83.5%) had achieved HCV cure as measured by sustained viral response 12 weeks post end-of-treatment. 10 were still receiving HCV therapy, and 3 had been lost to follow-up and had not completed treatment.

CONCLUSIONS

Over a 22-month period, the NICHE nurse successfully improved the HIV and HCV care cascades across southern Saskatchewan by facilitating diagnosis, linkage to care, and treatment for HIV and HCV for persons at-risk in community-based settings. No adverse outcomes were noted for those initiating HCV treatment, and cure rates for persons treated were comparable to those seen in ID Clinic with persons treated by conventional means through a specialist-based referral approach. Similar approaches for persons with and at-risk for HIV and HCV across Saskatchewan and other settings worldwide are needed to achieve the WHO targets for HIV by 2020 and HCV by 2030.

ACKNOWLEDGMENT

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Population Health

The Effect of Yoga on Decreasing the Risk of Fall-Related Injury in Peri- and Post-Menopausal Women

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INTRODUCTION

Yoga, a system of health that promotes physical, mental, emotional, social and spiritual well-being, is a popular health practice for women in their peri- and post-menopausal years.1 Yoga may offer unique benefits to decrease both fall and fall-related injury risk in the event a fall is unavoidable.2 A recent systematic review based on six trials found that the physical aspects of yoga, aside from meditation and breathing alone, resulted in small to medium effects on balance and mobility.3 A pilot study also supported 12 weeks of yoga decreasing fear of falls in older adults.4 These preliminary results are promising; however, there is no substantive evidence that these outcomes result in a decrease in fall rates or a reduction in the injuries associated with falling. Women over the age of 50 years are particularly vulnerable to sustaining fall-related injuries such as fractures.5 Evidence from our research team suggests that older women do not have the same capacity to control a safe forward fall landing and decrease force impact on their hands compared to younger women.6 Older women also demonstrate diminished activation of the abdominal core muscles prior to an unexpected simulated fall.7 Hatha yoga includes physical poses, mindful movement, breath awareness and regulation practices that can address balance, flexibility, and promote upper body loading and whole body strength training. This may consequently improve the ability to prevent a fall as well as decrease risk of injury.8,9 Yoga has multiple promising benefits to decrease the risk of fall-related injury in older women but there remain gaps in the literature to substantiate these claims. The primary purposes of this study were to determine the effect of 12 weeks of hatha yoga on: 1) fall risk factors (balance, balance confidence, functional mobility, muscle strength, incontinence (leakage, symptoms and bother), and 2) the capacity to land and control the descent of a forward fall (muscle activation, range of motion, reaction time, and energy absorption) in peri- and post-menopausal women. A secondary purpose was to determine the perceived changes in general health, fall risk, menopausal symptoms and incontinence for women who participate in yoga and the motivators, facilitators and barriers for participation in a regular yoga practice.

MATERIALS AND METHODS

Design and Participants:

This study was a repeated measures design where 39 eligible women entered the study, staggered over two Cohorts. There were three testing points: Baseline (12 weeks prior to program start, before the 'wait-control' period), Pre-Yoga (12 weeks after the 'wait-control' period, just prior to program start) and Post-Yoga (just after program). Cohort 1 (n = 23) entered the study in the fall of 2018, and Cohort 2 (n=16) entered the study in the spring of 2019. All testing was completed by the end of December, 2019. Thirty-two women completed all phases of the study.

Screening and Recruitment:
Women age 50 to 70 years were recruited via poster advertisements in the community, at the yoga studio, email distribution by community organizations, and electronic posting on community websites. Exclusion criteria included recent participation in yoga twice per week or more, any recent upper extremity (UE), neck or back injury, or other painful joint problem that significantly limits day to day activities, any fracture in the past year; a distal radius fracture in the past two years or a history of multiple wrist or forearm fractures; and any history of other conditions that would contradict maximum effort strength assessment or ability to safely participate.

Intervention:
The hatha yoga class was conducted at a local yoga studio (Ground Yoga, Saskatoon) and included physical poses and mindful movement integrated with breath. Two yoga instructors were trained and guided by our team experts to deliver the intervention. The yoga class components reflected the objectives of our study, focussing on addressing some of the physical, mental and emotional factors that potentially contribute to falls (i.e. balance, balance confidence, strength, coordination, proprioception, core training strategies, and breathing techniques) integrated with mindfulness practices and yoga philosophy. The practices incorporated a combination of seated, kneeling, UE weight bearing, standing and lying postures and transitions between positions with individual modifications provided on an as-needed basis. There were regular check-in meetings with the instructors as well as a final interview to determine their experiences of teaching the yoga classes and identify any variances in delivery between the two cohorts.

Testing Protocol:
Each participant was tested at the three time points, each 12-weeks apart (baseline, pre-yoga and post-yoga) with two different testing sessions at each time point (total of 6 testing sessions). In addition, nearly 75% of participants contributed to one of the five ancillary focus groups offered at the conclusion of the yoga intervention. Testing Session 1 included a comprehensive battery of functional tests for fall risk factors including upper and lower body muscle strength, gait velocity, balance, balance confidence, incontinence symptoms and pad test, timed up and go test, and posture. Testing session 2 included a complex assessment of upper body strength simulating the muscle activity required to control a forward fall, reaction time, and three trials of a controlled descent and an unexpected release simulating a forward fall conducted with 3D kinematics of the arms, and upper body, forces under the hands, and electromyography (EMG) recordings of arm and abdominal activation. Each participant kept a diary of fall and near fall events, other activities, injuries or new medical conditions.

Analysis:
Preliminary Analysis included repeated measures ANOVAs of change across three time points for a selected number of all of the outcome measures, including balance, muscle strength, and incontinence.

RESULTS AND DISCUSSION

Preliminary results reported below pertain to n = 32 women (mean age of 59.1 years ± 4.7 years, ranging from 51 years to 68 years) who completed all phases of testing. Repeated measures analysis found improvements in muscle strength, balance, and incontinence over three time points. Exploratory analysis with a p-value set at $p < .10$ (Pillai's trace) revealed multivariate significant changes across time for lower body strength and functional ability ($p = .011$; 30-second chair stand test), incontinence ($p = .003$; pad test urine leakage difference before and after stress inducing activities), arm strength ($p = .006$; hand held dynamometry left elbow extension), and balance ($p = .07$; one leg standing left leg). Pairwise comparisons found significant improvement after yoga training since both base and pre-yoga testing for chair stands ($p = .011, .042$) and for arm strength ($p = .001, .058$). Pairwise comparisons found significant improvement after yoga training since base testing for balance ($p = .021$) and for incontinence ($p = .092$). Further analysis will include repeated measures MANOVAs, with ITT analysis with all variables along with thematic analysis of focus group discussions. Two summer student projects have been secured to assist with further analysis.

CONCLUSION

This pilot study was successful in establishing a community yoga program that had high attraction to both older women and other public participants and continues to be offered and well attended. This study also contributed to the success of our team receiving a CIHR knowledge dissemination grant that will assist us to further analyse and disseminate the findings to health care providers, community partners and the public. Preliminary results are encouraging in supporting the benefit of a hatha yoga intervention to decrease fall and injury risk in older women.

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Shoulder Rehabilitation for Breast Cancer Survivors: Patient and Healthcare Professional Experiences and Perspectives

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INTRODUCTION

Although the number of breast cancer survivors (BCS) is increasing post-treatment shoulder issues are a formidable problem. A recent Saskatchewan based study found more than 80% of survivors experienced shoulder problems within the first five years following their treatments [1]. Shoulder symptoms such as pain, weakness, and stiffness were associated with difficulty in ability to work [2]. Despite the prevalence of shoulder problems and the known benefits of physical rehabilitation, specific programs for BCS with shoulder dysfunction do not exist in Saskatchewan and utilization of rehabilitation services to restore function and allow successful return to work is not clear. This study explored post-surgical shoulder rehabilitative experiences of BCS in Saskatchewan from the perspective of both the patient and healthcare professional (HCP).

MATERIALS AND METHODS

An exploratory and qualitative descriptive design was employed. We recruited 14 BCS who have experienced shoulder problems following surgery and 8 HCPs (i.e. who have experience and expertise in delivering care/treatment and services to BCS). Two separate 60 minute focus groups were conducted with the BCS, and one-on-one telephone interviews (40-60 minutes in length) were conducted with the HCPs. Transcripts were analyzed with inductive content analysis. NVivo12 Pro was used to assist coding qualitative data.

RESULTS AND DISCUSSION

Data were categorized under four main themes: (1) Awareness; (2) Existing Relationships; (3) Infrastructure; and (4) Communication. Findings indicate that every aspect of one's life is challenged through survivorship and individuals end up adjusting their expectations to fit a new set of personal and professional capabilities. Although there is a need for rehabilitation services to address the physical impacts to the shoulder and upper limb following cancer treatments, there is a general lack of awareness among both BCS and HCPs. Many BCS and HCPs are not aware that shoulder and upper limb problems are linked to cancer treatments nor the extent that these issues can last. Existing relationships with HCPs appear to be a facilitator for BCS receiving rehabilitation services. Personally knowing someone in the health care system helped women more rapidly receive medical attention and rehabilitation services. In the province of Saskatchewan, there is a lack of adequate infrastructure to ensure BCS receive timely rehabilitation; a multidisciplinary care pathway where BCS are followed pre and post operatively does not exist. There is also a lack of therapists with specialized training to address issues such as lymphedema. Finally, communication was a significant barrier to BCSs receiving rehabilitation services. BCS feel their concerns of shoulder or upper limb problems are not being heard by their specialists and communication between HCPs is fragmented. BCS are frustrated with having to find information about shoulder problems and services by themselves and in different places.

CONCLUSION

The journey that BCS must go through is extensive, painful, costly and life-changing. Current services in Saskatchewan are not adequate to address multifaceted barriers experienced by both BCS and HCPs. While some barriers may ultimately be addressed by more targeted funding by the health care system (i.e. more full-time positions, more training and specialization, more specialists to serve the province and distribute therapeutic services), others are harder to resolve (i.e. not feeling heard by the medical community, timely integration of services and a clinical pathway for rehabilitation to support survivorship). Better communication about shoulder problems and available rehabilitation supports, in a timely manner, is needed. Further, a centralized location where BCS can receive consultation about all potential supports (i.e. including rehabilitation) after their treatments and finding resources for different programs is lacking in Saskatchewan. Integrated policies are needed to overcome these barriers.

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Predictive Biomarkers of Cardiovascular Disease

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INTRODUCTION

Elevated levels of the enzymes gamma glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) have been shown to be correlated with increased risk of cardiovascular disease (CVD). (Kunter etal, 2014; Choi etal, 2018; van Beek, 2013; Wolfram etal, 2008;) Significant associations between GGT and incidence of CVD and CVD-related mortalities have been reported by several researchers. (Lee et al, 2006; Ruttmann etal, 2005; Kunter etal, 2014; Choi etal, 2018) Various atherogenic indices (function of triglycerides and HDL-cholesterol) have also been used for the diagnosis and prognosis of CVD. In conclusion, while there are some promising initial results that suggest the usefulness of GGT, ALT, AST, and ALP as biomarkers for increased risk of CVD-related health effects, most of this information is correlative in nature. The primary prevention of chronic diseases such as CVD (the second leading cause of death after cancer and a leading cause of hospitalization), diabetes and stroke is a public health priority in Canada. (Betancourt etal, 2014) The management of well established modifiable risk factors, e.g. standard lipid profile (total cholesterol, low-density lipoprotein cholesterol, high-density lipoproteins cholesterol, triglycerides); smoking; obesity, diet, and alcohol consumption, is a recommended approach to CVD prevention. (Wood D et al, 1998; Jackson, 2000; British Heart Foundation, 2004;) However, in the recent years several studies have reported an association between commonly used biomarkers (GGT, ALT, AST, and ALP) of liver dysfunction and an increased risk of CVD. (Kunter etal, 2014; Choi etal, 2018; van Beek, 2013) The reliability of these associations in the general Canadian population has yet to be fully analyzed and quantified and there is a lack of any comparable research in the Canadian population. To address these gaps, the objective of this proposed study was: To assess cross-sectional relationships between biomarkers GGT, ALT, AST, ALP and CVD in the Canadian population. In order to accomplish this objective we plan to test the hypothesis that GGT, ALT, AST, and ALP are predictive of the prevalence of CVD in adults using information available from the Canadian Health measures Survey (CHMS)- Cycles 1 through 5, Sanmartin, 2013; Day, 2014; Statistics Canada. 2016; Statistics Canada. 2017) taking into account individual (e.g. obesity) and contextual (e.g. socio-economic) factors.

MATERIALS AND METHODS

The CHMS are a series of cross-sectional national surveys led by Statistics Canada, in partnership with Health Canada and the Public Health Agency of Canada and details of these surevys can be found elsewhere. (Sanmartin, 2013; Day, 2014; Statistics Canada. 2016; Statistics Canada. 2017) Briefly, these surveys collect information on indicators of general health and wellness of Canadians. The CHMS has four components: The Household Component, the Mobile Examination Centre, the Laboratory Component, and the Biobank Component. For the present report, we used data from the first three components, Household Component includes a detailed interviewer-administered questionnaire, which collects information on participants' demographics; housing conditions; socio-economic factors; self-perceived physical and mental health status; any doctor-diagnosed acute or chronic conditions, family medical history, and medication use. Mobile Examination Center: Physical measurements include: anthropometric, cardiovascular, and musculoskeletal fitness measures, physical activity, oral health (cycle one only), spirometry, and biological specimens.

Laboratory Component: Participants' blood samples are assessed for diabetes, cardiovascular health, infectious disease markers, nutritional information, and other general information. Data from CHMS cycles 1 through 5 were used for this study. Study participants 18 years were include in the analysis. Statistical Methods: Statistical analysis were conducted to compare GGT, AST, and ALP levels, individual (e.g. Body Mass Index(BMI)) and contextual (e.g. socio-economic) factors and other population characteristics between two groups (presence or absence of CVD). Logistic regression technique was used to analyze CHMS data. Appropriate weight variables that have been computed by methodologists of Statistics Canada were used for all analyses. Balanced Repeated Replication(Thomas, 2008) method was used for variance estimation to account for clustering inherent in the study design of the CHMS. STATA software was utilized to perform all analyses.

RESULTS AND DISCUSSION

Based on univariable logistic regression, single associations of each predictor variables with the presence/absence of CVD revealed that GGT, AST (borderline), ALP, sex, age, BMI, education, income, ethnicity (white, Aboriginal, and others) were significant predictors of CVD prevalence. Based on multivariable logistic regression, we observed that immigration status [Odds ratio(OR)(95% Confidence Interval (95% CI))= 0.67 (0.53-0.85), reference category (RC)-no-immigrant] education [1.38(1.10-1.75), RC-higher than secondary education]; smoking status [ex-smokers: 1.16(0.89-1.51); current smokers: 1.41(0.98-2.05), RC-non-smoker]; and income [middle income: 0.69(0.43-1.10); high income: 0.49(0.29-0.83); RC-lower income] were significantly associated with CVD prevalence. Borderline association was observed between ALP and CVD prevalence. Age, sex, and BMI were effect modifiers in the relationship between GGT and CVD prevalence, while diabetes was an effect modifier in the relationship between AST and CVD prevalence. In this study, we aimed to explore the relationship between GGT, AST, and ALP along with individual (obesity) and contextual factors (income) and CVD. We observed positive relationships between biomarkers of primary interest (GGT, AST, ALP) with CVD prevalence in this sample of Canadian adults. Ours results align with previous findings of significant association with CVD prevalence and biomarkers (GGT, AST, ALP). (Kunutsor,2014;Kanbay, 2011;Li, 2014)

CONCLUSION

We observed that (i) ALP had borderline association with CVD prevalence; (ii) the relationship of GGT with CVD prevalence changed with age groups and BMI categories; and was different for males and females; and (iii) diabetes was an effect modifier in the relationship between AST and CVD prevalence. Socio-economic factors (education and income) were significantly associated with CVD prevalence.

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Inflammatory Bowel Disease Among Indigenous Peoples: Preliminary Results from Saskatchewan, Canada

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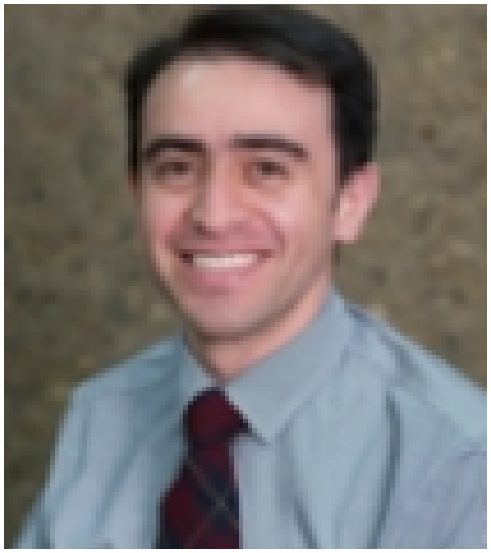
INTRODUCTION

Inflammatory Bowel Disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a chronic condition, usually diagnosed in early adulthood, with significant life-threatening disease-related complications and reductions in quality of life if left untreated. Despite available research about IBD in the general population, there is limited-to-no evidence about IBD among Indigenous peoples in Canada and around the world. We aimed to: 1) define a collaborative research framework, 2) determine the prevalence and incidence rate of IBD among First Nations (FNs) in Saskatchewan; and, 3) explore the perceptions of living with IBD, and barriers and facilitators to access healthcare among Indigenous peoples.



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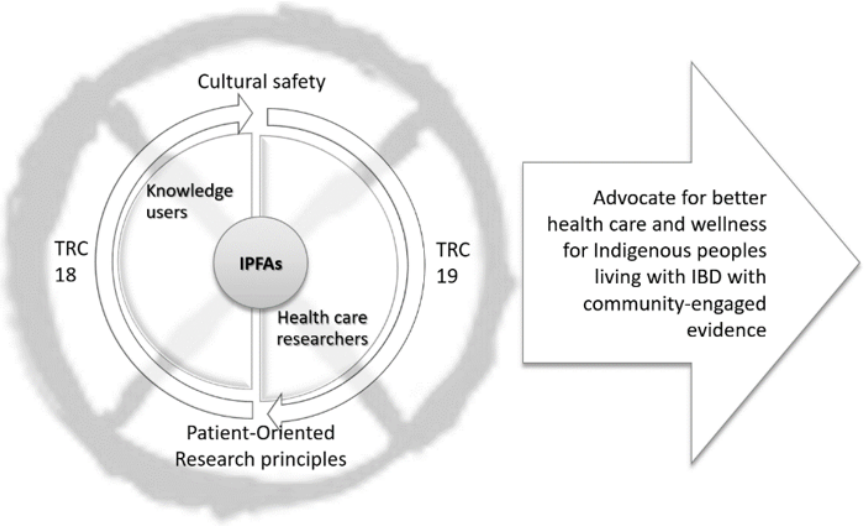
MATERIALS AND METHODS

This study began when Indigenous patients shared their health experiences with IBD with research team members. An interdisciplinary research team was formed including Indigenous patient and family advocates (IPFAs, Indigenous patients living with IBD and parents of an Indigenous person with IBD), an IBD gastroenterologist, knowledge users, and Indigenous and non-Indigenous researchers. Our research team committed to raise awareness of IBD among Indigenous peoples within Indigenous communities and among health care providers and to advocate for better healthcare and well-being by providing evidence of IBD among Indigenous peoples living with IBD in Saskatchewan. We defined a mixed methodology. The first phase of the study, we conducted a retrospective population-based study between 1999 and 2016 fiscal years using Saskatchewan administrative health data. A previously validated algorithm that required multiple health care contacts was applied to identify IBD cases. The "self-declared FNs status" variable in the Person Health Registration System was used to determine FNs meeting the IBD case definition and the population at risk. Generalized linear models (GLMs) with generalized estimated equations and a negative binomial distribution were used to estimate the annual prevalence of IBD, CD, and UC. Incidence rates and their corresponding 95% confidence intervals (95%CI) were estimated using GLMs with a negative binomial distribution. The GLMs were used to test trends overtime. The second phase is a photovoice study to gather "the voices" of Indigenous peoples with IBD, encouraging self-interpretation of pictures, engaging their communities, and empowering them with the study findings.

RESULTS AND DISCUSSION

The IPFAs play a critical role in the project by sharing their experiences and defining the directions of the project, as well as defining our research framework (Figure 1).



IPFAs: Indigenous patient and family advocates; TRC: Truth and Reconciliation Commission of Canada Calls to Action

Figure 1. A framework to study inflammatory bowel disease among Indigenous peoples.

The annual prevalence of IBD among FNs in Saskatchewan increased from 64 (95%CI 62-66) per 100,000 people in 1999 to 142 (95%CI 140-144) per 100,000 population in 2016. Also, the prevalence of CD and UC increased during the study period, with 53/100,000 (95%CI 52-55) for CD and 87/100,000 (95%CI 86-89) for UC in 2016. The average increase in the prevalence of IBD was 4.2% (95%CI 3.2-5.2) per year, with similar trends observed in CD (4.1% [95%CI 3.3-4.9]) and UC (3.4% [95%CI 2.3-4.6]). The incidence rates of IBD among FNs were 11 (95%CI 5-25) per 100,000 people in 1999 and 3 (95%CI 1-11) per 100,000 population in 2016. No statistically significant changes were observed in the incidence rates over time. Regarding the photovoice component of the study, we started the recruitment of Indigenous patients with IBD and family members of individuals with this chronic disease.

CONCLUSION

This ground-breaking patient-driven study is the first stage to improve health among Indigenous peoples living with IBD in Saskatchewan. We identified that FNs have increasing trends in the prevalence of IBD, which has also been described in the Canadian general population. In contrast to the general population of Canada and other developed countries that have shown decreasing trends, the incidence rates of IBD among FNs appear to be stable over time in Saskatchewan. Also, among FNs, UC appears to be more prevalent than CD; this pattern has been observed in the general populations of developing countries. These results illuminate the need to advocate for better health care and wellness for FNs living with IBD. Our study is generating community-engaged knowledge and expertise to inform the development of an Indigenous IBD framework that could promote better and knowledge-based healthcare for Indigenous peoples with IBD in Canada and worldwide.

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