SRTP - Project Description Form #206

PART I:

Name of Schulich faculty member who will supervise the project	Abhijit Biswas
Supervisor's Schulich, Western, Hospital or Lawson Email	abhijit.biswas@lhsc.on.ca
Schulich Department	Anesthesia & Perioperative Medicine

PART II - Project Description

Title of Project	"Cytokine alterations associated with persistent post-surgical pain and their regulation through epigenetic changes – A pilot study

Background

Chronic post-surgical pain (CPSP) is defined as a persistent state of discomfort that develops after surgery and extends beyond tissue healing. This is a biopsychosocial disorder involving complex interactions between genes and the environment. CPSP results from a combination of peripheral and central sensitization, and direct injury to somatic and visceral sensory nerves. Inflammation is an essential component of the surgical stress response and can facilitate peripheral and central sensitization through the production of cytokines, chemokines and growth factors. CPSP may occur following thoracotomy, amputation mastectomy, cholecystectomy, hernia repair, hysterectomy, hip replacement, and cesarean section.

CPSP negatively impacts postoperative quality of life and can be a significant financial burden on patients and the healthcare system. The pain associated with CPSP is difficult to treat with existing medications and pain treatment procedures.

Epigenetic mechanisms alter gene expression without modifying the underlying sequence of DNA. This is primarily achieved through histone modification, DNA methylation, and non-coding RNAs. Epigenetic changes facilitate rapid, stable and modifiable genetic responses to dynamic physiological and environmental factors. Several immune cell functions are regulated through epigenetic mechanisms, including immune cell activation, differentiation and inflammatory mediator production. Epigenetic mechanisms contribute to the development of several chronic inflammatory diseases, including chronic pain. Epigenetic changes like DNA hypomethylation have been with CPSP in children under going spine surgery to women following breast cancer surgery. Patients that developed milder chronic pain following breast cancer surgery demonstrated hypermethylation of the tumour necrosis factor (TNF)-α promoter, suggesting that epigenetic changes participate in the aberrant immune response during CPSP. However, our understanding of the complex epigenetic changes involved in CPSP requires further investigation.

The purpose of this study is to identify inflammatory biomarkers (cytokines, chemokines and growth factors) and epigenetic changes associated with the development of CPSP to proactively manage patient at risk.

Hypothesis

The pathophysiology of CPSP is complex, multifactorial and not well understood. Aberrant perioperative inflammation has been associated with the development of CPSP and epigenetic mechanisms are thought to contribute. In this study, inflammatory mediators involved in CPSP (cytokine, chemokine, and growth factor production) will be characterized in patients undergoing thoracotomy for non-metastatic lung malignancies. The mechanisms contributing to altered systemic cytokine, chemokine and growth factor production will be further explored through the assessment of DNA methylation within the promoters of associated genes. This innovative and novel characterization of CPSP at the biomolecular and genetic level, will aid in our understanding of the mechanisms responsible for the development of CPSP. It may also help to identify novel therapeutic targets for patient management and biomarkers for risk stratification that can guide the provision of personalized and improved pain management plans.

Hypothesis: Proinflammatory mediator production will be enhanced in patients that develop CPSP post-thoracotomy through promoter hypomethylation while anti-inflammatory mediator production will be reduced through promoter hypermethylation.
Objectives
1) Quantify the acute and chronic production of systemic cytokines, chemokines and growth factors in patients undergoing thoracotomy.
2) Identify differences in inflammatory mediator production between participants that develop CPSP and those that do not.
3) Characterize CPSP-associated differences in DNA methylation within the promoters of genes encoding inflammatory mediators found to be altered in Objective 2.

Proposed Methodology
This is a prospective longitudinal observational study to investigate the association between inflammatory mediator production, DNA methylation, and the development of CPSP in adults undergoing thoracotomy at London Health Science Centre and St. Joseph's Hospital in London, Ontario, Canada.

After REB approval, eligible patients will be identified through surgical registry and will be approached for recruitment and consent. Patient demographics, medical history, medication, and indication for surgery will be obtained. Patients will complete the self-administered comorbidity questionnaire (SCQ), the hospital anxiety and depression scale (HADS) and pain catastrophizing scale (PCS). Preoperative patient rated pain score will be obtained using the numerical rating scale (NRS) and blood sample will be obtained for the assessment of baseline inflammatory mediator production and DNA methylation (see below).

Participants will undergo unilateral thoracotomy under general anesthesia at Victoria Hospital, LHSC. All participants will receive standard of care for anesthesia and analgesia. Serial peripheral blood samples will be taken on postoperative day (POD) 2, postoperative month (POM) 3 and POM 6. At each timepoint, patient rated pain scores will be assessed using the NRS and the postsurgical pain questionnaire. Inflammatory mediators will be quantified using the Cytokine 30 Plex Human Panel. DNA extraction kit will be used to isolate genomic DNA.

All participants will be seen in the chronic pain clinic at POM 3 & 6 to identify patients who have developed CPSP. The pain DETECT score for neuropathic pain will be determined and sensory testing will be performed to assess nerve injury within affected areas. Patients will be stratified based on the presence or absence of CPSP post-thoracotomy for data analysis. Differences in inflammatory mediator production between groups will be identified and further investigated for altered DNA methylation within the promoters of genes encoding each affected cytokine, chemokine and growth factor.

Expected Outcomes
We speculate that CPSP will be associated with increased production of inflammatory markers, including cytokines, chemokine and growth factors. This project will specifically identify which inflammatory mediators are altered during CPSP. Through DNA promoter methylation analysis, we will understand the mechanism of such alteration.
A preoperative blood sample will be obtained for the assessment of baseline inflammatory mediator production and DNA methylation. Serial evaluations will take place on POD 2, POM 3 and POM 6 6. Peripheral blood samples will be obtained for the quantification of inflammatory mediator production and DNA methylation. The POD 2 timepoint will be used to assess acute perioperative inflammation and epigenetic changes. Chronic alterations in inflammatory mediator production and DNA methylation will be addressed using the POM 3 and POM 6 timepoints. All study participants will be seen in the chronic pain clinic in St. Joseph's Hospital at the postoperative month 3 timepoint. CPSP will be diagnosed and testing will be performed using the parameters as previously described.
Patients will be stratified based on the presence or absence of CPSP post-thoracotomy for data analysis. Differences in inflammatory mediator production between groups will be identified and further investigated for altered DNA methylation within the promoters of genes encoding each affected cytokine, chemokine and growth factor.
A positive correlation between changes in inflammatory markers, epigenetic changes and association with development of CPSP will determine success of the study. Logistic regression analyses will be performed to identify patient variables associated with pain group membership. Multivariate linear regression models will be developed to

better estimate the magnitude and precision of associations between specific inflammatory mediators and CPSP, and specific DNA methylation patterns and CPSP. Comparisons between groups will be performed using appropriate statistical tests.

Research Environment – Description of the number of research personnel, primary location of research, size of lab, etc

The Research environment will involve clinical perioperative and lab based environment t Victoria Hospital and Western University.

For the summer student, this will be an opportunity to learn and assist in writing for grant and ethics approval. The student should be able to work remotely and physical presence may be required only at times of supervisor meeting.

Names and titles of other individuals who will be involved with the research project?

Dr. Subrata Chakrabarti (Co-I) Professor, Department of Pathology and Laboratory Medicine, Western University.
Dr. Abubaker Sidahmed (Co-I) Assistant Professor, Division head, Transplant Immunology, director of Histocompatibility and Immunogenic laboratory. Department of Pathology and Laboratory Medicine, Western University.
Dr. Richard Malthaner (Co-I) Chair/Chief, Division of Thoracic Surgery, Director, Thoracic Surgery Research, Professor of Surgery, Epidemiology and Biostatistics:
Dr. Qutaiba Tawfic (Co-I) Associate Professor, Anesthesiologist
Dr. Collin Clarke (Co-I) Associate Professor, Anesthesiologist
Ms. Lee-Anne Fochesato (Research Coordinator) Department of Anesthesia and Perioperative Medicine

Can this project be done remotely?	Yes
Duration of Project	One Summer

Expected Objectives/Accomplishments for Student?

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A preoperative blood sample will be obtained for the assessment of baseline inflammatory mediator production and DNA methylation. Serial evaluations will take place on POD 2, POM 3 and POM 6 6. Peripheral blood samples will be obtained for the quantification of inflammatory mediator production and DNA methylation. The POD 2 timepoint will be used to assess acute perioperative inflammation and epigenetic changes. Chronic alterations in inflammatory mediator production and DNA methylation will be addressed using the POM 3 and POM 6 timepoints. All study participants will be seen in the chronic pain clinic in St. Joseph’s Hospital at the postoperative month 3 timepoint. CPSP will be diagnosed and testing will be performed using the parameters as previously described.

Patients will be stratified based on the presence or absence of CPSP post-thoracotomy for data analysis. Differences in inflammatory mediator production between groups will be identified and further investigated for altered DNA methylation within the promoters of genes encoding each affected cytokine, chemokine and growth factor.

A positive correlation between changes in inflammatory markers, epigenetic changes and association with development of CPSP will determine success of the study. Logistic regression analyses will be performed to identify patient variables associated with pain group membership. Multivariate linear regression models will be developed to better estimate the magnitude and precision of associations between specific inflammatory mediators and CPSP, and specific DNA methylation patterns and CPSP. Comparisons between groups will be performed using t-tests and repeated measures ANOVAs with Bonferroni post-hoc testing for parametric data. Mann-Whitney or Friedman tests with Dunns post-hoc testing will be used for statistical analyses of non-parametric data. 95% CIs for the differences between groups will be constructed using standard techniques for parametric data or bootstrapping of 10,000 replications for non-parametric data. Categorical outcomes will be analyzed using Fisher’s exact test or Chi-squared test, along with the relative risk and its two-sided 95% Confidence Interval.

PART III - Certifications

If the project will require any certification approvals from one or more of the following offices, please check the

appropriate box below.	- Human Ethics
Human Ethics: If you have the protocol information, please enter it below (or enter the status of the approval).	Pending - REB will be submitted and approved before summer 2024

Note: certification approval should be obtained prior to the start of the summer. Projects without this approval will not be a priority for funding.