Pain After Breast Cancer Surgery is Predicted by Pre-Operative Immunological and Psychological Factors

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ABSTRACT

This study identified risk factors for pain intensity at rest and with movement, pain qualities and neuropathic pain 24 hours post-breast cancer surgery (BCS). Before surgery 86 women completed demographic, health status, and psychological questionnaires and blood was drawn to measure baseline cytokine levels. Numeric Rating Scale-Rest (NRS-R), NRS-Movement (NRS-M), Short-Form McGill Pain Questionnaire (SF-MPQ) and Short-Form Neuropathic Pain Questionnaire (SF-NPQ) were completed 24 hours post-BCS. Backward regression models found significant correlates for NRS-R: younger age, increased pain catastrophizing and bilateral surgery; NRS-M: younger age, increased trait anxiety, bilateral surgery, and mastectomy; SF-MPQ: increased pain catastrophizing, bilateral surgery, and previous breast surgery; and SF-NPQ: decreased interleukin-10 and increased pain catastrophizing. These results support the biopsychosocial model of pain and the importance of measuring multiple pain outcomes. Variables accounting for the most variance in each outcome (pain catastrophizing [NRS-R; SF-MPQ], trait anxiety [NRS-M] and baseline IL-10 [SF-NPQ]) are potentially modifiable.

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ABBREVIATIONS

Abbreviation	Meaning
ACC	Anterior cingulate cortex
ACL	Anterior cruciate ligament
ALND	Axillary Lymph Node Dissection
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
APSP	Acute Post-Surgical Pain
ASA	American Society of Anesthesiologists Physical Status Classification System
BCS	Breast Cancer Surgery
BMI	Body Mass Index
BMSC	Bone Marrow Stromal Cells
CCI	Charlson Comorbidity Index
CES-D	Center for Epidemiological Studies – Depression Scale
COX-2	Cyclooxygenase -2
CRPS	Complex Regional Pain Syndrome
CSF	Cerebrospinal Fluid
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglion
ER	Estrogen Receptor
fMRI	Functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GCSF	Granulocyte colony stimulating factor
GCT	Gate control theory
GM-CSF	Granulocyte-macrophage colony stimulating factor
HC	Healthy Controls
HER2	Her2/neu receptor
ICAM-1	Intercellular adhesion molecule-1
ICBN	Intercostobrachial Nerve
IFN-γ	Interferon-γ
IL-1β	Interleukin-1β
IL-1Ra	Interleukin-1 receptor antagonist
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-17	Interleukin-17

KPS	Karnofsky Performance Status
LLOD	Lower Limit of Detection
MCP-1	Monocyte chemoattractant protein-1
mRNA	Messenger Ribonucleic Acid
MSD	Meso Scale Discovery
NeP	Neuropathic Pain
NK cells	Natural killer cells
NMDA	N-methyl-D-aspartate
NRS-M	Numeric Rating Scale for Pain with Movement
NRS-R	Numeric Rating Scale for Pain at Rest
PAG	Periaqueductal Grey
PCS	Pain Catastrophizing Scale
PGE2	Prostaglandin 2
PPSP	Persistent Post-Surgical Pain
PR	Progesterone Receptor
RA	Research Assistant
RVM	Rostroventral medulla
SF-MPQ	Short-Form McGill Pain Questionnaire
SF-NPQ	Short-Form Neuropathic Pain Questionnaire
SLNB	Sentinel Lymph Node Biopsy
SNI	Spared Nerve Injury
SNP	Single nucleotide polymorphism
STAI-S	State-trait anxiety inventory – state subscale
STAI-T	State-trait anxiety inventory – trait subscale
TGF-β	Transforming Growth Factor-β
TNF-α	Tumour Necrosis Factor-α
Treg	T regulatory cell
TRPA1	Transient receptor potential ankyrin 1
TRPV1	Transient receptor potential vanilloid 1
UD	Undetectable
UHN	University Health Network
VAS	Visual analog scale
VCAM-1	Vascular cell adhesion molecule-1
VIF	Variance inflation factor
WPT	Widespread Palpation Tenderness

1 INTRODUCTION

Breast cancer is the most common cancer affecting Canadian women with 26,300 expected diagnoses in 2017[1]. Mortality from breast cancer has declined since the mid-1980s, and the current five-year survival rate is 87%[1]. This increased survivorship warrants a focus on preventing and reducing treatment sequelae. Surgery remains frontline treatment, however, it is associated with acute post-surgical pain (APSP) in 15-60% of patients[2,3]. APSP can lead to complications involving multiple organ systems, psychological distress, reduced patient satisfaction, delayed discharge from hospital, unanticipated readmissions and persistent post-surgical pain (PPSP)[4].

Pain is a biopsychosocial construct and therefore, to elucidate risk factors for APSP considering multiple dimensions is essential. In the biological domain, various inflammatory cytokines have been implicated suggesting they could be effective biomarkers to identify those at risk for APSP. However, most research has examined chronic pain populations or the post-operative inflammatory response rather than baseline levels. Studies on APSP have mostly focused on demographic, surgical and psychological risk factors. The current study was the first to our knowledge to develop models of APSP intensity at rest, with movement, general pain qualities and neuropathic pain (NeP) qualities after breast cancer surgery (BCS) using pre-operative factors, including baseline cytokine concentrations, demographic, biological and health status, surgical, and psychological variables. Identifying patients at high-risk for APSP and modifiable risk factors will allow tailored analgesic, psychosocial and educational initiatives, which may reduce the burden of APSP.

2 CURRENT STATE OF KNOWLEDGE AND RATIONALE

2.1 Breast Cancer Surgery

Most patients with breast cancer undergo lumpectomy or mastectomy, and possibly sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND)[5,6]. Lumpectomy removes the tumour and some healthy tissue[5,6]. Mastectomy removes the entire breast and can be followed by immediate or delayed reconstruction[5,6]. SLNB removes the first lymph nodes from around the tumour and is performed to determine if there is involvement[5,6]. If there is, then ALND, the removal of 10-40 lymph nodes from the axilla, will be carried out to determine the extent of the spread[5,6].

Unfortunately, post-BCS pain has been reported to occur in 15-60%[2,3] of patients, and on average women report moderate pain in the post-anesthesia recovery room[3]. However, APSP varies widely with some patients reporting minimal pain and others experiencing severe pain[7].

2.2 Pain Definition, Mechanisms and Theories

Pain is a multidimensional experience defined by The International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[8]. Nociceptive pain is triggered by tissue damage and is usually throbbing, aching or pressure-like. NeP results from a lesion or disease of the somatosensory nervous system and is typically lancinating, shooting, electric-like or stabbing[9]. Often acute NeP occurs simultaneously with nociceptive pain[10]. Most attention to post-surgical NeP focuses on chronic NeP which occurs in 20-69% of patients[11]. Few studies distinguish between nociceptive and NeP in the acute period despite different etiologies and management strategies. Other terms commonly used to describe pain that do not imply an

underlying mechanism include: allodynia – pain from a stimulus that does not normally cause pain[12], and hyperalgesia – increased pain from a stimulus that normally causes pain[12].

Gate Control Theory

Melzack and Wall's (1965) Gate Control Theory (GCT), proposes that skin stimulation induces nerve impulses that are transduced by peripheral nociceptors to the dorsal horn[13]. At the dorsal horn, the impulse is integrated with signals from other afferent neurons, interneurons and descending modulatory signals[14]. This modulation affects the membrane potential of afferent fiber terminals and determines the excitatory effect of incoming impulses[13]. The balance between nociceptive and facilitatory signals with non-nociceptive and inhibitory signals in the dorsal horn will determine if the gate will be "open" or "closed", dictating whether the signal will be propagated to the brain[13,15]. Negative emotions, such as helplessness and anger, "open" the gate, while positive behaviours such as stress reduction "close" the gate[16]. Importantly, this theory proposes one possible mechanism for the influence of higher cortical functions on the subjective perception of pain.

The GCT continues to be the most widely accepted model of pain[17,18]. In 1999, Melzack updated the GCT, proposing the neuromatrix theory of pain, which defines pain as a multidimensional experience generated by a complex neural network in the brain rather than directly by sensory input[19]. In the following sections, I will first discuss the transmission of a nociceptive signals from the periphery to the central nervous system. Then I will explore the neuromatrix theory of pain and the complex interactions that occur within the central nervous system. Following that, I will explore how injury, such as surgery, may influence neurobiological changes in both the peripheral and central nervous systems and how cytokines influence these

processes. I will finish by exploring the evidence for various risk factors in the development of post-operative pain.

Nociception

The process of nociception begins when a noxious stimulus or tissue damage, such as that occurring during surgery, activates peripheral nociceptors[20]. Nociceptors are sensory afferent fibres that respond to external and internal stimuli but are normally silent, only transmitting signals when a given threshold is reached[21]. Afferent fibres, whose cell bodies lie predominantly in the dorsal root ganglion (DRG), enter into grey matter of the spinal cord as dorsal roots and terminate primarily in the dorsal horn where the incoming signals are modified by many interacting neurons[14]. The signal is then transmitted to projection neurons that ascend to the brain[22]. Aδ and C afferent fibres, which carry predominantly nociceptive signals, terminate mainly in the tip of the dorsal horn, which corresponds to the substantia gelatinosa or Rexed's laminae I-II[23]. These fibres principally release the excitatory neurotransmitter glutamate[14]. Low-threshold subsets of Aδ and C afferent fibres, that respond to non-noxious stimuli also exist[24]. Low-threshold Aβ fibres and low threshold Aδ and C fibres, responsible for transmitting tactile information, terminate primarily in laminae II-IV[25].

Some projection neurons ascend to nuclei in the thalamus while others ascend to other brain regions including the parabrachial nucleus, the midbrain periaqueductal grey (PAG), the reticular formation, the hypothalamus, the nucleus of the solitary tract, and the ventrolateral medulla[14,24]. The projection neurons that reach these regions in the brain then synapse with other neurons that extend to higher cortical areas of the brain, including the somatosensory cortices, insula, anterior cingulate cortex (ACC) and prefrontal cortex[22,26–28].

Dorsal Horn Integration

Afferent fibres interact with interneurons and directly with ascending projection neurons in the dorsal horn[14]. Interneurons may be excitatory or inhibitory, and these neurons receive input from primary afferent fibres, other interneurons, as well as from descending pathways[29–31]. Some inhibitory interneurons receive input from Aβ fibres and synapse with afferents innervating excitatory interneurons, causing presynaptic inhibition[32,33]. Other inhibitory interneurons receive input from nociceptive afferents and synapse with either excitatory or inhibitory interneurons[33,34]. Different types of excitatory interneurons also receive inputs from Aδ and C afferent fibres[35], while others respond to Aβ fibres[33,34]. Inhibitory interneurons mediate their effect primarily via gamma-aminobutyric acid (GABA) and glycine, while excitatory interneurons release glutamate[29,33–35].

Consistent with the conceptual GCT, the total input to projection neurons directly from primary afferent fibres and from a variety of interneurons, determines whether a signal will be transmitted along the ascending pathways[24]. Projection neurons found in lamina I contain most of the nociceptive-responsive ascending fibres[34]. Projection neurons in deeper laminae, particularly lamina V, have wide dynamic range neurons that respond to both nociceptive and innocuous stimuli[24]. The integration of signals in the dorsal horn is complex and various cell types and interactions continue to be elucidated however, current evidence continues to support the GCT.

Descending pathways

As suggested by Melzack & Wall in the GCT, descending pathways from the brain to the dorsal horn allow psychological factors to contribute to processing of nociceptive stimuli[36]. These descending inputs influence which signals are transmitted, ensuring information relevant to a given situation is received, while other less relevant signals are silenced[23]. Descending pathways, which contain serotonergic neurons, noradrenergic neurons, and dopaminergic neurons, as well as neurons that release GABA and endogenous opioids demonstrate both inhibitory and facilitatory effects directly on dorsal horn neurons and via interneurons in the dorsal horn[37]. This contributes to the "gating" function in the spinal cord and affects whether a nociceptive signal from Aδ and C fibres will be transmitted to nociceptive responsive ascending neurons[38].

Descending modulation may be influenced by context (pain beliefs, expectation, past experiences), cognition (attention, catastrophizing), mood (depression, anxiety), genetics and neurochemical changes[39]. The prefrontal cortex[40], ACC[41], amygdala[42,43], hypothalamus[44], PAG[43,45] and rostral ventromedial medulla (RVM)[43,46] have all been implicated in the descending modulatory systems. Importantly, many of these pathways are involved in or receive inputs from areas of the brain associated with emotion, fear, anxiety, and other higher order functions[37].

Some descending pathways have been well characterized while others are still being clarified. One of the most clearly described involves fibres descending from the RVM in the midbrain to the dorsal horn[46]. The RVM receives input from the PAG, the nucleus cuneiformis, prefrontal cortex, the amygdala and the ACC[28,47]. The RVM can have both an inhibitory and facilitatory effect, mediated through different cell types which is beyond the scope of this thesis[48,49] (see Heinricher et al. (2009)[46] for more details). The effect in the dorsal horn depends

on the balance of inputs from the inhibitory and facilitatory cells[28]. The various higher cortical areas with connections to the RVM descending pathway is one of many top-down systems implicated in the complex modification of nociceptive signals (see Millan (2002)[38] for an in-depth review).

Neuromatrix Model of Pain

Melzack later developed the neuromatrix model of pain which builds on the GCT[19,36]. The neuromatrix involves networks of neurons, with loops connecting the thalamus and cortex and cortex and limbic system[19]. This network is initially genetically determined and modified by sensory inputs[19]. These loops diverge allowing processing in different regions of the brain and converge leading to integration of different processing outputs[19]. Melzack proposed that inputs to the neuromatrix include: cognitive related brain areas (past experiences, attention, memory, anxiety), sensory signaling systems (cutaneous, visceral, musculoskeletal) and emotion related brain areas (limbic system and associated homeostatic and stress responses)[19,50]. These different dimensions of processing lead to outputs, referred to as the neurosignature, that travel to various brain areas to produce pain perception (sensory, affective, and cognitive dimensions), action responses (involuntary and voluntary movement) and stress regulation (cortisol, noradrenaline, endorphin and immune system responses)[50].

The pain neuromatrix is thought of as having two parts: a lateral component, which includes the somatosensory cortices, thalamus and posterior insula and is primarily responsible for sensory-discriminative aspects of pain; and a medial component, consisting of the anterior insula, ACC and prefrontal cortex, responsible for the affective-evaluative-cognitive aspect of pain[26,27,39,51]. However, this proposed 'matrix' is dynamic and different components may be

activated in different situations, which could contribute to some of the disparities reported in functional magnetic resonance imaging (fMRI) studies examining components of the pain matrix[39].

The primary regions involved in pain processing include somatosensory cortices[52–59], insula[52–54,56–61], ACC[41,56–58,61], prefrontal cortex[57,58,61,62] and thalamus[52,53,56–58,60,61,63] (see Apkarian et al. (2005)[64] meta-analysis). Other areas including the basal ganglia[65], cerebellum[58], amygdala and hippocampus[43,56,66] may also be activated depending on the individual and context[39]. Activation of motor areas was also observed [56–58,61,63] supporting that the output from the neuromatrix contributes to motor responses, as proposed by Melzack[19].

Importantly, many of the regions involved in the pain neuromatrix are also involved in emotional processing and cognitive functions and activity in various areas involved in the neuromatrix are differentially activated in different emotional states[67]. The outcome of these interacting factors that vary for each individual in different situations is proposed to, in part, explain varied pain experiences[16], such as seen after BCS[68]. Functional imaging studies have supported that varied activation and connectivity between different brain regions is related to different pain experiences[69].

2.3 Changes to Pain Processing in Injury

Tissue damage, such as surgical incisions, can lead to neurobiological changes at multiple levels of the pain processing pathway that result in increased pain sensitivity, which can be adaptive initially but may become problematic[70].

Peripheral sensitization

Peripheral sensitization is described as a reduced activation threshold in peripheral nociceptors and increased frequency of action potentials in response to stimulation[71]. Tissue damage results in the release of endogenous molecules, known as damage associated molecular patterns that activate innate immune cells[72]. Molecules released from damaged cells can also directly activate nociceptors[73]. Activation of nociceptors and local non-neural cells results in the release of endogenous mediators including neurotransmitters, substance P, bradykinin, prostaglandin, leukotrienes, neurotropic factors, cytokines etc.[74–76]. Importantly, nociceptors express receptors for many of these molecules leading to depolarization or activation of protein kinases that phosphorylate transducer proteins and ion channels, resulting in sensitization[20]. The presence of these inflammatory mediators also upregulates various sodium channels in DRG neurons[77–79]. In addition, N-methyl-D-aspartate (NMDA) receptors, important in excitatory neurotransmission, are upregulated and phosphorylated in peripheral nociceptors during inflammation, increasing excitability[80,81]. Ultimately, the outcome is decreased firing threshold and an amplified response to suprathreshold stimuli.

The substances released into the local area also: increase vascular permeability, allowing the escape of prostaglandins, bradykinin, growth factors and cytokines into the local area[71,82,83]; activate local immune cells that release more pro-inflammatory cytokines, chemokines, components of the complement cascade and vasodilators[70,75,76]; and they recruit circulating immune cells leading to an increase in immune cells at the site of injury[84–86]. These actions further increase the accumulation of inflammatory mediators at the site of damage.

In addition to the above mechanisms, peripheral nerve damage also induces additional changes leading to peripheral sensitization. Nerve damage induces changes in gene expression in damaged neurons which leads to changes in excitability, transduction and transmission properties[71,87]. For example, peripheral nerve injury leads to the upregulation of calcium channel subunits in DRG neurons which is associated with allodynia[88]. In addition, increased expression of neurotransmitters and receptors normally expressed in nociceptors are upregulated in other fibres resulting in a phenotypic switch with fibres that respond to light touch being recruited into the nociceptive circuit[71,87,89,90]. Nerve damage also leads to the recruitment of immune cells to the injured nerve and the DRG[91] as well as activation of glia in the dorsal horn[92,93].

All of these changes contribute to increased spontaneous nociceptor activity, decreased activation thresholds, amplified response to suprathreshold stimuli and recruitment of silent nociceptors[94]. The end result is increased input to the spinal cord[95].

Central sensitization

Peripheral tissue injury also induces changes in the central nervous system that contribute to increased sensitivity[96]. Central sensitization is associated with spontaneous activity, decreased activation threshold, increased responsiveness and increased receptive field size of dorsal horn neurons[97]. C fibres release glutamate, substance P, neurokinin-A and calcitonin gene related peptide into the dorsal horn[98]. The repetitive stimulation of dorsal horn neurons by C fibres and the substances they release trigger a range of changes in dorsal horn neurons. Firstly, phosphorylation and removal of magnesium block from NMDA glutamate receptors in the spinal cord increases their susceptibility to activation by glutamate[80,99]. The increased calcium entering the neurons strengthens the synapse between the nociceptor and the 2nd order neuron, leading

to hyperalgesia[100]. Secondly, activation of receptors for glutamate and substance P further increases intracellular calcium in dorsal horn neurons and leads to activation of voltage-gated calcium channels[20,74]. The increased intracellular calcium as well as binding of inflammatory and neurogenic mediators to neurons in the dorsal horn activates kinases that phosphorylate membrane channels increasing their excitability[20,101]. The increased responsiveness mediated by one kinase, phosphatidylinositol-3-kinase, is associated with phosphorylation of NMDA receptor subunits as well as translocation of an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subunit to the membrane in dorsal horn neurons[97,102]. Decreased GABA and glycinergic inhibitory regulation in the dorsal horn also contributes to central sensitization[101,103–105]. GABAergic and glycinergic descending projections represent a subpopulation of descending modulatory projections from the brain[15]. Increased Interferon-γ (IFN-γ) and Tumour Necrosis Factor -α (TNF-α) have both been shown to be involved in the reduction of GABA-mediated inhibition in the spinal cord[104,106]. Loss of inhibition by the descending pathways leads to increased transmission of excitatory nociceptive signals, including signals from low-threshold A fibers which may contribute to allodynia[107]. In addition, non-neural cells such as astrocytes, microglia, and T cells, are activated which triggers release of prostaglandins, cytokines (including Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-8 (IL-8), TNF-α) and other molecules that sensitize dorsal horn cells[20,100,108–111]. Overall, these changes result in increased synaptic strength, reduced activation thresholds, increased responsiveness to suprathreshold stimuli, expanded receptive fields and spontaneous activity in spinal cord neurons [112,113].

In addition to the above mechanisms of central sensitization, nerve damage also induces specific changes in spinal cord structures. It downregulates glutamate transporters that maintain glutamate levels in the synapse, leading to increased glutamate in synapses and therefore increased activation of glutamate receptors on neurons[114]. In addition, non-neural cells such as macrophages, neutrophils and T cells, migrate into the dorsal horn after nerve injury[70,101,115]. Nerve injury also induces proliferation of microglial cells in the spinal cord[108]. Microglia and astrocytes release cytokines and reactive oxygen species that excite spinal neurons and act in an autocrine and paracrine fashion creating a positive-feedback loop of pain-enhancing signals[116]. Sprouting of sympathetic neurons into the DRG and of Aβ neurons into lamina II of the dorsal horn also contribute to increased activity of dorsal horn neurons after peripheral nerve injury[117,118].

Injury also induces changes in a number of supraspinal structures involved in pain processing[119]. In mice, peripheral inflammation upregulated the NR2B subunit of the NMDA receptor in ACC neurons and injection of an NR2B inhibitor into the ACC or systemically, inhibited behavioural allodynia, supporting the relevance of these changes to inflammation induced hypersensitivity[120]. Tajerian et al. (2013)[121] reported global changes in DNA methylation in the prefrontal cortex and the amygdala after nerve injury which correlated with mechanical and thermal sensitivity. In addition, dendrites in the prefrontal cortex of rats subjected to spared nerve injury were longer and had more branches[122]. These changes were also accompanied by increased NMDA currents that were inversely correlated with paw tactile thresholds[122]. See Jaggi et al. (2011)[123] or Boadas-vaello et al. (2017)[124] for an in-depth review on the supraspinal changes induced after peripheral nerve injury and in pathological pain states.

2.4 Cytokines and Pain

Cytokines are important mediators in inflammation and the many sensitizing changes that occur after injury such as surgery[125,126]. Immune cells in the area of damage, including macrophages or mast cells, as well as those recruited to the site of injury secrete mediators including cytokines[127–129]. Activated or damaged nerves and activated glia release neuropeptides, neurotransmitters and cytokines, including IL-6, TNF-α, substance P and prostaglandins, into the central nervous system[93,127,130,131]. These mediators, released from immune cells and neurons, act directly on nerve terminals and immune cells to modulate the inflammatory response[127,128] and neuronal sensitivity[21,130].

Some effects that alter the inflammatory response include: activating other immune cells like macrophages[132], neutrophils[75,76] and mast cells[133], to release more mediators; recruiting and activating leukocytes via expression of adhesion molecules[84–86]; increasing vascular permeability allowing increased extravasation of other immune cells into the damaged area[82,83,134]; and inducing changes to chemotaxic signaling[84,135,136].

Direct effects on neurons by cytokines, in both the peripheral and central nervous system, lead to increased excitability of nociceptive pathways[21,105,137,138]. Cytokines trigger sympathetic sprouting in the DRG[117]; modify or change expression of ligand gated channels or voltage gated sodium channels in dorsal root ganglion neurons[77–79,138,139]; and alter inhibitory interneurons resulting in disinhibition of nociceptive pathways[105,106]. These changes lead to increased action potential generation and increased excitability of nociceptive pathways.

Pro-inflammatory cytokines also contribute to supraspinal changes that impact top-down control and processing of noxious stimuli after injury. Various cytokines, including IL-1β, IL-6 and TNF-α are increased in regions of the brain associated with pain processing, like the PAG and ACC, and contribute to changes in sensitivity[140–142].

The initial sensitivity mediated by the pro-inflammatory response may contribute to behavior aiming to protect the injured area to allow healing[100]. This is usually accompanied by an anti-inflammatory response to attenuate the disturbances and damage caused by excessive inflammation[126]. Alterations in the balance between pro-inflammatory and anti-inflammatory signaling is believed to be involved in the generation of increased sensitivity that outlives its usefulness, and which may manifest as chronic pain[126].

Given the importance of the balance between the pro-inflammatory and anti-inflammatory responses in sensitization, higher baseline levels of pro-inflammatory cytokines or lower baseline levels of anti-inflammatory cytokines may predispose patients to sensitization before surgery or to exaggerated responses after surgery, leading to more post-operative pain. This project explored eight of these mediators (IFN-γ, Interleukin-2 (IL-2), IL-6, IL-8, IL-10, TNF-α, Transforming growth factor-β (TGF-β)) and sought to determine if baseline levels, in combination with biomedical and psychological factors predict increased pain after BCS.

Many different signaling pathways activated by various cytokines have been identified and continue to be elucidated. While cytokines are important for many homeostatic processes, here I will present a select review of some of the downstream effects of each of the cytokines investigated in this project. First, I will discuss the cells that produce each cytokine and where the receptors are located. Then, I will discuss some of the effects of these cells in the body, with a

focus on changes that lead to inflammation or sensitization. Finally, for each cytokine I will describe animal and human data supporting the role of that cytokine in nociception, and where available, the role of that cytokine specifically in post-operative pain. See Table 1 for a table summarizing studies on cytokines and pain in humans.

Table 1. Literature Review for Inflammatory Cytokines and Pain

	Population	Sample	IFN-γ	IL-2	IL-6	IL-8	IL-10	IL-17	TGF-β	TNF-α	Notes
Acute											
Cueller et al. (2010)[143]	Acute, unilateral knee pain undergoing arthroscopic ACL reconstruction (n=12, painless knee=15)	Synovial fluid	↑	n.s	↑	n.s	n.s	n.s	____	n.s	
Cueller et al. (2009)[144]	Acute, symptomatic meniscal tear (n=39, asymptomatic=31)	Synovial fluid	↑	↑	↑	____	↑	____	____	____	IFN-γ and IL-6 positively correlated to reported pain. IL-8, IL-17 and TNF-α listed as tested but no results
Arthritic											
Hussein et al. (2008)[145]	Rheumatoid arthritis (n=24, HC =6)	Serum	____	____	____	____	↑	↑	____	↑	
Liu et al. (2012)[146]	Rheumatoid arthritis (n=18, HC= 18)	Serum	____	____	↑	____	____	↑	____	↑	IL-17 correlated with anxiety
Inflammatory											
Malhotra et al. (2012)[147]	Fibromyalgia (n=26, HC = 26)	Plasma	↓	↓	↑	____	↓	____	____	____	IL-2, IL-10 and IFN-γ negatively correlated with VAS score. IL-6 positively correlated with VAS score.
Mendieta et al. (2016)[148]	Fibromyalgia (n=15, HC=14)	Serum	UD	UD	↑	↑	UD	____	UD	____	IL-6 and IL-8 correlated with fibromyalgia impact questionnaire score
Wang et al. (2008)[149]	Fibromyalgia (n=20, HC= 80)	Serum	____	____	n.s	↑	____	n.s	____	↑	IL-8 correlated with VAS at the end of treatment
Üçeyler et al. (2006)[150]	Chronic widespread pain (n=40, HC= 40)	Serum	____	____	____	____	↓	____	n.s	n.s	This population included 26 patients with fibromyalgia
		mRNA	____	n.s	____	n.s	↓	____	n.s	n.s	
Lundh et al. (2013)[151]	Chronic prostatitis-chronic pelvic pain syndrome (n=32, HC=37)	Plasma	____	n.s	____	____	____	____	____	↑	TNF-α only significant when controls with health problems removed

Inflammatory Cont'd		**Sample**	**IFN-γ**	**IL-2**	**IL-6**	**IL-8**	**IL-10**	**IL-17**	**TGF-β**	**TNF-α**	**Notes**
Miller et al. (2002)[152]	Chronic prostatitis-chronic pelvic pain syndrome (n=48, HC=14)	Seminal plasma	↑	n.s	____	n.s	↑	____	____	____	IL-10 correlated with positively with pain intensity
Slade et al. (2011)[153]	Temporomandibular disorders w/ widespread palpation tenderness (WPT) vs without (n=84, no WPT= 115)	Plasma	UD	UD	n.s	↑	UD	UD	____	n.s	
Neuropathic											
Alexander et al. (2012)[154]	Complex regional pain syndrome (n=148, HC=60)	Plasma	↑	↑	n.s	↑	n.s	____	____	↑	
Üçeyler et al. (2007)a[155]	Complex regional pain syndrome (n=42, HC=34)	Serum	____	↑	____	n.s	↓	____	↓	n.s	
Backonja et al. (2008)[156]	Chronic pain from post-traumatic neuralgia or distal painful non-diabetic polyneuropathy (n=14, HC=6)	Plasma	____	____	n.s	n.s	↓	____	____	n.s	IL-10 inversely correlated with pain intensity
Bäckryd et al. (2016)[157]	Chronic peripheral neuropathic pain (n=14, HC= 17)	Plasma	____	____	↑	n.s	____	____	____	UD	Mostly failed back surgery associated radiculopathy
Pedersen et al. (2015)[158]	Lumbar radicular pain secondary to disc herniation, severe VAS≥3 vs mild pain VAS<3 (severe n=52, mild n =75)	Serum	____	____	↑	↑	____	____	____	____	IL-6 and IL-8 associated with pain intensity score on VAS

		Sample	IFN-γ	IL-2	IL-6	IL-8	IL-10	IL-17	TGF-β	TNF-α	Notes
Wang et al. (2016)[159]	Severe (VAS >3) sciatica pain secondary to lumbar disc herniation vs mild VAS (≤ 3) (n=58, mild = 50)	Serum	____	____	↑	n.s	↓	____	____	↑	
	Severe (VAS>3) sciatica pain secondary to lumbar disc herniation vs HC (n=58, HC=30)	Serum	____	____	↑	↑	n.s	____	____	↑	IL-10 was increased in mild sciatica compared to HC
Üçeyler et al. (2007)b[160]	Painful vs. painless neuropathy (painful n=32, painless =20)	Serum	____	↑	____	____	n.s	____	____	↑	
Üçeyler et al. (2010)[161]	Small fibre neuropathy (n=24, HC=34)	mRNA in blood	____	↑	n.s	n.s	↑	____	↑	n.s	
Mixed											
Koch et al. (2007)[162]	Chronic neuropathic, nociceptive or mixed pain (n=94, HC=6)	Plasma	n.s	n.s	↑	n.s	n.s	____	____	n.s	IL-2 and TNF-α elevated in patients with severe (NRS=7-10) vs. light pain (NRS=1-3) but only severe pain above level of sensitivity
Post-operative											
Ko et al. (2018)[163]	Hip fracture surgery in pts >60 yrs of age (n=40) Correlation with POD 3 resting pain and walking pain	Plasma	____	____	n.s	____	____	____	____	↑	
Si et al. (2017)[164]	Patients undergoing total knee arthroplasty (n=96) Correlation with NRS-R and NRS-M pre and 24-hrs post-op	Serum	____	____	↑	n.s	____	____	____	n.s	IL-6 also significantly associated with NRS-M 48 and 72 hrs post-op.
Other											
Dennis et al. (2014)[165]	Opioid addicted patients with comorbid pain vs. without (with pain n=58, no pain=177)	Serum	↑	___	n.s	n.s	n.s	___	____	n.s	IFN-γ only significant after adjusting for covariates

ACL: anterior cruciate ligament. HC: healthy controls. IFN-γ: Interferon-γ. IL- 2: Interleukin-2. IL-6: Interleukin-6. IL-8: Interleukin-8. IL-10: Interleukin-10. IL-17: Interleukin-17. mRNA: Messenger Ribonucleic Acid. n.s: not significant. NRS-R: Numeric Rating Scale at rest. NRS-M: numeric rating scale with movement. TGF-β: Transforming Growth Factor-β. TNF-α: Tumour Necrosis Factor-α. UD: undetectable. VAS: visual analog scale. WPT: widespread palpation tenderness

Pro-nociceptive

Interferon-γ

IFN-γ is produced primarily by T cells[166,167], including both cytotoxic T cells[168,169] and T helper cells[166], and natural killer (NK) cells[167,170–172]. It is also produced by astrocytes[173] and neurons[173,174]. There are two subunits that make up the IFN-γ receptor: IFN-γR1 is expressed on all cells[175]. IFN-γR2 is expressed at very low levels on all cells but can be induced[175] allowing the effect of IFN-γ to be closely regulated. Importantly, DRG neurons express both subunits[174,176], and receptors in the dorsal horn are most dense in the superficial layers, the location of nociceptive pathways[176,177], suggesting a direct effect on nociception is likely.

IFN-γ has diverse effects on different cell types. On macrophages, IFN-γ induces the release of IL-1, IL-6, TNF-α[178], reactive oxygen intermediates and reactive nitrogen intermediates[179], which increase the inflammatory response. On monocytes it induces complement protein production[180] and activates tumoricidal activity[181]. However, it also induces IL-10 production[182] and inhibits IL-8 production from monocytes, effects which may counter the inflammatory response.

In addition, IFN-γ activates NK cells which further increases IFN-γ release[183]. On the other hand, it inhibits proliferation of T_H2 cells and production of IL-4 and IL-5 by T_H2 cells[184]. Additional control of the inflammatory response is generated by IFN-γ mediated inhibition of IL-17 release from T helper cells[185].

Effects on neutrophils are also diverse. IFN-γ increases phagocytosis, increases reactive oxygen species production, increases release of enzymes from granules and increases TNF-α and IL-6, all pro-inflammatory effects[75]. However, evidence for its effect on neutrophil recruitment is

mixed and may suggest complex regulation. IFN-γ inhibits IL-8 production (chemokine for neutrophils)[186] and some reports indicate IFN-γ limits neutrophil recruitment[185], which would limit inflammation. Others, however, have found it is necessary for neutrophil attraction[136,187]. Bonville et al. (2009)[187] suggested it was not sufficient to recruit neutrophils but was required in combination with another cofactor.

Although the effects on neutrophils are unclear, IFN-γ does play a role in the recruitment of other immune cells. It increases cell surface adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), and antigen presentation molecules on keratinocytes[135]. It also induces keratinocytes to release chemokines including monocyte chemotactic protein-1 (MCP-1) (attracts monocytes, dendritic cells and T cells), and RANTES, which attracts T cells[135].

In addition to effects on a range of immune cells, IFN-γ is involved in signaling in the nervous system and is upregulated in the dorsal horn after nerve injury[115]. IFN-γ increases spontaneous activity in dorsal horn neurons and increases after-discharges[188] which may occur in part due to reduced GABA mediated inhibition[104]. It also directly induces neuronal dysfunction by enhancing glutamate neurotoxicity via changes to AMPA receptors that lead to increased calcium influx which upregulates nitric oxide synthase[189]. The hyperresponsiveness may also be related to increases in NMDA-induced currents[190].

Spinal-glial cell interactions also play a role in IFN-γ mediated hypersensitivity. IFN-γ activates microglia and astrocytes[189] and increases proliferation of microglia[108]. Activation by IFN-γ upregulates nitric oxide synthase which increases production of nitric oxide in glial cells[191] and increases cell surface adhesion molecules on microglia, such as ICAM-1 and vascular cell adhesion

molecule (VCAM-1)[192]. Activated microglia also release mediators that increase NMDA-induced currents on neurons, increasing reactivity of neurons in the substantia gelatinosa[190].

These diverse effects mostly serve to increase the inflammatory response and immune reaction at the site of injury and in the nervous system. The molecular mechanisms outlined above support an hypothesis regarding how IFN-γ may increase pain sensitivity after surgery. The pro-nociceptive effect of this cytokine is further supported by animal models. In knockout mice lacking IFN-γR1, mechanical allodynia after nerve injury was significantly reduced suggesting signaling through this pathway was essential for development of the neuropathic pain phenotype[115]. In addition, activation of the IFN-γ receptor on microglia induced tactile allodynia in rats, while ablating the receptor reduced allodynia[108]. Furthermore, intrathecal administration caused mechanical allodynia and hyperalgesia[108,188].

Human studies have also supported the role of this cytokine in pain processes. IFN-γ was elevated in the plasma of patients with complex regional pain syndrome (CRPS)[154] and a reduction in IFN-γ predicted a reduction in NeP in spinal cord injury patients[193]. In addition, it was elevated in patients with comorbid opioid addiction and pain compared to patients with opioid addiction but no pain[165]. It was also elevated in the seminal plasma of patients with chronic prostatitis-chronic pelvic pain syndrome[152]. On the other hand, IFN-γ in the plasma of patients with chronic nociceptive, neuropathic or mixed pain was not different from controls[162]. The heterogenous nature of the patient population in that study may have contributed to this discrepancy[162]. Interestingly, one study of patients with fibromyalgia reported a decrease in patients compared to healthy controls[147], however, a systematic review of cytokines in patients

with fibromyalgia compared to healthy controls found no difference in serum or plasma IFN-γ, although this included only two studies[194].

In the acute setting, in the synovial fluid of patients with acute knee pain undergoing anterior cruciate ligament reconstruction[143] or with symptomatic meniscal tear[144] IFN-γ was elevated relative to controls. There were few studies assessing IFN-γ in surgical settings. One study found non-neurological surgery did not correlate with a change in IFN-γ concentration compared to baseline but this study did not investigate pain[195]. In addition, IFN-γ was not increased in the serum of patients after orthopaedic trauma compared to healthy controls[196]. The lack of a response after surgery or trauma could be related to the time frame at which the effect was measured. Regardless, the involvement elucidated by molecular studies, animal studies and some human chronic and acute pain studies, suggests that baseline levels of IFN-γ could assist in identifying those at increased risk of sensitization and increased pain after BCS.

I was unable to identify any studies investigating IFN-γ concentrations in patients undergoing BCS. One study identified a short nucleotide polymorphism (SNP) in the gene for IFN-γ increased the risk of severe persistent pain after BCS[197] however, how this polymorphism impacts concentrations is unclear making conclusions difficult to draw.

While there is some conflicting evidence, IFN-γ clearly plays a significant role in inflammatory processes and in nociception and the balance of studies suggest it is pro-nociceptive. A better understanding of whether systemic levels of IFN-γ can predict for pain after BCS is warranted.

Interleukin-6

IL-6 is a 22-27kDa glycoprotein that can be produced by nearly all cell types[125] including immune cells such as macrophages[198], monocytes[199], T cells[200], B cells[201], neutrophils[75,202], dendritic cells[203], basophils and mast cells[204]. Cells in the nervous system such as neurons[205], specifically DRG neurons[206] and sympathetic neurons[207], and glial cells[206], also produce IL-6 providing a local source to effect changes in the nervous system. Non-immune cells such as hepatocytes[208], fibroblasts[209], endothelial cells[210], epithelial cells[211], and adipocytes[212] also contribute. Importantly, breast stromal cells secrete IL-6, and this may be altered in malignant breast tumors[213], although results have been mixed as to whether local IL-6 is protective or harmful in malignancy[214]. Serum levels in patients with breast cancer have consistently been shown to be elevated compared to controls, with levels associated with stage of disease[214]. In the current study, this could increase the risk of post-operative pain in patients with more significant or advanced disease prior to surgery.

There are two subunits to the IL-6 receptor complex, IL-6R and gp130[215,216]. The gp130 subunit, the signal transduction portion, is expressed on all cells[215,216], including on DRG neurons and glial cells[206]. The IL-6R component, which has a more limited expression pattern, exists as a membrane bound receptor and in a soluble form[216]. Membrane bound IL-6R is found on hepatocytes, monocytes[217], macrophages[218] and some lymphocytes[219]. It is also expressed on epithelial cells[211] and breast adipocytes[212]. In the nervous system, some have reported expression of IL-6R on neurons[206] while others have not found neural expression[220,221]. Microglia also express IL-6R[218,222] and astrocytes express IL-6R mRNA but no protein[218]. Cells that do not express the membrane bound version require soluble IL-6R in order to respond to IL-6. The

soluble receptor is released by cleavage of the membrane bound form[223] or by alternative splicing[224].

IL-6 binds either membrane bound IL-6R or soluble IL-6R, which then associates with gp130[225], triggering dimerization and the initiation of signaling[226]. Signaling via the soluble IL-6R is thought to be predominantly responsible for the pro-inflammatory[227] and the neuropathological effects of IL-6[228].

As a result of the widespread expression of both IL-6 and its receptors, and the ability of cells to respond to IL-6 in the presence of the soluble form of IL-6R, this cytokine has extensive, pleiotropic effects. Firstly, it contributes to the acute phase response[229] and angiogenesis[230], suggesting it would be important in the immediate post-operative reaction and in healing.

IL-6 also promotes T_H2 and T_H17 differentiation and inhibits T_H1 differentiation and T regulatory (Treg) cell generation[231,232]. Importantly, Treg cells are primarily responsible for dampening the immune response, so inhibition of Treg cell differentiation increases inflammation and the immune response[232]. IL-6 also contributes to T follicular helper cell induction and expansion[201]. In cytotoxic T cells, it increases development of effector functions, including release of TNFα and IFN-γ, two pro-inflammatory cytokines[111].

IL-6 also induces monocyte differentiation to macrophages[233]. Interestingly, it induces macrophage production of IL-1R antagonists (IL-1Ra) and soluble TNFα receptors, which dampen the inflammatory response[234], likely a mechanism to prevent damage from too great an inflammatory response.

IL-6 increases neutrophil proliferation[235] but inhibits neutrophil recruitment[236]. IL-6 is involved in the recruitment of leukocytes[237] and researchers have suggested it is involved in the switch from the initial neutrophil immune response to a more robust leukocyte response[238]. It recruits leukocytes by increasing ICAM-1 on endothelial vessels and increasing the expression of the chemokines MCP-1 and IL-8 from peripheral mononuclear cells[84]. It also increases MCP-1 from endothelial cells[84]. Some have shown IL-6 reduces expression of IL-8 from endothelial cells, but this is debated[84,239]. IL-6 also increases chemokine receptor expression on T cells[240] further assisting with recruitment of immune cells that increase the inflammatory response at the site of injury. Increased concentration of IL-6 systemically may therefore increase the inflammatory response during and after surgery.

In the nervous system, IL-6 activates microglia increasing inducible nitric oxide synthase mRNA[109] and expression of TNF-α in microglial cells[93], increasing inflammatory mediators locally within the nervous system. IL-6 also directly affects neurons in a number of ways. It increases intracellular calcium in DRG neurons acutely and increases neurokinin-1 receptors in DRG neurons after extended exposure[137]. It also alters voltage gated and receptor operated ion channels in various neural subsets, with some changes resulting in hyperexcitability and others having neuroprotective effects (see Vezzani & Viviani (2015)[138] for a review). IL-6 generates hyperexcitability in dural neurons by increasing a voltage gated sodium channel (Nav 1.7)[241]. IL-6 also increases transient receptor potential vanilloid- 1 receptor (TRPV1, responsible for heat sensitivity) in DRG neurons, which also increases excitability[79]. gp130, the signal transducer for IL-6 is required for expression of the transient receptor potential ankyrin-1 channel (TRPA1, important for chemical and mechanical sensitivity), so IL-6 signalling may also be involved in the

upregulation of this channel[242]. These changes may explain the heat sensitization in spinal neurons and mechanical sensitization of C-fibres reported with increased IL-6 or IL-6 treatment [221,243]. IL-6 also reduces GABA-induced currents by changes in lamina II of the spinal cord[105] and in the PAG of the brain[140], leading to disinhibition and increased excitability. All of these changes in ion channels result in increased excitability of neurons and therefore increased susceptibility to hyperalgesia. In addition, IL-6 induces sympathetic sprouting, with sympathetic fibres invading into the DRG, an effect that leads to cross-talk between the two systems[117].

On the other hand, IL-6 promotes neuronal survival[207,244,245]. For example, it can reduce the number of sodium channels in spinal cord neurons, reducing excitatory currents and protecting neurons by decreasing release of excitatory transmitters or by reducing energy consumption[244]. This has been proposed as a compensatory neuroprotective mechanism[138]. Although, IL-6 has a positive effect on neuronal survival and differentiation, its involvement in the physiology of nociception, suggests it may be an important mediator contributing to the post-operative pain experience[245].

In animal studies, IL-6 was increased in the DRG[205] and the spinal cord[246] after nerve injury, and this increase was associated with mechanical allodynia[247]. In addition, IL-6 knockout mice had reduced hyperalgesia and reduced plasma extravasation in response to an inflammatory stimulus suggesting a reduced inflammatory response[83]. Exogenous application of IL-6 *via* intraplantar injection in rats also caused hyperalgesia and injection of an IL-6 antibody attenuated this response[248]. Administration of an IL-6 antibody also delayed the nerve-injury-associated mechanical allodynia, supporting the role of IL-6 in the development of nerve-injury-related allodynia[247]. All of these studies suggest IL-6 is an important mediator of the behavioural

outcomes of both inflammatory and nerve injury related stimuli. As such, IL-6 is also likely important in the response to surgery, which includes both an inflammatory response[249,250] and potential nerve damage[6].

Studies in humans have also largely supported the pro-nociceptive effects of this cytokine. Most studies comparing patients with chronic pain to healthy controls have reported increased IL-6, including in patients with chronic peripheral neuropathic pain[157]; chronic nociceptive, neuropathic or mixed pain[162]; rheumatoid arthritis[146], and fibromyalgia[147,148]. In addition, a meta-analysis reported higher plasma IL-6 in patients with fibromyalgia compared to controls[194]. IL-6 was also positively correlated with pain intensity in patients with chronic pain receiving long-term intrathecal opioids[251]. Furthermore, it was found to be elevated in patients with more severe lumbar radicular pain secondary to disc herniation compared to those with less pain[158,159] or healthy controls[159].

Conversely, however, IL-6 was not associated with comorbid pain in patients with opioid addiction [165] and no difference was found in patients with chronic post-traumatic neuralgia or distal painful non-diabetic polyneuropathy compared to healthy controls[156]. There was also no difference reported in patients with temporomandibular joint disorder with widespread palpatory tenderness compared to those without widespread palpatory tenderness[153] or between patients with CRPS and healthy controls[154]. These differences could be related to different time points within a disease process, as reflected in the wide range of disease duration. For example, Backonja et al. (2008)[156] examined patients a mean of 10 years (range .8-20.2) after disease onset and Alexander et al. (2012)[154] examined patients a mean of 8.8 years (range .7-36) after disease onset. Both of these studies found no difference in IL-6 in patients compared to

controls. On the other hand, Pedersen et al. (2015)[158] examined patients 6 weeks and 12 months after disease onset, and Wang et al. (2016)[159] examined patients with mild sciatica on average 23 weeks after disease onset and patients with severe sciatica on average 48 weeks after disease onset. Both of these studies reported significant differences between severe pain and mild pain. Another study found increased serum IL-6 levels after disc herniation predicted increased disability at 1 year[252] supporting the importance of IL-6 levels in the acute or early period following disease onset or injury. A study by Bäckryd et al. (2016)[157] examined patients on average 7.8 years after disease onset and found increased IL-6 in patients however, they did not exclude patients with other painful comorbidities which may have affected IL-6 levels. Despite discrepant findings, overall IL-6 seems to be involved in chronic pain, especially in the early period after disease onset, suggesting it could be effecting changes in the nervous system that increase sensitivity to pain.

Consistent with this, elevations in IL-6 have also been reported in acute pain. Plasma IL-6 was positively correlated with pain qualities in a study of older women with acute low back pain[253]. It was also elevated in knee synovial fluid in patients with acute, symptomatic meniscal tears[144] and acute, painful anterior cruciate ligament tears[143] compared to non-injured knees.

Studies on IL-6 in post-operative pain are limited. However, IL-6 is considered a marker of the extent of tissue damage after trauma[254] and surgical procedures[255]. It is increased after abdominal surgery[256]. Interestingly, this increase was delayed and exaggerated in older patients compared to young adult patients[256]. In a study on total knee arthroplasty, serum IL-6 was positively correlated with resting and movement-related pain intensity pre-operatively and 24 hours after surgery[164]. Serum IL-6 in this study continued to be correlated with movement related

pain intensity three days post-operatively[164]. IL-6 was also locally elevated after surgical extraction of impacted third molars and levels correlated with pain intensity[257]. It was not however, associated with pain after acute hip fracture surgery in patients >60 years old[163]. This study had a small sample size (n=40) and only measured IL-6 three days after surgery which may not have captured the early effects of IL-6 on post-traumatic sensitization. Although IL-6 continued to be associated with walking pain three days after total knee arthroplasty, these patients had severe arthritis, a progressive chronic condition[258], while patients undergoing hip surgery had an acute condition[163], therefore the subsequent inflammatory responses may have differed. Only one study was available on BCS patients. This study identified an SNP in the gene for IL-6 that decreased the risk of mild persistent pain after BCS[259]. This gene is associated with lower serum levels of IL-6 protein[259], suggesting lower levels of IL-6 could be protective in the post-operative period. The potential protective effect of lower serum levels of IL-6 suggests exploring how baseline levels of IL-6 could affect sensitization processes before surgery or the degree of inflammatory response to surgery is warranted.

In addition to the above studies on both chronic and acute IL-6 in human pain, an examination of immunotherapy for painful inflammatory conditions further supports the involvement of IL-6 in pain processes. A review of therapeutics targeting cytokines found intrathecal administration of IL-6 neutralizing antibody reduced mechanical allodynia and an IL-6 neutralizing antibody is currently in use for patients with rheumatoid arthritis[260]. This anti-human IL-6R antibody, Tocilizumab, reduced the number of tender and swollen joints and improved health-related quality of life in patients with rheumatoid arthritis[261]. Case reports on

this neutralizing antibody have described reduced pain in other conditions including sciatica[260], further supporting the importance of this cytokine in pain conditions.

The balance of the findings on the role of IL-6 at the cellular level, the increases found in both animal and human pain studies, and the effect of anti-IL-6 immunotherapy suggest IL-6 could be an important mediator in increasing risk for post-operative pain after BCS. This study explored whether IL-6, in combination with other risk factors, could identify patients at elevated risk for significant acute post-operative pain

Interleukin 8

IL-8 is a chemotactic cytokine involved in inflammation and nociception[112]. It is produced by a wide range of cell types including monocytes[262], endothelial cells[263], lymphocytes[264], fibroblasts[265], epithelial cells[266], keratinocytes[267], and microglia [222,268]. Receptors for IL-8 include CXCR1 and CXCR2[269] and are expressed on immune cells including neutrophils[270], monocytes[271], T cells[169,272], mast cells[273], basophils[274], NK cells[275] and microglia[222]. Neurons in the substantia gelatinosa also express a receptor for IL-8[276].

IL-8 attracts neutrophils[277,278] and T cells to the site of damage[272]. In neutrophils, activation by IL-8 leads to degranulation, releasing enzymes and more chemotactic molecules[76]. Activation of mast cells by IL-8 results in cell migration[273]. IL-8 also regulates expression of leukocyte adhesion molecules on endothelial cells[86], facilitating the recruitment of leukocytes to the site of injury. These effects increase the presence of other immune cells at the site of injury, leading to increased accumulation of inflammatory factors that could influence pain.

Increased IL-8 in animal models supports the association between IL-8 and inflammation and nociception. IL-8 was increased in the DRG and dorsal horn in an animal model of lumbar disc herniation and intrathecal administration of an IL-8 receptor antibody reduced associated mechanical allodynia, supporting the importance of IL-8 signaling in the development of allodynia[279]. IL-8 was also increased in the spinal cord and ACC in an animal model of inflammatory pain[142]. In the ACC, IL-8 increased synaptic transmission, *via* both pre and post-synaptic mechanisms and blocking IL-8 with an antibody reduced the observed excitability as well as thermal hyperalgesia[142]. These studies of both a nerve injury model and an inflammatory pain model support the direct role IL-8 has on the excitability of neurons and on nociception. This suggests IL-8 could play an important role in pain after surgery, which could include both nerve injury[6] and inflammatory signals[249].

Human studies have demonstrated mixed results on the association between IL-8 and chronic pain. Increased IL-8 in fibromyalgia[148,149] and CRPS[154], compared to healthy controls, supports the pro-nociceptive effect of IL-8. In addition, patients with temporomandibular joint disorders and widespread palpatory tenderness had increased plasma IL-8 compared to those without widespread palpatory tenderness[153]. IL-8 was also increased in patients with more severe (visual analog scale (VAS)$\geq$ 3) lumbar radicular pain secondary to disc herniation compared to those with less pain (VAS<3)[158]. Wang et al. (2016)[159] however, found increased IL-8 in patients with lumbar radicular pain due to disc herniation compared to healthy controls but not compared to patients with less severe pain ($\leq$3). The reasons for the discrepant results in two studies investigating similar conditions is unclear, however, different cut-offs for mild and severe

pain were used and other methodological differences may have contributed. Regardless, IL-8 seemed to be involved in the pathology of this neuropathic condition.

Others have also reported no difference in IL-8 levels in patients with chronic pain conditions compared to healthy controls[152,155–157,162,165]. These differences could be related to a range of methodological dissimilarities between studies, such as inclusion criteria, and small sample sizes[194]. In addition, due to the chemotactic nature of the effects of IL-8, systemic levels may not be as informative as local levels[155,277].

Minimal human research has been completed investigating IL-8 and acute pain. A study on acute pain secondary to anterior cruciate ligament tear found no increase in IL-8 in synovial fluid from painful knees[143]. This study found increases in other chemotactic proteins including MCP-1. It is possible that in this specific condition, other chemotactic molecules are more important than IL-8 whereas, in other conditions IL-8 is a more important chemotactic molecule.

Despite these mixed results in chronic and acute human pain conditions, IL-8 may be involved in immune and inflammatory responses after surgery. IL-8 was increased in response to lower abdominal surgery[256], and the molecular mechanisms outlined above could affect subsequent pain experiences. The influence of IL-8 on post-surgical pain has been investigated in the local environment after impacted third molar extraction, where it was increased and correlated with pain intensity[257]. In another study however, serum IL-8 after total knee arthroplasty was not associated with resting or movement related pain[164]. No studies exploring IL-8 levels and post-BCS pain were identified, however one study found there was no association between 3 different SNPs of the IL-8 gene with severe pain after BCS[197]. This study however, did not investigate cytokine concentrations making it unclear if other mechanisms, aside from

genetic polymorphisms, such as post-translational changes, could influence IL-8 concentrations and post-BCS pain.

Notwithstanding some conflicting evidence, given the role of IL-8 in propagation of the inflammatory response and in sensitization, and the finding that IL-8 is increased after surgery[250,256], this study examined whether baseline levels are a risk factor for increased post-operative pain.

Interleukin-17

IL-17A is the prototypical cytokine in the IL-17 family. Some research specifies IL-17A while other studies report on IL-17, without specifying which member of the family. Although IL-17A was measured in this study, evidence regarding IL-17A and IL-17 will be reported here.

IL-17A is a 17-26 kDa protein with variable levels of glycosylation[280]. It is predominantly produced by T_H17 cells[281–283], however, others have shown that $\gamma\delta$ T cells[284], invariant natural killer T cells[285], lymphoid tissue inducer-like cells[286], neutrophils[283], B cells[287], microglia[192], and astrocytes[81,288] are also able to produce IL-17.

The IL-17RA receptor is made up of two subunits: IL-17RA and IL-RC[289,290]. IL-17R is expressed ubiquitously on all cells that have been tested in various studies including B cells, NK cells, peripheral blood mononuclear cells, endothelial cells, epithelial cells and fibroblasts[280,290–294]. Some have identified IL-17RA on C fibres in the DRG[295] and spinal neurons[81] while others have reported that IL-17 receptors are found on astrocytes and microglia but not on neurons[192]. Regardless, some direct effect on cells of the nervous system is likely.

IL-17 stimulates secretion of inflammatory mediators including IL-6, IL-8, granulocyte colony stimulating factor (G-CSF), prostaglandin E2 (PGE2), and adhesion molecules from synovial fibroblasts[296]; induces transcription of TNF-α and secretion of cytokines including TNF-α, IL-6, and PGE2 from macrophages[110]; induces IL-8 synthesis and release from epithelial and endothelial cells[297]; and increases production of nitric oxide and inducible nitric oxide synthase from chondrocytes[298]. IL-17 also stimulates granulopoiesis[299] and stimulates T cells to proliferate[280]. In addition to the above molecules directly increasing inflammation, the above changes also recruit neutrophils[283,292,300], macrophages and T cells[294,300].

IL-17 also affects CNS cells. It induces microglia to produce neurotropic factors such as nerve growth factor, IL-6 and adhesion molecules[192], leading to the accumulation of immune cells and inflammatory mediators that contribute to sensitization[100]. In addition, IL-17A activates signalling cascades resulting in phosphorylation of protein kinase B and ERK in C fibres, which alters voltage gated ion channels enhancing excitability[295]. Meng et al. (2013)[81] postulated that hyperexcitability of nociceptive neurons is mediated through IL-17 dependent phosphorylation of NR1, part of the NMDA receptor, on neurons.

The molecular changes induced by IL-17 result in an enhanced inflammatory response and sensitization of neurons. Data from animal models suggests that these molecular changes lead to pro-nociceptive changes. IL-17 was increased in injured nerves[300] and in astrocytes in the DRG after an inflammatory stimulus[81], and the elevated IL-17 resulted in increased mechanical allodynia and reduced withdrawal thresholds[81,300]. In addition, IL-17 deficiency decreased hypersensitivity normally observed after nerve injury[300] and local injection of IL-17 antibody reduced hyperalgesia[81]. These findings further support the involvement of IL-17 in both

neuropathic and inflammatory pain. Given that surgery causes tissue damage that induces inflammation[249] and may cause nerve damage[6], IL-17 could contribute to the mechanism behind post-operative pain.

Despite evidence for the role of IL-17 in pain processes, human research on IL-17 has been limited and focused primarily on autoimmune disorders, including those with pain as one of the primary symptoms such as rheumatoid arthritis. IL-17 was elevated in patients with rheumatoid arthritis compared to controls[145,146]; and a meta-analysis further supported this finding[301]. One study in a non-autoimmune population found IL-17 was elevated in patients with intervertebral disc degeneration compared to healthy controls, and IL-17 levels correlated with reported pain[302]. It was however, not elevated in fibromyalgia patients compared to healthy controls[149]. Given the limited number of studies in chronic pain conditions, it is difficult to draw conclusions about the effect of IL-17 on pain processes outside of autoimmune conditions like rheumatoid arthritis.

In addition, the only study on acute pain that was identified demonstrated no significant difference in IL-17 in the synovial fluid drawn from the symptomatic knee of people with acute knee pain secondary to anterior cruciate ligament tear compared to the asymptomatic knee or healthy controls[143]. No studies examining the effect of IL-17 on post-operative pain or post-BCS pain were identified. Despite limited evidence in human pain populations, the role of this cytokine on propagating the immune response and its direct effect on nociceptors, suggest an increased basal level of IL-17 could predispose patients to greater inflammatory responses and increased pain after surgery. A better understanding of the role of this cytokine in pain is needed and this study investigated whether baseline IL-17 levels contribute to post-BCS pain.

Tumour Necrosis Factor - α

TNF-α is another pro-inflammatory cytokine that has been implicated in pain processes and is an early mediator in the inflammatory response after surgical procedures, trauma or infection[125,303]. TNF-α exists as a 26 kDa transmembrane molecule as well as a soluble 17 kDa molecule[304]. It is produced by a wide range of cells including monocytes[305] and macrophages[132,306], dendritic cells[306], T cells[307,308], mast cells[309], endothelial cells, fibroblasts[132], synoviocytes[305,310] and supportive cells in the nervous system including glia[311] and Schwann cells[132]. TNF-α does not appear to be produced by neural cells and production in the DRG seems to be limited to non-neural cells[306].

There are two receptors for TNF-α, TNFR1 and TNFR2. Receptors are found on all nucleated cell types[312], including on immune cells[126]. The distribution of these receptors in the nervous system is debated. Some report expression of both TNFR1 and TNFR2 in DRG neurons[313–316], while others have found TNFR1 only on neural cells, and both TNFR1 and TNFR2 on non-neuronal cells in the DRG[91,306]. TNFR1 on primary sensory neurons may be primarily responsible for effects on nociception[317–319]. TNFR2 is expressed on Treg cells and may have an anti-inflammatory effect to prevent overactivation of inflammatory responses[320]. TNF-α signalling through TNFR1 upregulates TNFR2[310]. An upregulation of both receptor types has been observed after nerve injury[321] and in inflammatory models[91,306].

This cytokine's sensitizing effect is mediated via both direct and indirect mechanisms. Indirect effects lead to increased concentrations of other cytokines and an accumulation of inflammatory mediators. TNF-α induces IL-6 which activates PGE2 synthesis[248]. It also increases release of IL-8[265], nerve growth factor and IL-1β, other factors involved in propagating the

immune response and sensitization[322]. TNF-α increases vascular permeability[82], in part by upregulating vascular cell adhesion molecule-1[134] and E-selectin[236], allowing infiltration of macrophages[91] and neutrophils to the injury site[309]. In addition, TNF-α signalling activates glial cells which contribute to inflammation by releasing other mediators[116,191].

Not only does TNF-α increase immune cells and inflammatory mediators at the site of injury, but direct effects on neurons are also widespread. TNF-α signalling results in changes to a number of ion channels in neurons. Upregulation of Nav1.7[77], Nav1.3[78] and Nav1.8[78,139] in A and C fibre DRG neurons occurs in response to TNF-α. AMPA receptors are also inserted into neuronal membranes[323] and an increase in the number of DRG neurons expressing TRPV1, a channel that mediates thermal hyperalgesia, has also been reported[315]. In addition, TNF-α inhibits tonic firing of inhibitory interneurons in lamina II[106]. These changes result in increased responsiveness of C fibres[82,313,324] and ectopic activity[82,324]. Furthermore, TNF-α triggers the release of intracellular calcium stores and activates stress kinases in sensory neurons[314]. It is also involved in activating sympathetic tone observed in inflammatory states[325].

The effects of TNF-α in the peripheral and central nervous system are widespread but include indirect effects on immune cells that result in upregulation of various inflammatory mediators involved in enhancing neuronal sensitivity as well as direct actions on neural cells. Ultimately, TNF-α initiates changes in the peripheral and central nervous systems that could pre-sensitize individuals to increased pain after surgery. A higher baseline level could also result in an increased response to an insult such as surgery.

The connection between the changes observed in molecular studies with behaviour has been demonstrated in animal models. Animal models have demonstrated that TNF-α is upregulated after nerve injury[132], in both injured neurons and neighbouring intact neurons[326]. Increased TNF-α locally in inflamed skin[327], in non-neural DRG cells[306] and in cerebrospinal fluid[304] after inflammation has also been reported. In addition, increased TNF-α after an inflammatory stimulus was associated with hypernociception in mice and TNFR1 knockout mice displayed reduced hyperalgesia to inflammatory stimuli[327] supporting the importance of this cytokine in the behavioural response to inflammation. Furthermore, administration of exogenous TNF-α led to increased firing in sensory afferent fibres[324], hyperalgesia[322,327–329] and mechanical allodynia[329] in both rat and mice models. The increased TNF-α observed after nerve or inflammatory injury and association with hyperalgesia suggest this is an important mediator of pain responses after injury and thus, could be important in post-operative pain.

While molecular studies and animal models support the involvement of TNF-α in the response to injury, further evidence for the involvement of TNF-α in pain processes comes from studies on chronic pain patients. TNF-α was elevated in patients with a variety of chronic pain conditions including: CRPS[154]; chronic prostatitis-chronic pelvic syndrome patients[151]; and in patients with rheumatoid arthritis[145,146]. In addition, TNF-α inhibitors reduced pain in rheumatoid arthritis, supporting the role of this mediator in pain processes[330]. Furthermore, patients with painful neuropathies had elevated TNF-α compared to those with painless neuropathies or healthy controls[160]. Patients with diabetic neuropathy also had increased TNF-α[331] and serum TNF-α was associated with pain intensity[332]. Moreover, TNF-α was increased in patients with severe sciatica pain (VAS>3) compared to mild sciatica pain (VAS≤3) and healthy controls[159].

Others however have not found an elevation in patients with chronic pain[150,153,155,156,165]. Reasons for these discrepancies are unclear particularly since studies on similar patient populations demonstrate conflicting results (ex. CRPS). Methodological differences, such as the duration of disease may have contributed. For example, Alexander et al. (2012)[154] investigated patients with CRPS on average 8.8 years (range .7-36) after disease onset and found increased TNF-α while Üçeyler et al. (2007a)[155] investigated patients with CRPS on average 12 weeks (range 1-70) after disease onset and found no significant difference in TNF-α compared to controls. Therefore, different stages in disease pathology may have played a role in these discrepant findings. In addition, cross-study variability in the inclusion criteria for healthy controls may also have contributed to differences. For instance, Lundh et al. (2013)[151] found significant differences between patients with chronic prostatitis and controls but only after removing participants with comorbidities. Other studies did not explicitly exclude participants with health problems that could impact immune and inflammatory processes (for example see Slade et al. (2011)[153]) potentially obscuring any effects. Regardless, taken together, the evidence from molecular and animal studies and the large number of studies that have a found an association between TNF-α and chronic pain support the importance of TNF-α as a potential predictor of risk for post-operative pain.

Available research on acute pain and TNF-α in humans is limited. TNF-α in the synovial fluid from acutely painful knees secondary to anterior cruciate ligament tears was not significantly different than those with non-painful anterior cruciate ligament tears[143]. In post-operative pain, TNF-α was positively correlated with resting and walking pain after surgical repair of acute hip fracture in older patients (>60 years)[163]. However, it was not correlated with resting

or walking pain before or after knee arthroplasty[164]. Surgery for hip fracture was related to an acute traumatic event[163] while knee arthroplasty was performed in patients with osteoarthritis, a progressive chronic painful condition[258]. This difference in study populations may explain the conflicting results of these two studies. The only study identified on TNF-α and post-BCS pain found an SNP in the gene for TNF-α was associated with mild persistent pain after BCS[259]. These results require replication and at present, the effect of this polymorphism on protein concentrations is unclear, making conclusions difficult to draw.

Despite discrepancies in the literature, given the available evidence of the role of TNF-α in pain sensitization, in animal models and some findings in human pain populations, an examination of how baseline levels may impact post-operative pain is warranted. Baseline levels of this cytokine could result in heightened baseline sensitization or could increase the response to surgical injury, resulting in greater sensitization changes during and after surgery. As such, this study explored the relevance of this cytokine in predicting pain after BCS surgery.

Anti-nociceptive

Interleukin 10

IL-10 is an 18kDa anti-inflammatory cytokine involved in attenuation of the inflammatory response. It is synthesized mainly by monocytes[333,334], macrophages[334,335], and T helper cells, including T_H1, T_H2 and T_H17 cells [184,336,337]. It is also produced by other immune cells including: Treg cells[338], $\gamma\delta$ T cells[339], cytotoxic T cells[340], neutrophils[341], NK cells, B cells[333], mast cells[342], dendritic cells[343] and by microglia in the nervous system[344].

The receptor for IL-10 consists of 2 subunits: IL-10R1 and IL-10R2, both of which are necessary for signalling[345]. IL-10R1 is expressed predominantly on immune cells including macrophages[346], monocytes, NK cells, B and T cells[182,333], neutrophils[347], dendritic cells, and mast cells[182]. This receptor subunit is also expressed on non-immune cells but typically at lower levels requiring upregulation to be effective[348]. Some non-immune cells that express IL-10R1 include fibroblasts[349] and epidermal cells[350,351]. Expression has also been observed in DRG neurons[352] and on glial cells in the central nervous system[353]. IL-10R2 is expressed on most cells[333] and expression is not usually significantly impacted by induction signals[348].

Given the extensive receptor expression, IL-10 has a wide range of effects on immune cells including influencing immune mediators, antigen presentation and phagocytosis[354]. Here I will focus on the release of immune mediators that mediate inflammation and changes in nociception.

IL-10's effect appears to be strongest on macrophages and monocytes[354]. In macrophages and monocytes it inhibits release of immune mediators including TNF-α, IL-6[355], IL-1β, IL-8, granulocyte macrophage-colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (GCSF)[356]; stimulates release of IL-1Ra[357]; stimulates release of TNF-α soluble receptors (TNF-α antagonist) and downregulates surface TNF-α receptors[358]; inhibits cyclooxygenase-2 (COX-2) reducing production of PGE2[359]; and inhibits the release of reactive oxygen intermediates[360]. It also reduces the recruitment of macrophages[361]. In neutrophils it has similar effects including inhibiting release of TNF-α, IL-1β, IL-8[362]; increasing release of IL-1Ra[363]; and inhibiting COX-2 protein expression[341]. All of these changes dampen the inflammatory

response and reduce the amount and effectiveness of pro-inflammatory and pro-algesic molecules.

IL-10 acts differently on different classes of T cell. It inhibits the release of IFN-γ and IL-2 from T_H1 cells[336] and activates Treg cells to reduce T_H17 mediated inflammation[364]. It also decreases T_H2 differentiation and survival[365]. In addition, IL-10 reduces IL-8 mediated chemotactic responses of T helper cells[366]. These effects further dampen the inflammatory response by decreasing the presence of pro-inflammatory T cell subsets.

IL-10 also directly affects cells in the nervous system. It decreases IL-1β, TNF-α, IL-6 and CCL2 (chemotactic factor for monocytes and basophils) expression by astrocytes[367]. It also increases TGF-β expression by astrocytes, which in turn inhibits microglial activation[367]. IL-10 reduces IL-6 expression by microglia[367] and induces β-endorphin, an endogenous opioid peptide, expression by microglia[368]. Direct effects on neurons include reducing baseline expression of Nav1.6 and Nav1.8 at the mRNA and protein level in DRG neurons[352]. It also abolishes TNF-α induced upregulation of Nav1.3, Nav 1.6 and Nav1.8[352]. These changes reduce the inflammatory mediators present locally in the nervous system and directly reduce the excitability of the neurons in the DRG.

Data from animal models of pain link these molecular changes to behavioural responses, further supporting the anti-nociceptive effects of this cytokine. For instance, after partial nerve ligation and chronic constriction injury, rats exhibited significant mechanical allodynia and IL-10 levels were significantly decreased[369]. Further, blocking IL-10 signaling with intrathecal administration of an IL-10 antibody delayed resolution of inflammatory hyperalgesia[334] and prevented recovery from paclitaxel-induced allodynia[370] supporting the role of endogenous IL-10

signaling in the resolution of inflammatory and nerve-injury-induced sensitization. In addition, intrathecal delivery of IL-10 attenuated allodynia in rat nerve injury models[352,368,371] and inhibited spontaneous DRG discharges triggered by paclitaxel therapy, reducing allodynia[370]. These studies further support the importance of IL-10 in attenuating sensitization, particularly after nerve injury.

Although IL-10 seems to be important in recovery from nerve injury and preventing allodynia associated with nerve injury in animal models, studies on IL-10 in patients with chronic pain have reported conflicting results. In support of findings in animal models, IL-10 was decreased in patients with chronic pain from post-traumatic neuralgia or distal painful non-diabetic polyneuropathy[156], fibromyalgia[147], CRPS[155] and chronic widespread pain[150] compared to healthy controls. Some studies also reported IL-10 was inversely correlated with pain intensity[147,156,251]. In addition, patients with painful neuropathies had decreased IL-10 mRNA compared to patients with painless neuropathies[160]. IL-10 was also decreased in patients with severe sciatica pain secondary to lumbar disc herniation compared to those with mild pain[159].

Overall, the above studies support that IL-10 reduces pain, however, some studies did not find a significant difference in patients with pain compared to controls[154,162,165]. Surprisingly, a few studies found increased IL-10 in patients with certain chronic pain conditions including in patients with chronic prostatitis-chronic pelvic pain syndrome[152], in patients with small fiber neuropathy[161] and in patients with rheumatoid arthritis compared to healthy controls[145]. These increases could reflect protective mechanisms in which increased IL-10 was produced to counter the pro-inflammatory and pro-algesic responses, in order to re-establish homeostasis.

The regulatory role of IL-10 makes interpreting these discrepant findings in chronic pain conditions difficult. Findings on the acute effects of IL-10 were also mixed. IL-10 in the synovial fluid of acute, painful knees secondary to anterior cruciate ligament tear were not significantly different from levels in controls[143]. On the other hand, a study on IL-10 in synovial fluid from acute, painful knees secondary to meniscal tear found significantly higher levels in painful knees compared to non-painful knees[144]. Different timelines in these studies (examination within six weeks of injury[143] vs. within six months of injury[144]) may have influenced these differences. The fact that one study showed increased IL-10 and one showed no difference in the acute pain setting makes understanding if and how IL-10 may affect one's risk for post-operative pain unclear and suggests more research is needed.

The only study identified investigating IL-10 and post-surgical pain found an IL-10 haplotype that was associated with reduced risk for severe persistent post-surgical pain after BCS however, they did not report on the effect of the polymorphism on protein levels[197] making these findings difficult to interpret.

While evidence in animals strongly supports the anti-nociceptive effect of IL-10, human studies report discrepant findings. Gene vector therapies currently being developed to treat neuropathic pain support the anti-nociceptive effect of IL-10. Intrathecal delivery of plasmid IL-10 gene vector has been shown to successfully reduce allodynia in rats, and animal studies are ongoing to develop IL-10 gene therapy treatments to be used for neuropathic pain[371]. See Milligan et al. (2012)[372] for a more complete description of the IL-10 gene therapy options being explored. Importantly, this supports not only the anti-nociceptive effects of IL-10 but also that IL-10 is potentially modifiable.

Human data on IL-10 and pain is mixed, possibly due to the regulatory function of this cytokine. However, the strong evidence from cellular and animal models suggests increased research on IL-10 and pain in humans is needed. Understanding IL-10 as a risk factor for post-operative pain is particularly important as with the development of gene therapies, prophylactic treatment to prevent post-surgical pain may be possible[372]. This study aimed to determine if there is an association between baseline IL-10 and post-operative pain.

Immunoregulatory

In addition to cytokines that can be classified as primarily pro-inflammatory or anti-inflammatory, some cytokines have bimodal effects and immunoregulatory effects.

Interleukin-2

IL-2 is a 15 kDa protein produced primarily by T helper cells, particularly Th1 cells[373–375]. IL-2 is also produced by other T cells, including cytotoxic T cells[376], dendritic cells, NK cells[375,377], and B cells[378].

The IL-2 receptor has three subunits: IL-2Rα, IL-2Rβ and IL-2Rγ. IL-2Rβ and IL-2Rγ form a receptor with low affinity for IL-2. The low affinity receptor is expressed at low levels on memory T helper cells and high levels on memory cytotoxic T cells[375]. A trimeric receptor containing all three subunits forms the high affinity receptor[379]. The differential expression of the high affinity receptor is believed to contribute to the bimodal effects of IL-2 signalling[380]. High expression of the IL-2Rα subunit, which helps form the high affinity receptor, is associated with Treg cells while low expression is associated with T effector cells[380], indicating different sensitivities and providing a potential explanation for the different responses to low and high concentrations of IL-2 (see below). Expression of IL-2Rα on T and B cells and monocytes requires induction by antigen

presentation[380,381]. Functional receptors have also been identified in primary sensory neurons in the DRG[382], in the spinal cord[379], in higher brain structures such as the hippocampus[383], and in microglia[384].

IL-2 mediates a range of effects in the immune system that contribute to inflammatory responses. Firstly, it alters the expression of cytokine receptors on T helper cells, signalling cells to differentiate into subsets[385]. IL-2 plays a particularly important role in the balance between Treg cells and T_H17 cells. It inhibits T_H17 differentiation[386], promotes Treg development[387] and maintenance[376], and promotes T_H2 responses[388]. IL-2 also expands and activates cytotoxic T cells[376] and upregulates the IL-2Rα subunit on cytotoxic T cells[381,389], increasing the response to IL-2. It also induces the expression of chemokine receptors on T cells (CC-CKR1 and CC-CKR2) enabling recruitment[388,390]and induces proliferation of NK cells[171,391].

Different levels of IL-2 have differential effects on T cells which may contribute to the bimodal effects observed in animal and human studies. High doses of IL-2 preferentially activate NK cells and T effector cells while low doses selectively activate Treg cells[387] and T memory cell differentiation[380]. The balance between the activation of T effector cells (pro-inflammatory) and Treg cells (anti-inflammatory) will determine whether the effect will be predominantly proinflammatory or anti-inflammatory.

One important way IL-2 exerts pro-inflammatory effects is by activating a range of immune cells to release pro-inflammatory cytokines. It triggers IFN-γ and TNF-α release from peripheral blood mononuclear cells, including NK cells[171], cytotoxic T cells and T helper cells[166]. It also activates monocytes rendering them cytotoxic[181], and induces release of IL-6[392] and IL-8[393] from monocytes.

On the other hand, some anti-nociceptive effects are mediated by direct interaction with the nervous system. Peripherally, IL-2 triggers membrane hyperpolarization, reduced calcium currents and reduced intracellular calcium concentrations in DRG neurons by activating μ opioid receptors[394]. In the central nervous system, IL-2 exerts its anti-nociceptive effect at least partially by activating opioid receptors and also by increasing leu-enkephalin in the paraventricular nucleus and locus coeruleus, two regions of the brain involved in pain processing[395].

These bimodal effects make teasing apart the impact of IL-2 on nociception challenging. However, the effect of exogenous IL-2 or IL-2 gene therapy has helped to elucidate how this cytokine affects nociception. One study demonstrated different responses to different intrathecal doses of IL-2 with high intrathecal doses resulting in thermal and mechanical hyperalgesia and low doses resulting in thermal anti-nociception[396]. These differential effects at high and low doses could be related to the varied expression of the high affinity receptor on different cell types[380]. More recent research is investigating muteins or antibody complexes that specifically target IL-2R signalling on T effectors instead of T regulatory cells, which would preferentially inhibit pro-inflammatory signalling. However this research is in its infancy[373].

As a result of the immunoregulatory properties of IL-2, and the differential effects of different concentrations and signalling on different cell types, the effect of IL-2 on pain in humans is difficult to predict. Studies in human chronic pain populations have presented conflicting results. IL-2 was elevated in some chronic pain populations, including in patients with CRPS compared to controls[154,155], in patients with small fiber neuropathies compared to controls[161], and in patients with painful neuropathies compared to those with painless neuropathies[160]. A study on chronic neuropathic, nociceptive or mixed pain found no significant difference in the

patient group compared to the controls but did report IL-2 was increased in patients with severe pain compared to "light" pain[162]. All of these studies support a pro-nociceptive effect. However, others found evidence of an anti-nociceptive effect. IL-2 was decreased in fibromyalgia patients compared to controls,[147] and in spinal cord injury patients with neuropathic pain an increase in IL-2 was associated with a decrease in pain[193]. The bimodal effects of this cytokine exerted through differential expression of high affinity receptors may be involved in these discrepant results[380]. Some studies have also reported no difference between patients and controls including in patients with chronic widespread pain[150] and in patients with chronic prostatitis-chronic pelvic pain syndrome compared to controls[151,152]. The pathophysiology of both of these chronic pain syndromes is varied which could make it difficult to find differences between patients and controls in these studies.

In terms of acute injury, results are also varied. In one study of acute, meniscal tear, IL-2 was increased in the synovial fluid of painful knees compared to non-painful knees[144]. However, IL-2 did not differ between synovial fluid from knees with acute, anterior cruciate ligament tear and asymptomatic knees[143]. The reasons for these discrepancies are unclear however, different time frames were used in these two studies (onset within 6 months[144] compared to onset within 6 weeks[143]), thus the inconsistent findings may reflect a timing specific role for IL-2 in nociception and sensitization. The small number of studies on IL-2 and acute pain make drawing conclusions about how IL-2 may influence sensitization in the acute setting challenging.

Few studies have examined IL-2 and surgical responses. One study found IL-2 is increased during and after non-neurological surgery, but they did not investigate the correlation with reported pain[195]. No studies were available that investigated the relationship between IL-2 levels

and intensity of any type of post-operative pain, including after BCS. However, given the bimodal effects of this cytokine on immune and inflammatory responses, further research is warranted to better understand how changes in this cytokine may influence pain. The current study was the first to investigate the relationship of baseline IL-2 to pain after BCS.

Transforming Growth Factor- β

TGF-β is a 13 kDa cytokine that may also have both pro-nociceptive and anti-nociceptive effects depending on the context[130]. Many cells produce TGF-β including Tregs[397,398], T_H17 cells[397], monocytes[399] and macrophages[398], B cells[398,400], platelets[401], endothelial cells[402] and astrocytes in the nervous system[399]. Breast tumour cells can also produce TGF-β making this a particularly relevant cytokine in the context of the current study population[403].

Receptors are found on monocytes[404], B cells[400], T cells[405], NK cells[406], mast cells[133], neutrophils[407] and fibroblasts[408]. They are also found on cells of the nervous system including neurons (both ventral horn motor neurons and DRG sensory neurons) and glial cells[246,409] suggesting direct effects are likely.

One of the roles TGF-β plays in the immune response to infection or injury involves T cells. It inhibits T cell proliferation[405,410] and inhibits cytotoxic T cell maturation[410]. TGF-β also inhibits T cell differentiation into T_H1[410–412] and T_H2 cells[410,413] and prevents cytokine production by T_H1 (IFN-γ)[411] and T_H2 (IL-4)[413] cells. TGF-β also induces Treg differentiation[414], which suppresses the inflammatory response. On the other hand, TGF-β induces T_H17 differentiation[386,397] when IL-6 is present[412]. T_H17 cells are usually pro-inflammatory, however, strong activation of T_H17 cells by TGF-β also stimulates production of IL-10 inhibiting the

inflammatory properties of T_H17 cells[412]. It is unclear at what level of endogenous TGF-β this switch to anti-inflammatory effects on T_H17 occurs.

Effects on monocytes and macrophages also appear to be multimodal. In early stages of inflammation TGF-β appears to have pro-inflammatory effects. It increases monocyte chemotaxis and induces IL-1 expression from monocytes[404]. However, TGF-β also inhibits monocyte release of TNF-α, reactive oxygen intermediates, reactive nitrogen intermediates[179], and IL-1 and IL-2 induced production of IL-6[392]. Other pro-inflammatory effects of TGF-β include increased chemotaxis of mast cells[133], neutrophils[407], fibroblasts[408] and dendritic cells[415]. The accumulation of all these cell types contributes to an increased inflammatory response. TGF-β also induces COX-2 expression resulting in increased PGE2 in muscle cells[416]. Other anti-inflammatory effects include inhibiting IL-2-dependent proliferation and antigen presentation functions of B cells[400], inhibiting cytolytic functions of NK cells[406] and inhibiting IL-6 and IL-8 expression by endothelial cells[417].

In the nervous system, both pro-nociceptive and anti-nociceptive effects have also been described. TGF-β increases capsaicin-induced calcium influx in primary sensory neurons in the DRG[418] and results in a less negative resting membrane potential and reduced activation threshold of DRG neurons[409]. The mechanism behind these changes likely involves modulation of the expression of voltage-gated potassium channels in DRG neurons and downregulation of potassium currents[409].

On the other hand, TGF-β inhibits activation of microglia and astrocytes[246,419,420] and inhibits microglia proliferation[246,420]. It also reduces TNF-α production by astrocytes, microglia and neurons in the dorsal horn[419] and inhibits IL-8[268], IL-1, IL-6 and superoxide anion production

by microglia[420]. In addition, TGF-β increases expression and function of μ- and δ-opioid receptors[421] and increases synaptic release of enkephalins[421,422] in the spinal cord, reducing allodynia[421]. Furthermore, TGF-β upregulates glutamate transporters in the dorsal horn, responsible for clearing glutamate from synaptic clefts reducing excitotoxicity[419]. Together, this results in a reduction of pro-nociceptive mediators and an increase in anti-nociceptive mediators.

While TGF-β seems to be involved in changes relevant to nociception, the ultimate outcome of these pro-nociceptive and anti-nociceptive findings is unclear and may depend on the local environment. Effects may also differ in the peripheral nervous system vs the central nervous system and in healthy vs chronic inflammatory states[409].

Research on animal models has supported mixed effects of TGF-β. Increased endogenous TGF-β signaling in mice resulted in decreased mechanical allodynia[422]. In addition, increased TGF-β secreted from transplanted bone marrow stromal cells reduced mechanical allodynia and/or heat hyperalgesia and decreased neuroinflammation in the spinal cord and DRG[423]. Intrathecal infusion also prevented hypersensitivity from nerve injury and reversed mechanical allodynia and thermal hyperalgesia[246].

The above studies support an anti-nociceptive effect; however, TGF-β may have pro-nociceptive effects in the periphery and anti-nociceptive effects in the central nervous system[424]. In a rat model of chronic pancreatitis, increased TGF-β was observed in the pancreas, and this was associated with peripheral nociceptor sensitization and hyperalgesia[424]. Infusion of TGF-β into the pancreas induced sensitization while intrathecal administration of TGF-β reduced hyperalgesia[424]. Others have refuted this suggestion of different effects in the periphery compared to the central nervous system. Lantero et al. (2012)[425] found that systemic

administration of TGF-β reduced mechanical allodynia in mice, and administration of a systemic neutralizing antibody that does not cross the blood brain barrier increased mechanical allodynia, suggesting anti-nociceptive effects in both the periphery and the central nervous system[425]. Another study proposed the effects of TGF-β in chronic inflammation may differ when compared to healthy states[409]. They found in a rat model of chronic pancreatitis TGF-β antagonism decreased hypersensitivity; however, in healthy rats, antagonism increased hyperalgesia[409].

The above studies in animals indicate the effects of TGF-β on inflammation and nociception are complex and not easily categorized as pro-nociceptive or anti-nociceptive. Human studies have also reported varied conclusions about the effect of TGF-β on pain experiences. TGF-β was increased in migraine patients during headache free periods compared to controls[426] suggesting it could be involved in risk for migraines. It was also increased in patients with small fiber neuropathies compared to controls[161]. However, it was decreased in patients with CRPS[155] and was not significantly different between patients with chronic widespread pain and healthy controls[150]. Although the pathophysiology behind each of these conditions is poorly understood, these discrepancies could reflect differences in the pathologies of the conditions examined. Firstly, in small fiber neuropathy it has been proposed that the diseased fibres have increased sensitivity to pro-inflammatory cytokines so the increased TGF-β observed may have contributed to an anti-inflammatory compensatory process with IL-10[161]. CRPS on the other hand is believed to occur due to exaggerated post-traumatic neurogenic inflammation[155]. Therefore, the decrease observed in CRPS patients may have reflected ongoing inflammation with decreased anti-inflammatory cytokines (including IL-10). In addition, in patients with chronic widespread pain the finding that TGF-β was equivalent in patients and controls[150] may be due to

the heterogenous nature of the patient population. Chronic widespread pain includes multiple subgroups and these different groups may have different underlying etiologies[150].

Given that Zhu et al. (2012)[409] found different effects of TGF-β in healthy compared to chronically ill animals, understanding TGF-β's effects in the acute setting is important. However, most research on acute or post-operative pain has not examined TGF-β concentrations and no studies were identified that correlated TGF-β with reported pain after surgery. One study found it was significantly increased after orthopaedic trauma, although, they did not report pain ratings[196]. A study of BCS found TGF-β decreased after surgery[427]. However, TGF-β was elevated in patients with breast cancer before surgery, possibly due to secretion from the tumor itself and then decreased after the removal of the tumour[403]. Therefore, the observed decrease may not be an effect of tissue damage from surgery but rather could be related to the removal of the TGF-β secreting tumour. The effect of TGF-β on post-operative pain in the breast cancer setting is therefore particularly intriguing. The diverse effects on nociception described in cellular and animal studies, and the discrepant findings reported in humans make predicting how TGF-β levels before surgery may impact sensitization and inflammatory processes challenging. However, this cytokine seems to play a role in inflammation and nociception, and given that breast tumour cells can secrete it, it may be particularly relevant in pain responses after BCS. This study was the first to investigate the role of pre-operative TGF-β in predicting pain outcomes after BCS.

Summary

As this review has shown, a number of cytokines are important in sensitization and pain. While gaps exist, taken together, the balance of the available evidence suggests the pro-inflammatory cytokines IL-6, IL-8, IL-17, IFN-γ, and TNF-α contribute to increasing pain

perception, while the anti-inflammatory cytokine IL-10 is involved in decreasing pain perception. The evidence regarding IL-2 and TGF-β is mixed but overall supports the involvement of these cytokines in pain processes. In this study, we predicted the pro-inflammatory cytokines IL-6, IL-8, IL-17, IFN-γ, and TNF-α would be positively correlated with pain outcomes, while the anti-inflammatory cytokine IL-10 would be negatively correlated with pain outcomes. The direction of the relationship between IL-2 and TGF-β and pain outcomes was difficult to predict *a priori* based on the available literature. Nonetheless, we predicted IL-2 would be positively associated with pain outcomes, particularly neuropathic pain, given that several studies on patients with neuropathies demonstrated a positive association. We predicted TGF-β would be negatively associated with pain outcomes as in two out of three studies in humans, the difference between TGF-β in patients and healthy controls paralleled differences found in IL-10, suggesting an anti-inflammatory role.

Given the evidence for the changes induced by each of the cytokines described, a greater pro-inflammatory cytokine profile may facilitate pain susceptibility[428] as was found in PPSP after inguinal hernia repair[429]. Some studies have examined the post-surgical inflammatory response; however, baseline levels need to be further explored. Studies examining pre-operative cytokines have almost never considered psychological factors simultaneously. This was the first study, to our knowledge, to measure baseline levels of IL-2, IL-6, IL-8, IL-10, IL-17, IFN-γ, TGF-β and TNF-α, and other biopsychosocial variables simultaneously to identify risk factors for APSP.

2.5 Risk Factors for Post-Surgical Pain

The biopsychosocial model of pain was developed from the Gate Control Theory and is compatible with the Neuromatrix Model of Pain. It conceptualizes pain as a dynamic, bidirectional interaction between a range of biological, psychological and social factors[16]. Importantly, these contributing factors all influence one another, suggesting various complex interactions rather than unidirectional effects. According to this framework, post-surgical pain is likely to be predicted by a range of biopsychosocial factors.

Surgical factors

Surgical procedure is associated with APSP, partly due to differences in extent of tissue damage. Tissue damage and potential nerve damage lead to inflammation and sensitization which increases pain[98]. Patients undergoing breast reconstruction are at greater risk than those undergoing mastectomy without reconstruction[3] and patients having mastectomy are at greater risk for post-operative pain than those undergoing lumpectomy[430–432]. Breast reconstruction increases the risk of damage to nerves including the lateral pectoral, thoracodorsal and long thoracic nerves[433]. ALND, which is more invasive, increases risk of post-operative pain compared to SLNB[432,434,435]. Importantly, ALND increases risk of damage to the intercostobrachial nerve (ICBN), which innervates the skin of the axilla and the medial, proximal arm, and could lead to NeP[6]. Nonetheless, surgical procedure cannot fully explain the variance in APSP, necessitating consideration of other factors.

Demographic Factors

Age. The relationship between age and APSP is unclear with reports of no relationship[436–438] and decreases[3,7,430,432,434,435,439–443] with age. Mechanisms for any potential relationships are unclear[444] but may include changes in life stage and psychosocial factors[445] as well as changes in the immune system[446]. Younger women may show greater levels of distress compared to older women prior to surgery which could contribute to their post-operative pain[6,434].

Correlations between aging and cytokine levels have been extensively studied; however, conclusions vary due to differences in subject selection and assay method. IL-6 elevations with age are the most consistently reported[446–448] although some have found no significant difference[449]. Increased TNF-α has been reported[446,447] but not by all[449,450]; and increased IL-10 [446,447] with age has also been reported but not by all[448,449]. Baseline IL-8 is increased in older adults[446] in some studies but not all[449]. Greater increases in IL-6 and IL-8 in older than younger surgical patients suggest a more robust immune response with age that could influence pain[256]. While the relationship between age and APSP as well as the mechanism behind any relationship is unclear, the influence of age on APSP was examined in this study.

Biological and Health Status Factors

Elevated body mass index (BMI) has been linked to chronic inflammation[451] and may contribute to post-surgical pain by sensitizing the nervous system pre-operatively. Motaghedi et al. (2014)[452] found obesity was related to the severity of the post-surgical inflammatory response however they did not measure the association with pain. Some have proposed that obesity affects handling of the ICBN, and thereby, post-surgical NeP[453]. Although several studies have found no correlation between BMI and post-BCS pain[3,432], others have found increasing BMI was

associated with greater opioid consumption, even after correcting for weight[435]. Some studies of PPSP after BCS have reported positive associations with BMI[3,454,455] however, others have not[456,457]. In general surgery populations, results have also varied with reports of positive correlations[253,458,459] and no associations[429,437,442,443,452]. One study found BMI predicted APSP quality in younger but not older surgery patients[460]. Importantly, BMI may be positively correlated to movement-related pain but not pain-at-rest one year post-BCS[461] suggesting the outcome measure may contribute to the discrepancies. Other methodological differences, especially in pain measurement, may have also contributed to discrepancies[457,462]. Many APSP studies have not considered BMI, and a systematic review found only 2/48 studies included BMI as a possible predictor[441]. Given the inflammatory status associated with elevated BMI, clarification of how BMI, inflammatory cytokine levels, and psychological factors influence APSP is needed.

Psychological Factors

When nociceptive signals arrive at the brain, various regions are involved in formulating the perception of pain through a complex network of interacting pathways[19]. Crucially, many of these supraspinal structures are also involved in emotional and cognitive functions[67]. Emotional and cognitive factors may influence pain pathways in a number of ways: by contributing to changes in inflammatory cytokines; by activating descending modulation pathways, therefore affecting spinal processing; or by affecting cerebral processing. Various psychological factors have been consistently associated with post-surgical pain outcomes.

Anxiety. 75% of patients awaiting BCS experience anxiety[463], the most consistently reported psychological risk factor for severe APSP after BCS[430,431,435,464]. Anxiety was positively correlated with pain after other surgeries as well[7,437,441–443,465–467]. The association between pain and anxiety is well established[468] and dysregulation of cytokines implicated in pain processes have been observed in patients with anxiety. Liu et al. (2012)[146] found increased IL-17 in patients with rheumatoid arthritis and anxiety compared to those without anxiety. Others have reported increased IL-6 in anxious participants[469]. This group postulated that the increased threat perception associated with anxiety may lead to more frequent and amplified activation of the stress response[469]. Others however, did not find this association[470]. In patients with colorectal cancer, serum IL-6, IL-8 and TNF-α were found to be positively correlated with anxiety, and serum IL-10 was negatively correlated with anxiety[471]. This suggests the association between psychological factors and pain could be partially mediated through changes in inflammatory cytokines.

In addition, anxiety is related to dysfunction in the ACC and the prefrontal cortex, resulting in reduced top-down control of emotional regulation tasks, particularly in the amygdala[472]. Long term potentiation and changes in the ACC and prefrontal cortex in chronic anxiety conditions may result in neuronal activity that impacts normal sensory processing, resulting in increased sensitivity and activity in pain-related brain areas[468]. Activation in the prefrontal cortex, and ACC is observed with anxiety and with pain[473,474]. Increased activity in the entorhinal cortex (part of the hippocampus), an area important for memory consolidation, prior to pain predicted activity in areas of the brain associated with affective (perigenual cingulate) and intensity (mid-insula) processing[66]. Fairhurst et al. (2007)[56] also reported anticipation prior

to a noxious stimulus was correlated with increased activity in the entorhinal cortex in addition to the PAG and VTA and the activity in these regions predicted pain-related activation in the posterior insula. The more anticipatory anxiety the patient reported prior to the stimulus, the greater activation of the PAG and reported pain intensity during the stimulus[56]. Individuals demonstrating increased trait anxiety had greater activity in the anterior insula and the amygdala in response to an emotional processing task[475] and lorazepam, an anxiolytic drug, reduced activity in these brain regions, further supporting their involvement in anxiety[476]. The amygdala, a component of the limbic system associated with the emotional-affective dimension of pain[477], is particularly important for fear and anxiety processes[468], and may be involved in fear associated with pain[71,477]. The activation and dysfunction of multiple brain areas that are implicated in pain processes during anxiety supports the relationship between anxiety and pain. The relationship between anxiety and pain is bidirectional[474], and this study considered anxiety as a pre-operative predictor of post-surgical pain outcomes.

Depression has also been associated with increased APSP after BCS[431,464] and other surgeries[439,442,465–467]. The bidirectional relationship between depression and chronic pain is well established, with individuals who are pain-free and depressed at increased risk of developing chronic pain, and patients with chronic pain at increased risk of developing depression[474].

In 1991, Smith proposed the macrophage theory of depression which suggests excessive secretion of inflammatory cytokines, including TNF-α, is the underlying cause of depression[478]. Since, multiple studies have implicated a number of pro-inflammatory cytokines in depression, particularly IL-6 and TNF-α[479]. Depression has been positively correlated with IL-6[470,479–483] and a greater IL-6 response to experimental stressors has been found in depressed than non-depressed

patients[481,484], suggesting patients with depression may not only have pre-sensitized immune systems but may have greater post-surgical IL-6 increases. Cells from individuals with depressive symptoms have also shown increased production of IL-8 in response to inflammatory stimuli[485], so depression could predispose individuals to a greater inflammatory response after surgery, and therefore increased APSP. Increased TNF-α has also been found in depressed patients[69] but not consistently[480,486]. Another study found the magnitude of depressive symptoms in women after BCS correlated with TNF-α[482]. In colorectal cancer patients, serum IL-6, IL-8 and TNF-α were found to be positively correlated with depression and serum IL-10 was negatively correlated with depression[471]. Therefore, both the increased resting levels of inflammatory cytokines in patients with depression as well as increased inflammatory reactivity to stress[480] could increase the risk of APSP in depressed patients. (For an in depth review of the effects of cytokines on depression see Felger & Lotrich (2013)[480] or Slavich & Irwin (2009)[487]).

Dysfunction in the serotonin[488] and norepinephrine[489] neurotransmitter systems are also implicated in the proposed pathophysiological mechanism of depression[490] and these systems are involved in descending pain pathways[491]. A number of functional imaging studies have found depressed mood to be associated with changes in activity in brain regions associated with processing of nociceptive stimuli, particularly areas implicated in affective processing[492]. Giesecke et al. (2005)[492] compared cortical responses to painful pressure stimuli in the brains of patients with major depressive disorder compared to healthy controls and found no difference in activity in somatosensory cortices (sensory-discriminative dimension of pain) but found increased activity in the anterior insula and in the amygdala in patients with major depression. Increased activity in response to painful stimulation has also been reported in the thalamus, the

prefrontal cortex[57] and the amygdala[493] in patients with depression. Decreased activity in the ACC and PAG, regions involved in descending modulatory pathways, has also been observed in depressed patients exposed to painful stimuli[493]. Further evidence from fMRI studies suggests depressed patients may experience increased levels of affective processing before experiencing pain[57], demonstrated by increased activity in the amygdala, anterior insula and ACC[493]. Depression has also been linked to a reduction in inhibitory descending modulation[491] and malfunctioning in the ACC[477]. Taken together, depression could predispose patients to APSP by altering inflammatory cytokine levels or reactivity, or by supraspinal mechanisms in areas of the brain associated with both depression and pain.

Pain catastrophizing is an amplified negative response to actual or expected painful experiences[494]. It has been positively correlated with pain after BCS[430,434] and other surgeries[438,441,465,495,496]. It is one of the most important factors predicting pain intensity and disability and is associated with poorer pain treatment outcomes[497,498].

The association between inflammatory cytokines and pain catastrophizing has been less extensively studied however, pain catastrophizing was associated with greater pain-related increases in IL-6 in an experimental pain study[499] suggesting alterations in cytokines could play a role in the effect of pain catastrophizing on pain outcomes.

Pain catastrophizing is also believed to mediate increased pain via increased attention to stimulation and increased emotional responses[491]. In response to painful mechanical stimuli, high catastrophizers demonstrated increased activity in the medial frontal cortex and cerebellum (anticipation of pain), the dorsal ACC and prefrontal cortex (attention to pain) and the claustrum (closely linked to the amygdala; emotional processing of pain)[500]. According to an fMRI study,

during mild pain, activity in brain regions, including the ACC and the insula, were positively correlated to PCS scores, suggesting increased emotional processing[501]. During moderate pain, PCS scores were negatively correlated with activity in brain regions associated with top-down control, including the dorsolateral frontal cortex, which may suggest impaired descending control[501].

Pain expectations. Higher pain expectations have been associated with increased post-BCS pain intensity[432]. No studies were identified that examined the association between cytokine levels and pain expectations.

However, in brain imaging studies the anterior insula, ACC, thalamus and PAG are all activated during anticipation of pain[56]. In a study on healthy volunteers, expectations of reduced pain were associated with a reduction in the pain intensity reported after painful heat stimulation as well as a reduction in activity in brain areas associated with pain processing, including the thalamus, primary and secondary somatosensory cortices, insula, ACC, prefrontal cortex and cerebellum[502]. Based on nocebo experiments, negative expectations are mediated by hyperactivity of the hypothalamic-pituitary-adrenal axis, increased CCK, and decreased dopamine and opioid activity in the nucleus accumbens[503]. Activity in the PAG, a region important in descending pathways, is also associated with anticipation of pain and perceived pain intensity[477]. Increased anticipation of pain is associated with increased activity in the anterior insula, and the amount of activity correlates with ratings of pain intensity[477].

Pain and Treatment History Factors

Pre-operative pain has been reported as a risk factor for APSP in general surgical[7,437,439,441–443,465,466,496] and BCS populations[435,436]. Pre-operative pain could contribute to pre-operative nervous system sensitization due to alterations in pro and anti-inflammatory cytokines[504]. In addition, nerve or tissue damage by tumour growth could contribute to pre-operative pain and neuroplastic changes prior to surgery[504]. Pre-operative pain could also influence pain beliefs and expectations, and therefore, modulation in the spinal cord and brain, leading to more severe APSP[7]. Patients with pre-operative pain, particularly chronic pain, may also be predisposed to post-surgical pain due to shared genetic and psychosocial risk factors[455].

Previous breast surgery may also increase the risk of post-surgical pain due to changes in the patient's expectations and response shifts as a result of previous experience[505]. Neurobiological changes in the surgical area may also occur after previous surgery predisposing these patients to pain[506]. Previous surgical experience has been reported as a risk factor in a general surgical population[460].

Hormonal therapy is associated with musculoskeletal pain[507] and has been associated with increased risk of PPSP after BCS[454]. Researchers have suggested the association between hormonal therapy such as aromatase inhibitors with joint pain is related to reduced estrogen levels[508]. In one study, increased IL-4 and reduced IFN-γ were correlated with reduced pain intensity from aromatase inhibitors suggesting inflammatory mediators may contribute to the mechanism of aromatase inhibitor related pain[508].

Previous radiation or chemotherapy could also increase the risk of post-operative pain due to neurobiological changes. Studies have reported post-operative radiation or chemotherapy was associated with increased PPSP[456,509,510]. Both radiation and chemotherapy treatment, particularly regimens with taxanes, platinum agents or vinca alkaloids, are associated with neuropathic pain[511]. Chemotherapy induces axonal injury which activates an inflammatory response that is, in part, mediated by cytokines[512]. Different mechanisms of neurotoxicity have been reported for different types of chemotherapy, however all involve upregulation of gene expression associated with inflammatory and immune responses, including inflammatory cytokines[512]. In addition, radiation induces fibrotic changes, which are associated with increased IL-6 and TGF-β, and decreased IL-10[513]. These cytokine changes could affect excitability of neurons in the breast and axilla area.

Many patients receive chemotherapy, radiation or hormonal therapy after surgery. As a result, only one study was identified that investigated pre-operative chemotherapy and radiation and they did not find an association with APSP[3]. Since some women may have had a previous cancer that was treated with chemotherapy, radiation or hormonal therapy and some women receive neo-adjuvant chemotherapy in order to shrink the tumour to facilitate surgery, this may be an important variable to assess pre-operatively. Therefore, these variables were considered in the current study because of their association with both PPSP and non-surgical pain.

2.6 Pain Outcomes

Pain intensity, or how much it hurts[514], was considered both at rest and with movement. Different correlates have been reported for resting pain and movement-evoked pain in both APSP[442,515] and PPSP[461]. For example, signs of neuropathic pain 1 week after BCS, contributed to the model predicting pain at rest one year after surgery, but was not included in the model predicting movement-related pain[461]. BMI was included in the model for movement-related pain but was not included in the model for pain at rest[461]. Movement-evoked pain is also a proxy for mechanical hyperalgesia[8] and provides information on the impact of pain on physical functioning[516]. Opioid medications may be less effective at reducing movement-evoked pain than resting pain[4,517] so understanding risk factors for each could help tailor analgesia plans. Despite these differences, a systematic review found 39% of post-operative pain studies did not include movement-evoked pain as an outcome, and 52% did not specify whether they were measuring resting pain or movement-related pain[516]. The current study measured both resting and movement-related pain intensity to better elucidate differences in factors contributing to APSP.

Pain quality, or how the pain feels[518], was also assessed in this study. APSP is often described as aching, tender, throbbing, sharp and tiring-exhausting[519]. Changes in pain quality have been reported to impact interference beyond changes in intensity, supporting the importance of assessing both intensity and quality[520]. Evaluating pain qualities may provide information about the etiology of pain which could lead to more effective treatment[521]. For instance, Minocycline had no impact on overall pain intensity but reduced scores on the affective scale of the Short Form-McGill Pain Questionnaire, a measure of sensory and affective pain qualities[522]. IL-6 and depression symptoms explained 21% of the variability in pain quality in

patients with back pain[253] so understanding the impact of cytokines and psychosocial factors on pain qualities may further our understanding of those at risk for particular types of pain.

NeP was also investigated in the present study. Nociceptive pain and NeP may respond to different pharmacological interventions so determining risk factors for each is essential[523]. Given the importance of pain qualities, this study developed biopsychosocial models predicting pain intensity at rest and with movement, pain qualities and NeP separately.

2.7 Consequences of Unrelieved Post-Surgical Pain

The amplified sympathetic activity associated with severe APSP increases the risk of complications in the cardiovascular, gastrointestinal, immune, muscular, pulmonary and renal systems[4,524]. APSP also impacts psychological functioning and leads to reduced patient satisfaction, delayed discharge from hospital and post-anesthesia recovery units and unanticipated readmissions[4]. In older people, it can increase the risk of post-operative delirium and cognitive dysfunction[4]. Severe APSP is also a predictor for PPSP, which occurs in 22-70% of patients[3,455]. Improved management of APSP is essential to reduce the likelihood of these complications.

2.8 Relevance and Importance

Despite advances in surgical technique and our understanding of pain, post-operative pain control remains inadequate. Identifying high-risk patients prior to surgery will allow pre-operative and post-operative care to be adjusted based on risk. Modifiable factors identified in the pre-operative period will also provide a basis for research on targeted pre-operative interventions. While multiple studies have examined psychological, surgical and demographic

variables as risk factors for APSP, this is the first study, to our knowledge, that considered these factors simultaneously with baseline inflammatory cytokine levels to develop models predicting pain at rest, pain with movement, pain qualities and NeP after BCS.

2.9 Objectives

To develop biopsychosocial models of pre-operative risk factors to predict pain intensity at rest; pain intensity with movement; sensory and affective pain qualities; and neuropathic pain.

2.10 Hypotheses

1. At 24 hours after surgery, each pain outcome (NRS-R, NRS-M, SF-MPQ and SF-NPQ), will be predicted by a range of preoperative biopsychosocial factors. We predict that psychological factors will most strongly predict the outcomes, although surgical factors and inflammatory cytokine concentrations at baseline will also contribute significantly to each model.
2. There will be overlap between each of the models developed, however, we expect there to be differences in the variables that contribute to each model.
3. IFN-γ, IL-2, IL-6, IL-8, IL-17, TNF-α will be associated with increased pain, while IL-10 and TGF-β will be predictive of decreased pain.

3 METHODS

This study was part of a larger, ongoing longitudinal study of post-operative pain after BCS (Canadian Cancer Society Research Institute Grant no. 18367). Ethics approval was obtained from the University Health Network (UHN) and York University.

Sample, Inclusion and Exclusion Criteria. 300 women scheduled for unilateral or bilateral mastectomy at UHN were recruited to the larger study. The 86 women who consented to a pre-operative blood draw and who completed a post-operative follow-up were included in this analysis. Inclusion criteria: ≥18 years old, able to read and write English sufficiently to provide informed consent and complete questionnaires, Class 1-3 on the American Society of Anesthesiologists Physical Status Classification System[525]. Patients with a variety of breast cancer diagnoses (including ductal carcinoma in situ, invasive ductal carcinoma and invasive lobular carcinoma), stage 0-4, and those undergoing prophylactic surgery were recruited. Exclusion criteria: significant central nervous system, respiratory, cardiac, hepatic, renal or endocrine dysfunction and/or any significant associated sequelae; cognitive impairment or documented diagnosis of a DSM-IV Axis 1 disorder; contraindication to opioids or acetaminophen; documented substance abuse or dependence within one year; pregnant or breastfeeding within six months; use of exogenous hormones within three months; immunization within 30 days; blood donation within 60 days; acute or infectious illness, allergic reactions, herbal supplements, physical injuries or dental work within two weeks.

Procedure. Preadmission. Patients were recruited from the survivorship class at Princess Margaret Cancer Centre and the preadmission clinic at Toronto General Hospital. All patients approached were documented, and the reason for exclusions recorded. Informed consent was obtained, and the Short Orientation-Memory-Concentration Test was administered. No patients had scores suggesting cognitive impairment. Patients completed a comprehensive package including demographic information, menopausal status, pain expectations, history of chronic and ongoing pain problems, history of breast surgery and a measure of pre-operative pain at rest. A research assistant (RA) certified in phlebotomy requested permission to obtain a blood sample or to add additional vials to blood work ordered by physicians. Two lavender EDTA-coated Vacutainer® blood collection tubes were filled, allowing for a backup sample. Immediately after blood was drawn, it was centrifuged and plasma was separated and frozen at -80˚C until analysis. Patients were given a take-home package including measures of anxiety, pain catastrophizing, and depression, which they completed and returned on the day of surgery. The RA also completed a chart review for disease and health information (Charlson Comorbidity Index, Karnofsky Performance Status, diagnosis, stage and history of cancer related treatment). BMI was calculated based on patient's height and weight as measured at the preadmission assessment (BMI=weight (kg)/height^2 (m)).

Intraoperative Management. Intraoperative management followed standard practice and may have differed slightly between patients. Decisions regarding intraoperative anesthesia and analgesia were made at the discretion of the anesthesiologist.

Post-Operative Follow-up. Post-operative management followed standard practice. Decisions regarding management were made by the patient's care team and therefore may have differed slightly between patients. Typically, patients received IV morphine 2-4mg and 650-1000mg of acetaminophen every six hours as needed. On the first post-surgical day, an RA visited or called the patient if they had been discharged. The Confusion Assessment Method was completed to assess delirium. There were no cases of scores suggesting delirium that required nursing intervention. Measures of pain at rest, pain with movement, pain qualities, and NeP were completed. When a patient could not be reached, pain ratings were abstracted from the patient's chart. Only pain at rest and occasionally movement-evoked pain were available from patient charts. Surgical information (type and length, complications, intraoperative and post-operative medications) and adverse events were recorded. See Appendix 2 for a summary of measures collected for the larger, longitudinal study.

3.1 Measures

Measures of Cognitive Status. The Short Orientation-Memory-Concentration Test assessed cognitive impairment pre-operatively. Scores can range from 0 to 28 with <18 indicating cognitive impairment. It has been used in cancer patients[526]. The Confusion Assessment Method was used to assess for post-surgical delirium. It is valid and reliable[527].

Measures of Pain. Numeric rating scale at rest (NRS-R) and with movement (NRS-M). Patients selected a number from 0 (no pain), to 10 (worst possible pain). The NRS-R measured pain at rest. For movement-evoked pain, patients completed NRS-Ms after taking two maximal inspirations. NRSs are valid for post-surgical pain[528]. The Short-Form McGill Pain Questionnaire (SF-MPQ) was used as a multidimensional rating of the intensity of 11 sensory and 4 affective

pain qualities as none, mild, moderate and severe. It is valid and reliable in the assessment of cancer pain[529] and post-surgical pain[518,519]. Total scores can range from 0-45 with higher scores indicating increased total affective and sensory pain intensity. The Short-Form Neuropathic Pain Questionnaire (SF-NPQ) is a valid and reliable measure of intensity of NeP qualities[530]. Patients reported the intensity of tingling, numbness and pain due to touch from 0-100[530]. Total scores can range from 0-300, with higher scores indicating increased intensity of neuropathic pain qualities. Answers were scored in two ways: firstly, the sum of responses to the three questions was used for the model outcome as this study was primarily interested in the presence of any neuropathic pain qualities; secondly, a validated scoring system[530] which aims to categorize patients as experiencing neuropathic vs. non-neuropathic pain was used to better characterize the population of patients with probable neuropathic pain. Pain expectations were assessed by asking patients to answer three questions on a scale of 0 to 10: how intense do you expect the pain to be immediately following your surgery when you first wake up?; How intense do you expect the pain to be after you are given the pain medication?; How intense do you expect the pain to be one week following your surgery? The average of the answers to the three questions was used.

Measures of Health Status. Charlson Comorbidity Index (CCI) is a weighted index of 19 comorbid conditions that was used to obtain a total comorbidity score. Higher scores indicate more comorbidity. It is valid and reliable in breast cancer and surgical patients[531]. Karnofsky Performance Status (KPS) was used as a measure of functional status (0= ”dead” to 100= ”no evidence of disease”). It is valid and reliable in cancer patients[532].

Measures of Psychosocial Status. The Centre for Epidemiologic Studies – Depression Scale (CES-D) was used to measure depression. It is a 20-item measure that is valid and reliable in breast cancer patients[533]. Total scores range from 0-60 with higher scores indicating more depressive symptoms. The Pain Catastrophizing Scale (PCS) is a 13-item questionnaire that was used to measure pain rumination and magnification and helplessness in managing pain. Scores range from 0-52 with higher scores indicating increased pain catastrophizing. It is valid and reliable in adult pain populations[534]. The State-Trait Anxiety Inventory measured state anxiety (STAI-S), anxiety in response to specific situations, and trait anxiety (STAI-T), a general tendency towards anxious feelings, each with 20 items. Possible scores on each scale range from 20-80 with higher scores indicating increased anxiety. It is valid in surgical and cancer patients[535].

Plasma Cytokine Levels. The Meso Scale Discovery (MSD) V-PLEX Cytokine Panel 1 (human) kit measured plasma IL-17. Plasma TGF-β1 was measured using the MSD 96-Well Multi-Array® Human TGF-β1 Assay. Plasma IFN-γ, IL-2, IL-6, IL-8, IL-10 and TNF-α were measured using the MSD V-PLEX Proinflammatory Panel 1 (human) kit. The manufacturer's instructions were followed (MSD, Rockville, MD, USA). These enzyme-linked immunosorbent assays are validated. Plates came pre-coated with primary antibodies and calibrators, diluents, secondary antibodies and read buffer solutions were provided. Briefly, multi-analyte lyophilized calibrators were diluted to create seven solutions that were included on each plate. Plasma samples were thawed on ice. Two-fold dilutions of each sample were made. Each sample was tested twice and the mean was used. Samples were incubated for two hours in separate wells on each plate allowing cytokines to bind to primary antibodies on the well surface. After washing, detection antibodies conjugated with electrochemiluminescent labels were added to each well and incubated for two

hours. The secondary antibodies then bound to the bound analytes. Next the plates were washed and a read buffer, which creates the environmental conditions necessary for electrochemiluminescence, was added. The plates were read using an MSD SECTOR® Imager which applied a voltage to the plates causing the bound secondary antibodies to emit light. The MSD SECTOR® Imager measured the intensity of emitted light to quantify the analytes in the sample. Intensity of light from calibrators allowed for the development of a standard curve from which concentrations could be calculated. The use of this curve allowed samples below the detection limit but within the curve to be extrapolated. The lower limits of detection (LLODs) were: IFN-γ: 0.02pg/ml, IL-2: 0.09pg/ml, IL-6: 0.06pg/ml, IL-8: 0.04pg/ml, IL-10: 0.03pg/ml, IL-17: 0.74 pg/ml, TGF-β1: 17pg/ml and TNF-α: 0.04pg/ml. Interleukin-1β (IL-1β), Interleukin-4 (IL-4), Granulocyte-macrophage colony stimulating factor (GM-CSF) were also explored however, most samples were below the detection limit and are therefore not presented.

3.2 Data Analyses

Missing Data. All data were double checked for data entry errors prior to analysis. Missing data was assessed using Little's Missing Completely at Random test[536] and was found to be missing completely at random. The mean item response for a particular participant was imputed for questionnaires with less than 20% items missing[537]. When participants were missing more than 20% of items on questionnaires used as predictors, total questionnaire scores were imputed using maximum likelihood estimates[538]. No data were imputed for any pain outcome (NRS-R, NRS-M, SF-MPQ, SF-NPQ) missing >20% of items. These patients were excluded from the model for which their score was missing.

Cytokine values below the curve fit were imputed with zero. Analyses were conducted with pairwise deletion and with imputed data. No significant differences were found so data are presented with imputed data. The intra-assay coefficient of variation (%CV), a measure of precision between duplicate samples was examined. A %CV<25 was considered acceptable[539]. There were some samples above this limit: one sample for IFN-γ, one for IL-2, three for IL-6, three for IL-8, one for IL-10, one for IL-17, and three for TNF-α. These were excluded from subsequent analyses using pairwise deletion.

Descriptive Statistics. Means and standard deviations were calculated for normally distributed continuous variables, medians and interquartile range for non-normally distributed continuous variables, and frequencies for categorical variables. Data were examined for skewness and kurtosis. Z-scores for skewness and kurtosis were calculated by dividing by the corresponding standard error. A cut-off of 3.29 was used to determine normality[540]. Scores on the SF-MPQ were not normally distributed (z-score skewness= 6.46, z-score kurtosis = 6.77); therefore, a square root transformation was applied as the data were positively skewed and contained zero values[541]. After square root transformation, the z-score of skewness was 1.00 and the z-score of kurtosis was 0.69. Age-related differences in descriptive characteristics were assessed using Pearson's correlations for normally distributed continuous variables, Spearman's correlations for non-normal continuous and ordinal variables, and independent t-tests or one-way analysis of variance tests for categorical variables[541].

The proportion of patients selecting each pain quality on the SF-MPQ Sensory and Affective subscales and the SF-NPQ scale was determined. The average number of words chosen on each was also calculated. The SF-NPQ was also scored to distinguish between neuropathic and

non-neuropathic pain. Independent samples t-tests, Mann Whitney U-tests of medians and Fisher's Exact tests were used to identify group differences.

Bivariate Analyses. Models to identify pre-operative predictors of acute post-surgical NRS-R, NRS-M, SF-MPQ Total, and SF-NPQ were developed. Potential predictors included demographic (age), biological and health status variables (pre-operative plasma cytokine concentrations, BMI, menopausal status); psychological variables (anxiety, depression, pain catastrophizing and pain expectations); surgical variables (surgical procedure, time in hours from surgery to follow-up, surgical indication); pain and treatment history variables (pre-operative pain (NRS-R pre-op), chronic pain or analgesic use in previous 6 months, ongoing pain problem, core biopsy prior to surgery, previous radiation or chemotherapy, previous hormonal therapy). Independent t-tests assessed differences in age and pain outcomes for any categorical variable with small cell size (<15% of total sample). When no significant differences were found ($p>.05$), these variables were not included as potential predictors. Associations between continuous predictors and outcomes were assessed with Pearson's correlations for normally distributed data and Spearman's correlations for non-normal and ordinal variables[541]. Associations between outcomes and categorical predictors were assessed using independent t-tests. Levene's test for Equality of Variances was used to determine if the assumption of homogeneity of variances was met. When Levene's test was significant at $p<.05$, Welch's t-test was used to compare group differences on outcomes[541].

Model Building. Predictor variables that were correlated with any of the outcomes at a significance level of $p<0.1$ were considered for inclusion in all of the multiple regressions. In cases where predictor variables were highly correlated ($r>0.7$), the variable with the least missing data or greater literature to support its importance was included[538]. Backward multivariate regressions determined significant pre-operative predictors of acute post-surgical pain outcomes. Criteria for removal was $p>0.15$.

Assumptions of multiple regressions were investigated. Durbin-Watson's test was used to test for independence of residuals for each model. Partial regression plots of each independent variable with each dependent variable were visually analyzed to confirm the presence of a linear relationship. Standardized residuals were assessed for normality using the standard error of skew and kurtosis. Standardized residuals $>\pm 3.3$ were considered to indicate an outlier[538]. Leverage values were also examined to identify cases that may be influencing the model. The average leverage ((k+1)/n) times 3 was used as a cut off[541]. Cook's values were also analyzed as a second technique to identify influential points. A cut-off of 1 was used[542]. Variance inflation factor (VIF) values were examined to confirm there was no multicollinearity. All data were analyzed using SPSS Version 24.0 for Windows (SPSS. Inc., Chicago, IL).

4 RESULTS

Of the 300 patients included in the larger study, 105 consented to a blood draw. 19 of these patients did not complete any portion of the post-operative follow-up and were therefore excluded from this analysis. A flow diagram of recruited patients is presented in Figure 1. A total of 86 patients had scores on the NRS-R, 83 had scores on the NRS-M, 78 had scores on the SF-MPQ and 73 had scores on the SF-NPQ. Most missing data involved pre-operative questionnaires measuring anxiety, depression, pain catastrophizing and pain expectations. The largest amount of missing data was on the STAI-T with 15% of patients missing the entire scale and two patients missing one or two items.

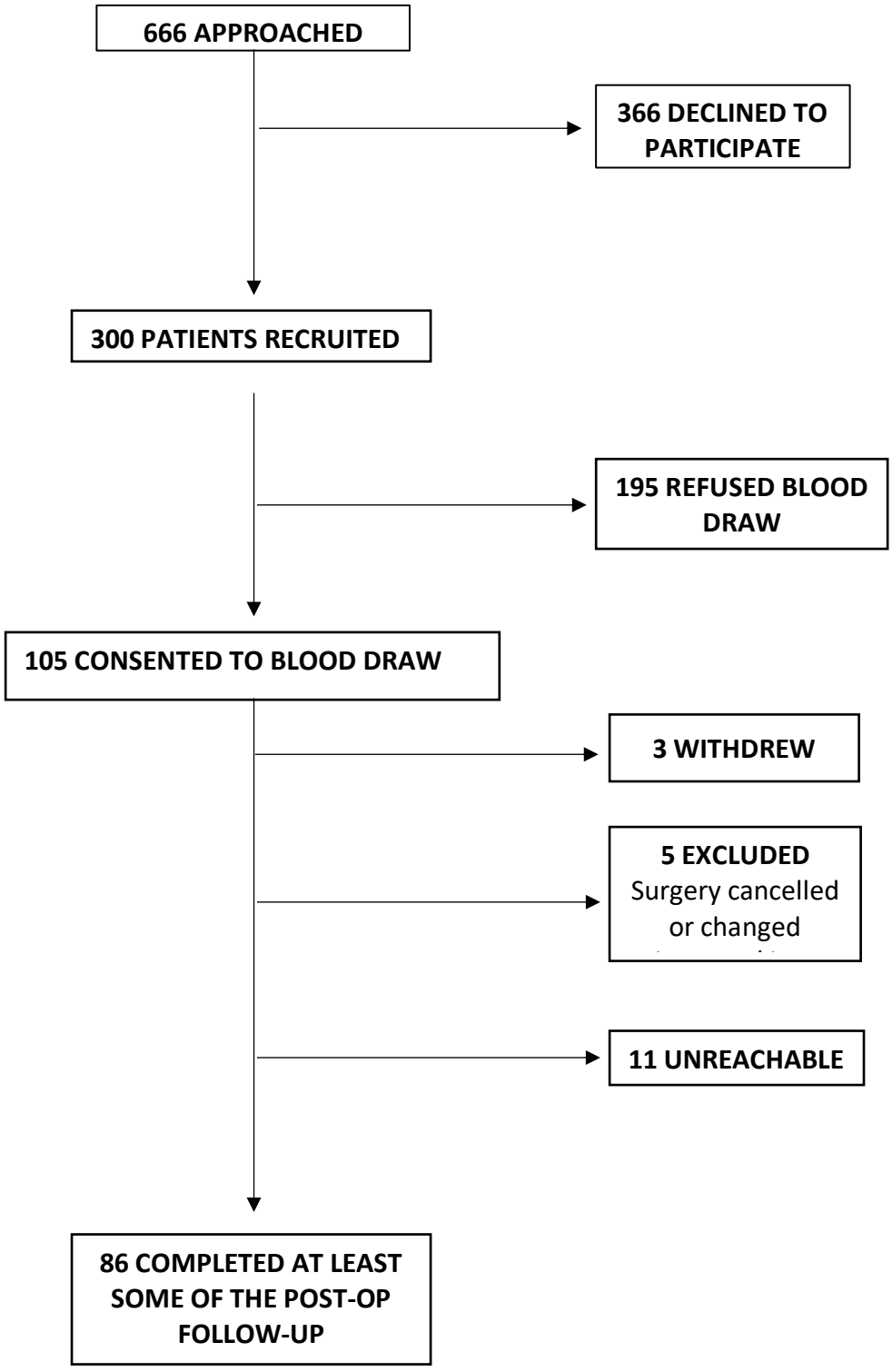

Figure 1. CONSORT diagram of participants.

4.1 Participant characteristics

61 patients were recruited from Toronto General Hospital and 25 patients were recruited from Princess Margaret Cancer Centre. Demographic information is presented in Table 2. Age ranged from 24-81 years. The sample was predominantly white (84.9%) and spoke English as their primary language (84.9%). 40 (46.6%) patients underwent prophylactic surgery, 27 (31.4%) had non-recurrent breast cancer, 16 (18.6%) had a recurrence to the same breast, 2 (2.3%) had residual disease from a previous surgery, and 1 (1.2%) had a recurrence to the opposite breast. The majority (84.9%) did not have pain prior to surgery, with only 13 people (15.1%) indicating NRS-R pre-op >0.

Table 2. Participant Baseline Characteristics

	Mean ± SD or Frequency (%)
Age	50.62 ± 10.96
BMI	27.39 ± 4.569
CCI>0	68 (79.1)
KPS	93.37 ± 5.972
Race	
Caucasian	73 (84.9)
Asian	5 (5.8)
South American	3 (3.5)
African	2 (2.3)
Missing	3 (3.5)
ASA	
I	7 (8.1)
II	49 (57)
III	29 (33.7)
Missing	1 (1.2)
Menopause	50 (58.1)
Chronic pain >6 mos /analgesic use	25 (29.1)
Ongoing Chronic Pain from past Sx.	15 (17.4)
Ongoing pain problem	38 (44.2)
Missing	1 (1.2)
Education	
High School or Less	13 (15.2)
Community College	18 (20.9)
Bachelor's Degree	36 (41.9)
Graduate Degree	13 (15.2)
Professional Degree	6 (7)
Living Arrangement	
Alone	4 (4.7)
w/ partner	25 (29.1)
w/ partner, children	46 (53.5)
w/ children	6 (7)
w/ other	4 (4.6)
Missing	1 (1.2)
Marital Status	
Common Law	14 (16.3)
Married	57 (66.3)
Single	4 (4.7)
Separated/Divorced	9 (10.5)
Widowed	2 (2.3)
Have Children	71 (82.6)

BMI: Body mass index. CCI: Charlson Comorbidity Index. KPS: Karnofsky Performance Status. ASA: American Society of Anesthesiologists Physical Status Classification System. Ongoing pain problem for which treatment has been sought.

Previous treatment, disease and surgical details are presented in Table 3. Most patients underwent a mastectomy (84.8%) and 73.3% of the sample underwent reconstruction. One patient had a lumpectomy on one side and a mastectomy on the other side. Duration of surgery ranged from 80 to 1034 mins in duration with a mean of 471.44 ± 268.71. Four patients had intraoperative complications. One patient experienced intraoperative premature ventricular contractions. One experienced venous congestion of a flap. In another patient, at the end of surgery flaps were found cool and a re-exploration was done. The fourth complication involved insufficient flow from venous anastomoses.

The median time between the end of surgery to the post-operative follow-up was 23.79 hours (IQR: 18.56, 41.01). Three patients underwent second surgeries before the post-operative follow-up was completed. Two were for hematoma evacuations and one was for failing free flaps. One patient experienced reduced levels of consciousness and oxygen desaturations post-operatively which resolved with Narcan® treatment.

Younger patients were more likely to have undergone bilateral surgery (p=.019), and reconstruction (p=.003) than older patients. Younger patients also had higher pain expectations than older patients (p=.001). Older patients were more likely to have had SLNB (p=.029). There were no age-related differences in KPS, CCI, other psychological (CES-D, STAI-S, STAI-T, PCS), pain or treatment history (pain medicine taken in past two weeks, chronic pain or analgesic use, previous chemotherapy or radiation, previous breast surgery, core biopsy prior to surgery), or surgical variables (mastectomy compared to lumpectomy, prophylactic surgery compared to cancer diagnosis). There was also no age difference in WHO analgesic ladder score at 24 hours.

Table 3. Treatment History, Disease and Surgical Details

	Frequency (%)
Prev. Hormonal Therapy	25 (29.1)
Prev. Chemotherapy/Radiation	39 (45.3)
Prev. Breast Surgery	57 (66.3)
Pre-surgery Core biopsy	40 (46.5)
Diagnosis	
DCIS	8 (9.3)
Invasive Ductal Carcinoma	30 (34.9)
Invasive Lobular Carcinoma	4 (4.7)
Other	4 (4.7)
Prophylactic	40 (46.5)
Stage	
0	9 (10.5)
I	21 (24.4)
II	11 (12.8)
III	5 (5.8)
Prophylactic	40 (46.5)
BRCA	
1	10 (11.6)
2	7 (8.1)
Not Tested	49 (57)
Missing	4 (4.7)
ER positive	31 (36)
PR positive	27 (31.4)
HER2 positive	3 (3.5)
Procedure	
Uni. Lumpectomy	12 (14)
Uni. Mastectomy	34 (39.5)
Bilat. Mastectomy	39 (45.3)
Mastectomy+Lumpectomy	1 (1.2)
SLND	43 (50)
ALND	11 (12.8)
Reconstruction	63 (73.3)

ER: Estrogen receptor. PR: Progesterone receptor. HER2: Her2/neu receptor. Note 40 prophylactic women and those diagnosed with DCIS did not have ER, PR or HER2/neu testing.

Baseline scores on psychological measures are presented in Table 4. 41.9% of the sample had scores 16 or higher on the CES-D, indicating a risk for clinical depression[543]. On the STAI-S, 44.2% of patients had state anxiety classified as low (20-39)[455], 45.3% had state anxiety levels classified as moderate (40-59)[455] and 10.5% of patients had high state anxiety levels (60-80)[455]. On the STAI-T, 61.6% of patients had low anxiety (20-39)[455], 30.2% had moderate anxiety (40-59)[455] and 7% had high anxiety (60-80)[455].

Table 4. Pre-operative Scores on Psychological Measures

	Mean ± SD
Average Pain Expectation	4.23 ± 1.63
CES-D	16.11 ± 11.75
PCS	14.51 ± 9.35
STAI-S	41.99 ± 12.52
STAI-T	38.09 ± 12.52

CES-D: Centre for Epidemiologic Studies-Depression Scale. PCS: pain catastrophizing scale. STAI-S: State-trait anxiety scale – state subscale. STAI-T: state-trait anxiety scale-trait subscale.

4.2 Cytokine concentrations

There were 5 patients with IL-2 values below the curve fit, 2 cases with IL-6 values below the curve fit and 1 case with IL-17 values below the curve fit. Results using pairwise deletion were the same as with imputation therefore medians and interquartile ranges with imputed data are presented in Table 5. GM-CSF, IL-1β and IL-4 all had less than 30% of samples in the detection range so these were excluded from all subsequent analyses. Data for these cytokines are not presented.

Table 5. Pre-operative Cytokine Concentrations

	Median Concentration (pg/ml) (IQR)
IFN-γ	3.248 (2.229, 5.350)
IL-2	.123 (.057, .221)
IL-6	.492 (.247,.818)
IL-8	3.317 (2.115, 4.380)
IL-10	.201 (.121, .320)
IL-17A	.516 (.318, .991)
TGF- β	2110.847 (1399.004, 3246.473)
TNF-α	1.353 (.933, 1.855)

Note: IFN-γ: interferon-γ. IL-2: interleukin-2. IL-6: interleukin-6. IL-8: interleukin-8. IL-10: interleukin-10. IL-17A: interleukin-17A. TGF-β: transforming growth factor-β. TNF-α: tumour necrosis factor-α.

4.3 Pain Outcomes

Mean post-operative NRS-R was 3.07 ± 2.2 with a range of 0-8. 6 patients (7%) had severe resting pain (NRS-R=7-10[544]), 28 (32.6%) had moderate resting pain (NRS-R=4-6[544]), 38 (44.2%) had mild resting pain (NRS-R=1-3[544]) and 14 (16.3%) had no pain (NRS-R=0). Mean post-operative NRS-M was 5.4 ± 3.05 (n=83) with a range of 0-10. 34 patients (41%) had severe movement-related pain (NRS-M=7-10), 26 (31.3%) had moderate movement-related pain (NRS-M=4-6), 15 (18.1%) had mild movement-related pain (NRS-M=1-3) and 8 (9.6%) had no movement-related pain (NRS-M=0).

The median (IQR) score on the SF-MPQ total scale was 6 (3.00, 10.63) and ranged from 0-36. The median (IQR) scores on the SF-MPQ affective and sensory subscales were 0.5 (0, 3.00) and 5 (2.00, 8.25), respectively. The median (IQR) number of words chosen on the SF-MPQ total scale was 3.5 (2, 6); on the sensory subscale, patients chose a median of 3 words (1, 5) and on the affective subscale patients chose a median of 0.5 words (0, 1). The frequency that each item on the SF-MPQ was selected is presented in Figure 2. Words chosen by ≥33% of patients have

been shown to be characteristic of a specific type of pain[545,546]. On the sensory subscale the words meeting the criterion of ≥33% were: tender (75.6%), aching (55.1%), heavy (38.5%) and sharp (34.6%). On the affective subscale only tiring-exhausting (44.95%) was selected by ≥33% of patients.

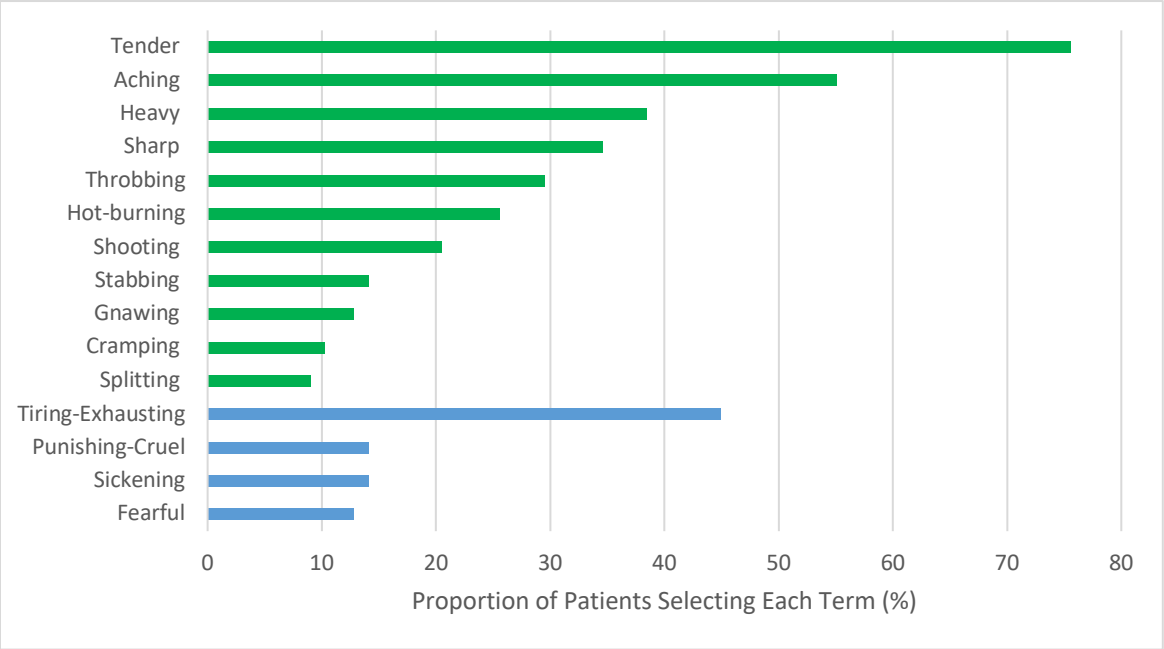

Figure 2. Proportion of patients selecting each item on the SF-MPQ

Note: Green= sensory subscale, Blue=affective subscale.

The mean score on the SF-NPQ was 72.2 ± 59.9 and ranged from 0-210. The SF-NPQ can also be scored to distinguish between neuropathic and non-neuropathic pain. Using this discriminant scoring method, 22 (30.1%) patients had scores suggesting NeP and 51 (69.9%) had scores indicating non-neuropathic pain. Those in the probable neuropathic group were more likely to have had previous chemotherapy or radiation (p=.041). Patients classified as having probable NeP were also more likely to have had a mastectomy (p=0.013) and to have undergone reconstruction (p=.012). The most commonly chosen item was “increased pain due to touch”

with 72.6% of patients endorsing this descriptor. Numbness was chosen by 43.8% of patients and tingling by 19.2% (see Figure 3).

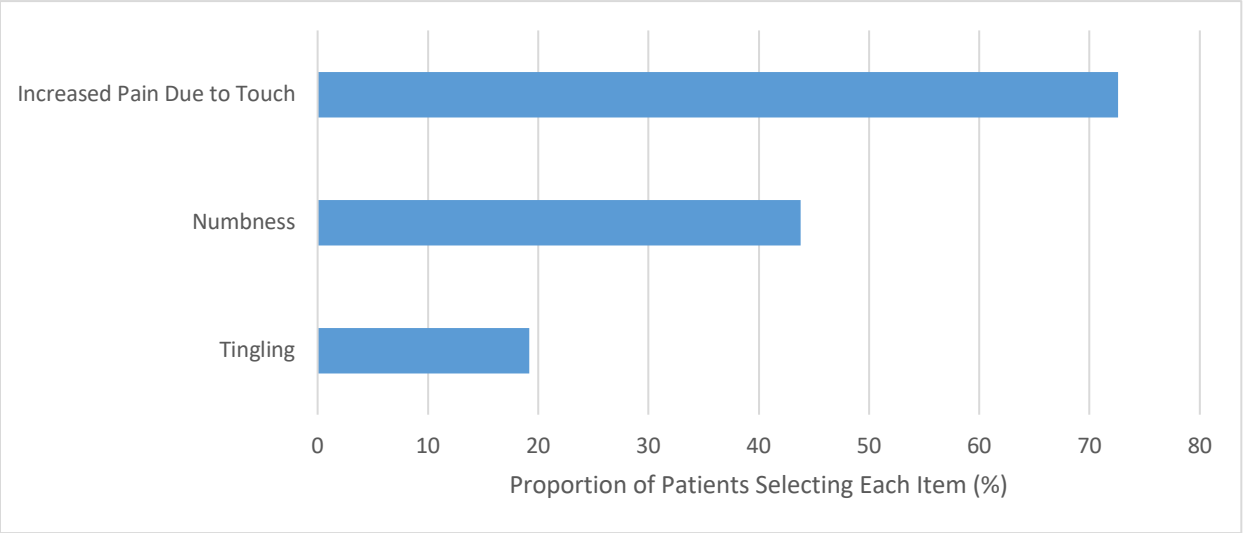

Figure 3. Proportion of patients selecting each item on the SF-NPQ

4.4 Bivariate Analysis

There were only 11 patients (12.8%) who underwent ALND. This group of patients was compared to the patients who did not have ALND using independent t-tests on the four outcome variables and age. There were no significant differences so this variable was not considered in the regression models. Only 12 patients (14%) had lumpectomies however; scores on post-operative NRS-R (p=.045), NRS-M (p=.001) and SF-NPQ (p=.027) were significantly different between patients who underwent mastectomy compared to lumpectomy so this variable was considered in the bivariate analysis. Results for the bivariate analyses with NRS-R, NRS-M, SF-MPQ and SF-NPQ are presented in Table 6.

Demographic factors. Younger age was associated with increased NRS-R, NRS-M and SF-MPQ but was not associated with SF-NPQ scores.

Biological and health status factors. Increased IL-10 was significantly associated with reduced SF-NPQ scores (p=.046). Increased IL-8 was non-significantly associated with reduced NRS-R scores. No baseline cytokines were associated with NRS-M or SF-MPQ. BMI, CCI, KPS, and menopausal status were not associated with any of the outcome variables.

Psychological factors had the greatest number of correlations meeting the criterion for inclusion (p=.10), with 17 out of 20 correlations reaching significance (85%). Average pain expectations, PCS and STAI-T were significantly positively associated with all four outcomes. STAI-S was only associated with increased NRS-R and SF-MPQ. CES-D was also significantly associated with all four outcomes however, due to significant multicollinearity with STAI-T (r=.76) and STAI-S (r=.70) it was not included as a potential predictor in the final models. Anxiety has been found to be a better predictor of acute post-operative pain than depression[431,441].

Surgical factors. Increased NRS-M had correlations meeting the criteria for inclusion with more surgical variables than the other outcomes (duration, time to follow-up, bilateral surgery, mastectomy, prophylactic surgery and reconstruction). SF-NPQ had the fewest significant correlations with surgical variables and was only associated with mastectomy (p=.002) and reconstruction (p=.023). Reconstruction and mastectomy were the only surgical variables associated with all four outcomes. Bilateral surgery, prophylactic surgery and surgical duration were correlated with increased NRS-R, NRS-M and SF-MPQ, however they were not significantly associated with SF-NPQ. Surgical duration was excluded as a potential predictor due to high correlation with reconstruction (r=.78). Reconstruction is more frequently reported in the literature on acute post-operative pain after breast cancer surgeries[3,197]. The number of hours

from the end of surgery to the follow-up was only significantly associated with increased NRS-M. SLNB was not associated with any of the outcome variables.

Pain and treatment history variables demonstrated correlations meeting the criteria for inclusion with several pain outcomes however, there were no variables associated with all four outcomes. The SF-MPQ was associated with more pain and treatment history variables in comparison to the other pain outcomes (57%). Core biopsy prior to surgery was associated with reduced NRS-R, NRS-M, and SF-MPQ. Previous hormonal treatment was associated with increased NRS-R, NRS-M, and SF-MPQ. Previous radiation or chemotherapy was associated with increased NRS-R, SF-MPQ, and SF-NPQ. Previous breast surgery was associated with increased SF-MPQ and SF-NPQ. History of chronic pain or analgesic use greater than 6 months, having an ongoing pain problem and NRS-R pre-op were not associated with any of the outcomes.

Table 6. Bivariate Analysis with Pain Outcomes and Potential Predictor Variables

	NRS-R (N=86) R (p)	NRS-M (N=83) R (p)	Sqrt SF-MPQ (N=78) R (p)	SF-NPQ R (p) (N=73)
Demographic				
Age	-.329 (.002)**	-.366 (.001)***	-.235 (.038)*	-.138 (.246)
Biological and Health Status				
BMI	-.017 (.880)	.003 (.981)	-.078 (.496)	-.068 (.566)
CCI	-.060 (.582)	-.079 (.477)	-.054 (.640)	-.089 (.455)
KPS	-.121 (.266)	.007 (.952)	.124 (.280)	-.117 (.324)
Menopause	-1.603 (.113)	-.245 (.807)	-.685 (.496)	.118 (.906)
IL-2	-.178 (.103)	-.006 (.956)	-.077 (.504)	-.077 (.519)
IL-6	-.151 (.173)	-.037 (.746)	-.143 (.221)	-.145 (.233)
IL-8	-.223 (.043)*	-.027 (.811)	-.159 (.174)	-.095 (.436)
IL-10	-.137 (.210)	.062 (.577)	.018 (.873)	-.238 (.044)*
IL-17A	-.098 (.374)	.053 (.635)	.004 (.974)	.012 (.921)
IFN-γ	-.054 (.623)	-.023 (.841)	.126 (.276)	.150 (.209)
TGF-β	-.050 (.515)	.039 (.726)	-.051 (.659)	-.033 (.779)
TNF-α	-.132 (.234)	.017 (.878)	.042 (.720)	-.008 (.949)
Psychological				
Avg. Expect	.311 (.004)**	.374 (<.001)***	.393 (<.001)***	.359 (.002)**
CES-D	.250 (.020)*	.299 (.006)**	.408 (<.001)***	.299 (.010)**
PCS	.330 (.002)**	.253 (.021)*	.329 (.003)**	.283 (.015)*
STAI-S	.210 (.053)	.177 (.110)	.277 (.014)*	.155 (.192)
STAI-T	.308 (.004)**	.331 (.002)**	.268 (.018)*	.253 (.031)*
Surgical				
Duration	.230 (.033)*	.374 (<.001)***	.201 (.077)	.167 (.159)
Time to f/up	-.05 (.517)	-.219 (.047)*	-.037 (.749)	-.011 (.928)
SLNB	-.488 (.627)	-1.485 (.141)	-.941 (.350)	.258 (.797)
Bilateral Sx.	3.349 (.001)***	3.66 (<.001)***	2.982 (.004)**	1.569 (.121)
Mastectomy	2.038 (.045)*	3.499 (.001)***	1.893 (.062)	3.402 (.002)**
Prophylactic Sx.	1.917 (.059)	2.798 (.006)**	2.628 (.010)**	.736 (.464)
Reconstruction	3.286 (.001)***	3.907 (<.001)***	2.987 (.004)**	2.318 (.023)*
Pain and Treatment History				
NRS-R pre-op	0.09 (.412)	-.031 (.779)	-.038 (.739)	.121 (.308)
Core biopsy	-2.565 (.012)*	-3.091 (.003)**	-1.943 (.056)	-.988 (.326)
Prev Hormonal Tx	1.947 (.055)	1.784 (.078)	1.725 (.089)	.510 (.611)
Prev Rads or Chemo	1.714 (.090)	1.158 (.250)	1.755 (.083)	1.954 (.055)
Prev. Breast Sx.	1.410 (.162)	1.543 (.127)	3.084 (.003)**	2.184 (.032)*
Chronic pain/analg	-1.273 (.206)	-.515 (.608)	.697 (.488)	.390 (.698)
Ongoing pain prob	-.116 (.908)	-1.392 (.168)	.742 (.460)	.093 (.926)

Green=met criteria for inclusion in multivariate models (p<.1). *p<.05, **p≤.01 ***p≤.001.

NRS-R: numeric rating scale – rest. NRS-M: numeric rating scale – movement. SF-MPQ: Short-form McGill Pain Questionnaire. SF-NPQ: Short-Form Neuropathic Pain Questionnaire. BMI: body mass index. CCI: Charlson Comorbidity Index. KPS: Karnofsky performance status. IL-2: interleukin-2, IL-6: interleukin-6. IL-8: interleukin-8. IL-10: interleukin-10. IL-17A: interleukin-17A. IFN-γ: interferon-γ. TGF-β: transforming growth factor-β. TNF-α: tumour necrosis factor-α. Avg. Expect: average pain expectation. CES-D: center for epidemiologic studies depression scale. PCS: pain catastrophizing scale. STAI-S: state-trait anxiety inventory – state subscale. STAI-T: state-trait anxiety inventory – trait subscale. SLNB: sentinel lymph node biopsy. NRS-R pre-op: pre-operative numeric rating scale at rest.

4.5 Models

The following variables met the criteria of p<.1 for entry into the backward regression models: age, baseline IL-8 and IL-10, average pain expectations, PCS, STAI-S, STAI-T, previous breast surgery, previous chemotherapy or radiation treatment, previous hormonal therapy, core biopsy prior to surgery, bilateral surgery, mastectomy, reconstruction, prophylactic surgery and hours to follow-up (see Table 6). Details on tests conducted to assess the assumptions of multiple regression for each model can be found in Appendix 3.

NRS-R. The model for NRS-R (see Table 7) explained 29.9% of the variance in scores. This model included three significant variables – one demographic variable, younger age; one psychological variable, increased PCS; and one surgical variable, bilateral surgery. There were two non-significant variables that were retained in the model: increased IL-8 predicted a decrease in NRS-R and previous radiation or chemotherapy predicted increased NRS-R.

NRS-M. The model for NRS-M (see Table 7) explained the greatest amount of variance, 32.3%, and included one significant demographic variable, younger age; one significant psychological variable, increased STAI-T; and two significant surgical variables, bilateral surgery and mastectomy. No pain and treatment history variables were retained in this model.

SF-MPQ. The model for SF-MPQ (see Table 7) explained 30.1% of the variance in the outcome. This model included one significant psychological variable, increased PCS; one significant surgical variable, bilateral surgery; one significant pain and treatment history variable, previous breast surgery; one non-significant demographic variable, younger age and one non-significant biological and health status variable, decreased IL-8.

SF-NPQ. Of the four models developed, the model for SF-NPQ (see Table 7) explained the smallest amount of variance, 17.4%. Only two variables were significant, one biological and health status variable, reduced IL-10 and one psychological variable, increased PCS. One surgical variable, bilateral surgery, and one pain and treatment history variable, previous radiation or chemotherapy, were non-significant but retained in the model.

Table 7. Multivariate Backwards Regression Models for Pain Outcomes

	NRS-R (N=86) β^a (p)	NRS-M (N=83) β^a (p)	SF-MPQ (N=78) β^a (p)	SF-NPQ (N=73) β^a (p)
DEMOGRAPHIC				
AGE	-.247(.013)*	-.214 (.034)*	-.171 (.100)	
BIOLOGICAL AND HEALTH STATUS				
IL-8	-.167 (.083)		-.163 (.112)	
IL-10				-.277 (.016)*
PSYCHOLOGICAL				
PCS	.319 (.001)***		.333 (.001)**	.237 (.038)*
STAI-T		.290 (.004)**		
SURGICAL				
BILATERAL SURGERY	.290 (.004)*	.243 (.022)*	.280 (.008)**	.201 (.076)
MASTECTOMY		.253 (.014)*		
PAIN AND TREATMENT HISTORY				
PREV RADS OR CHEMO	.154 (.107)			.189 (.095)
PREV BREAST SURGERY			.265 (.010)**	
ADJUSTED R^2 (P)	.299 (<.001)***	.323 (<.001)***	.301 (<.001)***	.174 (.003)**

*p ≤ .05, **p ≤ .01, ***p ≤ .001. a: adjusted β coefficient.

NRS-R: numeric rating scale – rest. NRS-M: numeric rating scale – movement. SF-MPQ: Short-form McGill Pain Questionnaire. SF-NPQ: Short-Form Neuropathic Pain Questionnaire. IL-8: Interleukin-8. IL-10: Interleukin-10. PCS: pain catastrophizing scale. STAI-T: State-trait anxiety scale – trait subscale. Rads: radiation. Chemo: chemotherapy.

5 DISCUSSION

As the number of patients undergoing breast surgery increases, a better understanding of those at risk for post-operative pain is essential. The post-operative pain experience, which includes both intensity and pain quality, varies widely with some patients experiencing minimal pain and others experiencing severe pain. The current study identified pre-operative predictors of pain intensity at rest, pain intensity with movement, pain qualities and neuropathic pain in the acute post-operative period. This is the first study, to our knowledge, that simultaneously identified biological, psychological, and medical correlates of multiple acute post-operative pain outcomes. Similarities were observed across models, however, each pain outcome also had unique predictors supporting the necessity of assessing multiple dimensions of the pain experience. Importantly, despite differences, each model included biological, psychological and surgical predictors. Younger age, decreased IL-10, increased pain catastrophizing, increased trait anxiety, bilateral surgery, mastectomy, and previous breast surgery each played a role in at least one of the models. Interestingly, no variable made a significant contribution to all four models.

5.1 Pain Outcomes

This study found a significant burden of acute post-operative pain despite analgesia. Specifically, 40% of patients experienced moderate-to-severe resting pain, and 72% had moderate-to-severe movement-related pain. The burden of post-operative resting pain was comparable to other studies of acute pain after breast cancer surgery[432,436], however, the proportion of patients with moderate-to-severe movement-related pain was higher in this study compared to others[436]. Bruce et al. (2012) assessed patients on the 7th post-operative day and

asked for an average rating over the previous week which may explain the discrepancy between the studies. There is extensive literature examining the difference between pain reports at the time pain is being experienced and reports based on recall and averaging over time. Other studies have found recalled ratings to be inconsistent with ratings made at the time[547,548]. Recall and averaging may introduce memory, recency and primacy biases into self-report[547,549,550]. Therefore, the different assessment time frames and tasks may have contributed to the discrepancies in the findings of these studies.

The most frequently selected SF-MPQ pain descriptors, tender, aching and tiring-exhausting, were similar to the most commonly selected in other surgical populations[518,519,551]. A study on inguinal hernia repair found tender and aching were also the most commonly selected, however the other frequently chosen words in that study, stabbing and punishing, were selected by <15% of participants in the current study[459]. This may reflect differences in pain qualities across different types of surgery.

Based on the selection of neuropathic pain qualities, which included numbness, tingling and pain due to touch, 30% of patients were classified as having probable neuropathic pain. A previous study found 10% of patients had acute neuropathic pain after mastectomy and 7% had neuropathic pain after breast conserving surgery[552]. The higher prevalence in the current study may be related to the measure used. In addition, the Jain et al. (2014)[552] study did not specify whether patients had reconstruction. Despite not being retained in the final model, patients classified as having neuropathic pain were more likely to have had reconstruction than those not undergoing reconstruction.

In summary, measures of both pain intensity and quality indicated a high prevalence of acute post-operative pain despite administration of analgesics, supporting the need to better understand and manage pain after breast surgery. Given that acute pain is one of the most consistently reported predictors of chronic pain[456] and that unrelieved post-operative pain increases the risk of various physiological and psychological adverse events[4], better management of acute pain will not only improve the post-surgical experience but could also improve long-term outcomes. Understanding who is at risk for higher pain levels is essential to tailor management strategies and to address modifiable factors in the pre-operative period.

5.2 Multidimensional Model of Pain

Specificity theory suggested there was a one-to-one relationship between tissue damage and pain[13]. This theory has not been supported by neurobiological evidence[50,553] and has largely been replaced by the Gate Control Theory. The Gate Control Theory explains the variable relationship between pain and injury by positing that nociceptive stimuli are modified in the spinal cord by a range of biopsychosocial factors including context, cognition, mood, genetics and neurochemical changes[13]. The interaction between the sensory-discriminative, the motivational-affective and the cognitive-evaluative dimensions, creates the perception of pain[554]. These different dimensions may not be mediated by the same physiological substrates[555] and could differentially contribute to different types of pain.

The models developed in the current study supported this biopsychosocial conceptualization. Pain intensity at rest was predicted by younger age, bilateral surgery and increased pain catastrophizing. Single measurements of pain intensity have been used

extensively in clinical practice and provide valuable information about pain[554]. Most post-operative pain research uses a unidimensional pain measurement scale as an outcome and often does not specify whether pain at rest or with movement is being measured[516]. Post-operative pain at rest is associated with a variety of negative outcomes including a reduced physical and mental quality of life in the immediate post-operative period[556] highlighting the importance of this outcome measurement, in combination with other outcomes, in post-operative pain research.

Similar to the biopsychosocial model of pain intensity at rest, movement-evoked pain also supported the biopsychosocial model of pain. Movement-evoked pain was predicted by younger age, bilateral surgery, mastectomy and greater trait anxiety. Movement-evoked pain is a proxy for mechanical hyperalgesia[8], which is associated with central sensitization[89], a key process in acute pain and the development of chronic post-surgical pain. Movement-evoked pain provides a measure of physical functioning and has been associated with delayed rehabilitation efforts[4]; therefore, the high proportion of patients experiencing severe movement-evoked pain warrants attention. Various studies have reported that certain pharmacological interventions are effective for resting pain but not movement-evoked pain[4] and the small proportion of post-operative studies assessing this outcome may contribute to the poor management of dynamic pain[516]. A recent systematic review also found acute movement-evoked pain is more frequently associated than resting pain with chronic post-surgical pain[557]. Therefore, inclusion of movement-evoked pain, measured separately from resting pain as a post-surgical outcome was essential.

The model developed to predict pain qualities also substantiated the biopsychosocial model of pain. Pain qualities were predicted by bilateral surgery, previous breast surgery and increased pain catastrophizing. Measures of pain quality, or how the pain feels, are also infrequently included in studies on acute post-operative pain in breast cancer despite being a principal component of the pain experience. Different pain qualities are characteristic of different types of pain[554] and certain pain treatments are more effective for some qualities than others[514]. For instance, morphine may be more effective for relieving "throbbing", "shooting" and "aching" pain qualities while gabapentin may be more effective for "tiring-exhausting" and "sickening" pain qualities[558]. Pain quality has been shown to predict the extent to which pain interferes with activity above the contribution of pain intensity alone[520]. This may impact ability to engage in rehabilitation efforts and return to normal activities of daily living. These differences support the need for post-operative care and research to consider pain qualities in addition to intensity, as was done in the current study.

Finally, prediction of neuropathic pain also corroborated the multidimensional nature of the biopsychosocial model of pain. Neuropathic pain qualities were predicted by reduced IL-10 and increased pain catastrophizing. Most of the post-operative literature on neuropathic pain has focused on chronic pain and research on acute neuropathic pain is lacking. This is the first study we are aware of that investigated pre-operative predictors for acute neuropathic pain symptoms after breast surgery. Neuropathic pain is defined as pain resulting from an injury or disease of the nervous system[12] and has often been described as burning, tingling and numb[552]. Neuropathic pain has been shown to be more severe and distressing than nociceptive pain[523] and

acute neuropathic pain symptoms have been reported as a risk factor for chronic pain[461,559]. Consequently, it was critical to also consider neuropathic pain in the acute post-operative period.

Taken together, our findings support the biopsychosocial model and suggest that while pain intensity at rest and with movement, pain qualities and neuropathic pain symptoms are overlapping constructs, their distinct features may be important for both pain theory and management. Each model included one psychological factor, at least one biological and health status variable, and at least one surgical variable, although the surgical and biological and health status variables were not significant in all models. While the models overlapped, movement-evoked pain, pain qualities and neuropathic pain were each predicted by unique variables, highlighting the need to consider multiple outcomes simultaneously to gain a complete understanding of the pain experience. In the next section, the contribution of each predictor is considered separately.

5.3 Predictors of Pain Outcomes

Demographic Correlates

Age is a biopsychosocial phenomenon that can impact all aspects of pain[444]. Younger age has been frequently reported as a predictor for post-operative pain after breast cancer surgery and other surgeries[3,431,434,443], however others have not found this relationship[436,441,464,560]. These discrepancies may stem from methodological differences including the assessment method used and consideration of confounding factors such as comorbidities and surgical and analgesic protocols[444]. In addition, the age range in different studies and degree to which the sample is

representative of an older population may vary[444]. For example, healthier older people may be more likely to consent to research studies[444].

In this study, younger age predicted greater resting and movement-evoked pain and made a non-significant contribution to predicting greater pain qualities. It was not predictive of neuropathic pain symptoms. Younger age likely served as a proxy for other demographic or psychosocial risk factors for acute pain[431] and a variety of biopsychosocial as well as life-stage factors may have been involved[444]. Some have reported greater emotional distress in younger women prior to breast cancer surgery which could impact their post-operative pain[6]. In this study, younger patients were more likely than older patients to have had reconstruction and bilateral surgery which could have contributed to the increased pain in younger patients. In addition, younger patients expected more pain than older patients which may have exacerbated pain perception after surgery. A factor not measured in the current study may also underlie the observed relationship. This factor could be biological, for example hormone-related, psychological, such as the meaning attributed to the cancer, or social, for instance availability of social supports. The impact of variables could also change over the lifetime[445] and longitudinal studies examining the role of various biopsychosocial factors at different life stages are needed to better understand the relationship between age and post-operative pain.

Biological and Health Status Correlates

The only baseline cytokine that was a significant predictor for any pain outcome was decreased IL-10, which predicted increased neuropathic pain. IL-10 is an anti-inflammatory cytokine that down-regulates pro-inflammatory cytokines involved in pain mechanisms[126,561]. A similar protective effect of IL-10 has been reported in chronic pain populations. In chronic pain

patients with a variety of conditions, plasma IL-10 was inversely correlated with pain intensity[251] and significantly lower in patients with chronic widespread pain in comparison to age and sex matched healthy controls[150]. Consistent with this, IL-10 was inversely correlated with the intensity of chronic neuropathic pain[156]. Patients with painless peripheral neuropathies also have higher IL-10 mRNA than patients with painful neuropathies[160]. Given these associations, research using animal models has investigated delivery of IL-10 protein, viral vectors or naked plasmid DNA. Delivery of IL-10 has successfully reversed neuropathic pain behaviours in animal models[126,371,561]. Therefore, the literature supports the role of IL-10 in neuropathic pain. The present study was the first, to our knowledge, to identify a role for pre-injury IL-10 in the development of acute neuropathic pain after surgery in a human model, highlighting the contribution of this cytokine to neuropathic pain mechanisms. The potential role of IL-10 in preventing neuropathic pain should be further explored.

IL-8 was retained as a non-significant predictor in the model for resting pain and pain qualities. Surprisingly, increased IL-8 predicted a decrease in pain scores. IL-8 is a pro-inflammatory cytokine involved in pain processes that is upregulated after surgery[562]. It has been shown to be increased in chronic pain populations[154] and has been correlated with pain intensity in a variety of populations[158,257]. Given that most of the evidence has suggested IL-8 increases pain (see Table 1), reasons for the non-significant reverse relationship observed here are unclear. It is possible that in this model that examined baseline cytokines in combination with psychosocial factors to predict pain at a later time point, the role of IL-8 is less clear. However, it is imperative to not over-interpret this finding without replication given the lack of statistical significance. This needs to be further investigated prior to drawing conclusions.

Despite the cytokines we tested being implicated in pain mechanisms in the literature (see Table 1), few were retained as predictors in the four models developed. This may be due to the methodological approach taken in the current study. Unlike most studies that have considered both cytokines and pain, we measured cytokine concentrations prior to injury rather than examining the change in concentrations after surgery[195,250,256,257,427,563–565] or once pain has become chronic[147,148,150,153–158,162,251,561,566]. It is possible that the timing of the cytokine assay in the trajectory of injury, recovery and chronicity is a key factor in understanding pain mechanisms. However, we were unable to identify any studies that have employed repeated longitudinal assays that consider the relationship between specific cytokines and pain. A strength of the current approach is in predicting risk for post-operative pain, and future studies should continue to explore the predictive role of baseline cytokine concentrations.

Other biological mediators that were thought to potentially contribute to post-operative pain based on a literature review were not found to be significantly associated with any outcomes in the bivariate analysis and therefore were not considered in the multivariate models. BMI has been previously reported by some to be an important predictor for acute[164,460] and chronic[3,454,455,462] post-operative pain outcomes. BMI is associated with an increased inflammatory state[567] which could contribute to baseline sensitization. While patients had a wide range of BMIs, it is possible that patients at the extremes were underrepresented leading to a lack of power to detect the impact of this factor. Patients with very low BMIs or very high BMIs are at increased risk for surgical complications[568] and are therefore less likely to undergo surgery. Comorbidity score and performance status have also previously been reported to predict post-operative pain in some studies[68,197], however, no significant relationship was found in the current

study. Those studies used a self-report measure of comorbidities that included common conditions such as hypertension that are not included in the CCI. This may have contributed to this difference. In addition, there was a relatively small range of performance statuses in the present sample, again related to surgical risk criteria[569], which may have contributed to the lack of significance of these variables. Menopause has also been associated with increased reports of pain in a community sample[570] and changes in inflammatory cytokines after menopause[571] could result in alterations in sensitization processes. However, similar to the current study, another study on breast cancer patients also found menopausal status was not an important predictor for post-operative pain[68].

Psychological Correlates of Acute Pain

In line with the biopsychosocial model, psychological factors are known to be involved in post-operative pain[441,572]. Models for all pain outcomes developed in this study included one psychological factor as a predictor. In three of the four models, the psychological factor accounted for the largest amount of variance in the pain outcome emphasizing the significance of the psychological dimension in the pain experience.

Pain catastrophizing, a cognitive variable that refers to the tendency to describe a painful experience in more exaggerated terms, to ruminate on the pain and to feel more helpless[494], was a significant factor in the models for resting pain, pain qualities, and neuropathic pain. Pain catastrophizing has frequently been reported as a predictor for post-operative pain in various surgical populations[438,441,495,496,573–575] including breast surgery[434,436,573]. Catastrophizing leads to a variety of fear responses including physiological, behavioural and cognitive responses[576]. The attentional focus on the pain stimuli and exaggerated threat value attributed to pain likely

contributes to pain facilitation and therefore increased pain perception[494]. In addition, pain catastrophizing leads to an aroused, negative emotional state and maladaptive pain responses, all of which can increase pain[577]. Studies have also reported that pain catastrophizing was associated with changes in supraspinal endogenous pain-inhibitory and facilitatory processes[578]. The diminished endogenous inhibition may have contributed to the increased post-operative pain seen in these patients. The role of pain catastrophizing in predicting three of the four pain outcomes explored in this study supports the importance of considering this variable in the pre-operative period.

Anxiety. Trait anxiety, a motivational-affective psychological factor that has frequently been reported as a correlate of post-operative pain[431,441,464], predicted the greatest amount of variance in movement-evoked pain after surgery. Trait anxiety is characterized by a general tendency to perceive situations as threatening[574] and is related to avoidance behavior, particularly with regards to anticipation of pain from certain activities[576]. This is consistent with the relationship between movement-evoked pain and trait anxiety observed in the current study. Individuals with high trait anxiety are generally hypersensitive to stimuli and psychologically more reactive[441,579]. The hypersensitivity to environmental threats may have contributed to the influence of trait anxiety on movement-related pain.

While there is some debate as to whether pain catastrophizing and anxiety are distinct constructs[578], they appear to be overlapping but separate components of negative affectivity. Anxiety relates primarily to the motivational-affective dimension of pain while pain catastrophizing is a component of the cognitive-evaluative dimension of pain[580]. A principal component analysis conducted by Mounce et al. (2010) found anxiety loaded on to a factor

designated as “general distress” while pain catastrophizing loaded on to a factor labeled “cognitive intrusion” supporting that these are two different constructs[580]. Avoidance behavior may have a greater impact on functional limitations while rumination and worry appear to be more important for resting pain states[581]. This may explain, in part, why in this study, anxiety was a predictor of movement-evoked pain while pain catastrophizing was a predictor of resting pain, pain qualities and neuropathic pain.

In the current study, pain catastrophizing and trait anxiety were correlated, however, below the designated cut-off for multicollinearity, supporting that these are distinct aspects of negative affectivity. A study on breast cancer surgery found catastrophizing was associated with resting pain but not movement-evoked pain and the magnitude of the association between anxiety and movement-evoked pain was greater than that between anxiety and resting pain[436]. Catastrophizing has been found to be a mediator for anxiety in the prediction of post-operative pain after hysterectomy[575]. That study examined worst pain scores and did not specify whether resting pain or movement-related pain was measured, complicating comparison to the current study.

The retention of a psychological factor in each model supports the Gate Control Theory, which proposes tissue damage stimuli are modulated by a variety of inhibitory and facilitatory signals in the spinal cord[13]. Sensory-discriminative, motivational-affective and cognitive-evaluative dimensions are all involved in this modulation that contributes to the perception of pain[555]. The role of pain catastrophizing and anxiety in prediction of pain intensity is well-established and this study extended the role of these variables to other pain outcomes including pain qualities and neuropathic pain. Additionally, and perhaps most importantly, these findings

suggest that psychological factors continue to play an important role in the prediction of pain even after controlling for surgical and biological factors.

Surgical Correlates of Acute Pain

Surgical variables are commonly reported as risk factors for post-operative pain. Not surprisingly, bilateral surgery was a predictor in three of the four models: resting pain, movement-evoked pain and pain qualities. An association between bilateral breast surgery and increased pain was also found by Schreiber et al. (2016)[430]. The larger surgical field may have led to a greater inflammatory reaction in patients undergoing bilateral than unilateral surgery which could have contributed to the increased pain responses in these patients. Many studies have excluded patients undergoing bilateral breast surgery[197,435,436,461,544,573,582], however, given that 45% of patients in this study underwent bilateral surgery, this group represents a significant portion of the breast surgery population. Therefore, it is essential to include these patients in research to enhance the external validity, representativeness and applicability of the research to clinical reality.

Mastectomy, a surgical predictor in the model for movement-evoked pain, has been reported to be correlated with acute post-operative pain by some[3,434] but not all[436]. Given that the predictive model for movement-evoked pain contained two surgical factors, surgical invasiveness may be particularly important for dynamic pain. More invasive surgery could elicit a greater inflammatory response, thereby leading to more significant sensitization and increased movement-evoked pain. As previously mentioned, movement-evoked pain is associated with mechanical hypersensitivity[8] which is related to central sensitization[89].

No surgical variable accounted for a significant portion of the variance in neuropathic pain, although bilateral surgery made a non-significant contribution to the model. Axillary surgery has been associated with acute sensory disturbances, a feature of neuropathic pain[436]. Some studies have also found ALND to contribute to the prediction of other post-operative pain outcomes[434,435]; however, Vilholm et al. (2008) did not find this[583]. Few women had ALND in this study so the predictive effect of this procedure could not be examined. Addressing the role of ALND in acute neuropathic pain is particularly important due to the increased risk of damage to the ICBN.

Treatment and Pain History Correlates of Acute Pain

Previous breast surgery, which predicted increased scores on the SF-MPQ, as a measure of pain qualities, was the only treatment and pain history variable that made a significant contribution to any of the models. This relationship has been previously reported in a general surgical population[460]. According to the Gate Control Theory, nociceptive inputs are modulated in the spinal cord by a variety of factors including past experiences[555]. Experience with previous surgery may have resulted in response shifts leading to changes in an individual's internal standard of measurement or a change in values[505]. Previous breast surgery could also have led to neurobiological changes in the tissue that impacted the qualities of pain experienced after subsequent surgery[506]. Taken together, these changes may have led to facilitation in the central nervous system, resulting in increased pain.

Previous radiation or chemotherapy made a non-significant contribution to predicting resting pain and neuropathic pain. The neurological changes resulting from radiation or chemotherapy, particularly taxane-based chemotherapy[6,584], could have predisposed patients to

neuropathic pain. Since most patients receive chemotherapy or radiation therapy after surgery, the majority of the acute post-operative literature on breast surgery does not examine the impact of previous chemotherapy or radiation. One study on acute post-operative pain that did consider history of chemotherapy or radiation, contrary to the current study, did not find an association with acute post-operative pain scores[3]. Neither the current study nor the Fecho et al. (2009) study obtained information on the dosage or location of radiation, the type of chemotherapy received or the time-period between radiation or chemotherapy delivery and surgery. The reasons for the different findings in these two studies are difficult to resolve without these details. In addition, the lack of statistical significance observed in the current study warrants caution when interpreting this finding and future research should consider these details to clarify the role of previous radiation or chemotherapy in acute post-operative pain.

Pre-operative pain. Contrary to some other reports[440,443], pre-operative pain was not a predictor of post-operative pain. Many studies reporting a relationship between pre-operative pain and post-operative pain have been conducted in populations where pre-operative pain is more common, such as in joint replacement surgery[437,442]. In addition, studies on post-operative pain that have found a positive association between pre-operative pain and acute post-operative pain, have reported a higher incidence of pre-operative pain[437,465,466,585], while studies finding no association have often described a population with a low burden of pre-operative pain[431,432]. For example, only 17.5% of participants in a study on breast cancer surgery had pre-operative pain and no association with post-operative pain was found[432]; however, in another study, 42% of patients had pre-operative pain and a significant relationship with post-operative pain was found[436]. In the current sample the burden of pre-operative pain was low with only 13 patients

(15.1%) reporting any pre-operative pain (NRS-R>0). The lack of association between pre-operative and post-operative pain in the current study is consistent with other studies that had a small burden of pre-operative pain[431,432]. The small burden of pre-operative pain in the current study may have presented a unique opportunity to observe the impact of other factors that are obscured in populations with a significant burden of pain prior to surgery.

In summary, each of the four models developed in this study included a range of biopsychosocial factors with similarities between the models but also differences. Younger age was a significant predictor for resting and movement-evoked pain intensity; IL-10 was a significant predictor for neuropathic pain qualities; pain catastrophizing predicted increased resting and movement-evoked pain intensity and neuropathic pain; trait anxiety predicted movement-evoked pain; bilateral surgery predicted resting and movement-evoked pain intensity as well as pain qualities; undergoing mastectomy instead of lumpectomy predicted increased movement-related pain; and previous breast surgery predicted increased pain qualities. The models developed supported the biopsychosocial model of pain and the necessity of considering factors outside of surgical procedure in understanding post-operative pain. In addition, the development of overlapping but unique models attested to the need to measure multiple pain outcomes in both post-operative research and clinical practice to improve understanding and management of post-operative pain.

5.4 Limitations

There were several limitations of this study that need to be acknowledged. Firstly, most patients were Caucasian so it is unclear whether these results would apply to other ethnic groups. Given that this study was conducted at a single institution, findings should be confirmed in multi-institution studies with more diverse patient populations.

In addition, the sample size in the current study was limited, likely related to general challenges with research in the acute setting such as logistics before surgery, patient burden associated with prospective studies, or patient fatigue after surgery. Regardless, the current study had a sample size larger than 65% of studies cited in Table 1, supporting the acceptability. The number of variables included in the models was limited to ensure adequate power for the sample size. Furthermore, despite inclusion of a wide range of potential predictors in the bivariate analysis, the model predicting neuropathic pain symptoms explained a relatively small amount of variance, only 17.6%. Other variables not considered in this study may be related to neuropathic pain and this warrants further study.

We did not correct for intra-operative or post-operative analgesia in this study. Standardized regimens were used at the discretion of the anesthesiologist. While patients were offered similar levels of "as needed" medications, challenges with both provision of analgesia by nursing[586] and patient education on analgesia[587] have been reported to contribute to poor post-operative pain control. Other studies on acute post-operative breast pain have also not corrected for analgesia[431,436] and this study design appropriately reflects patients' experiences in this setting. However, given the potential for bias, correction for analgesic use should be considered in the future.

Finally, causation could not be definitively determined from these results, however, this study was prospective and longitudinal, providing support for the predictive ability of the considered variables.

5.5 Implications and Future Directions

Each model developed in this study contained a range of variables including demographic or biological, psychological and surgical variables emphasizing the biopsychosocial nature of pain and the importance of considering factors outside of surgical procedure in identifying patients at risk for severe post-operative pain. Consideration of the biopsychosocial factors identified in these models pre-operatively may assist with the identification of patients at high risk for post-operative pain. Awareness of those patients at greatest risk should prompt careful monitoring in the post-operative period and the provision of additional resources to ensure these patients are adequately supported.

In addition, this study demonstrated that while different pain outcomes are overlapping constructs, they have critical differences. Most studies do not measure resting pain and movement-evoked pain intensity separately[516] and few post-operative studies assess pain qualities. While all four pain outcomes were significantly correlated, the identification of overlapping but distinct predictors for each pain outcome suggests different mechanisms may be involved in various components of the pain experience, consistent with the multidimensional model of pain[555]. It is essential that future post-operative pain assessment and research consider these different outcome measures to better understand, prevent and manage post-operative pain. Inclusion of only one unidimensional measure may not be adequate. In addition, whether

these factors also play a role in the transition to chronic post-operative pain should be investigated in a longitudinal study. This analysis is currently underway for the sample examined in the current study.

Another important finding was the protective effect of baseline IL-10 on post-operative neuropathic pain. This has implications for understanding pain mechanisms and should provide the basis for future research investigating how this cytokine may be used as a biomarker or in pain prevention. As previously mentioned, there is some evidence in animal models that IL-10 administration can prevent and reverse neuropathic pain[126,371,561,588,589] and this should be explored. In addition, investigating the effect of local cytokine concentrations in the surgical field may provide more information on the autocrine and paracrine effects of inflammatory cytokines. Pain mechanisms involve both peripheral and central neuroplastic changes and one of the early steps in pain generation is the release of inflammatory cytokines from peripheral immune cells[113]. Since most cytokines operate at low concentrations, widespread concentration changes in the systemic circulation may be hard to identify. In addition, the site of investigation is important as a localized pain state may not always lead to systemic cytokine alterations[428]. Exploring the local environment could identify concentration changes at the surgical site that may not be detectable in the systemic circulation which could ultimately contribute to our understanding of pain mechanisms. The generation of IL-10 in the local tissue environment is particularly intriguing given that studies have shown increases in IL-10 in breast tumour tissue while systemic changes in IL-10 are less frequently reported[590]. Understanding how this local increase associated with breast tumour cells influences post-operative pain should be investigated, particularly

considering the protective effect against neuropathic pain observed in this study. This type of analysis could be conducted on biopsy tissue samples.

Similarly, psychological factors have been shown to be modifiable and the inclusion of a psychological variable in each model has both clinical and theoretical relevance. The pre-operative period may be a better time to address modifiable factors associated with post-operative recovery[591]. Therefore, identifying patients with high levels of anxiety or pain catastrophizing and addressing these factors in the pre-operative period could be an effective way to improve post-operative pain experiences.

A variety of psychosocial interventions have been shown to reduce anxiety, distress and/or pain catastrophizing and pain or physical function in various chronic pain[592–596] and cancer populations[597,598]. Importantly, some of these interventions have shown sustained decreases in anxiety, pain catastrophizing and pain[592] suggesting teaching these skills could have long-term positive effects for patients. Other studies have only reported changes in psychological variables without reporting on pain intensity[599,600], however, this adds to the evidence supporting the modifiability of these factors.

One challenge with this type of intervention in the pre-operative period is the extended duration of the therapy, with many spanning between 4-10 weeks, making use in the pre-operative period, when the time frame between identifying a need for surgery and surgery is relatively short, difficult. However, some have found a single session successfully reduced pain catastrophizing in chronic pain patients and this reduction was maintained four weeks after the intervention[601]. They did not investigate the impact on pain however, the short duration of the intervention suggests this would be feasible in the pre-operative period. A review on pre-

operative psychosocial interventions also reported the effectiveness of both pharmacological and non-pharmacological interventions for reducing pre-operative anxiety[602].

A variety of studies have shown a decrease in anxiety after pre-operative psychological or educational interventions[603–605]. Lin et al. (2005) also reported a simultaneous decrease in pain and pain interference accompanying the decrease in anxiety supporting the value of further investigating pre-operative interventions to address psychological risk factors prior to surgery[603]. Similar results have been reported for interventions aiming to reduce pain catastrophizing in surgical patients[606] and chronic pain patients[607].

In terms of pharmacological interventions, Clarke et al. (2013) reported treatment with Gabapentin reduced both pre-operative anxiety and pain catastrophizing[608]. Other studies have also reported decreases in pre-operative anxiety using a variety of pharmacological agents[602,609].

The importance of anxiety and pain catastrophizing in predicting post-operative pain observed in the current study, in combination with the promising results from investigations on pre-operative psychosocial interventions, support the need to further explore psychosocial treatments to reduce pre-operative anxiety and pain catastrophizing as a preventative measure to reduce post-operative pain.

6 CONCLUSIONS

In conclusion, the findings of this study strongly support the biopsychosocial model of pain and the need to consider biological, psychological and social factors when predicting and managing pain. Each pain outcome, pain intensity at rest, pain intensity with movement, pain qualities and neuropathic pain, was predicted by a range of biological or demographic factors, psychological factors and surgical factors. Critically, while the models had similarities they also had differences, emphasizing the importance of considering multiple pain outcomes to effectively prevent and treat post-operative pain. The modifiable nature of some of the variables in each model, in particular baseline IL-10, anxiety and pain catastrophizing, suggests these could represent promising targets for interventions to prevent or reduce post-operative pain after breast cancer surgery. The findings of this study provide a basis for future research to confirm the role of these risk factors and investigate interventions targeting the modifiable variables identified in this study.

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APPENDICES

APPENDIX A: QUESTIONNAIRES

Short Orientation-Memory-Concentration Test (SOMC)

Ask each question. Score 0 for incorrect answer, and indicated score for each correct answer or part of answer correct. Self-correction is allowed. Indicate date of test at top.

Question	Scoring if correct		Score
What year is it now?	4		
Answer			
What month is it now?	3		
Answer			
Repeat this address			
Address chosen (A, B, C, D)			
About what time is it now? (Correct if within one hour)	3		
Answer			
Count backwards from 20 to 1 *Two points off for each error*	4 or 2		
Say the months in reverse order *Two points off for each error*	4 or 2		
Repeat the address given *Two points off for each error*	10, 8, 6, 4, or 2		
TOTAL SCORE			

Address A	**Address B**	**Address C**	**Address D**
Mr. John / Brown, 42 / West Street, Gateshead	Mr. Joe / Smith, 34 / Church Road Banbury	Mr. Tom / White, 26 / Station Road, Aylesbury	Mr. Philip / Jones, 18 / North Way, Oxford

/ = marks separate items within address

Researcher's Initials: _____

Confusion Assessment Method Instrument (CAM)

Acute Onset:

1. Is there evidence of an acute change in mental status from the patient's baseline? Yes / No

Inattention

2. A. Did the patient have difficulty focusing attention, for example, being easily distractible, or having difficulty keeping track of what was being said? *(check answer below)*

- ❑ Not present at any time during interview.
- ❑ Present at some time during interview, but in mild form.
- ❑ Present at some time during interview, in marked form.
- ❑ Uncertain.

B. (If present or abnormal) Did this behavior fluctuate during the interview, that is, tend to come and go or increase and decrease in severity? *(check answer below)*

- ❑ Yes.
- ❑ No.
- ❑ Uncertain.
- ❑ Not applicable.

C. (If present or abnormal) Please describe this behavior:

_____

_____

Disorganized thinking

3. Was the patient's thinking disorganized or incoherent, such as rambling or irrelevant conversation, unclear or illogical flow of ideas, or unpredictable switching from subject to subject?
Yes / No

Altered level of consiousness

4. Overall, how would you rate this patient's level of consciousness?

- ❑ Alert (normal).
- ❑ Vigilent (hyperalert, overly sensitive to environmental stimuli, startled very easily).
- ❑ Lethargic (drowsy, easily aroused).
- ❑ Stupor (difficult to rouse).
- ❑ Coma (unarousable).
- ❑ Uncertain.

Disorientation

5. Was the patient disoriented at any time during the interview, such as thinking that he or she was somewhere other than the hospital, using the wrong bed, or misjudging the time of day? Yes / No

Memory impairment

6. Did the patient demonstrate any memory problems during the interview, such as inability to remember events in the hospital or difficulty remembering instructions? Yes / No

Perceptual disturbances

7. Did the patient have any evidence of perceptual disturbances, for example, hallucinations, illusions, or misinterpretations (such as thinking something was moving when it was not)? Yes / No

Psychomotor agitation

8. Part 1:
At any time during the interview, did the patient have an unusually increased level of motor activity, such as restlessness, picking at bedclothes, tapping fingers, or making frequent sudden changes of position? Yes / No

Psychomotor retardation

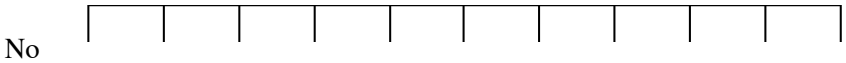
8. Part 2:
At any time during the interview, did the patient have an unusually decreased level of motor activity, such as sluggishness, staring into space, staying in one position for a long time, or moving very slowly? Yes / No

Altered sleep-wake cycle

9. Did the patient have evidence of the sleep-wake cycle, such as excessive daytime sleepiness with insomnia at night? Yes / No

Numeric Rating Scale – Rest (NRS-R)

Please circle the number below which best represents your ***present*** pain, the pain you are feeling right at this moment.



No pain 0 1 2 3 4 5 6 7 8 9 10 Pain as bad as it could be

Numeric Rating Scale – Movement (NRS-M)

Instructions: Patient is to roll from a supine to side-lying position and perform two maximal inspirations before rating their pain.

Please circle the number below which best represents your ***present*** pain, the pain you are feeling right at this moment.

No pain 0 1 2 3 4 5 6 7 8 9 10 Pain as bad as it could be

Short-Form McGill Pain Questionnaire (SF-MPQ)

Please indicate which words best describe your pain at present. I will read you each word and if the word describes your pain, I will ask you to rate the intensity of that characteristic as mild, moderate or severe.

	NONE	MILD	MODERATE	SEVERE
THROBBING	0)_____	1)____	2)____	3)____
SHOOTING	0)_____	1)____	2)____	3)____
STABBING	0)_____	1)____	2)____	3)____
SHARP	0)_____	1)____	2)____	3)____
CRAMPING	0)_____	1)____	2)____	3)____
GNAWING	0)_____	1)____	2)____	3)____
HOT-BURNING	0)_____	1)____	2)____	3)____
ACHING	0)_____	1)____	2)____	3)____
HEAVY	0)_____	1)____	2)____	3)____
TENDER	0)_____	1)____	2)____	3)____
SPLITTING	0)_____	1)____	2)____	3)____
TIRING-EXHAUSTING	0)_____	1)____	2)____	3)____
SICKENING	0)_____	1)____	2)____	3)____
FEARFUL	0)_____	1)____	2)____	3)____
PUNISHING-CRUEL	0)_____	1)____	2)____	3)____

PPI

Please choose the word which best describes your pain at the present moment.

0 NO PAIN ____
1 MILD ____
2 DISCOMFORTING ____
3 DISTRESSING ____
4 HORRIBLE ____
5 EXCRUCIATING ____

NEUROPATHIC PAIN QUESTIONNAIRE – Short Form

Please use the items below to rate your pain as it usually feels. Indicate a number which represents your pain on each scale. For example, if you have no tingling pain, you would rate the first item "0". If you have the worst tingling pain imaginable, you would rate it "100". IF neither of those fits your pain because it is in between, choose a number which fits your pain.

1. Tingling Pain

0 ← ═══════════ → 100

Please rate your usual pain: _____

No Tingling Pain

Worst Tingling Pain Imaginable

2. Numbness

0 ← ═══════════ → 100

Please rate your usual pain: _____

No Numbness Sensation

Worst Numbness Imaginable

3. Increased pain due to touch

0 ← ═══════════ → 100

Please rate your usual pain: _____

No Increase At All

Greatest Increase Imaginable

Charlson Comorbidity Index (CCI)
(Charlson et al., 1987)

Patient Age: _____ years

Does the Patient Have: (check appropriate response)

AIDS?	__ YES	__ NO
Cerebrovascular Disease?	__ YES	__ NO
Chronic Pulmonary Disease?	__ YES	__ NO
Congestive Heart Failure?	__ YES	__ NO
Connective Tissue Disease?	__ YES	__ NO
Dementia?	__ YES	__ NO
Hemiplegia?	__ YES	__ NO
Leukemia?	__ YES	__ NO
Malignant Lymphoma?	__ YES	__ NO
Myocardial Infarction?	__ YES	__ NO
Peripheral Vascular Disease?	__ YES	__ NO
Ulcer Disease?	__ YES	__ NO

Select the appropriate column for each condition; give only 1 answer per row

Diabetes Mellitus	__ NONE	__ Without End Organ Damage	__ With End Organ Damage	
Liver Disease	__ NONE	__ MILD	__ MODERATE	__ SEVERE
Renal Disease	__ NONE	__ MILD	__ MODERATE	__ SEVERE
Malignant Solid Tumour	__ NONE	__ MILD	__ MODERATE	__ SEVERE

Karnofsky Performance Index (KPI)

100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity, minor signs or symptoms of disease
80	Normal activity with effort, some signs or symptoms of disease
70	Cares for self. Unable to carry on normal activity or to do active work
60	Requires occasional assistance, but is able to care for most of his needs
50	Requires considerable assistance and frequent medical care
40	Disables, requires special care and assistance
30	Severely disabled, hospitalization is indicated although death is not imminent
20	Hospitalization is necessary, very sick, active supportive treatment necessary
10	Moribund, fatal processes progressing rapidly
0	Dead

CES-D

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

Rarely or none of the time (less than 1 day)
Some or a little of the time (1-2 days)
Occasionally or a moderate amount of the time (3-4 days)
Most or all of the time (5-7 days)

During the Past Week	Rarely	A little	Moderate	Most
1. I was bothered by things that usually don't bother me…….	0	1	2	3
2. I did not feel like eating; my appetite was poor…….	0	1	2	3
3. I felt that I could not shake off the blues Even with help from my family or friends….	0	1	2	3
4. I felt that I was just as good as other people...	0	1	2	3
5. I had trouble keeping my mind on what I was doing……...	0	1	2	3
6. I felt depressed……	0	1	2	3
7. I felt that everything I did was an effort…….	0	1	2	3
8. I felt hopeful about the future……	0	1	2	3
9. I thought my life had been a failure……	0	1	2	3
10. I felt fearful…….	0	1	2	3
11. My sleep was restless…….	0	1	2	3
12. I was happy…….	0	1	2	3

During the Past Week	Rarely	A little	Moderate	Most
13. I talked less than usual……	0	1	2	3
14. I felt lonely……..	0	1	2	3
15. People were unfriendly……	0	1	2	3
16. I enjoyed life……...	0	1	2	3
17. I had crying spells……	0	1	2	3
18. I felt sad……..	0	1	2	3
19. I felt that people disliked me……	0	1	2	3
20. I could not get "going"……	0	1	2	3

Pain Catastrophizing Scale (PCS)

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

0	1	2	3	4
Not at all	*To a slight degree*	*To a moderate degree*	*To a great degree*	*All the time*

When I am in pain…

1. I worry all the time about whether the pain will end.	**1**	**2**	**3**	**4**	**5**
2. I feel I can't go on.	**1**	**2**	**3**	**4**	**5**
3. It's terrible and I think it's never going to get any better.	**1**	**2**	**3**	**4**	**5**
4. It's awful and I feel that it overwhelms me.	**1**	**2**	**3**	**4**	**5**

0	1	2	3	4
Not at all	*To a slight degree*	*To a moderate degree*	*To a great degree*	*All the time*

5. I feel I can't stand it anymore.	**1**	**2**	**3**	**4**	**5**
6. I become afraid that the pain will get worse.	**1**	**2**	**3**	**4**	**5**
7. I keep thinking of other painful events.	**1**	**2**	**3**	**4**	**5**
8. I anxiously want the pain to go away.	**1**	**2**	**3**	**4**	**5**
9. I can't seem to keep it out of my mind.	**1**	**2**	**3**	**4**	**5**
10. I keep thinking about how much it hurts.	**1**	**2**	**3**	**4**	**5**
11. I keep thinking about how badly I want the pain to stop.	**1**	**2**	**3**	**4**	**5**
12. There's nothing I can do to reduce the intensity of the pain.	**1**	**2**	**3**	**4**	**5**
13. I wonder whether something serious may happen.	**1**	**2**	**3**	**4**	**5**

State-Trait Anxiety Inventory (STAI-S)

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then blacken the appropriate circle to the right of the statement to indicate how you *feel* right now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answers which seem to describe your present feelings best.

	not at all	somewhat	moderately so	very much so
1. I feel calm ..	○	○	○	○
2. I feel secure	○	○	○	○
3. I am tense	○	○	○	○
4. I am regretful	○	○	○	○
5. I feel at ease	○	○	○	○
6. I feel upset	○	○	○	○
7. I am presently worrying over possible misfortunes	○	○	○	○
8. I feel rested	○	○	○	○
9. I feel anxious	○	○	○	○
10. I feel comfortable	○	○	○	○
11. I feel self-confident	○	○	○	○
12. I feel nervous	○	○	○	○
13. I am jittery	○	○	○	○
14. I feel “high strung”	○	○	○	○
15. I am relaxed	○	○	○	○
16. I feel content	○	○	○	○
17. I am worried	○	○	○	○
18. I feel over-excited and “rattled”	○	○	○	○
19. I feel joyful	○	○	○	○
20. I feel pleasant	○	○	○	○

Researcher’s Initials: _____

STAI - T

Instructions: Read each statement and then select the appropriate response to indicate how you generally feel. Do not spend too much time on any one statement, but give the answer which seems to describe your present feelings best.

	Not At All	A Little	Somewhat	Very Much So
1. I feel calm				
2. I feel secure				
3. I feel tense				
4. I feel strained				
5. I feel at ease				
6. I feel upset				
7. I am presently worrying over possible misfortunes				
8. I feel satisfied				
9. I feel frightened				
10. I feel uncomfortable				
11. I feel self confident				
12. I feel nervous				
13. I feel jittery				
14. I feel indecisive				
15. I am relaxed				
16. I feel content				
17. I am worried				
18. I feel confused				
19. I feel steady				
20. I feel pleasant				

APPENDIX B: DATA COLLECTION TIMELINE

Assessment Time	Pre-admission visit	OR day before surgery	Intra-operatively	6 hours post-op.	24 hours post-op.	1 week, 6 weeks, 3 months, and 6 months post-op.
Pain Measures						
Pain History	X					
SF-MPQ	X	X			X	H
NRS-R	X	X		X	X	X
NRS-M	X	X		X	X	X
Satisfaction					X	X
BPI	H					H
Physical Function and Well Being Measures						
Arm Function (range of motion, grip strength, edema)	X				X	X
Menstrual Status	X	X				X
Wound healing						X
ESAS	X	X			X	X
SF-12	H					H
CCI	RA					RA
KPI	RA					RA
Cognitive Screen						
SOMC	X					X
CAM				X	X	
Psychological Measures						
PASS	H					
STAI	H (S & T)	X (S)			X (S)	H (S)
PCS	H					
Allodynia Measures (Quantitative Sensory Testing)						
Punctate, thermal & pressure pain threshold	X Baseline I	X Baseline II			X	X
Blood Work						
Estradiol		X			X	X (6 weeks excluded)
Progesterone		X			X	X (6 weeks excluded)
Cytokines	X	X			X	X (6 weeks excluded)
Morphine, M3G, M6G					X	
Analgesic Consumption						

APPENDIX C: MODEL ASSUMPTION TESTING

NRS-R

The Durbin-Watson statistic for the NRS-R model was 1.836. The average variance inflation factor (VIF) value, a measure of multicollinearity, was 1.057, indicating no multicollinearity. Outliers were assessed using standardized residuals. 7.2% of standardized residuals were above 2 and the largest standardized residual had an absolute value of 2.14 suggesting no outliers. The standardized residuals were normal based on a histogram and z-values of skewness and kurtosis. All Cook's distance values were below 1 and the highest value was 0.085. All leverage values were below the proposed cut-off suggesting no cases exerted undue influence on the model.

NRS-M

The Durbin-Watson statistic for this model was 1.657. The average VIF value was 1.1535. All standardized residuals were below the cut-off of 3.3. The model for movement-evoked pain had one individual with a standardized residual of 3.177. All leverage values were below the designated cut-off and all Cook's distances were below the threshold of 1. In addition, the standardized residuals were normal according to a histogram and z-scores of skewness and kurtosis. There were only two cases with standardized residuals greater than 2, so a total of 2.4% of cases were above 2.

SF-MPQ

The Durbin-Watson statistic for the model predicting SF-MPQ scores was 1.535. The average VIF value was 1.067. VIF values met the designated criteria to prevent multicollinearity. Standardized residuals were normal and all were below 3. The largest was 2.79. The largest Cook's distance was 0.154 and all leverage points were below the designated cut-off.

SF-NPQ

The Durbin-Watson statistic was 1.545. The average VIF value in the final model was 1.02, indicating no multicollinearity issues. Standardized residuals were normally distributed based on a histogram and z-scores of skewness and kurtosis. The highest standardized residual was 2.16. The highest Cook's distance was 0.11 and all leverage values were below the designated cut-off.