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The SYMBIOME Project: An Exploratory Investigation of the Biological, Psychological, and Social Mechanisms that Contribute to the Transition from Acute to Chronic Musculoskeletal Pain

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Abstract

This dissertation presents the initial findings of the SYMBIOME project; which attempts to combine the objective and subjective aspects of musculoskeletal pain to develop a prognostic clinical phenotype. Chapter 2 presents a moderator analysis of functional outcomes (pain interference and pain severity). Psychosocial moderators can affect the relationship between biomarkers and pain. For pain severity, TNF-α, TGF-β1, and CRP were moderated by employment status, pre-existing psychopathology, and sex. For pain interference, IL-1β, cortisol, TGF-β1, CRP, and IL-6 were moderated by pre-existing pain, peri-traumatic fear, region of injury, and peri-traumatic stress. Chapter 3 presents a latent growth curve analysis in determining the recovery trajectories of acute non-catastrophic musculoskeletal pain in the context of pain interference and severity over the course of 12 months. For pain interference, 3 distinct trajectories emerged: rapid recovery, delayed recovery, or minimal/no recovery. Pain severity favored a 2-trajectory model with rapid recovery or minimal/no recovery. Classification of recovery group depended on both baseline symptoms and relative rate of symptom decline. Recovery outcomes appeared to stabilize after a period of 3 months. Chapter 4 presents latent class analysis and growth mixture modeling as applied to a panel of 8 biomarkers (TNF-α, IL-1β, IL-6, CRP, IL-10, cortisol, BDNF, and TGF-β1). These markers may have the potential to discriminate between functional recovery outcomes. Using these markers, 3 meaningful groups or classes were identified. These groups could be adequately defined by using only 3 of the 8 markers (IL-1β, BDNF, and TGF-β1) where classes were organized by low concentration of markers in serum, average concentration, or high concentration of BDNF and TGF-β1. Those with high concentration of BDNF/TGF-β1 were more likely to score higher on self-report measures of pain and disability in their 6-month outcomes. These results support the claim that physiological factors are tied to pain through more than simple bivariate relationships. The context of the musculoskeletal trauma, both personal and social, can affect the behavior of biological systems.

Keywords

Musculoskeletal, trauma, pain, chronic, acute, recovery, trajectory, biomarker, prognosis

Summary for Lay Audience

Pain is a complex process that occurs in our body. It involves psychology, genetics, and a combination of the immune, nervous and endocrine systems. Short-term pain is important because it helps us survive. However, when something goes wrong in one of those systems, it can become a long-term problem. As pain goes on, it begins to affect the quality of life. For the 20% of Canadians who struggle with chronic pain, everything begins to suffer including health, finances, relationships, and work. Because pain is such a personal experience, it is very difficult to understand and treat.

The **SYMBIOME** project stands for the **SY**stematic **M**erging of **BIO**logy, **M**ental Health, and **E**nvironment. The purpose of this project is to understand how pain develops over time. After a traumatic accident or injury, people are invited into this year-long study. By using questionnaires and collecting tissue samples, we have monitored their recovery. After one year, some people fully recover while others develop chronic pain. When we compare the mental and physical changes that occur between these people, it helps us understand how pain becomes chronic. Your work status, education level, mental health, and physical injuries all contribute to how your body deals with trauma. These factors can influence your healing responses in a way that either increases or decreases your levels of inflammation and stress in the short-term. In the long-term, it may also affect your ability to recover as people tend to recover in 1 of 3 ways. Some people recover quickly and are functioning normally by 6 months. Some are delayed in their recovery but still reach normal function. And some people still struggle with pain and disability even after 12 months. Analyzing various groups of blood proteins may also shed some light on who tends to recover and who does not. With this information, it will be possible to develop new treatments that can help people who suffer from chronic pain. It may also help us treat pain in unique ways before it has a chance to become a chronic problem.

Co-Authorship Statement

The studies contained in this dissertation were co-designed, analyzed, interpreted, and written by Joshua Lee with invaluable collaboration and guidance from Dr. David Walton. Other essential collaborators including Dr. James Elliot, Dr. Paul Tremblay, Dr. Joy MacDermid, Dr. Ruth Lanius, Dr. Greg Gloor, Dr. Wanda Millard, and Mr. Curtis May provided pivotal feedback into study design and interpretation to sharpen the overall quality of chapter 3. Dr. Elliot also provided data via the Chicago cohort which was analyzed alongside the SYMBIOME cohort in chapter 3. Dr. Tremblay provided guidance and feedback regarding latent growth curve modeling (chapter 3) and latent profile analysis (chapter 4). Mohamad Fakhereddin and Maryam Ghodrati provided methodological guidance for correlation and regression analyses presented in chapter 2. The data were collected by myself, Paul Phares, and Ryan Power, with logistical support provided by Helen Phan.

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Dedication

To my Savior, the author and finisher of my faith (and this thesis). You carried me through this valley of great difficulty to show me that You are greater still. It's (still) You and me till the end.

"Call to Me, and I will answer you, and show you great and mighty things, which you do not know." –Jer 33:3

To my parents, who have walked with me through every challenge of my life. I have achieved nothing great or spectacular when measured against your sacrifices. This is the least I could have done when I consider everything you've given to me. If I know anything about love and dedication, it's because I first knew you.

Table of Contents

List of Tables

List of Figures

List of Appendices

Preface

Dear Reader,

This thesis is being submitted during a turning point in modern history as we are currently in the midst of an unprecedented health crisis. Due to the gravity of this event, I do not believe it to be out of place to say a few words here for the sake of posterity.

This dissertation was completed shortly after the World Health Organization declared the novel coronavirus (COVID-19) a pandemic on Mar 11, 2020. Many countries around the world are struggling to survive as hospitals are being overrun. The infection continues to spread at an alarming rate and resources are quickly being depleted. Governments are calling for former healthcare workers to come out of retirement and companies are being asked to repurpose their production facilities to meet the increasing demand for medical supplies. Thousands of people have already succumbed to this virus and this number increases by the day. Non-essential businesses have been forced to shut down and a nationwide protocol of self-isolation and social distancing has been put into effect.

We are still in the early days of this pandemic, but I have already seen some of the extremes of human nature. People have tried to hoard supplies and some refuse to isolate or quarantine themselves. Some are in complete denial of this entire situation and insist on indulging in their regular social pleasures despite the risk of infecting others. This has become a problem among so many that the government has had no choice but to make isolation a legal obligation. But then there is the other extreme. I have seen acts of heroic courage as healthcare workers voluntarily put themselves in harm's way for the sake of those who suffer and for those who are still unaffected. Thousands of retirees have answered their government's call to enter the fray and support their colleagues. Teachers and parents have demonstrated incredible compassion and resourcefulness as they continue to find ways to educate and provide for the younger generations. Countless volunteers have arisen to support frontline workers, employees work tirelessly to keep essential services running, and as scientists are toiling to develop a vaccine, they are volunteering themselves as test subjects to ensure its safety.

Although the world is trying to remain optimistic, it is uncertain how this will all end. Our lives have been disrupted in ways we could never have imagined, and this test has only begun. But even as I write this, I am reminded of a message written by a child on the sidewalk: “Stronger together”. We may not have seen the worst of this crisis, but our hope is greater still. People have united under these dire circumstances to work together, suffer together, cry together, and fight together. The world, it seems, has decided to teach us all a very important lesson. It is my sincere hope that the world in which you are reading this is better because we have learned it.

With hope,

Joshua Lee

“I remain confident of this: I will see the goodness of the Lord in the land of the living.” –Psalm 27:13

Chapter 1

1 Introduction

1.1 Preamble

The focus of this dissertation is to explore the biopsychosocial mechanisms which drive the transition from acute to chronic pain in non-catastrophic musculoskeletal trauma. The exploratory studies presented herein may help to characterize the complexity of pain to inform future research targeted towards prognostication and early intervention. The following introduction provides background information that will be necessary to understand relevant details behind each of the subsequent chapters. It will outline the burden of chronic pain and pain management, briefly describe the psychological and physiological processes of pain, and identify relevant dimensions for testing.

1.2 The Burden of Chronic Pain

Although pain is an important mechanism for survival, it is the persistence of pain that has a universal impact on our quality of life. Pain is by no means a stand-alone issue as it often carries financial, clinical and social consequences[1]. The effects of chronic pain are far-reaching as those affected also tend to struggle with depression, isolation, anxiety and anger; which, in turn, have detrimental effects on personal relationships and vocational roles[1, 2]. Taking into account both direct and indirect costs, the economic burden of pain has reached a staggering $125 billion per year in the US alone[2]. Currently, chronic pain affects 20% of all Canadian adults with an increasing prevalence in our aging population that exceeds even such well-known conditions as diabetes mellitus and asthma[2]. Medical interventions such as opioid drugs have seen a 347% increase in therapeutic use in the USA[3]. This, however, is only a temporary measure as studies have shown that opioids seem to be ineffective as a long-term solution to chronic, non-cancer pain; especially with the risk of complications such as tolerance, addiction and abuse[4, 5]. Furthermore, studies have shown that patients taking opioids were also susceptible to more severe pain resulting in a greater reliance on health care compared to patients who

were not taking opioids[6]. This myriad of complications is due, in part, to the complexity of chronic pain. Existing at the nexus between biology, mental health, and environment, pain has proven to be resistant to traditional medical and pharmaceutical treatments that seek to eliminate symptoms [7]. In the past, pain was regarded as the fifth vital sign, which created an increased emphasis on treating pain intensity [8]. This precipitated a program of management whereby opioid treatments were disproportionately prescribed in order to adequately address symptoms [9]. Treating patients based on pain intensity has not increased the quality of pain management [10], but has instead contributed to a crisis of opioid-related overdose and death [11]. Although much of the research in the fallout of this crisis has been directed towards reducing opioid use [12], research has begun to make the shift towards a more circumspect approach to pain.

1.3 Barriers to management

Despite the ongoing research in the field, the nature of pain is still fairly misunderstood and a number of professional organizations have agreed that the best course of action is to approach this issue from a multidisciplinary perspective [2, 13, 14]. Studies performed by Choinière et al[13] have provided a fairly recent picture of the issues surrounding pain management for people attempting to access multidisciplinary pain treatment facilities across Canada. Their findings have indicated that some clients were placed on wait-lists for months, even years before they were finally admitted to one of these facilities. Additionally, for many of these clients, the natures of their specific clinical needs were unclear and therefore, not adequately defined [2, 13, 14]. Since pain is both a sensory and emotional experience, it is highly variable between individuals[15]; which is perhaps what contributes to a lack of concrete, generalizable strategies for pain management and treatment. It may also explain why painkillers only function as an effective "magic bullet" in a select few populations of people.

Within the rehabilitation community, there is a wealth of literature that documents the psychological experiences of people who suffer from chronic pain. Although the validity of these studies is not in question, pain is a subjective experience and has thus remained a

fairly controversial condition [16]. This negativity is quickly transmitted to the public where it develops into stigmatization and stereotyping of individuals who suffer from chronic pain [17]. This has proven to be a difficult barrier to overcome as the concrete presence of pain is contested by co-workers, communities, friends, and even family [18]. As a result of this pervasive societal doubt, people with chronic pain experience a substantial decrease in both self-efficacy and willingness to seek treatment [18, 19]. From a biological standpoint, pain has become a well-established subject through intense and in-depth study. Current theories in Systems Biology suggest that pain is the result of a well-orchestrated balance between the nervous, endocrine, and immune systems. However, when one or more of these systems fails to resolve itself, the pain response (which normally promotes survival) falls into discord and becomes a chronic problem [20].

1.4 A Biopsychosocial Approach

With pain management becoming such a complex problem, researchers have begun investigating mechanisms that focus on the activity of entire systems, rather than specific areas. Although the development of drug targets still continues, there have been an increasing number of studies devoted to the understanding of structural and functional changes in the brain. This is of particular importance as pain involves a number of cognitive processes which are intimately linked with the perception of noxious stimuli. Over the years, a number of studies which have employed non-invasive brain imaging techniques have discovered a series of changes which occur in people who suffer from pain. While global functioning of the brain is still being defined, the involvement of higher neurological processes in chronic pain is undeniable.

One of the earliest accounts of pain control residing in the brain comes from surgical reports of soldiers on the battlefield. In 1946, Beecher published a number of intriguing findings from his experiences with soldiers that have suffered major wounds including compound fractures of lower limbs and penetrating wounds to the chest and thorax [21]. It was found that most of the severely wounded, but mentally alert soldiers, declined the offer of pain relief therapy upon admission to an army hospital. This was described as a

"puzzlingly low incidence of pain". Beecher goes onto describe how as with athletes who do not feel pain till after the competition, it is not uncommon for wounds received during fighting and anger to go unnoticed. Alternatively, it was suggested that being severely wounded represents an escape from all the horrors of the battlefield; which resulted not in the absence of pain, but the dismissal of its presence. In other words, strong emotional responses have the capacity to modulate pain. Additionally, Beecher criticized the automatic administration of morphine under the assumption of pain, rather than actual need. Soldiers experiencing excitement or hyperactivity upon admission were calmed significantly upon receiving a sedative, rather than an analgesic. It was also noted that small doses of sedative given in combination with small doses of analgesic provide both the mental depression and pain depression that the soldier actually needs. This treatment was much more effective than large doses of either treatment given individually.

Building upon years of foundational research in pain perception and emotional regulation, Melzack proposed the Neuromatrix theory [22]; that consolidated the pain experience into three different but integrated systems of mental functioning: cognitive-evaluative (attention or anticipation of a noxious stimulus), sensory-discriminative (intensity and location of a particular nociceptive input), and motivational-affective (emotional responses such as anxiety or fear). This revolutionary model depicted the formation of a "pain experience" that is specific to each individual and is dependent upon both innate and experiential information. Melzack was one of the first to elaborate on the concept that pain was more than a mere reflexive response to certain sensory inputs alone.

1.5 Stress-system reactivity

The Hypothalamic-pituitary-adrenal (HPA) axis, connects cognitive impulses in the brain with endocrine glands and hormonal control mechanisms in the body [23]. This neuroendocrine stress pathway represents an emotional nerve center for chronic pain, anxiety and depression [24]. Normally during stress, HPA axis activity involves the

hypothalamic release of corticotropin releasing hormone (CRH), which acts on nearby structures such as the locus coeruleus (LC - a brainstem nucleus and primary site for noradrenaline synthesis) the amygdala, and the pituitary [25]. Once it stimulates the pituitary, adenocorticotropic hormone (ACTH) is released into the bloodstream [24, 25]. ACTH causes the production and release of glucocorticoids (GCs) from the adrenal cortex to modulate immune responses, increase blood glucose, elevate blood pressure and activate the CNS [25, 26]. GCs stimulate the amygdala to release CRH, which is linked to elevated anxiety and fear [27]. Cortisol, a steroid hormone, is one of the primary GC products of the human HPA axis activity and is increased during both chronic and experimentally-induced pain [28]. Early-life stress also has long-lasting consequences for HPA axis activity. A recent review suggests that early-life adversity is linked to a long-term hypersensitivity to stress, elevated GCs and increased depression and anxiety-like behaviors [29]. Consistently high levels of GCs are potentially damaging to the hippocampus as they reduce its neurological structure [30] and prevent plasticity [31]. One of the primary ways this is thought to occur is through a reduction in brain-derived neurotropic factor (BDNF). This key protein, which is related to nerve growth factor (NGF), is active in the cortex and hippocampus and is vital for neuronal plasticity and long-term memory formation [32]. Reductions in hippocampal BDNF have been implicated in experimental pain [33], stress [34], and major mood disorders [35]. These changes suggest that early-life trauma, chronic pain and stress may prevent appropriate physiological responses to stressors later in life.

1.6 Immunity and pain

There is a significant link between the nervous system and the immune system especially in response to tissue damage and repair. The body's innate immune cells respond to injury with an inflammatory response that activates pain pathways. Infiltration of inflammatory cells, as well as activation of resident immune cells in response to nervous system damage, leads to subsequent production and secretion of various inflammatory mediators. Soluble mediators (particularly cytokines and chemokines) released by immune and glial

cells act on nociceptors (nerve fibers responsible for transmitting noxious stimuli), increasing synaptic strength, modulating sensitivity, and sensitizing primary afferents [36].

Once released, cytokines drive inflammation and are capable of influencing cell differentiation, gene transcription, and even cell survival [37, 38]. They can bind to specific receptors with such potency that only a handful of receptors need to be activated in order to initiate a massive signaling event [39]. Some of the most well-studied examples of pro-inflammatory cytokines are Tumor Necrosis Factor alpha (TNF-α), Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6). These cytokines function synergistically and are substantially elevated peripherally and centrally during injury, inflammation, and chronic pain [40]. They are extensively involved in the development of hyperalgesia (hypersensitivity to pain) and allodynia [41-43], generation of noxious nerve transmissions [44-46], prolonging pain sensitivity [40, 47], and neural degeneration and remodeling [48, 49].

Anti-inflammatory cytokines such as Interleukin-10 (IL-10) are crucial regulators of pain development. IL-10 [50] is a potent inhibitor of TNF-α, IL-1β, and IL-6 and it has been recognized for its ability to counter the sensitizing actions of the pro-inflammatory cytokines [51, 52]. These cytokine responses have been extensively studied in multiple animal models of injury, stress and chronic illness, and many of these observations have been verified in humans (reviewed in [53, 54]).

1.7 Chapter overview

This dissertation represents the initial findings of an ambitious exploratory study to investigate the mechanisms that contribute to the transition from acute to chronic pain. Since pain is very much an intersectional phenomenon between psyche and soma, the overarching theme throughout this body of work is an emphasis on deep clinical phenotyping. We have chosen to operate under the assumption that the body in pain is a complex system that demonstrates emergent properties that cannot be explained by single subsystem functions alone. To that end, the core of this dissertation will be presented in 3 distinct parts.

The aim of chapter 2 is to identify relationships between blood-based biomarkers and psychosocial dimensions of functioning. This provides a more inclusive look at physiological stress and immunity in an effort to go beyond simple bivariate relationships between biomarkers and pain. In this chapter, we outline an exploratory moderator analysis and hierarchical regression to find meaningful associations between cytokines and person-level variables such as sex, stress, fear, socioeconomic status, pre-existing comorbidity, and location of injury.

The aim of chapter 3 is to provide a longitudinal model of pain progression and recovery over the course of 12 months. Using latent growth curve modeling, statistically meaningful trajectories of recovery are identified in the context of both pain interference (a.k.a. functional disability) and pain severity (a.k.a. pain intensity). With pain interference as the primary functional outcome, people fall into 1 of 3 groups (or "recovery classes") where they demonstrate either a rapid recovery, delayed recovery, or minimal/no recovery by 12 months. With pain severity, people tended to fall into 1 of 2 groups where people either had a rapid recovery or minimal/no recovery. This work falls in line with previous studies of recovery trajectory modeling.

The aim of chapter 4 is to explore the potential of biomarkers to be used as prognostic tools for functional recovery outcomes. We introduce a panel of 8 different biomarkers, each with a previously identified association with pain. Using a similar analysis to chapter 3, latent class analysis and growth mixture modeling are used to identify 3 statistically meaningful classes based on biomarker concentration (low, average, and high concentration). For the sake of parsimony, this model is adequately represented by 3 of the 8 markers including IL-1β, BDNF, and TGF-β1. Although recovered groups do not tend to differ in their biomarker concentrations, those with persisting pain and disability may have higher levels of BDNF and TGF-β1 present in serum.

The objective of the overall SYMBIOME project is to use rigorously collected data and advanced longitudinal modeling techniques to identify and explore biopsychosocial

pathways of biological and cognitive functioning that can explain the transition from acute to chronic musculoskeletal pain. Also, by connecting the more personal indices of pain with biological mechanisms, we may gain a deeper understanding of this truly unique experience while identifying novel therapeutic targets. It is our hope that in doing so, we will provide biological evidence for the subjective, lived experience.

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Chapter 2

2 An exploration of blood marker-x-environment interaction effects on pain severity and interference scores in people with acute musculoskeletal trauma

2.1 Introduction

Pain is a complex experience that goes beyond simple sensory information. The neuromatrix model suggests that pain is not just a physical response. Through a combination of sensory, affective, and cognitive inputs, pain is the experience that is the end-result of a determination of actual or potential threat or harm [1]. The theory that pain goes beyond simple physical dimensions is also illustrated in the diathesis-stress model [2]; which suggests that certain psychosocial or physiological elements may render an individual more vulnerable to pain in the event of trauma. Due to the multifaceted nature of this experience, it is notoriously difficult to diagnose and treat [3]. Within the rehabilitation community, there is a wealth of literature that documents the psychological experiences of pain. Although the validity of these studies is not in question, pain in humans is measured largely through subjective self-report and has thus remained a highly debated condition [4].

From a biological standpoint, current theories in systems biology suggest that pain is the result of a well-orchestrated balance between the nervous, endocrine, and immune systems. The physiological stress response represents a powerful link between cognitive perception and biological action. A crucial regulator of this response is the Hypothalamic-pituitary-adrenal (HPA) axis, which connects cognitive impulses in the brain with key endocrine glands in the body [5]. This pathway incorporates such finely-tuned control mechanisms that even brief, experimentally-induced stress is sufficient to alter pain sensation and cause allodynia in healthy people [6]. Cortisol, a steroid hormone, is the primary product of HPA axis activity and people with either chronic or experimentally-induced pain show changes in cortisol production [7]. Another integral component of the pain experience is the involvement of the inflammatory response.

Inflammatory proteins such as c-reactive protein (CRP) have been implicated in modulating pain sensitivity and have been linked to other major health conditions such as diabetes, asthma and cardiovascular disease [8]. Other examples of inflammatory proteins in pain are pro-inflammatory cytokines Tumor Necrosis Factor alpha (TNF-α), Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6). These cytokines function synergistically and are substantially elevated during injury, inflammation, and chronic pain [9]. They appear to be involved in the development of hyperalgesia and allodynia [10-12], generation of noxious nerve transmissions [10, 13, 14], prolonging pain sensitivity [9, 15], and neural degeneration and remodeling [16, 17]. Anti-inflammatory cytokines Interleukin-10 (IL-10) and Transforming Growth Factor-beta (TGF-β1) also appear to play critical roles in pain development. Both IL-10 [18] and TGF-β1 [19] have been recognized for their ability to counter the pain-inducing actions of pro-inflammatory cytokines [20, 21]. The pain and sensitivity brought about by these proteins is relayed from the peripheral afferents to the spinal dorsal horn by factors such as brain-derived neurotrophic factor (BDNF); which is an essential neuropeptide involved in nerve growth, plasticity and sensitization [22].

As mentioned previously, the existence of pain does not rely solely on biological mechanisms. There are many examples of pain that exist in the absence of any obvious tissue damage or pathology [23]. There is enough research now to suggest that many chronic pain syndromes stem from a phenomenon known as sensitization; which involves dysregulation of the neural activity involved in pain perception [23, 24]. Many of the neural pathways of pain are shared by mood, depression, anxiety and fear, which are influenced by a variety of psychosocial variables [24]. Studies have demonstrated that socioeconomic status (SES) can affect levels of pain perception, where those with lower SES tend to report higher levels of pain intensity[25]. Even with the same level of pain intensity, those with lower SES may report anywhere from 2 to 3 times more perceived disability compared to those in a higher socioeconomic bracket [26]. Combining both the internal physiology and the external environment, pain is truly a complex phenomenon that requires balance between each system involved. When one or more of these systems fails to resolve itself, the whole response falls into discord and can become a chronic problem [27, 28]. What triggers this dysregulation, however, is still a topic of debate as the

transitional mechanisms between acute to chronic pain involves multiple domains that have not been definitively identified in humans [29, 30]. Current research emphasizes an interdependence of biopsychosocial domains such that pain may result from a combination of underlying biological vulnerability and/or induction via psychological or social triggers [31]. Prior work in the field of biomarkers indicates, for example, that processing of nociceptive information may occur through different receptors or pathways in males and females such that the magnitude of association between certain markers and pain could be moderated by sex [32, 33].

Therefore, the purpose of this paper was to add to this pool of knowledge by exploring potential interaction effects between relevant psychosocial variables and physiological mechanisms of pain (via biomarkers of stress, inflammation, and pain sensitivity) and perceived levels of pain within the acute stages of post-traumatic musculoskeletal injury.

2.2 Methods

2.2.1 Participant recruitment

Data for this study were drawn from the SYMBIOME (Systematic Merging of Biology, Mental Health and Environment) longitudinal cohort and data-banking study (clinicaltrials.gov ID no. NCT02711085). **Appendix C** outlines the overall data collection process for the study. Eligible participants were those presenting at an Urgent Care Centre in Ontario, Canada for reasons of non-catastrophic MSK trauma. Non-catastrophic referred to those with injuries that did not require surgery or hospitalization. Eligible participants presented to the centre within 3 weeks of the injury, were at least 18 years old, and could speak and understand conversational English. Study participants were admitted with injuries from a motor vehicle collision, fall/slip injuries, impact from another person or object, awkward lift/twist, or other context-specific causes that led to non-catastrophic (no surgery or inpatient admission required) injuries of the MSK system. Participants were free to indicate multiple areas of involvement. Participants were also asked to report whether they were taking any medications during the time of intake into the study. Excluded from this analysis were those with significant systemic or

neuromuscular comorbidity that would have been expected to affect physiological response to trauma or recovery, including active cancer, rheumatic conditions or other systemic inflammatory processes, significant organ disease, those with immunocompromised conditions (e.g. HIV/AIDS) or taking immunomodulatory drugs (e.g. high-dose steroids or disease-modifying anti-rheumatic drugs). Pregnancy was not an exclusion criterion, but no participant was pregnant during the study.

After being medically cleared and discharged, interested participants gave permission for a member of the research team to describe the study, answer questions, and enroll prior to leaving the hospital. Participants were provided a package of self-report questionnaires and serum samples were collected on-site from the median cubital vein by a phlebotomy-trained member of the research team. The questionnaires included tools to measure pain severity (Brief Pain Inventory - BPI, severity subscale [34]), pain-related functional interference (BPI, interference subscale [35]), and several questions pertaining to patient metadata (age, sex, work status, educational attainment, medicolegal status, household income and family status), and pre-existing health conditions (medications, comorbidities) variables. The BPI is one of the most widely used pain scales globally [36] and has adequate evidence of validity across many clinical populations including musculoskeletal pain [37]. Psychological and social elements have also been identified as influential outcomes in musculoskeletal trauma [38]. Key mental health dimensions including depressive symptoms and post-traumatic distress were captured in the SYMBIOME cohort. All participants provided informed, written consent prior to participation, and the study was approved by the local institutional review board prior to initiation.

2.2.2 Capture and analysis of serum biomarkers

The biomarkers chosen for this study are those associated with stress, pain, sensitivity, and inflammation (Table 2). Eight markers were chosen for this analysis: BDNF, TGF-β1, CRP, TNF-α, IL-1β, IL-6, IL-10 and cortisol. Analyte concentration in plasma (ng/g) was assayed using multiplexed biomarker immunoassay kits according to manufacturer

protocol for BDNF (Human Premixed Multi-Analyte Kit, R&D Systems Inc. cat. no. LXSAHM), TGF-β1 (TGFB11 Single Plex Magnetic Bead Kit, EMD Millipore cat. no. TGFB1MAG-64K-01), IL-1β, IL-6, IL-10 and TNF-α (Human High Sensitivity T Cell Magnetic Bead Panel Multiplex Kit, EMD Millipore cat. no. HSTCMAG-28SK). A BioPlex™ 200 readout System was used (Bio-Rad Laboratories, Hercules, CA) with Luminex® xMAP™ fluorescent bead technology (Luminex Corp., Austin, TX). Levels are automatically calculated from standard curves using Bio-Plex Manager software (v.4.1.1, Bio-Rad). Cortisol (Cortisol Enzyme Immunoassay Kit, Arbor Assays cat. no. K003-H1/H5), and CRP (C-Reactive Protein (human) ELISA Kit, Cayman Chemical Company cat. no. 10011236) were assayed following manufacturer protocol for Enzyme-Linked Immunosorbant assay (ELISA). All assays were performed in duplicate with the value for analysis being the mean concentration of the two. For the first 50 samples, assays were also conducted on two separate aliquots from each participant to monitor consistency in technique. Biomarker data were first explored for fidelity, removing any concentrations that, after duplicate runs, were not detectable or out of range of the kit. As correlations are sensitive to extreme values, we also identified any biomarker concentration that was >3SD above or below the sample mean and removed it to avoid spurious findings.

2.3 Analysis

Normality (skew and kurtosis) of biomarker data was statistically tested through Kolmogrov-Smirnov tests, and data were centered about the mean and square-root transformed where necessary. Descriptive statistics for the patient metadata were explored descriptively (mean, median, range).

For the first pass analysis a correlation matrix was created to estimate the simple bivariate associations between concentration of each of the 8 blood markers and scores on the two primary outcomes (pain and interference subscales of the BPI). As an exploratory analysis, the sample was then split into two sub-categories based on the level of participant metadata being explored (e.g. male/female, young/old age, high/low SES).

Where continuous data were used as a grouping variable (e.g. household income, body mass index, age), either a median split was used, or in the case of household income, the median Ontario income level as reported by StatsCan for 2016 of $80,000/year was used. The operationalization for each level of independent variable along with their definitions are outlined in **Table 3**. With the database split by level of moderator, bootstrapped linear correlations were again conducted. Bootstrapping using the n=1000 random sub-selection procedure in SPSS provided 95% confidence intervals for each of the correlation coefficients. Where the magnitude of correlation coefficient for one variable was outside of the confidence intervals of its categorical counterpart, that metadata variable was then retained for further analysis. Only those metadata variables for which each level included more than 10% of the total sample (minimum n = 8) were explored.

More rigorous exploration of potential moderators from the prior step occurred through hierarchical multiple regression analyses. Independent variables were (in order of entry): the individual biomarker for which the interaction may have been present, the metadata variable, and then the marker x metadata variable interaction term. After ensuring that assumptions of regression were satisfied, moderator analysis was conducted according to the methods previously described by Kraemer and colleagues [39]. This included centering the biomarker data on its mean to avoid multicollinearity and over-fitting of the models. Where the addition of the interaction term led to a significant change in F (significant improvement in model fit) after first controlling for the biomarker and metadata variable, the metadata variable was deemed a moderator of the association between biomarker and clinical pain or interference rating. If the inclusion of the interaction term led to significant change in model fit while the two base variables did not, the metadata variable was deemed to fully moderate the association. Where the interaction term was significant in addition to one of the two base variables, the moderation was considered partial. **Appendix D** depicts a simple schematic for moderator variables. All statistical analyses were performed with IBM SPSS Statistics 25.0 software.

2.3.1 Sample size estimation

Previous studies investigating biomarkers of pain performed by Cantón-Habas and colleagues have calculated their sample size based on a small to moderate potential correlation ($r = 0.3$) [40]. A power analysis was conducted using G*Power v3.1 [41] indicating that a total sample of 81 participants would be required to detect moderate effects ($f^2 = 0.10$) with $\beta = 80\%$ using multiple regression with $\alpha = 0.05$.

2.4 Results

From 2016 to 2018, a total of n = 109 participants provided blood samples used in the current analysis. Participant characteristics are reported descriptively in Table 1. When grouped by region as axial (head, neck or back) or peripheral (upper or lower extremities), 29.2% of the sample indicated axial injuries. Only 61.3% of participants reported taking medications, and 21.3% reported taking one or more medications for pain including NSAIDs, opioids, gabapentin, or pregabalin. Out of 109 participants, 3 participants were removed as all analytes were undetectable. Of the remaining 106 participants, some provided an incomplete data set as certain biomarkers were considered out-of-range. The proportion of assays that led to out-of-range results for the biomarkers were: IL-10 (3.7%), IL-1β (4.6%), TNF-α (2.8%), BDNF (0.9%), Cortisol (2.8%), TGF-β1 (0%), CRP (11.9%), IL-6 (11.9%). These values were >3SD beyond the mean and removed from the analyses. The results of Kolmogrov-Smirnov testing on the remaining data revealed significant deviation from normality in 6 of the 8 biomarkers, mostly due to positive skew. A square-root transformation was therefore applied to all 8 markers that significantly reduced the skew and saw all 8 markers achieve adequate normality for correlational analysis. Medicolegal Status was excluded from moderator analysis due to the number of participants involved in litigation on entry into the study (<3 weeks from injury) being below 10% of the sample. All other metadata variables could be split into two groups each of which included greater than 10% of the sample. Based on *a priori* sample size calculations, this required each group to contain more than 8 participants.

The first rows of Table 4 and 5 present the simple bivariate correlation coefficients between the 8 biomarkers and 2 clinical outcomes. None of the biomarkers on their own demonstrated a significant correlation with either BPI pain severity or interference. The Tables also present the correlation coefficients when the sample was first split by level of each of the 16 metadata variables. Bolded values are those that were explored through formal regression-based moderator analysis based on the difference in magnitude of correlation coefficient between the two levels.

2.4.1 Moderator analyses for Pain Severity

Results for the significant moderators are reported in Table 6A. Employment prior to trauma (Employed for pay/not employed for pay) fully moderated the association between TNF-α and pain severity, as the inclusion of the interaction term accounted for a significant 4.4% of the total variance in pain severity ($F(1, 90) = 4.39$, $p = 0.04$) (**Figure 1A**). Pre-existing psychopathology (diagnosed yes/no) fully moderated the association between TGF-β1 and pain severity ($F(1, 91) = 7.95$, $p < 0.01$) with the interaction term explaining 8.0% of the total variance (**Figure 1B**). Sex (male/female) fully moderated the association between CRP and pain severity ($F(1, 86) = 5.98$, $p = 0.02$), explaining 6.3% of the total variance **(Figure 1C**).

2.4.2 Moderator analyses for Pain Interference

Results for the significant moderators are reported in Table 6B. Reports of a pre-existing pain condition (yes/no) partially moderated the effect of IL-1β on pain interference, as it accounted for an additional 7.2% of variance beyond IL-1β alone ($F(1, 89) = 6.88$, $p = 0.01$). Inclusion of the interaction term accounted for an additional 6.9% (**Figure 2A**). Peri-traumatic fear (frightened at the time of the traumatic event, yes/no) also partially moderated the effect of IL-1β on pain interference, as it accounted for an additional 6.5% of variance beyond IL-1β alone, $F(1, 90) = 6.45$, $p = 0.01$ and the interaction term accounted for an additional 6.4% (**Figure 2B**). Region of injury fully moderated the effect of cortisol on pain interference, as the addition of the interaction term accounted

for 4.5% of the total variance, $F(1, 90) = 4.30$, $p = 0.04$ (**Figure 2C**). Region of injury also fully moderated the effect of TGF-β1 on pain interference, as the inclusion of the interaction term accounted for an additional 4.5% of the total variance, $F(1, 92) = 4.37$, $p = 0.04$ (**Figure 2D**). Peri-traumatic stress (general, life stress prior to the event) partially moderated the effect of TGF-β1 on pain interference, as it accounted for 8.9% of the total variance alone, $F(1, 91) = 8.91$, $p < 0.01$ and the interaction term explained an additional 4.4% (**Figure 2E**). Region of injury also fully moderated the effect of CRP on pain interference, as the inclusion of the interaction term accounted for an additional 10% of the total variance, $F(1, 87) = 10.28$, $p < 0.01$ (**Figure 2F**). Peri-traumatic fear partially moderated the effect of IL-6 on pain interference, as it accounted for 5.5% of the total variance alone, $F(1, 91) = 5.30$, $p = 0.02$ and the interaction term explained an additional 5.4% (**Figure 2G**).

2.5 Discussion

The purpose of this study was to determine whether various biological, psychological and social factors were associated with immune and neurological biomarkers in musculoskeletal trauma. The secondary purpose was to evaluate the extent to which these factors moderated any associations between biomarkers and pain. Similar to other research in the field of biomarkers, our study demonstrates the potential for psychological and social variables to influence agents of immunity. Our results showed that TNF-α, TGF-β1, and CRP form potentially meaningful associations with pain severity and IL-1β, cortisol, TGF-β1, CRP and IL-6 form associations with pain interference (disability) but only in the presence of important metadata variables. These associations were either partially or fully moderated by factors such as employment status, sex, pre-existing psychopathology, pre-existing pain, region of injury, peri-traumatic fear, and peri-traumatic stress.

Unlike prior work in pain biomarkers, none of the biomarkers on their own revealed a significant correlation with pain severity or interference. This is perhaps unsurprising as many of the studies indicating elevated levels of biomarkers in humans are shown under

chronic, not acute pain conditions [42]. It is also important to note that the present study analyzed serum levels of stress and inflammatory biomarkers. It is possible that trauma-related inflammation and stress in the acute phase may have been localized to the site of the injury. One of the few studies that measured biomarkers in acute pain and inflammation was performed by Angst and colleagues via microdialysis to track tissue-level cytokine changes after 24 hours [43]. Experimentally-inflamed skin did not affect TNF-α levels, but IL-1β, IL-10, and IL-6 were all significantly increased. They also found that administration of 400mg of the non-steroidal anti-inflammatory drug, ibuprofen, significantly increased heat and mechanical pain thresholds, while 800mg caused a decrease in IL-1β and IL-6 levels in the tissue. The microassays, along with experimental inflammation, both occurred in the thigh of their study participants which may suggest that the detected increase in cytokines was due to the proximity of the assayed tissues to the site of irritation.

The concept of external influences on biomarkers of stress and inflammation has been studied previously in the context of pain. Sibille and colleagues compiled a "risk factor composite" which included BMI, fibrinogen, CRP, and triglyceride levels [44]. Their results showed that generalized chronic pain was a significant predictor of this composite, but it was also significantly influenced by factors such as age, sex, education level, smoking habit, exercise, and alcohol consumption. Sterling and colleagues found that CRP and TNF-α were significantly different between severity groups in whiplash. They also showed that CRP was significantly different between acute and chronic phases of whiplash. However, in addition to IL-1β, those authors show that biomarkers were not significantly correlated with post-traumatic stress disorder or pain catastrophizing. In the study, they suggest that inflammatory biomarkers may be more associated with pain and disability rather than psychological constructs [45]. Our findings seem to be in agreement with previous studies in this area, however, we go on to suggest that the association between biomarkers and pain may actually depend on the presence of certain biopsychosocial factors.

Pain severity showed significant associations with only 3 different biomarkers, and these associations were dependent on specific variables. The relationship between TNF-α and pain severity was dependent on employment status prior to the trauma. Prior work suggests that inflammatory status can be influenced by productive activities such as employment and in particular, volunteering, can reduce the systemic inflammatory profile later in life [46]. Along a similar vein of research, Steptoe and colleagues reported differences in TNF-α and IL-6 blood levels between those in different grades of employment (high, intermediate, and low socioeconomic class). Their results suggested that intermediate grades have slightly elevated levels of TNF-α compared to high grade employees [47]. Results from the present study appear to agree with these findings as TNF-α displays other potential dichotomous relationships with pain severity in the context of return-to-work status, and with pain interference in the context of household income.

The relationship between TGF-β1 and pain severity was dependent on the presence or absence of psychopathology. Previous studies have suggested that psychopathologies such has depression and dementia involve a balance between 2 different classes of cytokines and that the TGF-β family (which represent a group of regulatory cytokines) can potentially influence this balance [48]. Those diagnosed with major depressive disorder, for example, have shown significant decreases in their TGF-β levels [49], and anti-depressive treatments for this disorder are associated with an increase in TGF-β [50]. With its anti-inflammatory properties, TGF-β1 has also been identified for its potential to influence key pathways associated with pain [51].

The relationship between CRP and pain severity seemed to be influenced by sex differences. It has been shown previously that men and women present differently with respect to pain. In osteoarthritis, for example, women experience a greater severity of pain and disability, and they appear to display more pain-related behaviors as well [52]. These differences may also extend to the levels of CRP in the blood as mean concentrations are also higher in females [53].

Pain interference, or disability showed significant associations with 5 different biomarkers, and once again these associations were dependent on the presence or absence of certain metadata variables. The relationship between IL-1β and disability was influenced by both pre-existing pain and peri-traumatic fear. Increases in IL-1β have been associated with allodynia (a feature of pathological pain), neuropathic pain, cognitive deficits and depression [54, 55]. As a well-known pro-inflammatory cytokine, this relationship has been fairly well-established in the literature so it comes as no particular surprise. What is interesting however is that IL-1β has also been implicated in conditioned fear memory [56] and enhancing the fear-learning response under stress [57]. These findings suggest that it is not unusual that IL-1β is influenced by both pain and fear. Peri-traumatic fear also influenced the association between IL-6 and disability. Like IL-1β, IL-6 is a pro-inflammatory cytokine that has also been shown to influence fear conditioning [58] and fear memory [59], along with being a key regulator of pathological pain states [60]. Both IL-1β and IL-6 have been shown to be involved with anxiety and fear-related pathologies such as PTSD and generalized anxiety disorder [61].

Another factor that appeared to be particularly influential in disability was the region of injury. The location of the trauma, either axial (head, neck or back) or peripheral (limb involvement), moderated the associations of cortisol, TGF-β1, and CRP with pain-related disability. Previous studies have shown that traumatic injuries involving axial structures result in more than twice the functional disability than peripheral injuries [62]. This may explain why the region of injury can influence these biomarkers of stress and inflammation. Cortisol, a primary stress hormone, is regulated by circadian rhythms, where dysfunctions in these rhythms have been associated with pain, fatigue, and coping in non-specific low back pain [63]. CRP has also been linked to chronic low back pain along with insomnia, which is a key contributor to disability [64]. With regard to TGF-β1, more recent studies have begun to investigate its role as a potential target for preventing degenerative disc disease, which can involve both the neck and back. It is has been shown to activate genes related to tissue growth and repair in the intervertebral discs, making it a potential therapeutic target in the future [65].

The regulatory role of TGF-β1 appears to extend even further as peri-traumatic stress also influenced its association with disability. Through a series of elegant experiments, Zhang et al showed that chronic stress has an immunosuppressive effect which is modulated by the TGF-β1 signaling pathway. A stress-induced imbalance of pro- and anti-inflammatory mediators was restored by blocking this pathway [66]. Taken together, each of these studies, along with our findings suggest that both pain and disability are fairly nuanced constructs that involve more than a simple binary association with immune and neurological biomarkers.

Some limitations to the present study will be addressed here. First, blood was drawn within the same time-frame that participants presented to the urgent care or emergency medical centres. This represents an important potential confound as it did not allow for consistency in sample collection times. Different biomarkers have known diurnal variations in their activity and these variations could not be accounted for as extraction occurred at various times throughout the day. Second, although we were able to demonstrate moderator effects of different variables between biomarkers and pain, the nature of these effects is still unknown. Based on an a priori sample calculation, we were sufficiently powered to show moderate effects, but within a single variable, some of the sample sizes may have been too small to detect the magnitude and direction of the different associations. For example, the region of injury group was approximately divided into 29.2% spinal and 70.8% peripheral injury. The medicolegal status group was excluded for this exact reason, despite being a potentially relevant moderator.

In conclusion, we have presented an exploratory study that demonstrates the potential involvement of biopsychosocial factors and their ability to influence the relationship between biomarkers and pain. This was done in the context of non-catastrophic musculoskeletal trauma using correlational analysis and regression. Our results show that employment status, pre-existing psychopathology, and sex emerge as relevant moderators of TNF-α, TGF-β1, and CRP respectively with regard to pain severity. Other factors such as pre-existing pain, peri-traumatic fear, region of injury, and peri-traumatic stress are relevant moderators of IL-1β, cortisol, TGF-β1, CRP, and IL-6 with regard to pain

interference/disability. This study adds to a pre-existing body of knowledge that suggests that the mere presence or absence of biomarkers is insufficient to adequately capture their effects on pain as their activity may be contextual. Studies that do not take these moderator variables into consideration may be at risk of underreporting the involvement of stress and inflammation in pain. Future research may involve investigating the downstream consequences of these psychophysical associations. Longitudinal studies may provide some insight into which associations are relevant in predicting the development of chronic pain in the future.

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Table 1 – Participant characteristics

Sample size	109
Mean Age	43.7 (±14.6)
Spinal injury	29.2%
Mean BMI	26.7 (±6.2)
Sex (% female)	57.7%
Mean pain severity (out of 10)	4.3 (±2.1)
Mean pain interference (%)	38.4 (±23.9)

Table 2 – Relevant immune and neurological biomarkers

Biomarker	Type	Rationale
TNF-α, Il-1β, Il-6	Pro-inflammatory cytokines	Increased during pain, generates hyperalgesia/allodynia, stimulates noxious signaling, prolongs pain sensitivity, contributes to neural degeneration and remodeling, influences long-term changes in neuronal signaling patterns
Il-10, TGF-β	Anti-inflammatory cytokines	decreased during pain, Inhibits TNF-α/Il-1β/Il-6, counter-acts pain sensitization activity of pro-inflammatory cytokines
BDNF	Secreted protein / neurotransmitter	Associated with sensitivity, depression, and anxiety. Facilitates interaction between peripheral and central nervous system
CRP	Inflammatory peptide	Increased during major pathology such as cardiovascular disease, diabetes, asthma. Increased levels implicated in increased pain sensitivity.
Cortisol	Steroid hormone	Primary product of HPA axis, associates with pain and stress, elevated in pain and stress, influences immune activity

Table 3 – Psychosocial variables with binary category designation

Metadata variable	***Category designations***
Sex	Male / Female
Region of Injury	Spinal (head, neck, or back) / peripheral (limb involvement)
Body-Mass Index (BMI)	>25.09 / <25.09 (since 25 indicates "overweight")
Employment prior	Employed / Unemployed for pay prior to trauma
Return-to-work status (RTW)	Full return / Partial or no return to previous employment
Medicolegal status	Involved / Not involved in litigation due to injury
Post-Secondary Education	post-secondary / no post-secondary (post-secondary includes college, university, trade school or other certification programs)
Household Income	<$80,000 / >$80,000 (where $80,000 represents the Ontario median)
Pre-existing Psychopathology	Present / Absent (any pre-existing condition that has been formally diagnosed and/or is being treated)
Pre-existing Pain	Present / Absent (any pre-existing condition that has been formally diagnosed and/or is being treated)
Peri-Traumatic Fear	Yes / No (whether or not the participant was frightened by the event)
Depression	Likely depressed / Not likely depressed (based on meeting the depression threshold on the PHQ-9 questionnaire)
Peri-Traumatic Stress	<2 / ≥2 (general, pre-trauma life stress was measured on a Likert scale where <2 indicates little or no life stress)
Any Adverse Childhood Experiences (ACE)	Absent / present (either direct or indirect exposure to abuse or neglect)

Table 4 – Correlations of biomarkers with pain severity in the presence of moderators

	TNFα (97)	**IL-10 (102)**	**BDNF (99)**	**IL-1β (94)**	**Cortisol (95)**	**TGFβ (97)**	**CRP (91)**	**IL-6 (94)**
Full sample (R (p))	-0.05 (0.61)	-0.08 (0.77)	-0.04 (0.72)	-0.03 (0.77)	-0.05 (0.65)	-0.05 (0.66)	-0.09 (0.39)	-0.12 (0.24)
Sex								
Female	(56) -0.03 (-0.32, 0.25)	(53) -0.09 (-0.31, 0.12)	**(58) 0.06 (-0.18, 0.30)**	(53) -0.07 (-0.36, 0.21)	(57) -0.02 (-0.29, 0.26)	(57) -0.01 (-0.25, 0.23)	**(54) -0.30 (-0.52, -0.04)*^^**	**(56) 0.06 (-0.26, 0.37)**
Male	(40) -0.02 (-0.32, 0.30)	(39) -0.07 (-0.39, 0.25)	**(40) -0.19 (-0.48, 0.16)**	(40) -0.01 (-0.23, 0.25)	(37) -0.15 (-0.45, 0.14)	(39) -0.18 (-0.50, 0.15)	**(36) 0.22 (-0.08, 0.46)**	**(37) -0.31 (-0.58, 0.01)**
Region of Injury								
Spinal	(24) 0.08 (-0.26, 0.37)	**(22) -0.27 (-0.62, 0.06)**	(24) 0.01 (-0.42, 0.49)	(21) 0.03 (-0.43, 0.48)	**(22) -0.43 (-0.76, 0.04)***	(24) 0.02 (-0.39, 0.49)	**(20) 0.14 (-0.31, 0.63)**	(21) 0.06 (-0.49, 0.67)
Peripheral	(73) -0.08 (-0.32, 0.16)	**(71) -0.03 (-0.23, 0.16)**	(75) -0.06 (-0.28, 0.18)	(73) -0.04 (-0.24, 0.16)	**(73) 0.05 (-0.18, 0.27)**	(73) -0.05 (-0.26, 0.16)	**(71) -0.16 (-0.37, 0.07)**	(73) -0.17 (-0.39, 0.08)
BMI								
≤25.09	(46) -0.09 (-0.43, 0.26)	(41) -0.17 (-0.42, 0.08)	(43) 0.02 (-0.28, 0.32)	**(42) -0.18 (-0.45, 0.10)**	(41) -0.13 (-0.42, 0.15)	(42) -0.12 (-0.39, 0.19)	(41) -0.05 (-0.36, 0.26)	(41) -0.22 (-0.53, 0.13)
>25.09	(39) 0.01 (-0.29, 0.31)	(46) -0.01 (-0.24, 0.23)	(50) 0.03 (-0.24, 0.29)	**(47) 0.16 (-0.10, 0.40)**	(48) -0.04 (-0.33, 0.24)	(50) -0.01 (-0.31, 0.28)	(45) -0.07 (-0.35, 0.24)	(48) 0.06 (-0.27, 0.37)
Employment Prior								
Employed for pay	**(70) -0.20 (-0.40, 0.02)**	(69) -0.05 (-0.24, 0.14)	(72) -0.08 (-0.31, 0.16)	**(68) -0.03 (-0.22, 0.19)**	**(69) 0.08 (-0.15, 0.31)**	**(70) 0.05 (-0.17, 0.26)**	(68) -0.14 (-0.34, 0.08)	(69) -0.15 (-0.41, 0.13)
Not employed for pay	**(24) 0.28 (-0.13, 0.61)^^**	(22) -0.04 (-0.44, 0.36)	(24) -0.03 (-0.47, 0.37)	**(23) -0.24 (-0.58, 0.12)**	**(23) -0.20 (-0.56, 0.19)**	**(24) -0.35 (-0.68, 0.03)**	(22) 0.01 (-0.42, 0.46)	(23) -0.04 (-0.44, 0.33)
Return-To-Work Status								
Full RTW	**(52) -0.25 (-0.47, -0.01)**	(50) -0.16 (-0.41, 0.09)	(53) -0.11 (-0.35, 0.16)	(50) -0.12 (-0.37, 0.17)	(53) 0.03 (-0.20, 0.29)	(51) 0.04 (-0.22, 0.27)	(52) -0.18 (-0.40, 0.06)	(53) -0.18 (-0.48, 0.16)
Partial or no RTW	**(37) 0.16 (-0.18, 0.49)**	(35) -0.02 (-0.28, 0.26)	(38) 0.08 (-0.24, 0.37)	(36) 0.08 (-0.20, 0.39)	(34) -0.09 (-0.44, 0.27)	(38) -0.13 (-0.41, 0.16)	(33) -0.02 (-0.41, 0.36)	(34) -0.04 (-0.37, 0.24)
Post-Secondary Education								
No post-secondary ed.	(25) -0.01 (-0.54, 0.50)	(24) -0.18 (-0.53, 0.16)	**(24) 0.18 (-0.19, 0.48)**	**(25) 0.12 (-0.29, 0.50)**	**(22) 0.19 (-0.23, 0.59)**	(24) -0.08 (-0.49, 0.35)	(21) -0.12 (-0.57, 0.34)	(22) -0.13 (-0.60, 0.28)
Post-secondary ed.	(71) -0.07 (-0.27, 0.15)	(68) -0.05 (-0.25, 0.15)	**(74) -0.09 (-0.30, 0.14)**	**(68) -0.09 (-0.28, 0.12)**	**(72) -0.13 (-0.36, 0.11)**	(72) -0.05 (-0.25, 0.17)	(70) -0.08 (-0.30, 0.15)	(72) -0.11 (-0.36, 0.16)
Household Income								
<$80k	(46) -0.14 (-0.46, 0.17)	(45) -0.16 (-0.39, 0.07)	(48) -0.11 (-0.37, 0.14)	**(45) -0.20 (-0.49, 0.11)**	(45) -0.12 (-0.39, 0.20)	(47) -0.13 (-0.37, 0.13)	(43) -0.12 (-0.40, 0.21)	**(45) -0.34 (-0.64, -0.03)***
≥$80k	(47) 0.02 (-0.23, 0.27)	(45) 0.02 (-0.24, 0.28)	(47) 0.00 (-0.31, 0.30)	**(45) 0.14 (-0.14, 0.39)**	(46) 0.10 (-0.16, 0.35)	(46) 0.01 (-0.30, 0.29)	(46) -0.05 (-0.31, 0.25)	**(46) 0.02 (-0.33, 0.34)**
Pre-Existing								

Psychopathology Yes No	(26) 0.16 (-0.20, 0.46) (69) -0.10 (-0.39, 0.17)	**(25) -0.24 (-0.49, 0.00)** **(66) 0.02 (-0.19, 0.22)**	(26) -0.18 (-0.62, 0.18) (71) -0.01 (-0.25, 0.23)	**(25) -0.33 (-0.62, -0.02)** **(67) 0.05 (-0.16, 0.28)**	(25) 0.05 (-0.37, 0.47) (68) -0.10 (-0.34, 0.15)	**(26) -0.48 (-0.70, 0.17)*^^** **(69) 0.10 (-0.12, 0.30)**	(23) 0.02 (-0.36, 0.37) (67) -0.15 (-0.38, 0.10)	(23) -0.29 (-0.78, 0.16) (70) -0.05 (-0.33, 0.22)
Pre-Existing Pain Yes No	**(17) 0.41 (-0.06, 0.81)** **(76) -0.11 (-0.34, 0.14)**	**(16) 0.19 (-0.20, 0.79)** **(73) -0.12 (-0.32, 0.06)**	(17) -0.13 (-0.57, 0.32) (78) -0.04 (-0.25, 0.19)	**(17) 0.23 (-0.21, 0.59)** **(75) -0.10 (-0.29, 0.10)**	**(17) 0.25 (-0.16, 0.56)** **(74) -0.06 (-0.29, 0.19)**	**(16) -0.33 (-0.70, 0.25)** **(77) 0.00 (-0.19, 0.20)**	(14) 0.07 (-0.41, 0.51) (75) -0.11 (-0.34, 0.11)	**(15) 0.27 (-0.64, 0.86)** **(77) -0.20 (-0.43, 0.03)**
Peri-Traumatic Fear No Yes	(50) -0.17 (-0.45, 0.14) (46) 0.08 (-0.24, 0.37)	**(50) -0.17 (-0.40, 0.10)** **(42) 0.05 (-0.21, 0.30)**	(52) -0.05 (-0.30, 0.19) (46) 0.01 (-0.31, 0.36)	**(49) -0.15 (-0.41, 0.10)** **(44) 0.14 (-0.12, 0.38)**	(51) -0.02 (-0.30, 0.24) (43) -0.08 (-0.39, 0.24)	(50) 0.03 (-0.23, 0.26) (46) -0.14 (-0.39, 0.16)	(49) -0.17 (-0.45, 0.12) (42) 0.01 (-0.27, 0.31)	(50) -0.28 (-0.54, 0.03) (44) 0.02 (-0.30, 0.34)
Depression Likely depressed Not likely depressed	(27) -0.02 (-0.43, 0.40) (69) -0.08 (-0.33, 0.17)	**(25) -0.36 (-0.66, -0.09)** **(67) 0.03 (-0.20, 0.24)**	(27) 0.09 (-0.27, 0.46) (71) -0.08 (-0.29, 0.16)	**(26) -0.36 (-0.61, -0.12)** **(67) 0.07 (-0.16, 0.31)**	(25) 0.01 (-0.38, 0.42) (69) -0.03 (-0.28, 0.22)	(27) -0.13 (-0.45, 0.24) (69) -0.04 (-0.28, 0.19)	(22) -0.01 (-0.46, 0.42) (69) -0.10 (-0.32, 0.12)	**(24) -0.41 (-0.72, -0.10)*** **(70) -0.04 (-0.30, 0.25)**
Peri-Traumatic Stress Low stress (<2) High stress (2 or more)	**(45) 0.09 (-0.22, 0.38)** **(49) -0.23 (-0.46, 0.06)**	(44) 0.03 (-0.21, 0.27) (46) -0.17 (-0.42, 0.09)	(47) -0.14 (-0.39, 0.12) (49) -0.02 (-0.33, 0.25)	(44) -0.01 (-0.27, 0.28) (47) -0.15 (-0.42, 0.10)	(44) -0.08 (-0.38, 0.23) (48) -0.02 (-0.33, 0.27)	(46) -0.18 (-0.46, 0.13) (48) -0.01 (-0.22, 0.22)	(43) -0.09 (-0.41, 0.23) (46) 0.04 (-0.15, 0.25)	**(46) 0.04 (-0.30, 0.39)** **(46) -0.37 (-0.64, -0.07)***
Any ACE At least one None	(59) 0.06 (-0.24, 0.35) (36) -0.22 (-0.54, 0.11)	(56) -0.05 (-0.25, 0.13) (35) -0.12 (-0.46, 0.25)	(60) 0.04 (-0.21, 0.26) (37) -0.09 (-0.40, 0.26)	(57) -0.01 (-0.24, 0.23) (35) -0.07 (-0.38, 0.27)	(56) 0.03 (-0.23, 0.28) (37) -0.14 (-0.47, 0.24)	(60) -0.03 (-0.26, 0.21) (35) -0.04 (-0.38, 0.30)	(55) -0.17 (-0.42, 0.10) (35) -0.08 (-0.39, 0.21)	(57) -0.03 (-0.32, 0.26) (36) -0.30 (-0.59, 0.06)
Any ACE (direct) At least one None	(39) 0.02 (-0.26, 0.30) (56) -0.10 (-0.40, 0.19)	(37) -0.17 (-0.40, 0.05) (54) -0.05 (-0.32, 0.23)	(40) 0.04 (-0.29, 0.32) (57) -0.06 (-0.30, 0.20)	**(38) -0.21 (-0.49, 0.04)** **(54) 0.08 (-0.18, 0.34)**	(38) -0.04 (-0.33, 0.30) (55) -0.03 (-0.33, 0.24)	(40) -0.07 (-0.36, 0.23) (55) 0.01 (-0.27, 0.27)	(37) -0.03 (-0.34, 0.29) (53) -0.18 (-0.42, 0.09)	(38) -0.10 (-0.48, 0.25) (55) -0.16 (-0.41, 0.14)
Any ACE (indirect) At least one None	(51) 0.02 (-0.28, 0.34) (44) -0.12 (-0.41, 0.15)	(49) -0.09 (-0.33, 0.14) (42) -0.07 (-0.35, 0.22)	(52) 0.06 (-0.21, 0.30) (45) -0.10 (-0.38, 0.22)	(49) -0.02 (-0.29, 0.25) (43) -0.05 (-0.35, 0.24)	(48) 0.07 (-0.22, 0.35) (45) -0.15 (-0.45, 0.16)	(52) 0.02 (-0.23, 0.24) (43) -0.09 (-0.38, 0.24)	(47) -0.13 (-0.42, 0.18) (43) -0.12 (-0.37, 0.18)	(49) -0.06 (-0.40, 0.26) (44) -0.21 (-0.51, 0.12)

Bolded items indicate a significant correlation and/or one value exceeding the confidence intervals of its categorical counterpart. Grayed out regions represent excluded variables.

* = Correlation significant at the $p<0.05$ level

** = Correlation significant at the $p<0.01$ level

^ = partial moderation (interaction term retained with other variables)

^^ = full moderation (only interaction is retained)

Table 5 – Correlations of biomarkers with pain interference in the presence of moderators

	TNFα (96)	**IL-10 (92)**	**BDNF (98)**	**IL-1β (93)**	**Cortisol (94)**	**TGFβ (96)**	**CRP (91)**	**IL-6 (94)**
Full sample (R (p))	0.06 (0.56)	-0.04 (0.69)	-0.02 (0.88)	-0.00 (0.97)	-0.10 (0.33)	-0.05 (0.66)	-0.19 (0.07)	0.08 (0.47)
Sex								
Female	(56) 0.08 (-0.19, 0.33)	(53) -0.08 (-0.31, 0.15)	(58) 0.01 (-0.24, 0.26)	(53) 0.02 (-0.25, 0.31)	(57) -0.17 (-0.40, 0.09)	(57) -0.09 (-0.32, 0.15)	(54) -0.13 (-0.38, 0.15)	(56) 0.09 (-0.19, 0.38)
Male	(39) 0.04 (-0.24, 0.31)	(38) 0.01 (-0.29, 0.27)	(39) -0.07 (-0.36, 0.24)	(39) -0.05 (-0.33, 0.23)	(36) -0.02 (-0.43, 0.31)	(38) -0.01 (-0.31, 0.36)	(36) -0.25 (-0.51, 0.07)	(37) 0.057 (-0.25, 0.38)
Region of Injury								
Spinal	**(23) 0.38 (-0.18, 0.72)**	**(21) -0.25 (-0.67, 0.18)**	**(23) 0.32 (-0.20, 0.72)**	**(20) 0.32 (-0.16, 0.67)**	**(21) -0.52 (-0.80, -0.09)*^^**	**(23) 0.38 (-0.07, 0.79)^^**	**(20) 0.44 (-0.01, 0.81)**	(21) 0.25 (-0.14, 0.70)
Peripheral	**(73) 0.01 (-0.20, 0.23)**	**(71) -0.02 (-0.21, 0.18)**	**(75) -0.09 (-0.28, 0.10)**	**(73) -0.07 (-0.27, 0.14)**	**(73) 0.01 (-0.19, 0.25)**	**(73) -0.15 (-0.34, 0.06)**	**(71) -0.35 (-0.54, -0.14)**^^**	(73) 0.04 (-0.19, 0.28)
BMI								
≤25.09	(48) -0.05 (-0.34, 0.27)	(41) -0.02 (-0.28, 0.25)	(43) -0.08 (-0.32, 0.17)	**(42) -0.19 (-0.47, 0.07)**	**(41) -0.32 (-0.55, -0.08)***	(42) -0.12 (-0.38, 0.17)	(41) -0.23 (-0.50, 0.08)	(41) -0.03 (-0.37, 0.32)
>25.09	(39) 0.15 (-0.11, 0.38)	(46) -0.05 (-0.31, 0.24)	(50) 0.09 (-0.17, 0.35)	**(47) 0.19 (-0.13, 0.45)**	**(48) 0.03 (-0.28, 0.34)**	(50) -0.01 (-0.31, 0.25)	(45) -0.15 (-0.39, 0.14)	(48) 0.25 (-0.03, 0.48)
Employment Prior								
Employed for pay	(70) 0.04 (-0.18, 0.28)	(69) 0.00 (-0.19, 0.20)	(72) -0.10 (-0.29, 0.11)	**(68) 0.06 (-0.16, 0.28)**	**(69) 0.00 (-0.23, 0.23)**	(70) -0.02 (-0.23, 0.19)	(68) -0.13 (-0.33, 0.10)	(69) 0.02 (-0.19, 0.25)
Not employed for pay	(24) 0.02 (-0.38, 0.41)	(22) -0.11 (-0.52, 0.38)	(24) 0.10 (-0.39, 0.52)	**(23) -0.39 (-0.71, 0.03)**	**(23) -0.25 (-0.67, 0.17)**	(24) -0.19 (-0.57, 0.20)	(22) -0.31 (-0.66, 0.02)	(23) 0.18 (-0.25, 0.60)
Return-To-Work Status								
Full RTW	(52) -0.01 (-0.26, 0.26)	(50) -0.15 (-0.42, 0.10)	(53) -0.05 (-0.28, 0.22)	(50) -0.04 (-0.35, 0.27)	**(53) 0.04 (-0.22, 0.27)**	**(51) 0.14 (-0.11, 0.38)**	(52) -0.18 (-0.38, 0.07)	(53) -0.07 (-0.34, 0.21)
Partial or no RTW	(37) 0.14 (-0.19, 0.47)	(35) 0.05 (-0.22, 0.28)	(38) -0.02 (-0.32, 0.33)	(36) 0.07 (-0.23, 0.35)	**(34) -0.26 (-0.57, 0.15)**	**(38) -0.24 (-0.47, 0.01)**	(33) -0.22 (-0.54, 0.16)	(34) 0.16 (-0.13, 0.45)
Post-Secondary Education	(25) -0.07 (-0.47, 0.37)	**(24) -0.29 (-0.61, 0.10)**	(24) 0.01 (-0.29, 0.36)	**(25) 0.14 (-0.31, 0.60)**	(22) -0.04 (-0.41, 0.40)	(24) -0.08 (-0.44, 0.32)	**(21) -0.51 (-0.73, -0.18)***	(22) 0.06 (-0.34, 0.50)
No post-secondary ed.	(71) 0.12 (-0.10, 0.32)	**(68) 0.05 (-0.16, 0.25)**	(74) -0.02 (-0.25, 0.20)	**(68) -0.07 (-0.29, 0.14)**	(72) -0.14 (-0.38, 0.10)	(72) -0.03 (-0.26, 0.19)	**(70) -0.09 (-0.31, 0.13)**	(72) 0.09 (-0.15, 0.33)
Post-secondary ed.								
Household Income								
<$80k	**(46) -0.13 (-0.44, 0.17)**	(45) -0.09 (-0.32, 0.19)	(48) -0.06 (-0.32, 0.22)	**(45) -0.18 (-0.45, 0.11)**	(45) -0.16 (-0.43, 0.15)	(47) -0.04 (-0.31, 0.22)	**(43) -0.27 (-0.53, 0.05)**	(45) -0.04 (-0.34, 0.27)
≥$80k	**(47) 0.23 (-0.01, 0.46)**	(45) -0.00 (-0.33, 0.26)	(47) -0.07 (-0.30, 0.19)	**(45) 0.15 (-0.14, 0.40)**	(46) 0.08 (-0.18, 0.32)	(46) -0.12 (-0.37, 0.15)	**(46) -0.01 (-0.26, 0.25)**	(46) 0.12 (-0.17, 0.45)
Pre-Existing								

Psychopathology Yes No	(26) -0.06 (-0.44, 0.31) (69) 0.10 (-0.15, 0.35)	**(25) -0.36 (-0.67, -0.04)** **(66) 0.07 (-0.15, 0.27)**	(26) -0.11 (-0.43, 0.22) (71) 0.01 (-0.21, 0.24)	**(25) -0.37 (-0.67, 0.05)** **(67) 0.07 (-0.16, 0.28)**	(25) 0.02 (-0.31, 0.31) (68) -0.15 (-0.38, 0.09)	**(26) -0.31 (-0.62, 0.11)** **(69) 0.06 (-0.16, 0.27)**	(23) -0.26 (-0.63, 0.09) (67) -0.16 (-0.36, 0.09)	**(23) -0.19 (-0.64, 0.31)** **(70) 0.15 (-0.08, 0.37)**
Pre-Existing Pain Yes No	(17) 0.24 (-0.16, 0.71) (76) 0.06 (-0.14, 0.26)	**(16) 0.32 (-0.10, 0.66)** **(73) -0.12 (-0.35, 0.09)**	**(17) 0.12 (-0.34, 0.62)** **(78) -0.10 (-0.28, 0.10)**	**(17) 0.49 (-0.00, 0.86)*^** **(75) -0.13 (-0.36, 0.09)**	(17) -0.20 (-0.67, 0.19) (74) -0.02 (-0.25, 0.20)	**(16) 0.13 (-0.33, 0.70)** **(77) -0.10 (-0.30, 0.12)**	(14) -0.30 (-0.65, 0.21) (75) -0.17 (-0.38, 0.07)	**(15) 0.42 (-0.24, 0.81)** **(77) 0.02 (-0.21, 0.26)**
Peri-Traumatic Fear No Yes	**(50) -0.08 (-0.33, 0.19)** **(46) 0.19 (-0.08, 0.47)**	**(50) -0.21 (-0.46, 0.04)** **(42) 0.16 (-0.09, 0.41)**	(52) -0.03 (-0.25, 0.22) (46) 0.04 (-0.28, 0.34)	**(49) -0.23 (-0.48, 0.05)** **(44) 0.29 (0.04, 0.51)^**	(51) -0.04 (-0.33, 0.23) (43) -0.19 (-0.51, 0.14)	(50) -0.02 (-0.27, 0.22) (46) -0.04 (-0.33, 0.26)	(49) -0.26 (-0.47, 0.00) (42) -0.12 (-0.42, 0.16)	**(50) -0.20 (-0.45, 0.09)** **(44) 0.28 (0.02, 0.52)^**
Depression Likely depressed Not likely depressed	(27) -0.07 (-0.44, 0.36) (69) 0.11 (-0.09, 0.32)	(25) -0.24 (-0.60, 0.15) (67) -0.03 (-0.25, 0.19)	**(27) 0.22 (-0.18, 0.55)** **(71) -0.09 (-0.30, 0.10)**	(26) -0.15 (-0.48, 0.21) (67) 0.00 (-0.24, 0.23)	(25) -0.21 (-0.57, 0.13) (69) -0.02 (-0.25, 0.20)	(27) 0.01 (-0.33, 0.37) (69) -0.11 (-0.32, 0.14)	**(22) -0.11 (-0.56, 0.32)** **(69) -0.18 (-0.37, 0.02)**	**(24) -0.21 (-0.55, 0.19)** **(70) 0.15 (-0.09, 0.38)**
Peri-traumatic Stress Low stress (<2) High stress (2 or more)	(45) 0.03 (-0.27, 0.32) (49) 0.07 (-0.18, 0.34)	(44) -0.01 (-0.30, 0.27) (46) -0.06 (-0.28, 0.15)	**(47) -0.24 (-0.43, -0.01)** **(49) 0.15 (-0.11, 0.41)**	(44) -0.01 (-0.31, 0.28) (47) -0.05 (-0.33, 0.25)	(44) -0.14 (-0.41, 0.16) (48) -0.08 (-0.35, 0.18)	**(46) -0.31 (-0.54, -0.03)*^** **(48) 0.13 (-0.12, 0.39)**	(43) -0.17 (-0.42, 0.11) (46) -0.08 (-0.35, 0.22)	(46) 0.14 (-0.16, 0.47) (46) -0.02 (-0.28, 0.28)
Any ACE At least one None	(59) 0.00 (-0.26, 0.25) (36) 0.18 (-0.13, 0.44)	(56) -0.11 (-0.33, 0.12) (35) 0.050 (-0.29, 0.38)	(60) 0.02 (-0.22, 0.26) (37) -0.02 (-0.31, 0.29)	(57) -0.06 (-0.30, 0.17) (35) 0.10 (-0.23, 0.45)	(56) -0.02 (-0.28, 0.24) (37) -0.21 (-0.48, 0.09)	(60) -0.02 (-0.26, 0.24) (35) -0.07 (-0.42, 0.28)	**(55) -0.27 (-0.47, -0.04)*** **(35) -0.12 (-0.42, 0.22)**	(57) 0.07 (-0.21, 0.35) (36) 0.10 (-0.22, 0.43)
Any ACE personal At least one None	(39) -0.04 (-0.33, 0.25) (56) 0.16 (-0.08, 0.38)	(37) -0.15 (-0.42, 0.12) (54) 0.01 (-0.25, 0.24)	(40) -0.02 (-0.31, 0.25) (57) 0.00 (-0.26, 0.28)	(38) -0.13 (-0.40, 0.17) (54) 0.08 (-0.19, 0.35)	(38) -0.20 (-0.50, 0.13) (55) -0.04 (-0.31, 0.23)	(40) -0.01 (-0.26, 0.26) (55) -0.06 (-0.34, 0.24)	(37) -0.19 (-0.44, 0.09) (53) -0.22 (-0.45, 0.03)	(38) -0.02 (-0.34, 0.28) (55) 0.14 (-0.12, 0.44)
Any ACE other At least one None	(51) -0.04 (-0.32, 0.26) (44) 0.17 (-0.06, 0.39)	(49) -0.15 (-0.42, 0.11) (42) 0.07 (-0.20, 0.33)	(52) 0.02 (-0.23, 0.31) (45) -0.03 (-0.28, 0.24)	(49) -0.11 (-0.37, 0.15) (43) 0.09 (-0.21, 0.37)	**(48) 0.06 (-0.26, 0.34)** **(45) -0.25 (-0.49, 0.04)**	(52) 0.00 (-0.23, 0.26) (43) -0.09 (-0.37, 0.22)	(47) -0.28 (-0.50, -0.04) (43) -0.14 (-0.40, 0.13)	(49) 0.02 (-0.28, 0.33) (44) 0.11 (-0.19, 0.41)

Bolded items indicate a significant correlation and/or one value exceeding the confidence intervals of its categorical counterpart. Grayed out regions represent excluded variables.

* = Correlation significant at the $p<0.05$ level

** = Correlation significant at the $p<0.01$ level

^ = partial moderation (interaction term retained with other variables)

^^ = full moderation (only interaction is retained)

Table 6 – Significant Moderators identified for Hierarchical multiple Regression Analyses

A – *Pain Severity*

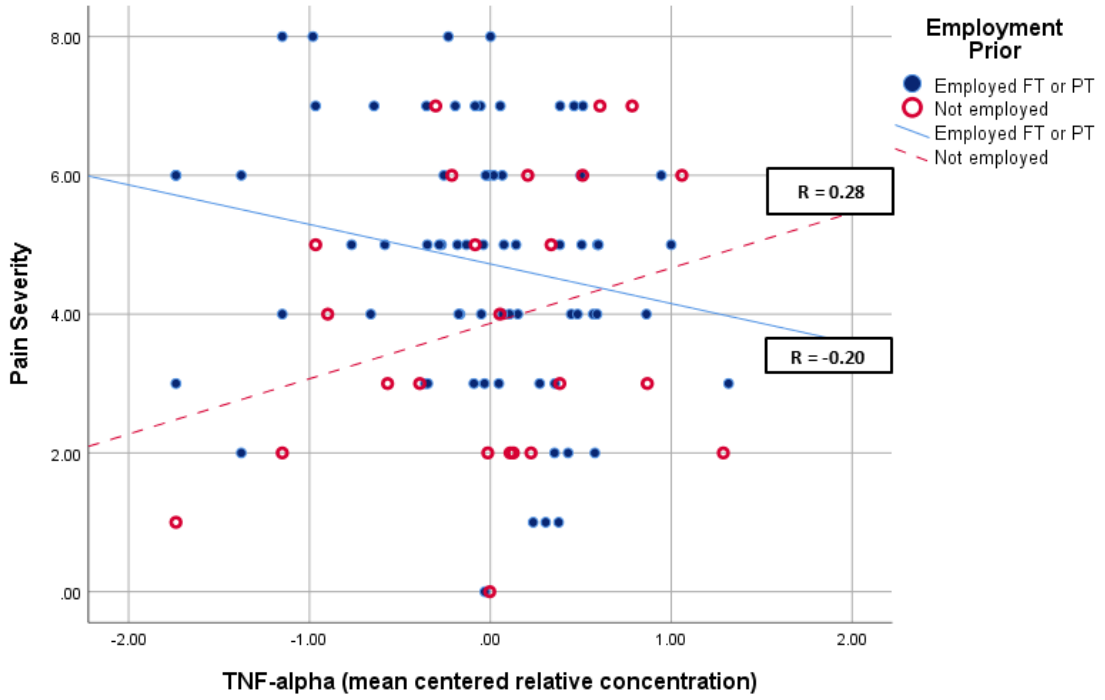
	β (95%CI)	R^2 (Δr^2)	F change (p)
TNF-α	-1.94 (-3.72, -0.16)	0.01 (0.01)	0.42 (0.52)
Employment Prior	-0.86 (-1.75, 0.04)	0.04 (0.04)	3.63 (0.06)
TNF-α x Employment Prior	**1.37 (0.07, 2.67)**	**0.09 (0.04)**	**4.39 (0.04)**
TGF-β	-0.06 (-0.09, -0.02)	0.01 (0.01)	0.56 (0.46)
Pre-Existing Psychopathology	0.05 (-0.81, 0.90)	0.01 (0.00)	0.14 (0.71)
TGF-β x Pre-existing Psychopathology	**0.03 (0.01, 0.05)**	**0.09 (0.08)**	**7.95 (<0.01)**
CRP	0.06 (0.00, 0.11)	0.01 (0.01)	0.56 (0.46)
Sex	0.46 (-0.36, 1.27)	0.03 (0.02)	1.68 (0.20)
CRP x Sex	**-0.04 (-0.07, -0.01)**	**0.09 (0.06)**	**5.98 (0.02)**

B – *Pain Interference*

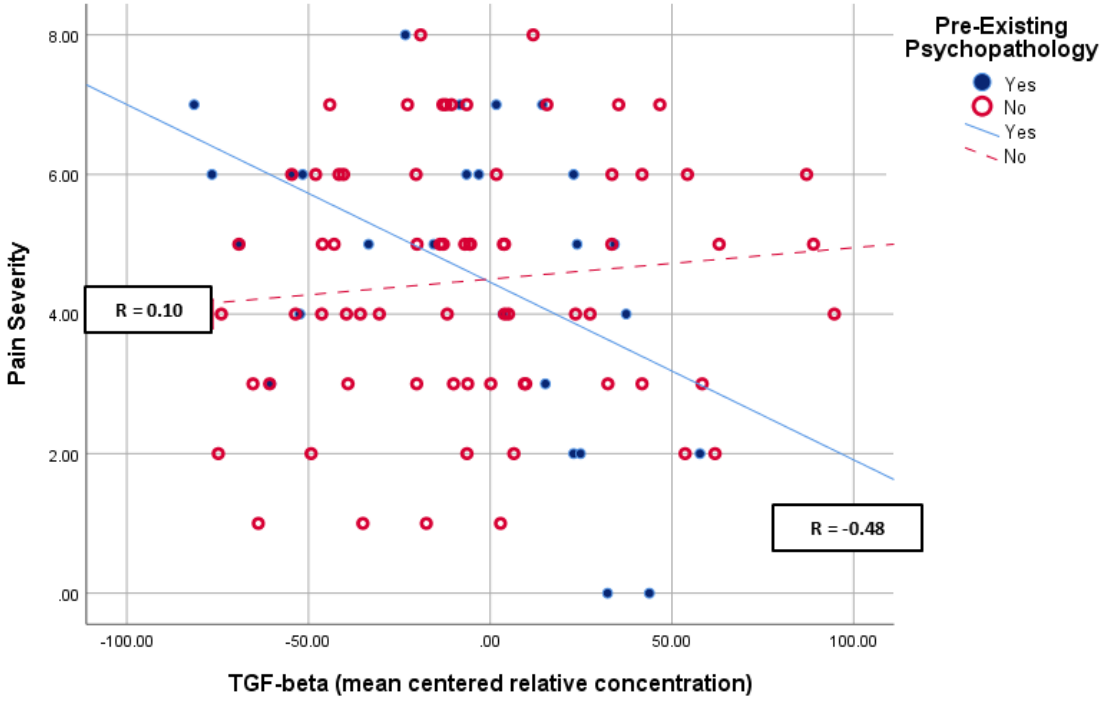
	β (95%CI)	R^2 (Δr^2)	F change (p)
IL-1β	59.96 (13.65, 106.27)	0.00 (0.00)	0.01 (0.94)
Pre-Existing Pain	**-9.92 (-18.32, -1.52)**	**0.07 (0.07)**	**6.88 (0.01)**
IL-1β x Pre-Existing Pain	**-32.03 (-56.07, -8.00)**	**0.14 (0.07)**	**7.01 (0.01)**
IL-1β	29.79 (6.25, 53.33)	0.00 (0.00)	0.00 (0.97)
Peri-Traumatic Fear	**-9.18 (-15.83, -2.53)**	**0.07 (0.07)**	**6.45 (0.01)**
IL-1β x Peri-Traumatic Fear	**-18.94 (-33.58, -4.29)**	**0.13 (0.06)**	**6.60 (0.01)**
Cortisol	-0.18 (-0.35, -0.02)	0.01 (0.01)	0.95 (0.33)
Region of Injury	4.56 (-3.67, 12.78)	0.02 (0.01)	0.51 (0.48)
Cortisol x Region of	**0.09 (0.00, 0.18)**	**0.06 (0.05)**	**4.30 (0.04)**

Injury			
TGF-β	0.41 (-0.01, 0.82)	0.00 (0.00)	0.19 (0.66)
Region of Injury	2.51 (-5.28, 10.30)	0.01 (0.01)	1.08 (0.30)
TGF-β x Region of Injury	**-0.23 (-0.45, -0.01)**	**0.06 (0.05)**	**4.37 (0.04)**
TGF-β	-0.28 (-0.53, -0.04)	0.00 (0.00)	0.14 (0.71)
Peri-Traumatic Stress	**10.58 (4.13, 17.04)**	**0.09 (0.09)**	**8.91 (<0.01)**
TGF-β x Peri-Traumatic Stress	**0.17 (0.01, 0.32)**	**0.13 (0.04)**	**4.56 (0.04)**
CRP	0.66 (0.17, 1.15)	0.04 (0.04)	3.38 (0.07)
Region of Injury	5.74 (-1.70, 13.19)	0.06 (0.02)	1.96 (0.17)
CRP x Region of Injury	**-0.44 (-0.71, -0.17)**	**0.16 (0.10)**	**10.28 (<0.01)**
IL-6	4.38 (0.71, 8.05)	0.01 (0.01)	0.54 (0.47)
Peri-Traumatic Fear	**-8.14 (-14.56, -1.72)**	**0.06 (0.06)**	**5.30 (0.02)**
IL-6 x Peri-Traumatic Fear	**-2.84 (-5.26, -0.42)**	**0.11 (0.05)**	**5.45 (0.02)**

A


Employment Prior
Employed FT or PT
Not employed
Employed FT or PT
Not employed
R = 0.28
R = -0.20
Pain Severity
8.00
6.00
4.00
2.00
.00
-2.00
-1.00
.00
1.00
2.00
TNF-alpha (mean centered relative concentration)

B


Pre-Existing Psychopathology
Yes
No
Yes
No
R = 0.10
R = -0.48
Pain Severity
8.00
6.00
4.00
2.00
.00
-100.00
-50.00
.00
50.00
100.00
TGF-beta (mean centered relative concentration)

C

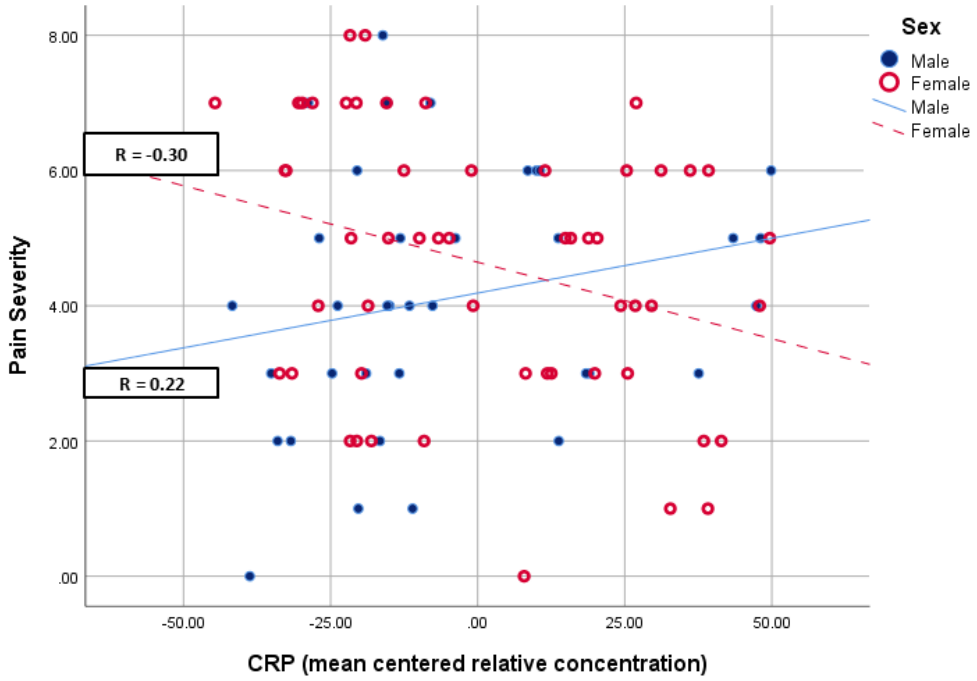
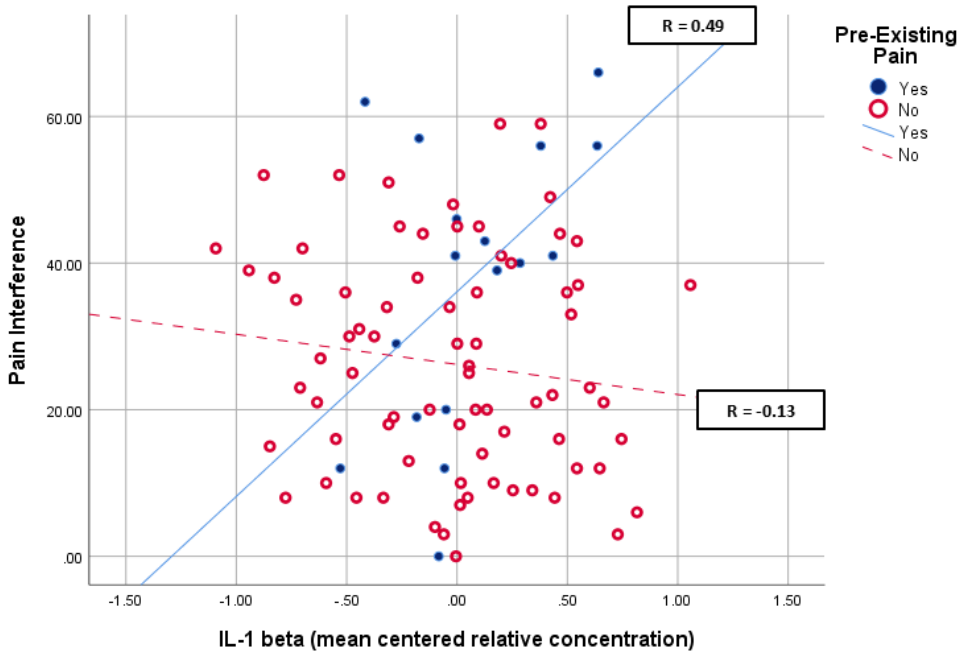
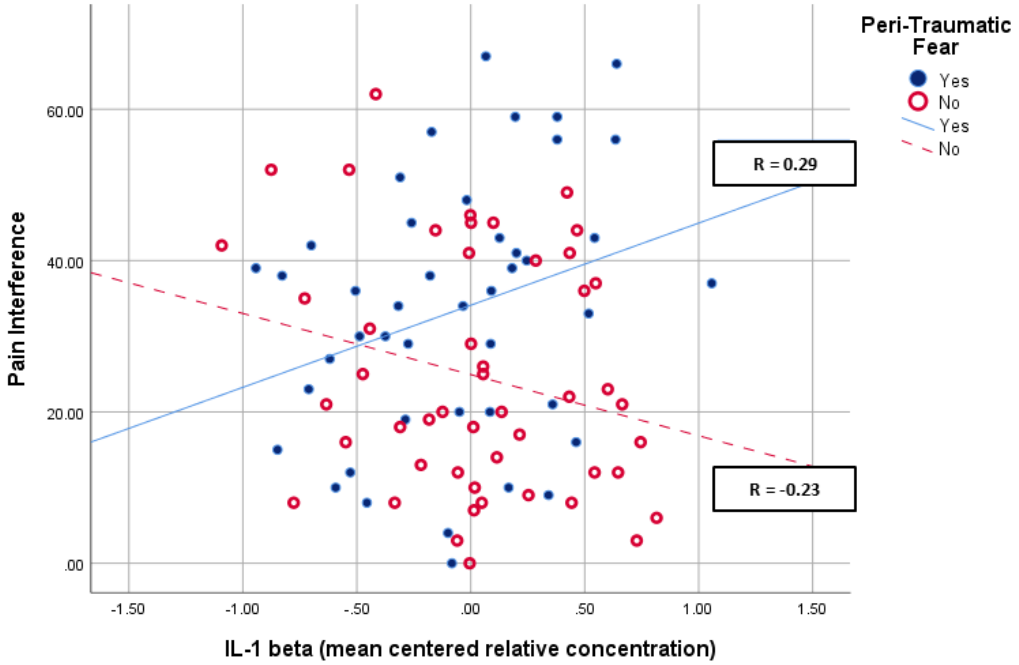


Figure 1 – Graphical Representation of Pain Severity Moderators

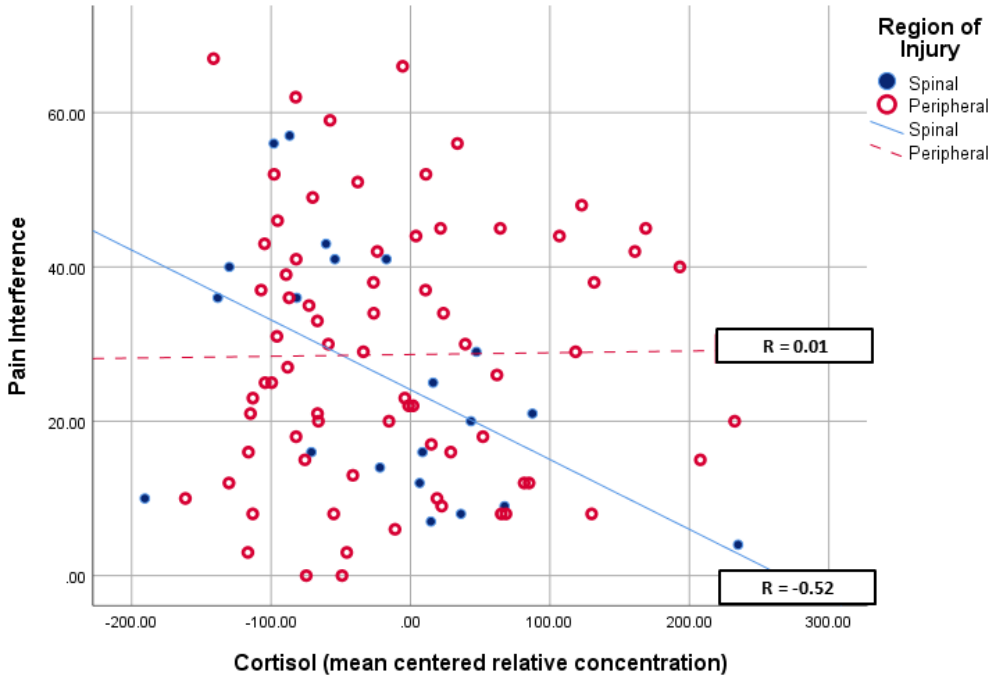
A



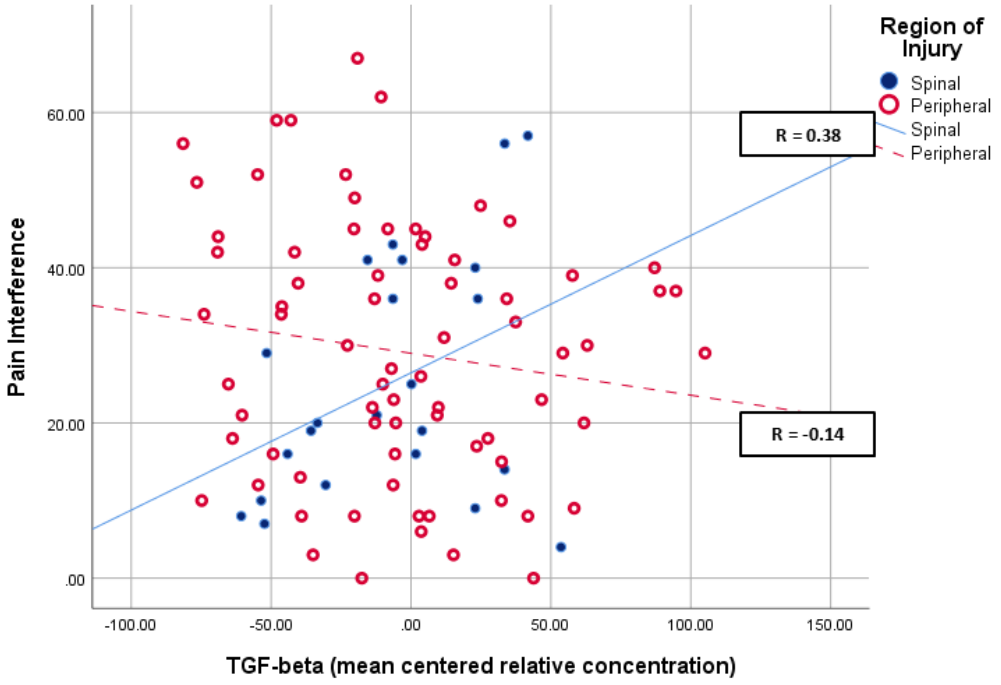
B


Peri-Traumatic Fear
Yes
No
Yes
No
R = 0.29
R = -0.23
Pain Interference
60.00
40.00
20.00
.00
-1.50
-1.00
-.50
.00
.50
1.00
1.50
IL-1 beta (mean centered relative concentration)

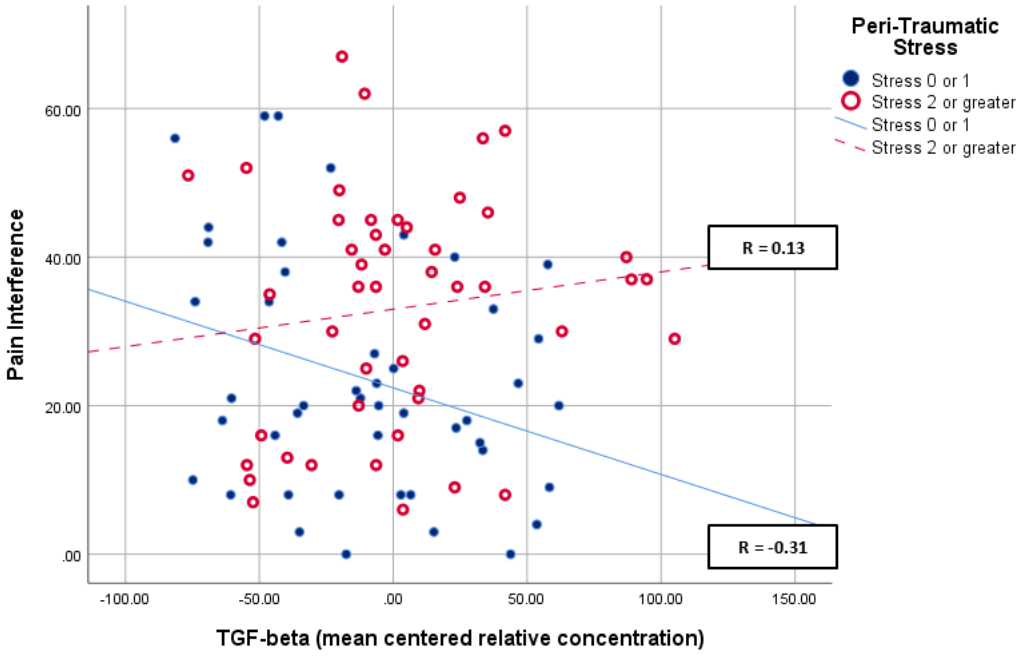
C


Region of Injury
Spinal
Peripheral
Spinal
Peripheral
R = 0.01
R = -0.52
Pain Interference
60.00
40.00
20.00
.00
-200.00
-100.00
.00
100.00
200.00
300.00
Cortisol (mean centered relative concentration)

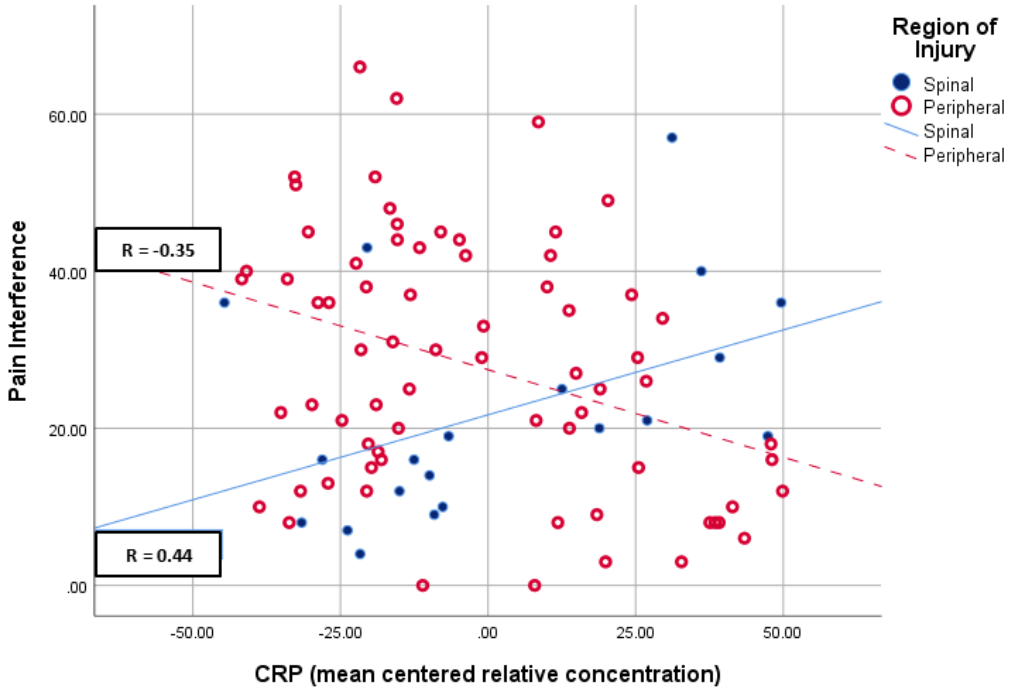
D


Region of Injury
Spinal
Peripheral
Spinal
Peripheral
R = 0.38
R = -0.14
Pain Interference
60.00
40.00
20.00
.00
-100.00
-50.00
.00
50.00
100.00
150.00
TGF-beta (mean centered relative concentration)

E


Peri-Traumatic Stress
Stress 0 or 1
Stress 2 or greater
Stress 0 or 1
Stress 2 or greater
R = 0.13
R = -0.31
Pain Interference
60.00
40.00
20.00
.00
-100.00
-50.00
.00
50.00
100.00
150.00
TGF-beta (mean centered relative concentration)

F



G

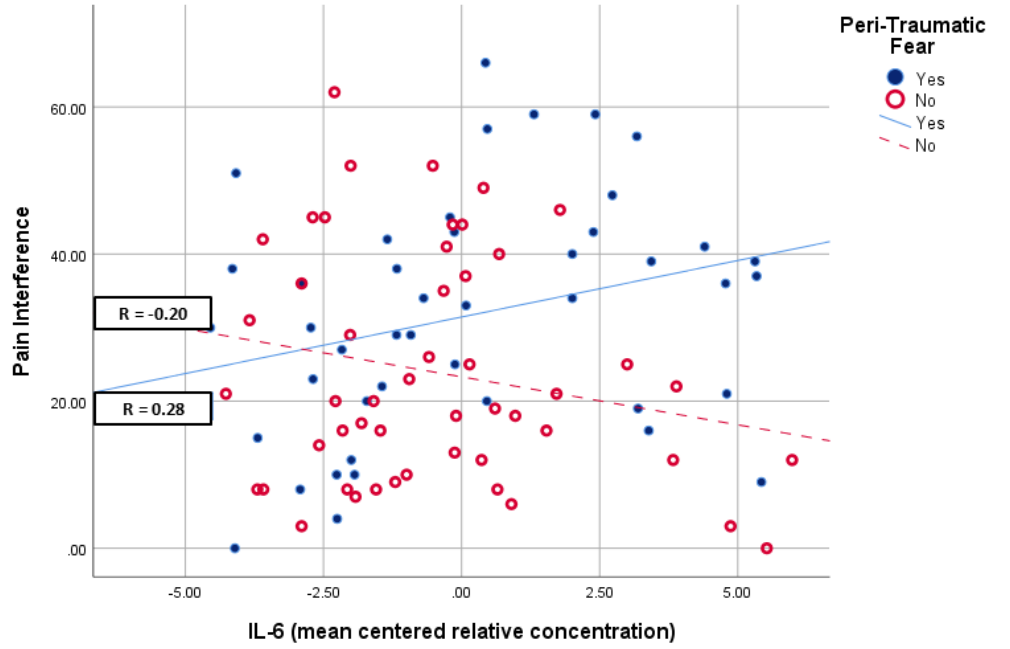


Figure 2 – Graphical Representation of Pain Interference Moderators

Chapter 3

3 Exploring recovery trajectories and predicting outcomes of acute musculoskeletal trauma using latent growth curve analysis

3.1 Introduction

Pain following musculoskeletal trauma is a complex phenomenon. While early models conceptualized pain as a direct result of the magnitude of tissue damage, newer models have re-conceptualized it as a highly subjective experience influenced by interactions of biology, psychology and social influences [1]. The experience of pain is a nearly universal phenomenon, and is widely recognized as essential for survival learning in most organisms including humans [2]. However, its inconsistent relationship to key physiological mechanisms [3] has made its regulation difficult. Unresolved pain can disrupt multiple aspects of life [4] and without proper control, pain has been considered its own pathological condition [5]. The incidence and prevalence of chronic pain is estimated to be nearly 20% of adults in Canada [6] and the United States [7] with staggering economic and social burden [8]. With pain being such a complex and integrated experience, health care providers can struggle to navigate the various interactions that lead to the development of chronic pain.

Many scholars in the field have argued that better mechanism-based prognostic models are needed to identify key intervention targets in the acute stage of injury and pain to prevent poor outcomes [9,10]. There has yet to be consistent evidence that any intervention strategy effectively prevents the transition to chronicity rendering the field no further ahead than it has been over the past several decades. Additionally, the use of opioids in acute pain management or as a long-term solution has been heavily scrutinized in recent years, driven largely by findings that prolonged administration (> 3 months) significantly increases the likelihood of physical or psychological dependence [11]. This general lack of effectiveness in preventing chronic or persistent pain is driven at least in part by poor

understanding of the mechanisms that explain the acute-to-chronic or acute-to-recovery transitions [12].

Pain prognosis as a field of study has evolved considerably over the past two decades, with emphasis added during the mid-1990s from groups exploring acute whiplash-associated disorder [13] and acute low back pain [14]. However, considerable challenges persist today, including the nature of the outcomes to be predicted and the multitude of confounding influences that very likely exist when creating prognostic models [15]. Traditionally, pain intensity (or severity) has been the most common outcome predicted in prognostic research following musculoskeletal trauma [16] and is not coincidentally one of the most reliable predictors of a poor outcome [17]. More recently however, psychological and physical function outcomes have been included in these models. For example, Sterling and colleagues conducted latent growth curve modeling in a sample of 155 people with acute (<1 month) whiplash and found that a 3-class model (mild, moderate, severe) best described the trajectory of outcomes for both physical (Neck Disability Index [18]) and emotional (Post-traumatic Stress Diagnosis Scale [19]) functioning. Panken and colleagues [20] similarly conducted latent growth curve analysis to again identify 3 emergent trajectories that best described the progress of pain intensity in 622 participants with low back pain of median 5.8 weeks duration (2 to 780 weeks). The 3-class trajectories model appears to be showing consistency in this literature, though outstanding questions persist including the translation of these findings to injuries affecting other parts of the body, and other relevant physical and psychological outcomes. That is, how consistent are these models in more heterogeneous samples when different outcomes are used? A better understanding of recovery trajectories will facilitate prognostic assessment of patients and help to direct healthcare resources to those who would benefit most while preventing overtreatment of those who are likely to recover quickly.

Therefore, the purpose of this study was to investigate the recovery trajectories following non-catastrophic MSK trauma in a general population which includes both axial and peripheral trauma. This was conceptualized as a first step towards a body region-

agnostic approach to prognostic phenotyping of people with acute, non-catastrophic MSK trauma.

3.2 Methods

3.2.1 Participant recruitment

Data for this analysis were drawn from two longitudinal cohort studies, one in London Ontario, Canada (SYMBIOME, Systematic Merging of Biology, Mental Health and Environment, clinicaltrials.gov ID no. NCT02711085) and one in Chicago Illinois, United States (ID no. NCT02157038). Eligible participants were identified by emergency or acute-care nursing or medical clinicians, all within 4 weeks of musculoskeletal (MSK) trauma. All participants were 18 to 65 years, had to have suffered a non-catastrophic MSK injury that did not require inpatient admission or surgical correction, and could speak and understand conversational (at least grade 8) English. Exclusion criteria were those with one or more prior motor vehicle collisions (Chicago cohort only), any nervous system or major systemic disorders that would be expected to otherwise impair recovery independently of the trauma, and any metabolic systems disorders (Chicago cohort only). Co-treatment or other chronic comorbidities were captured as part of the intake and follow-up packages. The London cohort included participants with non-catastrophic musculoskeletal (MSK) injury affecting any body region, while the Chicago cohort included only those with whiplash-associated symptoms about the neck arising from motor vehicle collisions. After being medically cleared and discharged, interested participants gave permission for a member of the research team to describe the study, answer questions, and consent to enroll prior to leaving the hospital. Participants were provided a package of self-report questionnaires to be completed within 24 hours of discharge. Biomarker data were also collected from participants though these differed considerably between the two cohorts so could not be combined and are not being described here. Follow-up in the two cohorts occurred within 1 month from inception, and again 3 and 12 months after injury.

3.2.2 Psychometric variables and metadata

The questionnaires differed slightly between the two cohorts, though the constructs being captured were similar enough to allow meaningful pooling. Both studies captured demographic and social data including age, sex, body mass index (BMI, kg/m^2), work status, medicolegal status, and significant comorbidities (e.g. depression or other mood disorders, existing pain conditions). The primary outcome for defining recovery trajectory was pain-related functional interference as measured by the Interference subscale of the Brief Pain Inventory (BPI [21], London cohort) or the Neck Disability Index (NDI [18], Chicago cohort). The BPI is one of the most widely used pain interference scales globally [22] and has considerable evidence of validity across many clinical populations including musculoskeletal pain [23]. The NDI is one of the most widely-used region-specific scales for capture of neck-related disability specifically and is more relevant to that population. The two tools share several items including work ability, sleep, and recreation, but the NDI excludes items irrelevant to those with neck pain like walking interference. Both the NDI and the BPI have demonstrated acceptable reliability, validity and responsiveness for capturing interference [18,21,23-26]. Both can easily be converted into a percentage score of the total scale range (0% = no interference, 100% = complete interference), allowing meaningful combination of the two databases. The NDI shows a moderate ($r = 0.58$) [27] to strong ($r = 0.71$) [28] correlation with the NPRS dependent on the time-frame explored. The NDI was also shown to have a strong correlation to the visual analogue scale ($r = 0.64$) [29]. Our database demonstrated a similar correlation between the BPI interference subscale and the NPRS ($r = 0.67$).

Pain severity as a secondary outcome was captured from all participants at each data collection period using a standard 0-10 Numeric Pain Rating Scale (NPRS) in both cohorts.

Intervention between follow-up periods, if any, occurred at the discretion of the participant and healthcare providers. Type of intervention was captured (e.g. physical therapy, pharmaceuticals, massage therapy, work hardening) though the balance of evidence available in the field does not support the superiority of any treatment modality

over others, including type of intervention, frequency, dosage or intensity, when compared to simple advice and education [30-33]. As such, intervention type was captured in general terms only. Ethics approval was obtained by the respective research ethics boards prior to recruiting participants into the study. Participants were reimbursed up to the equivalent of $240 Canadian dollars for expenses and time incurred during participation across all follow-up periods.

3.3 Analysis

3.3.1 Pre-Analysis

Participant metadata (age, sex, BMI, medicolegal status, work status) and baseline scores on each of the outcomes were evaluated descriptively (frequencies, means, ranges). The primary (% pain interference) and secondary (pain severity) outcomes were first explored for missing data and normality. Region of injury was coded according to the primary area of symptoms; presence of any head, neck or back injuries (regardless of additional peripheral injuries) were classified as “axial” while those affecting the upper or lower extremities (shoulder, elbow, wrist, hip, knee, ankle) were classed as “peripheral”. Where necessary, data were square-root transformed in order to reduce the skewness of the distribution to within acceptable limits.

3.3.2 Latent Growth Curve Analysis

Latent growth curve analysis (LGCA) was conducted on the square-root transformed data to identify the number of trajectories definable by each of the 2 primary outcomes following the steps of DiStefano and Kamphaus [34] using the Growth Mixture Modeling (GMM) function in MPlus v6.12 software (Muthen & Muthen, Los Angeles USA). For each, a series of models were constructed, starting with a single trajectory (termed ‘class’) and increasing until model fit no longer improved, the model could no longer be mathematically defined, one of the latent classes possessed fewer than 10% of participants, or the class structure did not make clinical sense. The fit indicators of interest were the Akaike Information Criterion (AIC) [35-37], the Bayesian Information

Criterion (BIC) [35-37], entropy [36], and the adjusted Lo-Mendell-Rubin likelihood ratio test (LMR-LRT)[35,37]. While no set criteria exist for deeming model fit acceptable [37], the cluster solution that provides the lowest AIC and BIC and the highest entropy value (ideally >0.80) that also conforms to theory is generally considered optimal [38]. An additional statistical analysis was conducted using the k-means approach, where the Lo-Mendell-Rubin Adjusted Likelihood Ratio Test (LMR-LRT) is used to statistically compare the fit of the k cluster solution (e.g. 3) with that of the k-1 class solution (e.g. 2). When fit no longer statistically improves ($p>0.05$) with the addition of a new class, the solution with the smaller number of classes is generally accepted for reasons of parsimony [37,39]. Both linear and quadratic (non-linear) models were tested.
After the optimal model solution was identified, each participant received an assignment to the most likely trajectory based on posterior probabilities from the modeling procedure. Owing to >20% missing data across all time points, a validation step was undertaken to improve confidence in the model solution. A repeated measures ANOVA with 4 levels of repeated variable (time: 0, 1, 3, and 12 months) and trajectory class as the between group variable was conducted on the raw interference or NPRS scores to ensure main effects of group and time were statistically significant and meaningful. Significant main effects were further explored using Tukey's post-hoc test or t tests with Bonferroni correction.

Prior research has suggested that that axial injuries (i.e. head, neck, and back) have more than twice the functional interference than injuries in other areas [40]. As such, a planned disaggregated sub-analysis was also conducted where the trajectories were explored in the axial and peripheral groups separately. Consistencies in trajectory shapes were expected, but proportions within each trajectory were hypothesized to be different, with proportionately greater representation of the axial traumas in the more severe pain groups.

As exploration of quadratic functions with 12-month outcomes was a planned analysis, only those participants with at least 3- or 12-month outcome data were included for each analysis. If participants were missing data for both 3 and 12 months, they were excluded

from outcome analysis. In those with a single missing value, data were estimated using maximum likelihood estimation based on all the available data in order to generate an acceptable class solution for recovery. This is an acceptable method for dealing with missing values which makes use of all available data to estimate an appropriate model that can be used to describe the entire sample [41,42]. As an additional measure of trustworthiness in model-estimated values, observed 12-month values for both pain severity and interference were compared against the MPlus-generated estimated values via t-test to ensure that no significant differences existed.

3.3.3 Sample size estimation

Previous studies investigating pain recovery trajectories using latent growth curve modeling and ANOVA-based approaches have identified distinct classes with a medium effect size [43]. A power analysis was conducted using G*Power v3.1 [44] indicating that a total sample of 189 participants would be required to detect moderate effects ($\eta_p^2 = 0.03$) with $\beta = 80\%$ using RM ANOVA between means with $\alpha = 0.05$.

3.4 Results

3.4.1 Participant characteristics

A total of 231 participants were recruited within 28 days (4 weeks) of non-catastrophic MSK trauma. Of those, 134 were from the London Ontario sample and 97 from the Chicago Illinois sample. The sample was 54.9% male, mean age of 39.7 years, average BMI of 26.1kg/m^2, and the modal cause of injury when the two databases were combined was motor vehicle collision (50.5% of responses). **Table 7** presents the remaining participant characteristics including baseline mean values on each of the 4 primary outcomes. Participants described a mix of axial (59.9%) or peripheral (40.1%) injuries.

3.4.2 Latent Growth Curve Analysis

The dataset for the base model included all 241 participants (axial and peripheral combined). **Table 8** presents the results of the 1, 2, 3, and 4-class models, for both the linear and the quadratic functions. In reviewing the fit indicators and clinical utility, the 3-class quadratic model for *square-root percent interference* was deemed optimal (AIC = 2911.63, BIC = 2973.59, Entropy = 0.68, LMR-LRT = 46.12, $p<0.01$ vs. the 2-class model). The 3 trajectories were labeled according to the intercepts, slopes, and quadratic functions of the curves: Curve 1 = Rapid recovery (lowest intercept, full or near full recovery by 3 months, 34.9% of the sample); Curve 2 = Delayed recovery (higher intercept, near linear recovery through to 12 months, 19.2% of the sample); Curve 3 = Little or no recovery (higher intercept, slight downward curve at later follow-up but persistently high interference scores, 45.9% of the sample).

Fit indicators for the *pain severity* outcome were optimal for a 2-trajectory quadratic model (AIC = 2935.51, BIC = 2983.58, Entropy = 0.79, LMR-LRT = 81.03, $p<0.01$). These were labeled: Curve 1 = Rapid recovery (lower intercept, recovery by 3 months with a flattened curve thereafter, 83.4% of the sample); and Curve 2 = Minimal or no recovery (higher intercept, persistent higher pain severity ratings at 12 months despite some improvement, 16.6% of the sample). While the inclusion of the quadratic term led to more meaningful model results for both the interference and pain outcomes, we had to constrain variance in that term to zero to achieve adequate convergence (essentially forcing each participant within a trajectory to have the same non-linear trajectory). Figures 3 (Percent Interference) and 4 (Pain Severity) present the trajectories graphically. Planned disaggregated analysis of axial vs peripheral injury yielded qualitatively similar trajectories with slight differences in proportions; where the axial injuries contained a higher proportion of Minimal or No Recovery and peripheral injuries contained a higher proportion of Rapid Recovery in accordance with our *a priori* hypotheses. The similarities in these trajectories lend support to maintaining a combined injury model that incorporates both axial and peripheral injury.

3.4.3 Validation

An RM ANOVA was performed with time as the repeated factor and 'class assignment' as the between-subjects factor. The results demonstrate a significant main effect of group ($F(2,231) = 517.14$, $p<0.01$) and time ($F(1,231) = 3779.41$, $p<0.01$) for pain interference in the expected directions. The interaction term between group and time was also significant ($F(2,231) = 518.47$, $p<0.01$). Mean percent interference was significantly lower in Curve 1 (rapid recovery) than the other two, and was significantly higher in the Curve 3 (little or no recovery) than the other two. Tukey's post-hoc revealed that mean interference was not different between the delayed and minimal recovery trajectories until the 3 month follow-up, supporting the label of 'Delayed Recovery' ($p<0.01$). At 12 month follow-up, Rapid and Delayed Recovery trajectories were not significantly different ($p=0.07$). The same RM ANOVA analysis was also performed for pain severity. As a 2-class combined injury model, there was significant main effect of group ($F(1,227) = 357.93$, $p<0.01$) and time ($F(1,227) = 366.13$, $p<0.01$). There was also a significant interaction effect of group and time ($F(1,227) = 302.68$, $p<0.01$), with significant differences at each time point based on independent samples t-tests with Bonferroni correction ($p<0.0125$). Mean severity was significantly lower in Curve 1 (rapid recovery) than in Curve 2 (minimal/no recovery). Further, the predicted models appeared sound even despite any missing data as there were no significant differences between 6-month predicted and observed values (all $p>0.40$, Tables 10A and B).

To better describe the relationship between *trajectory* and actual *distal outcome*, we first trichotomized the 12-month interference scores using simple thresholds informed by the nature of the data: those scoring 0-5% interference at 12 months were considered to be experiencing no clinically meaningful ongoing problems, those score 5-20% were considered to be experiencing mild ongoing problems, and those scoring over 20% were considered persistent and clinically relevant problems, in general accordance with widely accepted thresholds for NDI scores [45] (Table 10A and 10B). Owing to missing data by 12 months, 184 complete datasets (76.3% of baseline) were available for this analysis. A 3 x 3 cross-tabulation was established, shown in Table 10. No participants in the Rapid or Delayed interference recovery trajectories rated >20% interference at 12 months, though a combined 9 participants rated mild persistent problems. Similarly, only 1 participant

classed in the Minimal or No Recovery trajectory rated <5% interference at 12 months, while 56 (59.6% of those in that trajectory) rated 5-20% interference. These proportions indicated a clear distinction between the Rapid and Minimal or No Recovery trajectories in accordance with the labels given them, but some ambiguity in the middle trajectory and mild interference outcome. With regard to pain severity, all of the participants in the Minimal or No Recovery trajectory reported persisting pain of at least 4/10 severity at 12 months. In the Rapid recovery trajectory, 74 participants (51.0% of the class) indicated having no pain (0 out of 10) at 12 months, however 71 participants (49.0%) indicated some degree of pain severity still present at 12 months (ranging from 1 to 3 out of 10).

3.5 Discussion

This study provides evidence for the general decrease in disability and pain intensity over the course of 12 months in a mixed adult population following non-catastrophic axial or peripheral trauma. These dimensions appear to stabilize at approximately 3 months post-injury. Our results also suggest that classification of recovery following non-catastrophic injury is determined by both the intercept (baseline symptoms) and slope (relative rate of symptom decline). This is in keeping with known associations between pain perception and the severity of future pain in both pediatric [46] and adult populations [47-49].

In accordance with previous research by Sterling and colleagues [12, 50], we have identified 3 distinct classes of recovery for axial injuries in disability/pain interference. In addition, we have also demonstrated that these same recovery classes may also include perceived disability in peripheral injuries as our 3-class model was a mixed injury group, and disaggregated analyses showed qualitatively similar trajectories exist in both groups. With respect to pain interference, the majority of participants seemed to belong to the minimal/no recovery class (45.9%), however, the majority of our sample consisted of axial injuries (52.7% neck injuries and 9.1% lower back). This may have resulted in a higher number of participants experiencing persistent interference from their pain. This is in keeping with prior research that suggests a greater level of disability among axial injuries [40]. Pagé et al have also identified 3 predictive trajectories of pain intensity and disability in a study of over 1800 participants with mixed conditions enrolled in the

Quebec Pain Registry [51]. That registry however contains only those who have already been diagnosed with chronic pain and trajectories were based on recovery while in a multidisciplinary pain program. Studies investigating the trajectories of post-operative pain have also identified 3 distinct groupings based on initial severity of symptoms [52, 53]. Other studies investigating hip [54] and low back pain [55] recovery have identified 4- and 5-class models, respectively. However, in both studies, three categories of stable symptoms were always identified (low/mild, moderate persistent, and severe persistent); where additional classes were reflective of those who experienced fluctuating pain over the course of years.

Based on our data, pain severity seemed to favor a 2-class model. This differs from our 3-class model of pain interference. Although severity and interference are related, they are still considered separate constructs of pain measurement [21]. These dimensions can present differently such that it is possible to have a high level of pain intensity with a relatively low level of interference or disability [56]. Table 1 shows that 19.2 % of our participants scored over threshold for likely depression and 19.7% scored over threshold for likely PTSD at entry into the study. Approximately 16.6% of participants were also placed into the minimal or no recovery group for pain severity upon conclusion of the study. Although no conclusions can be drawn from these observations, chronic pain is often closely associated with a number of psychological disturbances including PTSD, depression, and anxiety [57, 58]. Whether or not these factors are linked in our study is up for debate, but the literature suggests that there is a connection between the changes in pain, depression, and anxiety over time. Gerrits and colleagues have investigated this synchronicity by measuring longitudinal changes in these symptoms in more than 2000 participants [59]. Over the course of 4 years, participants that had either a chronic or incident anxiety/depression diagnosis reported significantly higher pain compared to healthy controls. The participants who were in remission from their depression or anxiety at follow-up reported decreased levels of pain compared to chronic cases but were still significantly higher than healthy counterparts [59]. From a psychosocial perspective, Bonanno and colleagues investigated the trajectories of depression following traumatic spinal cord injury over the course of 2 years [60]. They identified 2 stable classes

that emerge from the beginning with 2 additional classes that fluctuate over time. Their results suggest that after 1 year there is a gradual trend toward the 2 stable classes [60]. Psychological comorbidity is closely tied to the overall experience of pain. Radiofrequency neurotomy in whiplash patients has shown that general psychological distress detected upon intake was resolved with the successful post-operative resolution of pain symptoms [61]. Similarly, disability and pain intensity can be affected to some degree by the resolution of tissue damage; whereas PTSD and depression (relating to emotional reactivity) may be more closely associated with pre-existing and ongoing psychosocial factors. It has been shown in post-traumatic distress that the occurrence of an initial traumatic event can psychologically sensitize the brain to subsequent trauma [62, 63]; which may explain why those with higher symptom severity at intake, continued along an elevated trajectory. More recently, Sterling and colleagues demonstrate a considerable agreement between trajectories of neck disability and post-traumatic distress. They also assert that membership in the disability and PTSD groups are determined by similar factors [12].

As table 10A and 10B suggest, our trajectories, while achieving acceptable fit to the data, still showed considerable inter-individual variation. Even in those who are on a rapid or delayed recovery process, 4.86% still present with mild-moderate levels of pain interference and 49.0% present with pain severity greater than 0 at their 6-month outcome. Our study population consists of many different types of MSK trauma and it is possible that this variety of traumatic injuries may be contributing to the variations in each recovery class. It is more likely however that this variability is a product of inherent, inter-individual differences. In a controlled environment with experimentally-induced pain (i.e. the same noxious stimulus being applied to everyone), healthy participants can indicate anywhere between 2 to 9 out of 10 on the visual analog scale of pain measurement [64]. These ratings were also proportional to the amount of neurological activity recorded in the pre-frontal cortex (executive function), anterior cingulate cortex (emotional reaction to pain, goal setting), and the primary somatosensory cortex (incoming sensory information) [65]. This suggests that the variability is not simply an error involved with subjective reporting, but that it is indicative of actual neurological

differences in the experience of pain [64, 65]. Regardless of the mechanism of injury, the perception of pain is highly specific to the person. Although we have demonstrated the emergence of distinct recovery classes in pain severity and interference, the information gleaned from these trajectories should be used with caution. As we have shown, the recovery class alone is insufficient to capture individual variability and completely predict 6-month outcomes. Both the pathway and the eventual outcome should be taken into consideration when defining recovery.

A few limitations need to be taken into consideration. The data used in the analysis were entirely generated via self-report for all constructs tested. This creates the potential to overestimate symptom severity. However, there is correlation between self-report and neural activity, and despite advances that have been made in pain neuroimaging, the most reliable measurement of pain over time in healthy individuals continues to be subjective self-report data [66]. The data itself were also compiled from two separate sites, one in Canada and one in the US, using constructs that were related but not identical. Despite the high level of agreement between the constructs being used, the exclusive focus on non-catastrophic neck trauma in the Chicago cohort compared to the multi-region trauma in the London cohort will inevitably add variation within the data. The use of two different scales for capturing pain-related interference between the two cohorts is definitely a limitation of this study, however both the NDI and the BPI interference subscale correlate strongly with standard measures of pain severity (NRS). From previous work on recovery trajectories [54, 55], it is possible that additional classes do exist and that our study lacks the adequate power to detect these smaller, more labile classes. However, our findings are in agreement with other studies of similar design [12, 50], and although no strict guidelines exist for adequate sample size for growth curve modeling, it is suggested that at least 100 participants are required in order to avoid underreporting the "correct" number of classes [67]. Other classes may also represent smaller, more statistically uncommon proportions of the population which may not be as clinically relevant as the broader groups.

In conclusion, findings of previous groups of a 3-class recovery trajectory model were replicated in our more heterogeneous sample of non-catastrophic acute MSK trauma, though pain severity tended to favor a 2-class recovery model. Graphical depiction of individual trajectories and crosstabulation of both recovery trajectory and 6-month outcome suggest a general agreement between course and state of recovery, though some symptom variability within each recovery class suggests that future researchers should consider both the trajectory and distal outcome when conducting longitudinal research. Recovery trajectories may help to guide prognosis and treatment but are insufficient on their own to dictate total recovery or eventual outcomes.

3.6 References

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Table 7 – Participant characteristics

Sex (% male)	54.9%
Cause (%)	
Motor vehicle collision	50.5%
Fall / Slip	14.2%
Hit by person or object (not MVC)	9.4%
Awkward lift or twist	8.0%
Other	17.9%
Body Region Injured (%)[1]	
Neck	52.7%
Shoulder	9.1%
Elbow	3.6%
Wrist or Hand	15.5%
Lower Back	9.1%
Hip	2.3%
Knee	8.6%
Foot or Ankle	16.4%
Employment (%)	
Full-Time	58.8%
Part-Time	14.9%
Off Work (temporary)	2.6%
Not Employed for Pay	23.7%
Current Work Status (%)	
Full Return	56.6%
Partial Return	25.4%
No Return	18.0%
Educational Attainment (%)	
High School or Less	25.0%
Community College or Trade School	31.9%
University Undergraduate Degree	29.3%
University Graduate Degree	12.1%
Other	1.7%
Household Income	
≤$20,000	7.1%
$21,000 - $80,000	45.1%
$81,000 - $150,000	36.3%
>$150,000	11.5%
Pre-Existing Pain (% yes)[2]	17.1%
Continuous Variables	
Variable	Mean (SD, range)
Age (years)	39.7 (13.8, 18 to 66)
Body Mass Index (kg/m^2)	26.1 (5.4, 14.4 to 51.5)
Post-Traumatic Distress (% of total score)[3]	19.7% (19.7%, 0 to 94)
Depressive Symptoms (% of total score)[4]	19.2% (19.1%, 0 to 95)
Pain Interference (% of total score)[5]	37.6% (21.0%, 0 to 96)
Pain Severity (0-10 NRS)	4.6 (2.2, 0 to 10)

1: The total proportions will exceed 100% as participants were free to choose more than one body region.

2: Pre-existing pain was identified by reviewing medication lists for any analgesic medication and co-morbidity lists for any pain-related condition (e.g. fibromyalgia, osteoarthritis, repetitive strain injuries, neck or back pain, etc…).

3: Post-Traumatic distress was captured using two different but related tools: The PTSD Checklist, The PTS Diagnosis Scale. Results have been reported as a percentage of total scale score.

4: Depressive symptoms were captured using the depression subscale of the HADS (HADS-D) and the PHQ-9. Results have been reported as a percentage of total scale score.

5: Disability, or functional interference was captured using the interference subscales of the Neck Disability Inventory or the Brief Pain Inventory and have been reported as a percentage of total scale score.

Table 8 – Latent Growth Curve Analysis for pain severity and interference dimensions

Model	AIC	BIC	Entropy	LMR-LRT adj (p)
2-class Pain Severity	**2935.51**	**2983.58**	**0.79**	**81.03 (<0.01)**
3-class Pain Severity	2927.64	2979.14	0.79	46.91 (0.21)
4-class Pain Severity	2911.76	2977.00	0.67	22.83 (0.53)
2-class Pain Interference	2952.30	3000.49	0.59	41.20 (<0.01)
3-class Pain Interference	**2911.63**	**2973.59**	**0.68**	**46.12 (<0.01)**
4-class Pain Interference	2874.52	2950.25	0.70	43.13 (0.09)

Table 9 – Counts and proportions for pain interference trajectory based on (A) Pain Interference % with 95% confidence intervals (axial and peripheral injuries) and (B) Pain severity scores (out of 10) with 95% confidence intervals (axial and peripheral injuries)

A

Trajectory	N (%)	Baseline*	1-month*	3-month*	12-month*
Rapid	34.9	4.68 (4.45, 4.91)	1.78 (1.62, 1.94)	0.27 (0.15, 0.38)	0.14 (0.05, 0.23)
Delayed	19.2	6.59 (6.23, 6.95)	5.66 (5.36, 5.95)	3.63 (3.37, 3.89)	0.51 (0.24, 0.77)
Minimal	45.9	6.39 (6.22, 6.56)	5.34 (5.19, 5.50)	4.69 (4.53, 4.85)	4.44 (4.25, 4.63)

B

Trajectory	N (%)	Baseline	1-month	3-month	12-month
Rapid	83.4	4.33 (4.15, 4.52)	2.99 (2.83, 3.15)	1.18 (1.06, 1.30)	0.65 (0.55, 0.75)
Minimal	16.6	5.70 (5.24, 6.16)	5.42 (5.00, 5.84)	5.20 (4.84, 5.55)	5.67 (5.35, 5.99)

Table 10 – Cross-tabulation table of trajectory and 12-month BPI pain interference outcomes (A) and BPI pain severity outcomes (B)

A

12-month outcome	Rapid recovery (%)	Delayed recovery (%)	Minimal/No recovery (%)	Total
<5% interference (mild)	53 (91.4%)	29 (87.9%)	1 (1.1%)	83
5-20% interference (mild-moderate)	5 (8.6%)	4 (12.1%)	56 (59.6%)	65
>20% interference (moderate-high)	0 (0.0%)	0 (0.0%)	37 (39.3%)	37
Total	58	33	94	185

B

12-month outcome	Rapid recovery (%)	Minimal/No recovery (%)	Total
Pain = 0	75 (51.0%)	0 (0.0%)	75
Pain > 0	72 (49.0%)	38 (100%)	110
Total	147	38	185

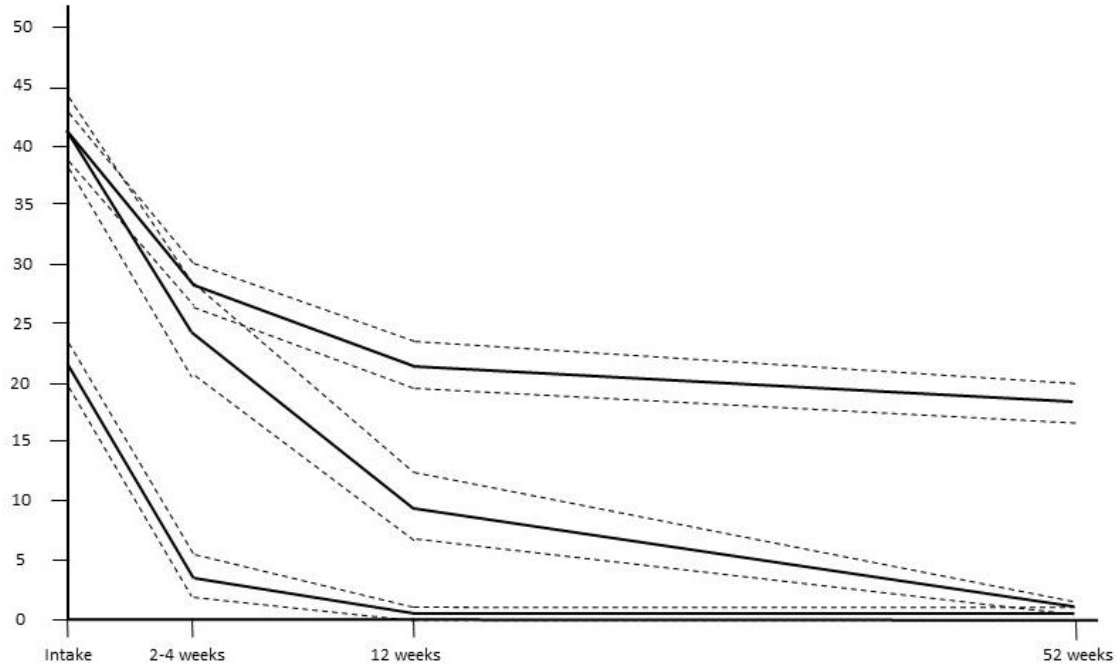

Figure 3 – Recovery Trajectories for Pain Interference in Axial and Peripheral Injuries

Graphical representation of a 3-class LGCA model of pain interference recovery for axial and peripheral injury over a 6-month follow-up period, where dashed lines indicate 95% confidence intervals for each class. The x-axis denotes time and the y-axis denotes pain interference expressed as a percentage out of 100. Rapid recovery (34.9%) is depicted as having a moderate intercept and rapidly declining slope. Delayed recovery (19.2%) is depicted as having a high intercept and steadily declining slope. Minimal or No Recovery (45.9%) is depicted as having a high intercept and minimally declining slope.

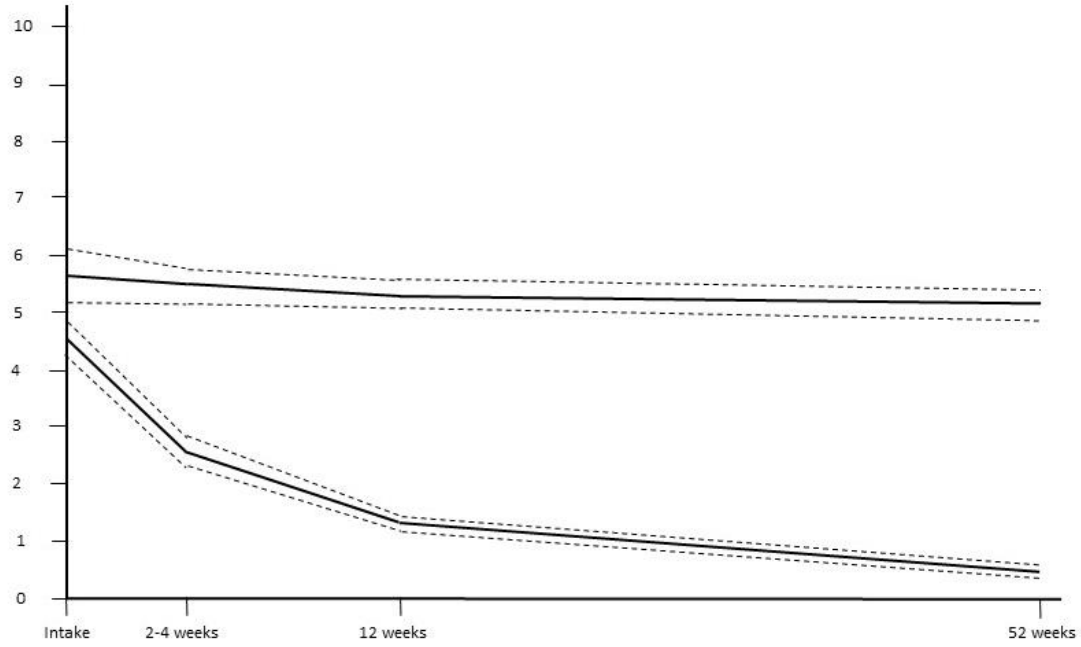

Figure 4 – Recovery Trajectories for Pain Severity for Axial and Peripheral Injuries

Graphical representation of a 2-class LGCA model of pain severity recovery for axial and peripheral injury over a 6-month follow-up period, where dashed lines indicate 95% confidence intervals for each class. The x-axis denotes time and the y-axis denotes their pain severity score out of 10. Rapid recovery (83.4%) is depicted as having a moderate intercept and steadily declining slope. Minimal or No Recovery (16.6%) is depicted as having a high intercept and minimal slope.

Chapter 4

4 Latent profile analysis of blood marker phenotypes and their relationships with clinical pain and interference reports in people with acute musculoskeletal trauma

4.1 Introduction

Chronic pain represents a substantial burden on patients and health systems, due in part to its complexity and resistance to traditional medical and pharmaceutical treatments [1]. While progress in interdisciplinary care strategies has been made, effective pain management remains a unique challenge [2]. With chronic pain becoming a problem of epidemic proportions [3], healthcare researchers and providers have turned their attention towards the identification of mechanisms for early detection and intervention [4, 5].

Longitudinal modeling studies in both clinical [6] and population-level [7] samples have identified trajectories of pain and recovery that most commonly indicate 15-25% of participants report long-term, chronic or persistent pain and functional interference after musculoskeletal trauma seemingly regardless of the body region affected [6, 8-11]. In a prior study (see chapter 2) we identified a 3-trajectory model of functional recovery from musculoskeletal (MSK) trauma representing trajectories of rapid recovery (32.0% of the sample), delayed recovery (26.7%), and minimal or no recovery (41.3%). Of note is that Sterling and colleagues followed post-traumatic stress outcomes and also found a qualitatively-similar 3-trajectory model as the best fit to the data. The identification of consistent recovery trajectories provides new opportunities to characterize predictive mechanisms.

Advances in research and technology has led to the re-emergence of a search for biomarkers that may explain the onset or persistence of pain, though these have moved from traditional approaches such as static structural imaging to more dynamic ‘omics’ approaches (e.g. genomics, transcriptomics, proteomics, metabolomics). The results of such work has been mixed though evidence is mounting that dysfunction in some aspect

of the omics cascade may represent a valuable biomarker of acute or chronic pain. In a recent review of biomarkers of low back pain (LBP) [12], inflammatory mediators such as high sensitivity C-reactive protein (hsCRP), tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) were identified as having a potential role in the acute phase of LBP. In the chronic phase Li et al [13] found that IL-10 was decreased while IL-6 was increased in people with low back pain compared to matched controls. Conversely, Klyne et al [14] showed that IL-6 levels do not significantly differ between those with low back pain and controls. They did, however, report a significant difference in IL-6 within the low back pain group between those reporting high levels of pain and those reporting low levels of pain. These studies suggest that there may be value in exploring blood-based proteins as markers of distress and/or pain but that simple bivariate associations may not yield consistent results.

The purpose of this study was to explore a theoretical position that 8 previously-identified blood-based protein/hormone biomarkers will explore meaningful variance in pain-related outcomes after trauma but only when considered as clusters rather than single bivariate associations. A secondary outcome was to explore the utility of the biomarker clusters for predicting previously-derived clinical recovery trajectories.

4.2 Methods

Data from this observational cohort study were drawn from the longitudinal SYMBIOME (Systematic Merging of Biology, Mental Health and Environment) databanking study (clinicaltrials.gov ID no. NCT02711085). The study was approved by the office of Human Research Ethics at Western University and the Lawson Health Research Institute, and written, informed consent was obtained from all participants. Eligible participants were identified by emergency or acute-care clinicians from an urgent care centre in London, ON, Canada. After being medically discharged, a member of the research team described the study, answered questions, enrolled and screened potential participants prior to leaving the hospital. Two samples of antecubital blood were drawn into 4mL K2 EDTA BD vacutainer tubes by a trained phlebotomist and immediately stored on ice for

transfer and storage at an immunity and proteomics lab. Prior to freezing the samples were centrifuged for 10 minutes at 2000 x g, had plasma pipetted into up to 6 x 50μL aliquots, and then both supernatant and pellet were stored at -80°C.
Participants were concurrently provided a package of self-report questionnaires that included demographic metadata (age, sex, education level, work status, household income, pre-existing pathology, BMI, and region of injury) and pain intensity and functional interference through the Brief Pain Inventory [15]. All participants provided informed, written consent prior to participation.

Follow-up occurred at 1, 2, 3, 6 and 12 months from injury, with the biological samples collected at baseline, 3, 6 and 12 months only. Participants were paid up to $300 in total compensation for participation. For the purposes of this study, only the baseline blood samples were analysed and interpreted for biomarker classes, and owing to attrition recovery up to the 6-month follow-up was used as the final end point. Functional recovery was measured using the pain and interference subscales of the BPI. The BPI is one of the most widely used pain-interference scales globally [16] and has adequate evidence of validity across many clinical populations including musculoskeletal pain [17].

4.2.1 Analysis of serum biomarkers

The target markers for this analysis were those shown previously to be associated with pain, distress, or inflammation [18-24]. Through a collaborative consultative process, eight markers were specifically chosen: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGFβ1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF-α), Interleukins 1-beta (IL-1β), 6 (IL-6) and 10 (IL-10), and the stress hormone cortisol. Analyte concentrations in plasma were assayed using multiplexed biomarker immunoassay kits according to manufacturers' instruction for Brain Derived Neurotrophic Factor (Human Premixed Multi-Analyte Kit, R&D Systems Inc. cat. no. LXSAHM), Transforming Growth Factor-Beta 1 (TGFB1 Single Plex Magnetic Bead Kit, EMD Millipore cat. no. TGFB1MAG-64K-01), Interleukins 1-β, 6, and 10 and TNF-α (Human High Sensitivity T Cell Magnetic Bead Panel Multiplex Kit, EMD Millipore

cat. no. HSTCMAG-28SK). A BioPlex™ 200 readout System was used (Bio-Rad Laboratories, Hercules, CA), that uses Luminex® xMAP™ fluorescent bead-based technology (Luminex Corp., Austin, TX). Levels were automatically calculated from standard curves using Bio-Plex Manager software (v.4.1.1, Bio-Rad). Cortisol (Cortisol Enzyme Immunoassay Kit, Arbor Assays cat. no. K003-H1/H5), and C-Reactive Protein (C-Reactive Protein (human) ELISA Kit, Cayman Chemical Company cat. no. 10011236) were assayed following industry-standard approaches for Enzyme-Linked Immunosorbant assay (ELISA). All assays were performed in duplicate with the value for analysis being the mean concentration of the two runs.

4.3 Analysis

Participant characteristics were summarized descriptively (means and distributions or proportions).

4.3.1 Pre-analysis of analytes

Prior to primary analyses we explored the distribution of the data both qualitatively and statistically. Concentrations of all 8 analytes were significantly positively skewed and in violation of normality via Kolmogorov-Smirnov tests. High outliers (>4SD above the mean) or those for which the assay resulted in non-detectable (too low or too high) concentrations were first removed. All concentrations were then square-root transformed to reduce skewness, and then Z-transformed to place all concentrations on the same scale with a mean of 0.0 and standard deviation of 1.0.

4.3.2 Bivariate associations

A matrix of all cross-product Pearson correlations between the 8 markers was created as an exploratory step and to identify potential problems with collinearity in cluster analysis ($r > 0.80$). There was no statistical correction for multiple comparisons, accepting the

potential for alpha error rather than prematurely rejecting potentially important findings at this exploratory stage.

4.3.3 Profile Analysis

Meaningful clusters in the data were identified with maximum likelihood estimation (MLE)-based latent profile analysis (LPA) as previously described [25] using MPlus software v6.12 (Muthen and Muthen, Los Angeles, USA). Using all 8 target biomarkers, a series of models were constructed, starting with a single profile (termed 'class') and increasing until model fit no longer improved in a meaningful way, the LPA estimation could no longer derive a mathematically definable model, one of the latent classes possessed fewer than 10% of participants, or the class structure did not make clinical sense. The fit indicators of interest were the Akaike Information Criterion (AIC) [26-28], the Bayesian Information Criterion (BIC) [26-28], entropy [27], and the adjusted Lo-Mendell-Rubin likelihood ratio test (LMR-LRT)[26, 28] while considering solutions that provide generally strong posterior classification probabilities (ideally ≥0.85). While no set criteria exist for deeming model fit acceptable [28], the cluster solution that provides the lowest AIC and BIC and the highest entropy value (acceptably >0.70, ideally >0.80) that also conforms to theory is generally considered optimal [29]. The LMR-LRT is used to statistically compare the fit of the k cluster solution with that of the k-1 class solution. When fit no longer statistically improves ($p>0.05$) with the addition of a new class, the solution with the smaller number of classes is generally accepted [28, 30].

In the interest of parsimony, once an overall class solution was determined biomarkers were then systematically eliminated to obtain the simplest discriminatory model. To start, mean differences in square-root transformed marker concentration were explored across the identified classes using one-way analysis of variance (ANOVA). The marker with the smallest interclass differences was eliminated first, followed by the next smallest, and so on until the simplest model remained that still showed good fit indicators in LPA. The intention was that each of the blood markers defining the final class solution should show a significant difference between the groups.

4.3.4 Recovery and outcome analysis

After LPA each participant was assigned to one of the identified classes based on relative blood marker concentration. Pain-related 12-month outcome data was trichotomized for pain interference (<5% mild interference, 5-20% moderate interference, >20% severe interference) and dichotomized for pain severity (pain = 0, pain > 0). Biomarker classes were then compared against these pain-related outcome groups using χ^2 analysis.

4.3.5 Sample size estimation

There is little guidance in the literature for optimal sample size in MLE-based LPA. Prior to the exploratory analyses described herein there was also no clear existing evidence to inform the likely number of clusters or the relative proportions or communalities to assist with sample estimation. Therefore we adopted the general position in the field that a minimum of 100 samples is a minimum for meaningful results, and continued to position the analyses as discovery (exploratory) in nature, that is, hypothesis-generating rather than hypothesis-testing.

4.4 Results

Table 11 provides the characteristics of the study population. There were 109 participants in the SYMBIOME database who provided blood samples within 3 weeks of MSK trauma. After assay, data for 3 participants were removed as all analytes were not detectable or out of range of the kits. Mean age of the remaining n=106 was 44.6 years and 58.5% of the sample was female. The modal mechanism of injury was reported as ‘other’ and 74.3% of the sample reported the primary region of injury as the upper or lower extremity (vs. the axial spine). Pain severity and interference at inception was moderate (Mean Severity = 4.5/10, SD = 2.0; Mean Interference = 28.6/70, SD = 16.8).

Table 12 is the cross-product correlation matrix between all biomarker pairs after removal of outliers and square root transformation. BDNF and TGF-β1 demonstrated the strongest association ($r = 0.74$, $p<0.01$). Cortisol and CRP did not appear to be associated with any other biomarker while IL-6 and IL-1β were significantly correlated with all markers except those two.

Table 13 shows the results of the LPA models with associated fit indicators for the models tested. The final class solution was a 3-class model as it showed a meaningful improvement over a 2-class solution based on relevant fit indicators (AIC = 2257.31, BIC = 2348.82, Entropy = 0.83, LMR-LRT = 28.08, p=0.08). Figure 5 show the relative concentrations of all 8 markers in the 3 class model. After settling on the 3-class model, analytes were removed in a systematic fashion based on total interclass differences. CRP ($F(2,108) = 0.14$, $p=0.87$) and cortisol ($F(2,108) = 2.34$, $p=0.10$) displayed the smallest interclass mean differences (Fig.1) and were eliminated first. Table 13 also shows the model fit adjustment of the 3-class latent profile solution with the sequential elimination of biomarkers. TNF-α ($F(2,108) = 10.65$, $p<0.01$), IL-6 ($F(2,108) = 11.40$, $p<0.01$), and IL-10 were also removed, in that order, each time retesting model fit and posterior classification probabilities. The remaining 3 markers were BDNF, TGFβ1 and IL-1β. BDNF and TGFβ1 were both discriminative across the 3 classes, while IL-1β provided improved discrimination between the two lower concentration classes. The decision to retain IL-1β despite acceptable model fit is described in the discussion section. The final model indicated a 3-class solution that could be adequately described by 3 of the 8 markers (AIC = 827.41, BIC = 865.09, Entropy = 0.80, LMR-LRT = 34.08, p=0.03). The 3 classes were labeled according to the relative concentrations of the 3 markers as: Class 1 = Low concentration of all markers (33.9% of the sample), Class 2 = Average Concentration of all markers (47.7%), and Class 3 = High concentration of BDNF and TGFβ1 (18.3%). Figure 6 shows relative (Z-transformed) concentrations graphically and Table 14 shows the raw (non-transformed) values with 95% confidence intervals.

With each participant assigned to the most likely biomarker class based on posterior probabilities, the sample was split into 3 groups. BPI Pain Severity and Pain Interference

scores captured at 6-month follow-up were compared across groups using chi-square analysis. Table 15 shows the frequencies of the final biomarker classes across relevant recovery outcomes of pain interference/disability (Table 15A) and severity (Table 15B). The majority of participants seemed to recover from their injuries with respect to both pain disability (75.5%) and severity (62.4%). In Table 15A, both mild and moderate interference contain a small minority of participants that show high BDNF/TGF-β1 (13.8% and 14.3%, respectively). Those with severe, persisting disability however, display an increased trend (40%) towards high BDNF/TGF-β1 ($\chi^2 = 5.85$, df = 2, p = 0.06, *Fisher's exact test*). Similarly in Table 15B, those with persisting pain at 12 months (pain > 0) have a greater proportion of high BDNF/TGF-β1 (26.3%) compared to those who have no pain (10.3%) at 12 months ($\chi^2 = 3.86$, df = 1, p = 0.049).

4.5 Discussion

We have presented a first step towards derivation of a potentially useful panel of immunological, neurotrophic, and endocrine markers assayed from serum for use in post-traumatic pain research. Through a multi-step approach to latent profile analysis, a 3-class solution was identified that could be adequately described by 3 of 8 markers: BDNF, TGFβ1 and IL-1β, though at least two other markers (IL-6 and IL-10) also showed some significant discriminative accuracy between the classes. Further, participants assigned to the class representing the highest mean BDNF and TGFβ1 concentrations also tended to rate higher on self-rated scales of pain-related functional interference when measured 12 months post-trauma. Although the difference between pain interference groups was non-significant from a traditional standpoint, it would be premature to dismiss these findings considering a similar result in pain severity and the exploratory nature of this study.

As shown in Figures 1 and 2, an argument could have been made for removing IL-1β from the final model and retaining only TGFβ1 and BDNF, though the strong correlation between these two markers (Table 12) led us to retain a third marker for better discriminative accuracy between Class 1 and Class 2, and to allow greater opportunities

for exploration of potential mechanisms behind the biomarker/clinical outcome associations found here. Both IL-10 and IL-6, and to a lesser extent TNF-α, could also have been retained as they too discriminated between the two lower concentration classes, but IL-1β provided the greatest discriminative accuracy (largest between-class mean difference) and was therefore chosen as the third marker. To our knowledge this is the first time that these 3 markers, arguably up to 6 markers, have been shown to interact as a panel that may have clinical utility if the findings can be replicated in an independent sample. It is notable that the only two markers that showed no between-class differences (CRP and Cortisol) were also those that showed no meaningful association with any of the other 6 markers (Table 12). This should not be mistaken as indicating that these markers are unimportant in research into pain and trauma, rather that through cluster analysis they did not contribute important explanatory utility to the classes identified herein.

BDNF is a small peptide that is involved in myriad functions related to survival, growth and plasticity of neurons and it acts as a key regulator of learning and memory [31]. It carries out this activity by binding to its receptor tyrosine kinase B (TrkB) and activating signalling cascades involved in gene transcription for proteins of stress and plasticity [31-33]. TGF-β1 is a ubiquitous, pleiotropic cytokine that, along with its immunomodulatory function, is involved in cell growth, development, angiogenesis, and wound healing [34]. TGF-β1 has been shown to play a role in the long-term facilitation of neuronal activity and transmission [35]. Both BDNF and TGF-β1 do not seem to display any significant short term effects on sensory neurons, but they appear to have a role in facilitating long-term signaling by affecting new growth at sensory neuron synapses [35, 36]. With regard to pain, Sikandar and colleagues have demonstrated that primary afferent-derived BDNF may be involved in the transition from acute to chronic pain. By applying an inflammatory stimulus to mice, they showed that conditional BDNF knockout mice do not develop an ongoing mechanical hyperalgesia [24]. Similarly, Richner and colleagues have shown that BDNF, via TrkB receptors, can reduce inhibition at the spinal dorsal horn by downregulating the expression of a protein known as KCC2 [37]. By inhibiting this BDNF-regulated pathway, they were able to prevent the decrease of KCC2 and impair

mechanical allodynia. TGF-β1, with its ability to suppress immune activity and promote endogenous opioid signaling, appears to have a protective effect against the development of chronic neuropathic pain [38]. The association between BDNF and TGF-β1 appears to have prior empirical support, at least in animal models. Sometani et al have shown that TGF-β1 administered to cortical neurons of the rat increases BDNF and TrkB expression, suggesting that BDNF may require TGF-β1 in order to carry out its neurotrophic effects [39]. Both BDNF and TGF-β1 also appear to regulate the *Gadd45* family of enzymes which have been implicated in psychiatric diseases [40]. Although it is unclear in what capacity BDNF and TGF-β1 are exerting their influence in persistent disability and pain in humans, their association is at least biologically plausible.

Despite the significance of BDNF and TGF-β1, at this early stage of research it is advised that future studies consider incorporating all of the biomarkers explored here. Cytokines often act synergistically such that their effectiveness is substantially increased when working in concert with one another [41]. Together they can affect multiple systems through peripheral and central crosstalk mechanisms to influence immune, endocrine and neuronal functioning [42, 43]. For example, prior work by Sterling and colleagues demonstrated a potential role for both TNF-α and CRP, wherein the latter appeared to show some discriminative accuracy in identifying those with more severe symptoms following whiplash injury [44]. Additionally, Li et al [13] and Klyne et al [14] found that IL-6 may also be involved in discriminating between control and low back pain, and within low back pain groups, respectively.

The effects in our study may be related to the simultaneous consideration of multiple markers in the same class. Many prior studies, including a recent companion manuscript from the same dataset (see chapter 1), we showed that in isolation none of the 8 markers explored here were associated with clinical pain or interference levels, though several potential moderating effects of psychosocial variables were identified. We believe however that it is the multivariate cluster nature of the results from this latent profile analysis that will prove more valuable. In the same way that a single genetic polymorphism is unlikely to explain important variance in a clinical outcome but gene x

gene interactions are more likely, the expression of certain proteins, at certain levels, in the same person appears as though it may be a more fruitful direction for exploration. In exploring this hypothesis we are working at the 'proteomics' level of the 'omics' cascade, downstream from genomic and transcriptomic processes but upstream from metabolomics. Future research directions could use these results and then move along that cascade in either direction to further explain these findings. It is important to reiterate that this has been considered exploratory research and needs replication, and that despite some biological plausibility, association is not causation.

There are some important limitations of this study to consider. First, blood was drawn using venipuncture which may involve increased anxiety for some. All participants were notified at screening and prior to consent of the requirement for repeated blood draws which may have been sufficient to eliminate those with needle-based anxieties. Second, blood was drawn as participants presented to the urgent care centre regardless of the time of day. This allowed for a more accurate "baseline" sample to be taken as close to the time of trauma as possible, but it does not take into account the known diurnal variations in some of these biomarkers, specifically cortisol [45] and CRP [46]. If sample collection had occurred at the same time each day, this may have shown a greater overall effect of the 8 biomarker model. Lastly, as this was an exploratory study, we have not attempted to build more complex multivariate models, including for example sex, age, or psychological distress. Our prior work supports the notion that the associations shown here may be moderated by other important person-level variables that require larger datasets to properly explore. This represents an important step for future studies as analyzing biomarker concentrations in isolation may be an oversimplification of their role in persistent pain.

In conclusion, we have presented an exploratory study of immune, neurotrophic and endocrine biomarkers in a population of people in the acute stage of non-catastrophic musculoskeletal trauma using latent profile analysis. Our results show that a 3-class profile solution appears to be the most statistically sound. Interestingly 6 out the 8 biomarkers showed some potential to discriminate between different classes, with cortisol

and CRP being the only exceptions. Classes were organized based on increasing serum biomarker concentration where the third class was characterized by high BDNF/TGF-β1. Although recovered populations are not significantly different in their levels of BDNF and TGF-β1, those who experience persisting disability or pain are more likely to have higher levels in serum. These findings, if used in combination with other self-report measures of pain and distress, may provide a simple biopsychosocial approach to phenotyping pain in a clinical population.

4.6 References

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Table 11 – Characteristics and baseline values of SYMBIOME participants in this analysis

N = 109	
Sex (% female)	58.5%
Age (mean, range)	44.6 years (18 to 66)
BMI (mean, range)	26.4 kg/m^2 (14.4 to 51.5)
Primary Region of Injury (%)	
Axial	25.7%
Extremity	74.3%
Mechanism of injury (%)	
Motor vehicle injury	7.1%
Fall	28.6%
Hit by person or object	19.4%
Awkward lift or twist	14.3%
Other	30.6%
Brief Pain Inventory at Inception (mean, range)	
Pain Severity (/10)	4.5 (0 to 8)
Pain Interference (/70)	28.6 (0 to 67)

Table 12 – Cross-product correlation matrix of all 8 analytes (Pearson's r) after square-root transformation

	IL-6	IL-10	TNF-α	TGF-β1	BDNF	CRP	Cortisol
IL-1β	**0.47****	**0.53****	**0.42****	**0.34****	**0.31****	0.03	-0.06
IL-6		**0.47****	**0.34****	**0.25***	**0.21***	-0.01	0.01
IL-10			**0.42****	**0.19***	0.17	-0.09	-0.10
TNF-α				-0.01	0.18	0.02	0.11
TGF-β1					**0.74****	-0.16	0.11
BDNF						-0.01	0.16
CRP							-0.05

*: correlation is significant at the $p<0.05$ level, **: correlation is significant at the $p<0.01$ level.
Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGF-β1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF-α), Interleukins 1-beta (IL-1β), 6 (IL-6) and 10 (IL-10), and cortisol.

Table 13 – Fit Indicators for latent profile analysis and class assignment

	AIC	BIC	Entropy	LMR-LRT (p)
2 class	2298.77	2366.06	0.78	90.80 (0.07)
3 class	**2257.31**	**2348.82**	**0.83**	**58.08 (0.08)**
4 class	2231.06	2346.79	0.89	43.23 (0.30)
3 class (- CRP)	1986.71	2067.45	0.83	57.81 (0.058)
3 class (- cortisol)	1678.22	1748.20	0.82	56.22 (0.054)
3 class (- TNFα)	1384.94	1444.15	0.81	47.06 (0.053)
3 class (- IL6)	1121.54	1169.99	0.80	39.44 (0.029)
3 class (- IL10)	**827.41**	**865.09**	**0.80**	**34.08 (0.033)**
3 class (- IL1β)	539.24	566.16	0.81	27.44 (0.025)

Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), Entropy and Lo-Mendull-Rubin Adjusted Likelihood Ratio Test (LMR-LRT). Values highlighted in **BOLD** indicate the preferred class for analysis. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGF-β1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF-α), Interleukins 1-beta (IL-1β), 6 (IL-6) and 10 (IL-10), and cortisol.

Table 14 – Mean (raw, untransformed) concentrations of the analytes across the 3 classes identified through LPA

	Overall mean (95%CI)	Class 1 (n = 42)	Class 2 (n = 47)	Class 3 (n = 20)	F (p)
IL-1β (pg/mL)	2.71 (2.43, 2.99)	1.32 (1.07, 1.58)	3.46 (3.14, 3.77)	3.19 (2.52, 3.87)	19.75 (<0.01) [1]
BDNF (ng/mL)	3.55 (3.00, 4.09)	1.78 (1.22, 2.34)	3.08 (2.71, 3.46)	8.65 (7.51, 9.80)	182.92 (<0.01) [2]
TGF-β1 (ng/mL)	24.45 (21.11, 27.79)	16.96 (12.22, 21.70)	21.78 (19.02, 24.54)	46.67 (35.74, 57.60)	67.14 (<0.01) [2]
IL-10 (pg/mL)	21.12 (18.08, 24.16)	15.7 (11.9, 19.5)	23.1 (18.5, 27.7)	27.8 (18.2, 37.4)	6.06 (<0.01)[1]
IL-6 (pg/mL)	92.17 (80.05, 104.29)	70.1 (56.9, 83.2)	101.9 (81.5, 122.2)	115.6 (80.3, 150.8)	4.81 (0.01)[1]
TNF-α (pg/mL)	5.61 (5.08, 6.13)	4.9 (3.9, 5.8)	6.0 (5.4, 6.7)	6.1 (4.6, 7.5)	2.77 (0.07)
CRP (mg/L)	3.34 (2.65, 4.01)	3.22 (2.24, 4.21)	3.36 (2.29, 4.44)	3.48 (1.41, 5.54)	0.00 (1.00)
Cortisol (µg/dL)	12.04 (10.58, 13.49)	10.44 (8.65, 12.22)	13.42 (10.75, 16.08)	12.05 (8.68, 15.43)	1.99 (0.14)

1: The mean concentration was significantly lower in Class 1 compared to the other two groups. 2: The mean concentrations of both BDNF and TGF-β1 were significantly different across all 3 groups. Statistical tests were one-way ANOVA with Tukey's post-hoc test using square-root transformed data to reduce deviations from normality. **BOLD** are the 3 markers retained in the final model solution. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGF-β1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF-α), Interleukins 1-beta (IL-1β), 6 (IL-6) and 10 (IL-10), and cortisol.

Table 15 – Crosstabulation of biomarker classes with 6-month outcomes in pain interference (A) and severity (B)

A

BPI Pain Interference	**Low-Mod BDNF/TGF-β1**	**High BDNF/TGF-β1**	**Total**
Mild interference (<5%)	69	11	80
Mod Interference (5-20%)	18	3	21
Severe Interference (>20%)	2	3	5
Total	89	17	106

B

BPI Pain Severity	**Low-Mod BDNF/ TGF-β1**	**High BDNF/TGF-β1**	**Total**
No Pain (Pain = 0)	61	7	68
Persisting Pain (Pain > 0)	28	10	38
Total	89	17	106

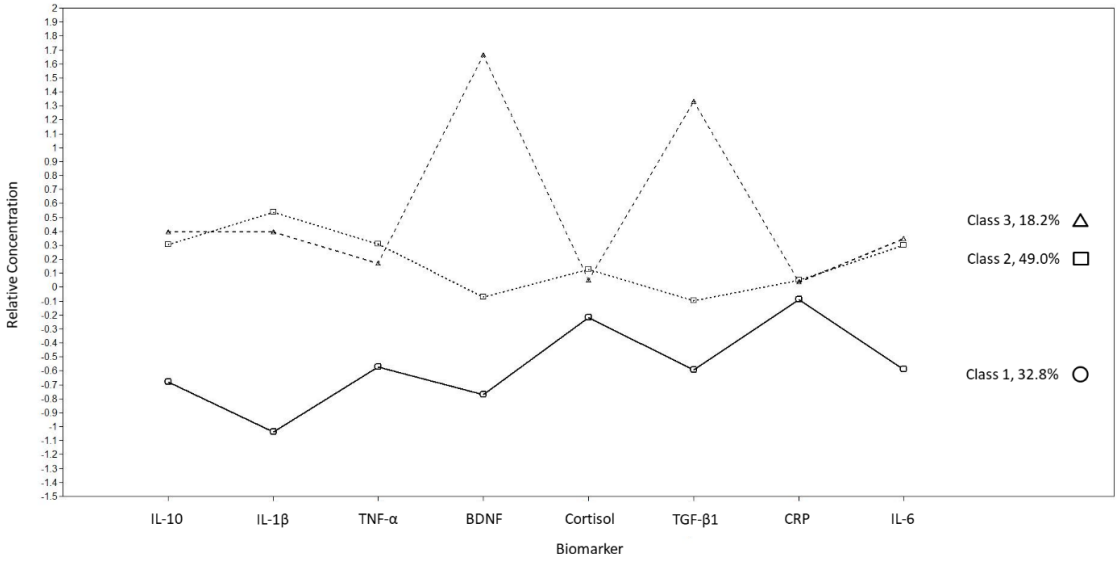

Figure 5 – Graphical representation of the 3-class latent profile solution along with the frequencies of each class.

All 8 target markers presented in a 3-class profile solution were labeled accordingly: Class 1 = Low concentration of all markers (32.8% of the sample), Class 2 = Average Concentration of all markers (49.0%), Class 3 = High Concentration of BDNF and TGF-β1 (18.2%). Relative concentration represents z-transformed values. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGFβ1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF-α), Interleukins 1-beta (IL-1β), 6 (IL-6) and 10 (IL-10), and cortisol.

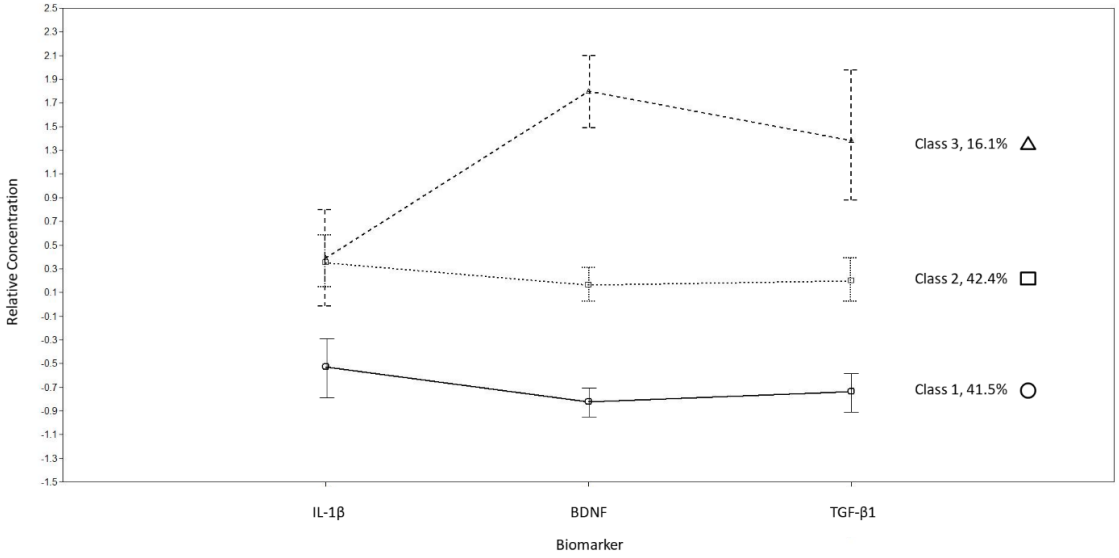

Figure 6 – **Graphical representation of the 3-class latent profile solution adequately described by 3 of the 8 markers.**

Classes were labelled accordingly: Class 1 = Low concentration of all markers (41.5% of the sample), Class 2 = Average Concentration of all markers (42.4%), and Class 3 = High concentration of BDNF and TGF-β1 (16.1%). Relative concentration represents z-transformed values. Biomarkers: Interleukin-1β (IL-1β), Brain-Derived Neurotrophic Factor (BDNF), and Transforming Growth Factor β1 (TGF-β1).

Chapter 5

5 Summary

In an essay entitled "Meditation in a toolshed"[1], author C.S. Lewis describes standing inside a shed and observing a beam of sunlight that has entered through a crack in the doorway. Standing beside the doorway, only the beam itself is visible inside the darkened space with specks of dust illuminated along its path. When he steps into the path of the beam however, and looks along the light through the crack in the door, he sees the tree outside, the sky, and the sun far off in the distance. These are two very different experiences that result in separate conclusions. He uses this example to illustrate the difference between "looking at" and "looking along", while making the comparison between objective and subjective experience. But which one represents the truth? Lewis argues that both perspectives are essential to understanding the greater questions of human existence.

The idea of addressing things in a holistic fashion is certainly not a new one. Lewis' essay was first published in 1970, and though it spoke to questions of a more transcendental nature, the underlying concept was also beginning to emerge in the medical field. Engel first proposed the biopsychosocial model as an alternative to the biomedical model in 1977; where Engel outlines the importance of considering the whole individual rather than giving automatic preference to the objectivity of a medical expert[2]. Even Melzack's neuromatrix model[3], upon which this dissertation is conceptually based, is now almost three decades old. Yet chronic pain remains a prevalent issue in our society with no single solution, which emphasizes both its universality and subjectivity. The purpose of this dissertation was to introduce only the initial phase of the SYMBIOME project, whose overarching goal is to reconcile the biological, psychological and social dimensions of the experience we know as pain. These chapters represent the first of our attempts to combine the perspectives of "looking at" and "looking along" musculoskeletal pain in an effort to better understand this very intersectional problem.

Chapter 2 demonstrates the results of a wide-ranging moderator analysis in the context of functional outcomes (pain interference and pain severity). Taking psychological and social moderators into consideration has an effect on the relationships between biomarkers and pain. In relation to pain severity, TNF-α, TGF-β1, and CRP were moderated by the effects of employment prior to trauma, pre-existing psychopathology, and biological sex, respectively. In relation to pain interference, IL-1β, cortisol, TGF-β1, CRP, and IL-6 were moderated by the effects of pre-existing pain conditions, peri-traumatic fear, the region of injury (axial vs peripheral), and peri-traumatic stress. These results support the claim that physiological factors are tied to pain through more than simple bivariate relationships. The context of the musculoskeletal trauma, both the personal and social context, can affect the behavior of biological systems. The greater implication of this work is that it advocates for a comprehensive phenotyping when dealing with pain, as opposed to the simple presence or absence of inflammatory regulators as can be done with certain arthritic conditions.

Chapter 3 demonstrates the results of latent growth curve analysis in determining the recovery trajectories of acute non-catastrophic musculoskeletal pain in the context of pain interference and severity over the course of 12 months from the time of the initial trauma. With pain interference as the relevant outcome, 3 distinct trajectories emerged wherein people would either rapidly recover, have a delayed recovery (but still reach full recovery by 12 months), or experience minimal to no recovery at all. Pain severity favored a 2-trajectory model with rapid recovery or minimal to no recovery. The classification of recovery group depended on both the initial baseline symptoms and the relative rate of symptom decline. Both recovery outcomes appeared to stabilize after a period of 3 months. The implications of this work are that recovery cannot adequately be measured by initial symptom severity alone. Recovery trajectories may help guide prognostication efforts but it is recommended that they be used in association with other measures. Future longitudinal research may wish to consider both the recovery trajectory and distal outcomes together in order to develop accurate groupings or classes.

Chapter 4 demonstrates the results of latent class analysis and growth mixture modeling when applied to relevant biomarkers of pain. This chapter presents the initial derivation

of a panel of 8 biomarkers (TNF-α, IL-1β, IL-6, CRP, IL-10, cortisol, BDNF, and TGF-β1) that may have the potential to discriminate between functional recovery outcomes. Using this panel of immune, neurotrophic and endocrine regulators, 3 meaningful groups or classes were identified. These groups could be adequately defined by using only 3 of the 8 markers (IL-1β, BDNF, and TGF-β1) where classes were organized by low concentration of markers in serum, average concentration, or high concentration of BDNF and TGF-β1. Participants assigned to the high concentration class at baseline were more likely to score higher on self-report measures of pain and disability in their 6-month outcomes. These results suggest that biomarkers of immune, nervous and endocrine function may provide a useful prognostic tool of persistent symptoms when used in combination with other measures of pain in the context of acute musculoskeletal trauma.

A clinical summary of this work suggests that person-level variables such as pre-existing psychological conditions, employment status, and sex may play a role in the perceived severity of pain. However, pre-existing pain conditions, the location of trauma (spinal vs peripheral), and presence of fear or stress at the time of trauma may be more relevant to one's perceived disability. Unlike what has been suggested in previous literature, initial symptom severity alone may not be adequate to predict long-term outcomes. Although people who rapidly recover will likely have lower levels of initial severity or disability, it is still possible to experience a full recovery with higher baseline symptoms. These individuals (as indicated by the "delayed recovery trajectory" in chapter 3) may recover at a slower rate, but still be fully functional by 12 months. Although it is not entirely clear which factors will distinguish between delayed and minimal recovery, the data suggests that significant improvements in interference can occur by 1-3 months for those in the delayed trajectory. Close monitoring of the aforementioned physical and psychosocial variables in the initial 3 months will be relevant for interdisciplinary treatment and the potential for early intervention. There also appears to be more variability in pain interference compared to severity as recovery tends to occur in 3 (as opposed to 2) different trajectories. This suggests that there may be more change associated with functional ability over the course of rehabilitation even if symptoms of

severity remain relatively stable. Although physical therapy cannot directly benefit from detecting clusters of biomarkers in serum at this time, it may be possible in the future to develop a composite score of pain that includes specific clusters of biomarkers, relevant person-level variables, and current pain measurement scales.

Although this dissertation is by no means a definitive formula on a systems-based approach to pain research, it represents our efforts to lay the groundwork for the deep clinical phenotyping of pain. By understanding the nature of the interactions between systems and the relevant risk factors therein, it may be possible to develop a predictive algorithm that can accurately identify those at risk of developing chronic pain. This knowledge may also be used toward the development of individualized treatment plans for those who suffer from chronic pain and require strategic, interdisciplinary management.

5.1 Future Directions

As stated previously, this project is merely the initial stage in the development of a "pain phenotype". As a simple strategy to further develop this prognostic phenotype, future research may consider a deeper exploration of sex and gender as it relates to the development of pain. Previous studies in the field of pain and gender have shown that females bear a greater burden of chronic pain than males[4]. This difference is only partially accounted for by the contributions of social factors such as education, economic stability, and age[5]. Further research may link differences in internal functioning with the age and sex of the participant. Other considerations that should be taken into account are gender roles and expectations. A common, pre-conceived notion is that the female role is associated with lower pain tolerance and greater likelihood to report pain[6]. These notions however are influenced by gender constructs and gender itself can be considered a continuum[6]. This area represents another relevant dimension in capturing the experience of pain especially since women are underrepresented in pain medicine[7].

From an intervention perspective, cannabinoids represent a class of compounds which may possess the potential to modulate a number of processes related to pain. Research has shown that cannabinoid compounds have significant modulatory effects on both the immune [8, 9] and nervous systems [10], along with higher brain functions [11]. External cannabinoids are able to exert their effects due to the presence of an internal system of cannabinoid receptors and endogenously-produced cannabinoid compounds (i.e. endocannabinoids) [12, 13]. This endocannabinoid system is closely linked with major physiological functions as cannabinoid receptors are located throughout the body[14]. Once these receptors are activated by either external cannabinoid compounds or endocannabinoids, they affect a number of different tissues to influence anxiety, pain, nausea, and inflammation [13, 15] . Despite this extensive involvement in regulating function, a number of genetic variations have been identified in the genes responsible for cannabinoid activity and cannabinoid breakdown in the body (i.e. metabolism). Although the results are not conclusive, these variations may influence the efficacy of cannabinoids in different individuals [16]. To address these individual variations, pharmacogenetics represents a promising new tool in the development of personalized medicine. It involves the screening of specific genes associated with a given drug's activity and metabolism to determine a person's potential responsiveness to the drug itself [17]. This technology has been used to develop personalized pain treatments, but has yet to be implemented on a wide scale due to a lack of knowledge on translating genetic profiles into clinical action [17] and the associations between gene targets and functional traits [18]. Application of this genetic screening technology within known recovery groups in musculoskeletal pain may help to identify novel gene-trait associations and inform cannabinoid treatment decisions going forward.

5.2 References

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18. Ting S, Schug S. The pharmacogenomics of pain management: prospects for personalized medicine. *J Pain Res.* 2016;9:49-56.

Appendices

Appendix A: Ethics approval for ongoing review of the SYMBIOME project

Western Research

Date: 4 November 2019

To: Dr. Dave Walton

Project ID: 106140

Study Title: Modeling post-traumatic pain and recovery: The SYMBIOME longitudinal cohort study

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 19/Nov/2019

Date Approval Issued: 04/Nov/2019

REB Approval Expiry Date: 17/Nov/2020

Dear Dr. Dave Walton,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix B: Letter of Information

Western HealthSciences

May 28, 2015

Principal Investigator: Dr. David Walton

Funding source: Western internal funding, CIHR bridge grant, Canadian Pain Society

Letter of Information

Modeling recovery after traumatic injuries

Dear Sir/Madam,
Thank you for your time in reviewing this letter of information and for considering participation in our study. Please be sure to read this letter in its entirety and have any questions you may have answered to your satisfaction before consenting to participate.

Why am I being invited to participate?
You are being invited to participate because you have indicated that you are a male/female between the ages of 18-65 and are seeking care from emergency, medical or rehabilitation services for a recent accident or injury to your muscles, bones or ligaments, or because you have responded to one of the posted advertisements for this study.
You are not eligible for this study if any of the following apply to you. Please tell the research coordinator if any of these apply:

1. Severe gingivitis, periodontal disease, active dental caries (tooth decay), or any other active oral condition
2. Actively undergoing cancer treatment
3. Are currently experiencing an infection or illness (cold, flu, fever, etc.)
4. Are currently taking antibiotics or have taken antibiotics within the past week
5. You are a smoker or have been a smoker within the past year
6. You have Diabetes, either Type I or Type II
7. You currently have stomach ulcers, Celiac or Inflammatory Bowel Disease (Ulcerative colitis or Crohn's Disease)

What is this study about?
We are trying to understand the process of recovery over the 6 months following a traumatic injury, and to identify things (factors) that may explain why people differ in how they recover after these events. We will be collecting information including the nature of your injury, your biology, psychology, and past experiences all in the same period. Our goal is to not only improve understanding of *how* people recover following different types of injuries, but what factors influence that recovery. By identifying important factors we will start to work on developing new ways to treat those factors and eventually improve the likelihood of successful recovery for people injured in the future.
What will I be asked to do?
If you agree to participate, you will be provided with a package that includes almost all of the data collection instruments that you will be asked to complete on your own at home starting at least 48 hours

after your injury. The procedures include questionnaires for you to complete and different vials into which you will provide saliva and a stool sample. Once collected, the samples can be stored in your home freezer until a member of the research team comes to pick them up. The questionnaires will be repeated monthly for 6 months after your injury and the biological sampling will be repeated after 3 and 6 months. After the 6th month, your participation in the study will be complete. Below you will find more detailed information on the types of data instruments in this study.

1. **A set of questionnaires** that will ask you about a variety of different things. These include: i) your age, sex, work and educational status, ii) the nature of your injury (type of injury, when it occurred, how long ago it occurred, a brief description of the injury itself), iii) your medical and legal involvement (if any), iv) experiences from your childhood, including bullying and home environment, v) recent stressors you may have experienced, vi) the stress you have experienced as a result of your injury, vii) the type and amount of symptoms and interference you have experienced as a result of your injury.
2. **Drool/Saliva (part 1)** – You will receive 3 specialized test tubes with sterile cotton swabs in each. You will start on a day that is convenient to you, preferably within 5 days of completing your questionnaires. A pamphlet explaining all procedures is included with the instruments. This pamphlet should be read in its entirety. The tubes with the cotton swabs are to be used 3 times during the same day – once immediately upon waking, again 20-30 minutes after waking, and again mid-afternoon between 2pm and 4pm. This will require you to chew the cotton swab for about 10 seconds before returning it to the test tube, sealing it and placing it in your freezer.
3. **Drool/Saliva (part 2):** You will receive a specialized test tube into which you will spit or drool a small amount of saliva BEFORE your nightly (bedtime) routine, before brushing but at least 2 hours after eating. Once completed, this and the other samples can be stored in your residential freezer until retrieved by a member of the research team.
4. **Serum:** A trained researcher will draw 3cc of blood from the vein on the front of your elbow.

The following two components are optional.

5. **Stool**: This is an optional part of the study. You will provide a sample of stool using a specialized, sterile tube with a Q-tip type cotton swab. This will simply require you to twirl the end of the swab in a piece of used bathroom tissue, sealing it in the test tube and placing in your freezer. Only a small sample is required, and this can be collected at any time of day.
6. **Hair:** This is an optional part of the study for which you will be compensated if you choose to participate. As long as you have at least 3cm of hair on your head, we will cut approximately 100 hairs from the back of your scalp in a manner that minimizes any obvious physical change in your hair style using sterile scissors. This will be done by a member of the research team, and will only be done once at the beginning of the study.

We are collecting saliva samples in order to analyze the levels of specific proteins, which we are calling "biomarkers", that are typically present in the body and that may change during times of stress. Specifically, these are classed broadly as the stress hormone *cortisol*, the gonadal hormone *testosterone*, and immune or inflammatory markers that are referred to as '*cytokines*'. Stool samples, on the other hand, will provide us with specific information regarding the different bacterial populations that inhabit your intestines. The types of bacteria in your intestines may be influenced as a result of significant stressors, such as trauma or injury. We will be looking to see if any major shifts in the types of bacteria occur in your system as you are recovering. There is some research that suggests certain genes play a role in the speed and effectiveness of recovery from an injury. The blood is being drawn primarily for exploratory and data redundancy reasons. If the other tissues/fluids fail for any reason, the blood will allow us to evaluate the same chemical markers without having to reconnect with you to collect more data. Finally, from your hair we will be able to determine the presence of different hormones that have been stored in your hair from the time before your injury.

It is important to understand that everyone's body is different and it's currently difficult to say what is 'good' or 'bad' in these analyses. For that reason, these tests should not be considered diagnostic of any specific diseases or conditions.

Once all samples have been collected, contact the research team at Western University. These samples will then be retrieved from you by a member of the research team at a day, time and location that is convenient for you. A subset of the questionnaires will be completed again at 1 month intervals (approximately 10 minutes to complete), and the biological samples will be collected at 3 and 6 months. After the 6th month, your participation in the study is complete.

What are the risks and benefits of participating?
There are no immediate anticipated benefits to you from participating in this study. If you request it, we will provide you with the results of the different system tests that we conduct, although they may be difficult to interpret in isolation until the rest of our data have been collected. However, if our predictions are correct and we are able to identify dysfunction in key systems that can explain at least part of the pain experience, this may open new avenues to treatment that may have benefit to you or others in the future.
All participants may receive a final report of the study in which the results (using only group data) will be presented. If you wish to receive this report, you will need to indicate this on the consent form and include contact information to which the report should be sent. Those participants who wish to receive their own individual results will be required to contact the Lead Researcher Dr. David Walton directly to make that request. His contact information can be found at the end of this letter. Keep in mind that the data associated with this study <u>is not a medical record</u> and shouldn't be used as such. We will keep the Master List that links your name with your ID number for 6 months after your completion of the study after which it will be shredded for confidentiality and privacy protection reasons. This means that we will not be able to provide your individual results beyond 1 year from your injury.

The risks to participation are minimal and are largely inconveniences due to time. The salivette (saliva collection tube with cotton swab) samples must be performed at three separate times throughout a single day which may be a mild disruption to your daily routine for that day. Improper collection and handling of stool samples MAY pose a risk of bacterial contamination/infection, however, if carefully performed (including washing your hands afterwards), this risk is quite minimal. The blood will be drawn using a standard protocol that you have likely experienced before in a doctor's office or the Red Cross. Completion of the questionnaires may lead to some people experiencing emotional distress, especially those that ask you to recall and reflect upon childhood experiences if yours were not positive. We have provided suggestions for managing emotional distress, should you experience it, at the end of this letter. We will do everything in our power to ensure your data, including the biological specimens and your questionnaires, are kept secure and confidential. However, we cannot guarantee against a data breach regardless of how good our physical and virtual security is. Your data will be stored with only a random ID number in order to mitigate any potential risk, nonetheless the risk of data breach or loss is possible and we want to ensure you're aware of this. Should this happen you will be quickly informed.

Will I be compensated for my participation?
You have different options for the degree to which you wish to participate in this study. The minimum level of participation is to complete the paper forms, saliva, and blood draw. This would be done once when you enter the study, then at 1, 2, 3, 6 and 12 months later. Each follow-up will likely take about 45 minutes of your time, and you will receive $30 total for participating in this level of the study. The hair and stool are optional components, and for each one you will receive an additional $15 ($30 for both). We recognize that collecting these samples is no small commitment, but can be completed in its entirety in a single day and a total anticipated time commitment of approximately 1 hour at each collection period. Out of respect for your time, you will be therefore be reimbursed a minimum of $180 total for participating in each phase of this study (intake and 1, 2, 3, 6, and 12 months). If you complete the two

additional components you are eligible for an additional $30 per session, up to an additional $180 for the entire study.

Who will have access to my information?
A unique randomly-generated 6-digit ID number will appear on all forms belonging to you for the sole purpose of connecting all of the data you provide at each period. The lead researcher at Western University, Dr. David Walton, will collect all of the data provided by all participants and will analyze it as an anonymous group. Once transcribed, all data are stored on the secure, password protected and firewalled server of Western University and the paper forms are shredded. Western University's REB and representatives from Lawson's Quality Assurance and Education Program will have access to participant's data to ensure that it is following the proper laws and regulations. Outside of these groups, your specific information will not be shared with anyone without your express written consent to do so.

Note some of the tools to be completed are meant to measure severity of symptoms related to depression or anxiety. *IF* your responses lead to a score that is suggestive of either depression or anxiety, your family doctor will be contacted to inform him/her of the results of the scale and what they may mean. It will ultimately be up to your family physician to decide how and when he/she should follow up with you if at all.

Data will be retained in anonymous form indefinitely as an ongoing database.

Voluntary participation
Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time. If you choose to withdraw from the study, you may request to have your contributions to that point removed, at any time up until 6 months after you are done the study. Withdrawal from the study or refusal to participate is your decision, and may be done without the requirement of explanation on your part. Withdrawal will in no way affect your current or future relationship with any of the research team or clinicians associated with the study.

What if I want more information?
You may contact the lead researcher, Dr. David Walton, at Western University (London, Canada) if you require any further clarification. If you have any questions about your rights as a research participant or the conduct of the study you may contact the Office of Research Ethics. You are encouraged to keep this letter of information for your own records.

If you wish to receive a summarized copy of the results of this study and/or your individual results, you may leave your email address on a separate sheet. The sheet will be held by the research coordinator, and the email addresses will only be used to provide the results, after which the list will be destroyed. We thank you in advance for considering participation in this study. *You do not waive any legal rights by signing this consent form*.

Sincerely,

David Walton BScPT, PhD
Lead Researcher

Co-researchers:
Ruth Lanius MD PhD
Stan Van Uum MD, PhD
Greg Gloor PhD
Walter Siqueira DDS, PhD
Melanie Colombus
Kristine Van Aarsen
Joshua Lee

If you are experiencing emotional distress:

This research study does NOT include treatment recommendations. However, while completing the questionnaires about your emotional state or past experiences, you may find that you experience emotional distress (e.g. sadness or anxiety) by virtue of thinking about and answering the questions. If this should happen, it is most commonly short-lived and may be a sign to take a break from the questionnaires until you settle down enough to come back to them.

However, in the distress can last longer than a day or can be quite severe in some people. If this happens to you, you are encouraged to seek professional assistance to help deal with your emotional state. The Canadian Mental Health Association includes several resources on their website as a good place to start: http://www.cmha.ca/mental-health/find-help/. TeleHealth Ontario can also offer support or direction, they can be reached 24 hours, 7 days per week. The London Mental Health Crisis Service offers 24-hour, 7 days per week support to those in acute mental distress. Finally, if you feel you are in significant emotional distress and require more immediate help, you can call your family doctor or emergency services (9-1-1). In that case you should refrain from completing any further questionnaires and let the researchers know that you are unable to continue.

January 9, 2015

Consent form

Modeling recovery from traumatic injuries

Principal Investigator: Dr. David M. Walton PT PhD

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction. I also consent to being contacted by the Lead Researcher in the case any of my scale scores suggest possible problems with depression or anxiety.

Please indicate the level of study participation to which you are consenting by placing a check in the appropriate circle:

□ Paper forms only (monthly forms, approximately 10 minutes each, $25 total compensation)

□ Paper forms *and* biological specimens *but not hair* (saliva, stool, serum) at intake, 3 and 6 months (approximately 1 hour each), paper forms at 1, 2, 4, and 5 months (approximately 10 minutes each). $100 total compensation

□ Paper forms *and* biological specimens *including a sample of about 100 hairs from the back of your head at intake*. Other data and intervals as described directly above (approximately 10 minutes in months 1, 2, 4 and 5, approximately 1 hour in months 3 and 6). $125 total compensation.

_____

Participant name (print)

_____ _____

Participant signature Date

_____

Person obtaining consent (print)

_____ _____

Signature of person obtaining consent Date

Request for Summary of Results

◯ I would like to receive a copy of the group average results from this project (Note: these results will not have any clinical application and will not affect your medical treatment)

If you would like to receive a copy of the group results, please provide your preferred method of delivery:

◯ Electronic (email); Email address: _____

OR

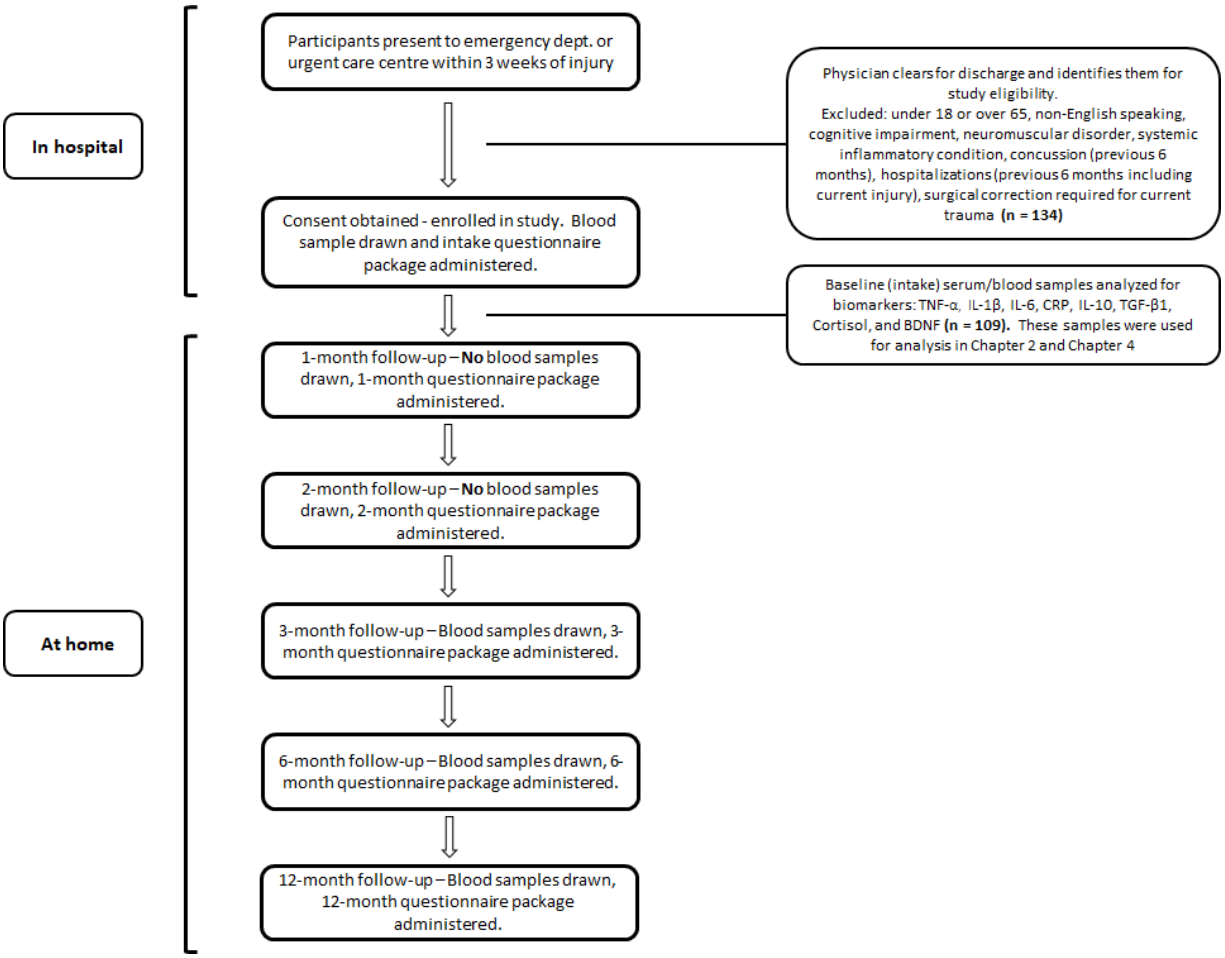
◯ Postal mail; Mailing address (incl. Street, City, and Postal code):

_____

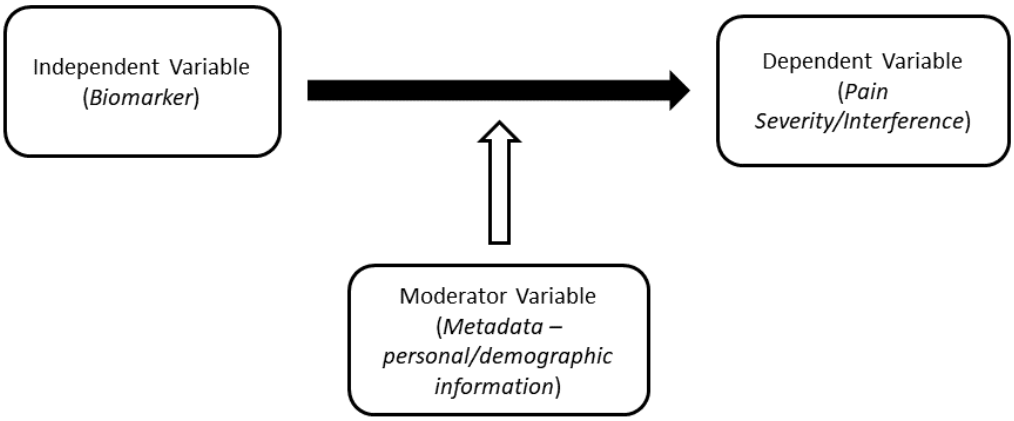
_____

_____

Appendix C: SYMBIOME data collection flow diagram

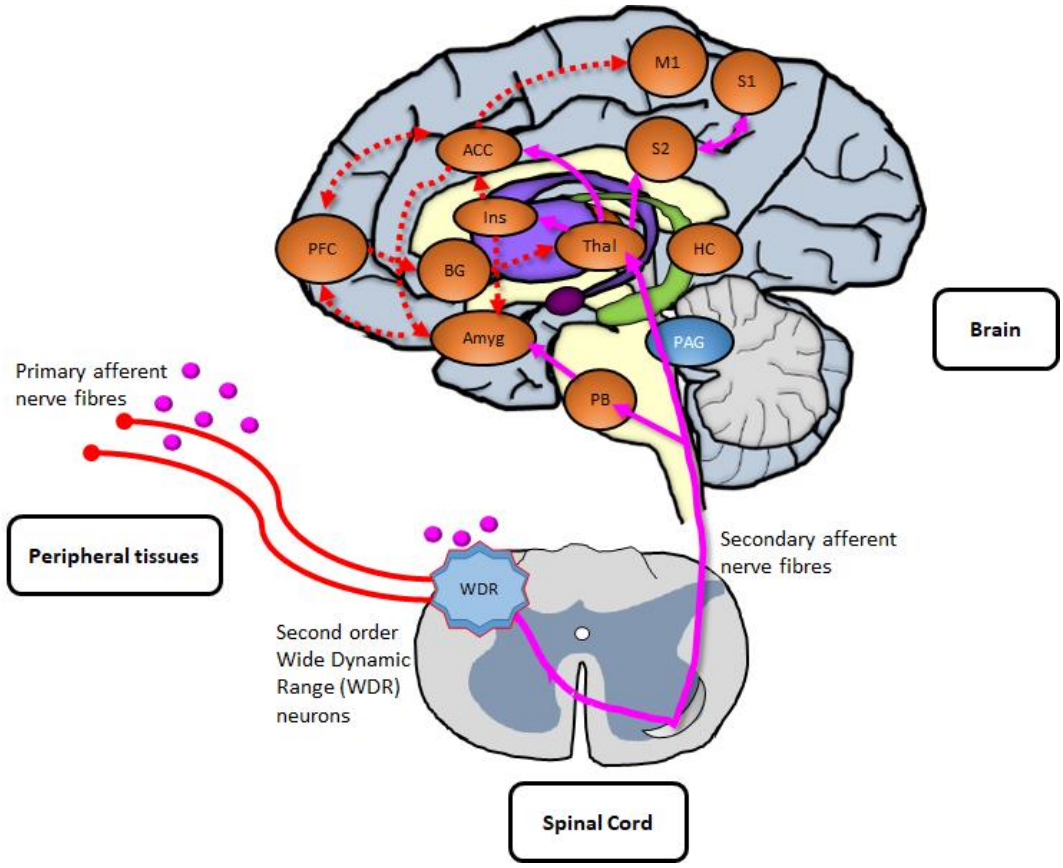


Appendix D: Simple Schematic of Moderator Variables



Moderator variables provide key context that can change both the magnitude and direction of the relationship between the independent variable (one of the 8 biomarkers) and the dependent variable (pain-related outcomes of severity and interference). The implications of these moderators are that biomarkers may behave differently in the context of pain-related outcomes depending on which metadata subgroup is being analyzed.

Appendix E: Conceptual Summary Figure



Tissue trauma can occur in the peripheral tissues (affecting primary afferent pathways) or spinal tissues (affecting secondary afferent pathways) resulting in an immune inflammatory repair response. Inflammatory factors (TNF-α, IL-1β, IL-6, IL-10, TGF-β1, and CRP) can affect neural activity of tissues which include increased noxious signaling (hyperalgesia) and heightened sensitivity to benign stimuli (allodynia). Inflammation can also affect sensitivity of second order WDR neurons resulting in increased sensitivity and neurological drive in the spinal cord. Cortisol, TGF-β1, and in particular, BDNF may be implicated in their ability to facilitate the long-term synaptic plasticity in spinal afferents that results in chronic sensitization. Progressing from peripheral to central signaling, IL-1β and IL-6 may be involved in facilitating fear-learning and fear-memory in the context of stress. This may contribute to the associative mechanism between trauma, fear, adaptation and learning. TGF-β1 and BDNF are associated with growth arrest and DNA damage enzymes (*Gadd45*) which have been implicated in a number of different psychiatric diseases. All of the above mentioned mechanisms have been previously described in the literature and cited throughout chapters 2, 3, and 4 of this dissertation.

Higher level cortical plasticity in the brain (from Mansour et al. 2014)[1] – Acute pain evokes nociceptive pathways (solid arrows) in the brain which involves the thalamus (Thal), parabrachial nucleus (PB), amygdala (Amyg), insula (Ins), anterior cingulate cortex (ACC), and 1° and 2° somatosensory regions (S1 and S2). Under normal circumstances, these nociceptive associations dissipate with time. Prolonged stimulation can result in the transition towards chronic pain and "emotional suffering" which involves higher brain regions that generate our motivation and sense of self (dotted arrows) including the basal ganglia (BG), pre-frontal cortex (PFC), and 1° motor cortex (M1). This transition leads to the persistence of memory with regard to pain, thereby perpetuating the experience.

1. Mansour AR, Farmer MA, Baliki MN, Apkarian AV. Chronic pain: the role of learning and brain plasticity. Restorative neurology and neuroscience. 2014 Jan 1;32(1):129-39.

Curriculum Vitae

Name: Joshua Lee

Post-secondary Education and Degrees:

Western University
London, Ontario, Canada
2015-2017 MPT Physical Therapy

University of Calgary
Calgary, Alberta, Canada
2008-2011 MSc. Biochemistry and Molecular Biology

University of Calgary
Calgary, Alberta, Canada
2002-2007 BSc. Biological Sciences

Honors and Awards

- **May 2019 –** Connaught Summer Institute in Pain (University of Toronto) -– "Individual Experience of Pain" **–** Registration **+** $500 (travel and accommodation)
- **May 2018 –** Top Research Poster Presentation (MSK Research to Advance Lifelong mobility) – Canadian Bone and Joint Conference 2018
- **Oct 2017** – UWO MPT - Western University School of Physical Therapy Valedictorian Award (2017)
- **Oct 2017** – UWO MPT - St. Joseph's Healthcare Award (2017)
- **Oct 2017** – UWO MPT - Nominee for the Troy Seely Clinical Excellence Award (2017)
- **Aug 2017** – Canadian MSK Rehab Research Network Trainee Award (2017-2018) - $5000
- **May 2017** – Ontario Graduate Scholarship award (2017-2018) - $15,000
- **Feb 2017** – Earl Russell Trainee Grant in Pain Research (2016-2017) - $10,000
- **May 2016** – Ontario Graduate Scholarship award (2016-2017) - $15,000
- **May 2015** – Ontario Graduate Scholarship award (2015-2016) - $15,000
- **Apr 2015** – 3-minute thesis competition – faculty finalist (Western University)
- **May 2014** – Ontario Graduate Scholarship award (2014-2015) - $15,000
- **Apr 2014** – 3-minute thesis competition – 2nd place (Western University) - $500
- **Feb 2014** – 1st place oral presentation – 1st year PhD category 2014 Health and Rehab Science Graduate Research Conference - $100

Related Work Experience

- **Physiotherapist - Family Physiotherapy Centre / London Intercommunity Health Centre -** Jul 2018 – ongoing
- **Course manager and lecturer for Western University PT 9511** – Foundations of Physical Therapy – Sept 2019 – Dec 2019

Ontario Physiotherapy Association (London chapter)

- Apr 2019 – OPA Interaction conference 2019 – **voting delegate** for the London chapter
- Apr 2018 – OPA Interaction 2018 conference – session **moderator**
- Sept 2017 – ongoing – Director of Research and Knowledge Translation – **Co-Founder** and **coordinator** of the IRIS initiative (Integrating Research Into Students)

University of Western Ontario – Graduate Studies (MPT/PhD)

- Oct 2015 – Aug 2017 – Physical Therapy student newsletter ("The Humerus") – **contributor** and **co-editor** of the UWO PT newsletter
- Oct 2015 – Aug 2017 – "Integrating Research Into Students" (IRIS) – **Co-Founder** and **coordinator** of research seminar series for UWO Physical Therapy students
- July 2015 – **International Collaboration** in Physical Therapy research – University of Western Sydney (Sydney, AUS) – Mapping the motor cortex with Transcranial Magnetic Stimulation
- Apr 2014 – Apr 2015 – Health and Rehab Science Grad Student Society (HRSGSS) – **President** – Organizing events and seminars + primary liaison between faculty and students
- Oct 2013 – Feb 2014 – HRSGSS annual research conference – organizing **committee member**
- Sept 2013 – ongoing – Rehabilitation Science **Researcher** – Multi-disciplinary chronic pain research in the field of biopsychosocial health and rehabilitation.

Professional Development

Memberships

- College of Physiotherapists of Ontario – Active status, in good standing – Since Jun 2018
- Canadian Physiotherapy Association – Since 2015
- Ontario Physiotherapy Association – Director of Research and Knowledge translation for London chapter – Since Sept 2017

Certifications

- Level 1 Orthopedic Certification – Canadian Physiotherapy Association (Orthopedic Division) – Aug 2017
- First Aid and CPR - Health Care Provider – St. John's Ambulance – Aug 2018
- Phlebotomy – Medix College – Mar 2016

Supplementary Education

- Jul 2019 - Connaught Summer Institute in Pain (University of Toronto) – selected (based on competitive international application) to participate in an intensive 1-week summer program on "The Individual Experience of Pain".

Intellectual Property and Patents

- "Blood Profile to Predict Rate of Recovery Following Acute Musculoskeletal Trauma" – WORLDiscoveries Tech ID: W-19-010 (Jun 2018) – Developer (75% - Dr. David Walton, 25% - Joshua Lee)

Review and Evaluation

- Jun 2019 - Western University School of Physical Therapy Admissions committee – Masters of Physical Therapy program application reviewer
- Jun 2018 – Article reviewer for "Musculoskeletal Science & Practice" journal.

Publications

Papers

- **Lee JY**, Guy SD, Lukacs MJ, Letwin ZA, Fakhereddin MF, Al-Nasri IJ, Salim S. Management of Fibromyalgia Syndrome: Cognitive-Behavioral Therapy (CBT) for healthcare professionals. University of Western Ontario Medical Journal. Mar 2018.
- **Lee JY**, Ready EA, Davis EN, Doyle PC. Purposefulness as a critical factor in functioning, disability and health. Clinical rehabilitation. 2017 Aug;31(8):1005-18.
- Walton DM, Elliott JM, **Lee J**, Loh E, MacDermid JC, Schabrun S, Siqueira WL, Corneil BD, Aal B, Birmingham T, Brown A. Research priorities in the field of posttraumatic pain and disability: Results of a transdisciplinary consensus-generating workshop. Pain Research and Management. 2016;2016.

Educational Materials

- Advanced Integrated Musculoskeletal Physiotherapy Education Program – Educational Support Materials – Leading contributor to the Pain Theory section. Jun 2019.

Peer-reviewed Abstracts

- **Joshua Y. Lee**, David M. Walton, Paul Phares, Curtis May, Wanda Millard. Identification of relevant blood markers in acute non-catastrophic musculoskeletal trauma and association with 6-month outcomes through latent class analysis. *Accepted*. IASP 2020.
- **Joshua Y. Lee**, David M. Walton, Paul Tremblay, Curtis May, Wanda Millard, James M. Elliott, Joy C. MacDermid. Defining pain and interference recovery trajectories after acute non-catastrophic musculoskeletal trauma through Growth Mixture Modeling. *Accepted.* IASP 2020.
- **Joshua Y. Lee**, Mohamad F. Fakhereddin, Maryam Ghodrati, David M. Walton. 2018. Exploring non-linear and interactive relationships between psychological and physiological markers of post-traumatic pain and distress: toward a biopsychosocial model of pain. Canadian Bone and Joint Conference 2018. London, ON, Canada.
- Mohamad F. Fakhereddin, **Joshua Y. Lee**, Maryam Ghodrati, David M. Walton. 2018. Exploring Recovery Trajectories and Predicting Outcomes of Acute Musculoskeletal Trauma: Further Exploration of the Prognostic Validity of the Traumatic Injuries Distress Scale (TIDS). Canadian Bone and Joint Conference 2018. London, ON, Canada.
- **Joshua Lee** and David Walton. 2014. The Determination of the Specific Biopsychosocial Mechanisms that Drive the Transition from Acute to Chronic Musculoskeletal Pain. Health and Rehabilitation Sciences Graduate Research Symposium 2014. London, ON, Canada.

Lectures and Presentations

- **Sept 2019 – Dec 2019** – Course manager and lecturer - Western University School of Physical Therapy (~40 teaching hours – tissue physiology, pain, gait, therapeutic exercise)
- **Jun 8, 2019** – Invited speaker – National Orthopedic Symposium 2019 – Cannabinoids and Pain (Toronto, ON)
- **Jun 7, 2019** – Invited speaker – National Orthopedic Division Instructor's Meeting – Pain, Allostasis, and Sensitization (Toronto, ON)
- **Dec 4, 2018** – Guest Lecturer – Western University – Physical Therapy (PT9514 – Neuroscience for Physical Therapists) – Microbiota and Pain
- **Oct 16, 2018** – Guest Lecturer – Western University – Physical Therapy (PT9514 – Neuroscience for Physical Therapists) – Understanding Pain

- **Oct 2, 2018** – Guest Lecturer – Western University – Physical Therapy (PT9512 – Integrated Assessment) – Balance Assessment
- **Sept 6, 2018** – Clinical in-service – Family Physiotherapy Centre of London – Pain, Allostasis and Management (A top-down approach to chronic pain)
- **July 10, 2018** – Guest Lecturer – McMaster University – Physical Therapy (Unit 6 Persistent Pain Lab) – Microbiota and Pain
- **May 12, 2018** – Canadian Bone and Joint Conference 2018 – Poster presentation (London, ON)
- **Jul 4, 2017 –** Guest Lecturer – McMaster University – Physical Therapy (2nd year class – Unit 6 Persistent Pain Lab) – Microbiota and Pain
- **Jan 25, 2017** – Guest Lecturer – Rehabilitation Sciences 3360 – Overview of Pain Systems – Health Sciences Building, Western University (London, ON)
- **Mar 21, 2016** – Guest Lecturer – Rehabilitation Sciences 3061 – Pain in rehabilitation - Elborn College, Western University (London, ON)
- **Nov 30, 2015** – "PIRLs of Wisdom" – Education and fundraising event – Oral Presentation (London, ON)
- **Nov 2, 2015** – "Integrating Research Into Students" seminar series – Oral presentation (London, ON)
- **Apr 9, 2015** – 3-Minute Thesis Competition – Oral presentation (London, ON)
- **Apr 10, 2014** – 3-Minute Thesis Competition – Oral presentation (London, ON)
- **Feb 5, 2014** – Health and Rehab Science Grad Student Symposium – Oral presentation (London, ON)