EVALUATING LOCAL SKIN HEATING AS AN EARLY DETECTION METHOD FOR SMALL-FIBER NEUROPATHY IN WOMEN WITH BREAST CANCER RECEIVING PACLITAXEL (TAXOL®)

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To my mother, H.K.Z, for her unwavering support and love.

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EVALUATING LOCAL SKIN HEATING AS AN EARLY DETECTION METHOD FOR SMALL-FIBER NEUROPATHY IN WOMEN WITH BREAST CANCER RECEIVING PACLITAXEL (TAXOL®)

The purpose of this prospective, observational study was to determine if a technique used to detect early signs of small-fiber neuropathy (local skin heating) could detect signs of small-fiber taxane-induced peripheral neuropathy (TIPN) in breast cancer survivors (BCS) during the first 6 weeks of Taxol®. Aims of the study were to compare the mean size of (1) axon reflexes and (2) axon flares (both markers of small fiber nerve function) in BCS receiving Taxol® to the size of reflexes/flares in healthy female controls (HCs). A third aim was to determine whether the size of axon reflexes/flares correlated with (a) overall TIPN severity and (b) severity of individual signs/symptoms of TIPN during early Taxol®.

Data for the study was collected from nine BCS and 20 HCs ($N = 29$). All BCS had first-time, non-metastatic cancer and received weekly or bi-weekly Taxol®. Data was collected at 3 time-points: Time 1 (day 0, before Taxol®), Time 2 (day 14), and Time 3 (day 42). Axon reflexes and flares were generated using a validated 40-minute skin heating protocol. Axon reflexes were measured using laser Doppler Flowmetry. Axon flares were measured using full-field laser perfusion imaging. TIPN was measured using the 5-item Short Form of the Total Neuropathy Score (Reduced Version).

Results identified potential signs of small-fiber TIPN in BCS after 6 weeks of Taxol®. Contrary to expectation, axon reflexes were larger for BCS at Time 3 than HCs, suggesting that Taxol® may be associated with an increase in small-fiber nerve function like that seen in pre-clinical studies. Clinical signs/symptoms of TIPN were not significantly correlated with axon reflexes or axon flares at the same time point. Analyses of axon flare size were confounded by issues with the data.

These results add to the growing body of evidence showing that Taxol® affects small-diameter sensory nerves and provides the first evidence in humans that changes in small-fiber nerve function may be detectable after just 6 weeks of Taxol® therapy. Studies in larger samples are needed to validate these findings.

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LIST OF ABBREVIATIONS

Term	Abbreviation
Anthracycline + Cyclophosphamide followed by Taxol®	AC+T
A-Alpha	A-α
A-Beta	A-β
A-Delta	A-δ
Breast Cancer Survivor (Female)	BCS
Body-Mass-Index	BMI
Calcitonin Gene-Related Peptide	CGRP
Chemotherapy-Induced Peripheral Neuropathy	CIPN
Cutaneous Vascular Conductance	CVC
Cutaneous Vascular Conductance, Maximum	CVC_{MAX}
Cutaneous Vascular Conductance, Percentage of Maximum	$\%CVC_{MAX}$
Dorsal Root Ganglia/Ganglion	DRG
Eutectic Mixture of Local Anesthetics (2.5% lidocaine/prilocaine cream)	EMLA
Full-field Laser Perfusion Imager/Imaging	FLPI
Food and Drug Administration	FDA
Healthy Controls	HCs
Isolated Sensory Neurons	ISN
Laser Doppler Flowmetry	LDF
Laser Doppler Imaging	LDI
Laser Doppler Imaging, maximum (hyperemic) response	LDI_{MAX}
Laser-Speckle Contrast Imaging	LSCI
Mean Arterial Blood Pressure	MAP
Non-Steroidal Anti-Inflammatory Drugs	NSAIDS
Selective-Estrogen Receptor Modulator	SERM
Skin Blood Flow	SkBF
Surface Area Covered by Axon Flare, Percentage of Maximum Toe Size	$\%Toe_{MAX}$
Taxane-Induced Peripheral Neuropathy	TIPN

Tissue Perfusion Units	TPUs
Time of Day	TOD
Total Neuropathy Score, Reduced Version (Short Form)	TNSr-SF
Transient Receptor Potential Cation Channel, Sub-family V, Member 1	TRPV.1

CHAPTER ONE

INTRODUCTION

Introduction

The purpose of this research is to describe my efforts to test a potential early detection method for *taxane-induced peripheral neuropathy* (TIPN). TIPN is a form of peripheral nerve damage associated with the antineoplastic drug *paclitaxel* (Taxol®) (Stubblefield et al., 2009), which is used to treat breast cancer (National Comprehensive Cancer Network, 2016). Following approval by the FDA in 1998 (Center for Drug Evaluation and Research, 1998), Taxol® quickly became recognized as one of the most effective treatments for breast cancer to emerge in decades. Unfortunately, Taxol® (like the other drugs in the taxane family) is neurotoxic, causing signs and symptoms of peripheral neuropathy for an estimated 60-90% of women that receive the drug (known as *breast cancer survivors* (BCS) by the oncology community) (Argyriou et al., 2005; Lam et al., 2016; Osmani, Vignes, Aissi, Wade, Milani, Levy, et al., 2012).

Neurologically, TIPN is classified as a distal polyneuropathy (Han & Smith, 2013; Hausheer, Schilsky, Bain, Berghorn, & Lieberman, 2006), with signs and symptoms typically beginning in the tips of the fingers and toes, and moving up the limbs as exposure to Taxol® increases (Dougherty, Cata, Cordella, Burton, & Weng, 2004; Mielke et al., 2003; Mielke et al., 2005; Pachman et al., 2016)). The neurological changes associated with TIPN are primarily sensory in nature, although motor and autonomic symptoms, including changes in coordination, balance, and strength, can be observed (Chico et al., 2001).

Studies show that for BCS, the neurological changes associated with TIPN can be intensely distressing and disabling for some (Bakitas, 2007; Kuroi et al., 2009; Tanay, Armes, & Ream, 2016). TIPN can interfere with survivor's ability to work, drive, and achieve restful sleep (Bao et al., 2016; Dougherty et al., 2004; Jansen, Cooper, Dodd, & Miaskowski, 2011; Miaskowski et al., 2017; Tian, Chen, & Zhang, 2015; Tofthagen, Overcash, & Kip, 2012). In addition, research has also shown that the combination of numbness, muscle weakness, and changes to proprioception associated with TIPN can significantly increase BCS' risk for falling (Tofthagen et al., 2012), which remains a major source of preventable injury for BCS following treatment (Sweeney et al., 2006; Winters-Stone et al., 2011). In addition, recent economic analyses indicate that TIPN is associated with increased used of healthcare, higher

healthcare expenditure, and greater use of medications to manage neuropathy symptoms in the months and years after BCS finish cancer treatment (Calhoun et al., 2001; Pike, Birnbaum, Muehlenbein, Pohl, & Natale, 2012).

For all these reasons, there is a growing consensus among clinicians and researchers in the oncology community that something needs to be done to address TIPN (Stubblefield et al., 2009; Stubblefield, McNeely, Alfano, & Mayer, 2012). Despite this impetus, attempts to reduce both the incidence and the impact that TIPN has for the thousands of BCS who receive Taxol® each year has been largely unsuccessful. Current data indicates that use of Taxol® is on the rise; Taxol's ® low cost, wide availability, and efficacy have made it a pillar of treatment for many systemic breast cancer treatment regimens, but this has increased the number of BCS developing TIPN. Complicating efforts to curb the impact of TIPN, options for treating TIPN remain limited. Decades of research have yet to produce FDA-approved therapies to manage this toxicity (Hershman et al., 2014), and existing therapies for pain and neuropathy including opioids, non-steroidal anti-inflammatory drugs, anticonvulsants, anti-depressants, and calcium-channel blockers show little efficacy for preventing or managing TIPN (Hershman et al., 2014; Majithia, Loprinzi, & Smith, 2016; Mitchell et al., 2006; R. D. Rao et al., 2007).

Together, the strong reliance on Taxol® as a first-line therapy in the breast cancer setting and few treatment options for TIPN (one of the principle toxicities associated with the drug) can leave BCS with few options but to endure their TIPN or consider altering cancer treatment if symptoms continue to worsen. While the lack of clinical trials testing potential therapies for TIPN remains an important barrier to developing ways to manage TIPN more effectively (Hershman et al., 2014), basic issues about which mechanisms to target to prevent or treat TIPN, how best to target these mechanisms, and most importantly, whether any of these can be targeted without interfering with patient's cancer therapy continue to prevent faster progress on the therapeutic side (Miltenburg & Boogerd, 2014).

In addition to these concerns, unresolved questions about what type(s) of nerves are affected during Taxol® therapy, and in what ways, continue to slow the search for therapies for TIPN. Until recently, it was thought that Taxol® preferentially affected larger, myelinated nerves (Dougherty et al., 2004; Loprinzi et al., 2011b; Sahenk, Barohn, New, & Mendell, 1994). However, recent pre-clinical work has called this view into question, showing that

Taxol® is associated with changes to both large- and small-diameter sensory nerves (Gracias, 2011; Saad et al., 2016b; Sharma, Venkitaraman, Vas, & Rayman, 2015; Xiao & Bennett, 2008). Unfortunately, methods for evaluating small-fiber nerve damage in humans are limited, making it difficult to confirm that the changes in small-fiber nerve function observed in pre-clinical models of TIPN hold true for BCS. This is especially true for early detection methods that would make it possible for nurses to test small-fiber nerves, which cannot be measured using current methods available to nurses.

Therefore, the purpose of this research was to test a potential early-detection method for small-fiber TIPN capable of detecting signs of the toxicity using a physiological endpoint. In this study, I investigated whether a physiological technique successfully used to detect early signs of small-fiber neuropathy in other populations – local skin heating – could be used to detect early signs of small-fiber neuropathy in BCS receiving Taxol®.

Results of this study describe previously undocumented changes to small-fiber nerve function in BCS during the first 6 weeks of Taxol® therapy. Results of the study also report on the association between subclinical signs of small-fiber nerve function and overt (i.e., clinical) signs and symptoms of TIPN reported by BCS, helping to fill a gap in current symptom science around TIPN. These results, which include a detailed discussion of the benefits and drawbacks of using local skin heating to screen patients for TIPN in the clinical environment, provide important perspectives on whether local skin heating is likely to be an effective early detection method for TIPN.

Background and Significance

One of the most important advances to emerge in the past century for the treatment of female breast cancer was the discovery of the microtubule-stabilizing agent *paclitaxel* (Taxol®). Paclitaxel was first discovered in the bark of the Pacific Yew tree in the early 1960's. However, it would be more than 30 years until the finished drug, now known as Taxol®, would be approved for use in breast cancer (Center for Drug Evaluation and Research, 1998). Once approved though, Taxol® emerged as one of the most effective and versatile treatments for breast cancer in decades (Carbognin et al., 2015; Gandhi et al., 2015; Sparano et al., 2008).

Even while newer, more targeted therapies for breast cancer have entered the market, Taxol® has remained a backbone of systemic treatment regimens for breast cancer because of its efficacy, affordability, and unique mechanism of action (Becker et al., 2013). The recent

discovery that shorter, more frequent dosing with Taxol® can boost the efficacy of this already effective drug while reducing its impact on the immune system (a potentially lethal side effect of the drug) has only increased reliance on Taxol® (Carbognin et al., 2015; Gandhi et al., 2015; Sparano et al., 2008; Williams & Bryant, 2011).

Unfortunately, Taxol® is neurotoxic, leading to peripheral neuropathy for a high percentage of BCS who receive the drug. In the case of Taxol®, an estimated 60–90% of BCS who receive Taxol® develop TIPN during treatment. Symptoms are primarily sensory in nature and can lead to a range of painful and non-painful neuropathy symptoms, including different qualities of pain, burning or "shock-like" sensations, tingling, or areas of numbness that begin in the distal extremities and worsen as exposure to Taxol® increases (Dougherty et al., 2004; S. L. Wolf et al., 2012a). Partial or complete loss of deep-tendon reflexes, changes in coordination and balance, and weakness in the lower extremities (leading to "foot drop") are common for BCS with TIPN as well (T. Berger, Malayer, et al., 1997).

Studies in BCS show that the signs and symptoms associated with TIPN, alone or in combination with other symptoms patients face, can significantly increase BCS' risk for developing poor healthcare-related outcomes during and after treatment (Stubblefield et al., 2009). Examples of negative outcomes associated with TIPN for BCS include:

- Up to a 10-fold increased risk of falling due to changes in balance and strength (Bao et al., 2016; Tofthagen et al., 2012).
- Difficulty sleeping due to TIPN symptoms (Hong, Tian, & Wu, 2014; Kim et al., 2014; Tian et al., 2015; Tofthagen, McAllister, & McMillan, 2011; Tofthagen, McAllister, & Visovsky, 2013).
- Difficulty performing daily tasks, including those needed for performance at work (A. J. M. Beijers et al., 2016; Zanville et al., 2016).
- Thousands of dollars in additional expenses related to lost work and increased use of healthcare resources (Calhoun et al., 2001; Hess et al., 2015; Pike et al., 2012).
- Greater reliance on medication, including opioids, to manage symptoms (Hirayama, Sasaki, Dosaka-Akita, & Ishitani, 2016; Loprinzi et al., 2011b; Pike et al., 2012; Reeves et al., 2012).

Even more troublingly, several studies indicate that 30% or more of BCS receiving Taxol® may have to deviate from the recommended course of therapy because of their TIPN

symptoms (Bhatnagar et al., 2014; Speck et al., 2013), potentially jeopardizing the efficacy of their cancer treatment. The seriousness of these risks is made worse by the lack of FDA-approved interventions to prevent or treat TIPN, which often leaves providers with little choice but to monitor BCS closely to determine if TIPN is serious enough to justify altering treatment. Because of this, routine screening for TIPN is recommended for all BCS during Taxol® therapy (Mielke et al., 2003)

Nurses play a critical role in screening BCS for TIPN. In many practice settings, nurses perform the majority of TIPN screening (E. M. Smith et al., 2014), and are responsible for monitoring BCS' symptoms and communicating findings to the health team. Screening BCS for TIPN is important for many reasons. The primary goal of screening BCS for TIPN during treatment is to make it possible for nurses to detect signs and symptoms of neuropathy before they can pose a risk to their safety (Tofthagen, Visovsky, & Hopgood, 2013a; Visovsky, Meyer, Roller, & Poppas, 2008b). Screening BCS for TIPN can also help identify women that begin to show signs of TIPN earlier than others, which may signal BCS who are likely to develop particularly severe or long-lasting cases of TIPN (Baron, Haendler, & Schulte, 1997). Finally, detecting TIPN early in treatment can help both patients and providers to plan for potential interruptions to Taxol® therapy that may be needed.

Despite the importance of detecting TIPN early, results of a recent report by the National Comprehensive Cancer Network's Task Force on the Management of CIPN concluded that underassessment of TIPN is "…a significant problem" (Stubblefield et al., 2009). Research indicates that lack of time and lack of support are reasons why TIPN is under-assessed (E. M. Smith et al., 2014)). Studies show that another barrier nurses face in meeting the recommended screening guidelines for TIPN is the lack of accurate methods for detecting TIPN in the clinical setting (Cavaletti et al., 2010; Griffith, Dorsey, Renn, Zhu, Johantgen, Cornblath, Argyriou, Cavaletti, Merkies, Alberti, Postma, Rossi, Frigeni, Bruna, Velasco, Kalofonos, Psimaras, Ricard, Pace, Galie, Briani, Torre, et al., 2014; Hershman et al., 2014; Markman, 2006b; Ocean & Vahdat, 2004; E. M. Smith, 2013b). In particular, experts underscore the need for an "…easily obtainable monitoring test for the potential development of symptomatic peripheral neuropathy…" (Markman, 2006a) (p. 276), as well as the need for biomarkers that "…facilitate… monitoring of [neuropathy]…" during and after treatment (Kiernan, 2012) (p. 1346). Current screening tools for CIPN rely heavily on patient self-report

or provider judgement. Although these approaches to screening are fast and cost-effective, these methods often lack the reliability, sensitivity, and specificity needed to detect TIPN before the onset of symptoms (i.e., at the subclinical level), or to predict the trajectory that TIPN will take once symptoms begin.

Statement of the Problem

BCS are one of the populations most at-risk for developing TIPN. Currently, more than half of all BCS who receive Taxol® are estimated to develop some degree of TIPN during their cancer treatment (Seretny et al., 2014; Song et al., 2017). Studies show that the risk of developing TIPN is especially high for the BCS receiving their Taxol® on a weekly or dose-dense basis, compared to traditional Taxol® dosing (which is given every 3 weeks). Because of this, experts now recommend that, at a minimum, BCS be screened for signs of peripheral neuropathy before and routinely during cancer treatment (Tofthagen, Visovsky, & Hopgood, 2013b).

Before starting Taxol®, neuropathy screening is used to identify BCS who may be at-risk for TIPN and to determine BCS' level of risk. During treatment, neuropathy screening is used to detect early signs of TIPN (i.e., before symptoms begin to interfere with treatment or daily activities). It is also used to document the location, severity, and type of TIPN BCS are experiencing and to rule out other potential causes of neuropathy that may require different treatment e.g., nerve compression, metastases, diabetic or immunological neuropathy (Backonja & Galer, 1998; Park et al., 2008a; Stubblefield et al., 2009; Tofthagen, Visovsky, et al., 2013a; Visovsky et al., 2008b). Routine neuropathy screening during treatment is also important to guide clinical decision-making about which symptoms to prioritize, to develop a tailored plan for managing symptoms, what medications (if any) to consider prescribing to reduce symptom bother, and whether to seek assistance from other disciplines (e.g., physical therapy, pain management). By tracking the severity of patients' symptoms, routine neuropathy screening also helps identify BCS that may need more frequent or involved neurological testing (Hausheer, 2008; Wald, 2001).

However, options for detecting signs of TIPN before the onset of symptoms are limited. Currently, the majority of neuropathy screening performed by oncology nurses is performed using neuropathy grading scales, questionnaires, and simplified testing protocols for peripheral nerve function (Paice, 2009; Stubblefield et al., 2009). Studies show that, when

performed properly, these methods can be helpful for monitoring TIPN. However, because these approaches require patients to be showing symptoms before they can detect signs of TIPN, these approaches are poorly suited for detecting early subclinical signs of TIPN that may precede symptom(s) (Tofthagen, Visovsky, et al., 2013a).

More physiologically-based approaches for detecting neuropathy such as nerve conduction velocity (NCV) and needle electromyography (EMG) (both types of *electrodiagnostic* tests) can be valuable for identifying subtle signs of nerve damage in other types of neuropathy (e.g., metabolic disturbance, autoimmune disorders, nerve entrapments) (England et al., 2005; Li Pi Shan et al., 2016). However, evidence supporting the effectiveness of electrodiagnostic testing to detect signs of TIPN before the onset of symptoms is mixed. Studies show that, in some cases, electrodiagnostic testing failed to find evidence of neurological disruption in patients with overt clinical symptoms, or that electrodiagnostic testing could only detect TIPN after symptoms had started (Park et al., 2008b; Tavee & Zhou, 2009a). In addition, because electrodiagnostic testing requires specialized equipment and cannot be performed by nurses at the bedside (where the bulk of TIPN screening is performed) because of scope-of-practice issues, electrodiagnostic testing is a poor choice as an early detection method for TIPN.

In addition to the limitations just described, an important limitation of electrodiagnostic testing is that testing can only detect damage to larger myelinated nerves that innervate the extremities (T. Berger, Malayer, et al., 1997; Latov, 2007). As outlined in Table 1-1, sensory nerves can be classified by characteristics such as their size (i.e., diameter), conduction speed, degree of myelination, as well as the role they play in sensation. Large-diameter sensory nerves such as A-α and A-β, which are involved in the perception of touch and limb-position, are coated in myelin, which acts as an insulation for incoming signals (known as action potentials) (Campbell, 2013). BB contrast, small fiber nerves such as A-δ and C-fibers, (which are involved in the sensation of noxious stimuli, and heat/cold), have little or no myelination (Tavee & Zhou, 2009b). Because the electricity seeks the path of least resistance, the electrical impulses used during electrodiagnostic testing preferentially seek out larger fibers (which have less resistance due to their larger dimeter and better insulation). As a result, routine electrodiagnostic tests cannot be used to screen BCS in evaluating smaller

unmyelinated nerves sensory nerves such as A-δ and C-fibers (Casanova-Molla, Grau-Junyent, Morales, & Valls-Sole, 2011; Siao & Cros, 2003).

Table 1-1

Classification of Sensory Nerve Fibers

Fiber Type	Diameter	Conduction Speed (m/s)	Degree of Myelination	Role in Sensation
A-α	Large	80-120	Full	Proprioception
A-β	Large	35-75	Full/Mostly	Proprioception, light touch, vibration, pressure
A-δ	Small	12-36	None/Light	Cold, pain
C	Small	0.2-1.5	None	Heat/warmth, pain (slow), itch

Notes. Conduction speed values courtesy of: (Warren, 2018)

Potential Early Detection Method for Small-Fiber TIPN

Researchers studying another potential cause of small-fiber neuropathy ─ diabetes mellitus ─ have also been searching for ways to detect damage to small-fiber nerves in its early stages (Tavee & Zhou, 2009b). In the case of diabetes, early detection is vital because the damage to peripheral nerves is often insidious, becoming noticeable only after damage is extensive (Deshpande, Harris-Hayes, & Schootman, 2008). Recently, a method capable of detecting small-fiber neuropathy before the onset of symptoms in diabetic patients was identified. The technique, known as *local skin heating,* uses heat delivered through a small heat probe to stimulate temperature-sensitive small-fiber nerves (i.e., A-δ and C-fibers) embedded in the skin surface (Minson, 2010). In individuals with healthy small-fiber nerve function, the heat from the heat probe causes an initial response that is driven by the release of vasodilatory neuropeptides onto nearby capillaries, causing a sharp increase in skin blood flow (SkBF). This reflexive response to skin heating is known as *axon reflex-mediated vasodilation*.

There are two basic approaches used to measure axon reflex-mediated vasodilation during local skin heating: axon reflexes and axon flares (described below).

Axon Reflexes

One approach used to detect subclinical signs of small-fiber neuropathy is to use a Laser Doppler Flowmeter (LDF) to measure the increase in cutaneous blood flow that occurs in response to local skin heating. As depicted in Figure 1-1, in individuals with intact small-

Figure 1-1. Example of Axon Reflex Generated in the Cutaneous Skin in Response to 40-minute, 42°C Local Skin Heating.

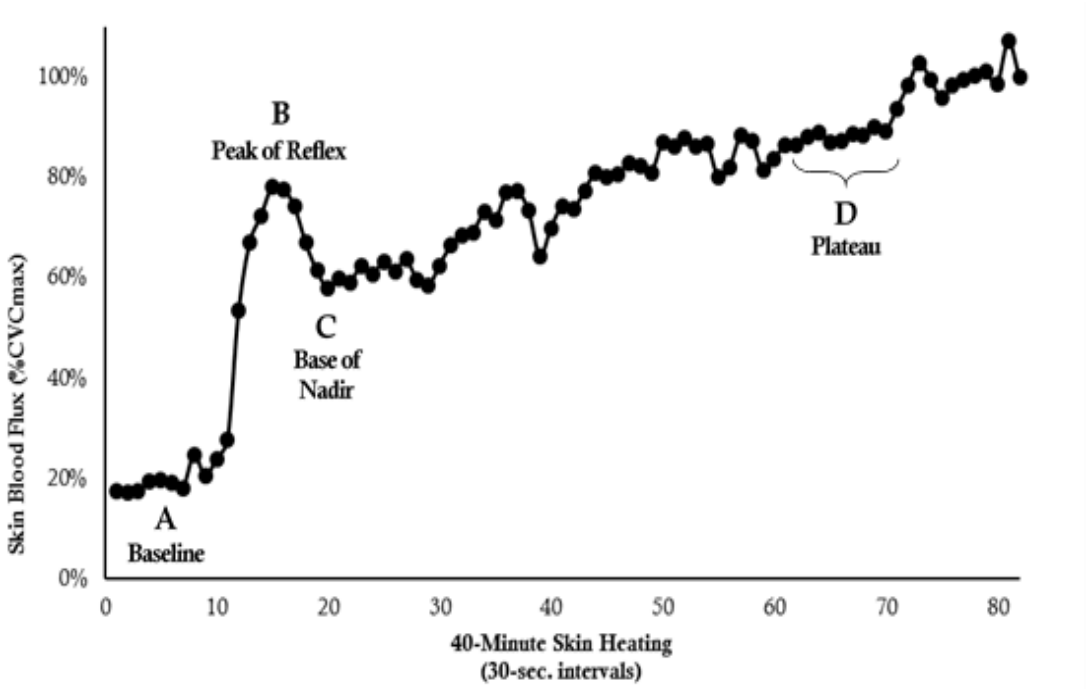

Figure 1-1. Points represent 30 seconds of skin blood flow (SkBF) data in the right toe surface during local skin heating. (A) Initial SkBF during 33 °C skin heating (used to establish baseline. (B) Peak of axon reflex after temperature of heat probe turned up to 42 °C (rate: 0.1 °C/1 sec). (C) Brief nadir in SkBF. (D) If 42 °C skin heating continues, SkBF continues to rise in slower fashion, typically plateauing near participant's theoretical maximum SkBF.

fiber nerve function, heating the skin between 39-44 °C causes SkBF to increase in a well-characterized pattern (Minson, Berry, & Joyner, 2001). During the first few minutes of local skin heating, SkBF rises quickly, often increasing three- to four-fold during the first 1-2 minutes.[1,2]. This initial peak in SkBF is known as the *axon reflex* (B, figure below), and it is primarily initiated by the (reflexive) release of neuropeptides such as calcitonin gene-related peptide and substance P from small-fiber sensory nerves located near blood vessels in the skin surface (S. T. Krishnan & G. Rayman, 2004; Mahe, Humeau-Heurtier, Durand, Leftheriotis,

[1] Like *circulatory vascular resistance* (which is an index of the amount of resistance the blood encounters as it passes through the vessels), *circulatory vascular conductance* (CVC) refers to the lack of resistance, or ease, with which blood passes through the vasculature. CVC is calculated by dividing the blood flux measured by the laser Doppler (which is usually expressed in arbitrary tissue perfusion units) by the participant's mean arterial blood pressure (MAP).

[2] Research shows that the in individuals with healthy nerve function, the axon reflex typically reaches 75–85% of the participant's *maximum circulatory vascular conductance* (CVC_{MAX}), which describes how much blood flow is passing through the vessels relative to the vessels' theoretical max.

& Abraham, 2012; Minson et al., 2001; P. R. Vas & G. Rayman, 2013b). Studies also show the transient receptor vanilloid-1 (TRPV.1) channel, which are widely-expressed on small-diameter sensory nerves, and which are activated at temperatures >42 °C and during noxious stimuli, also mediate the axon reflex response (B. J. Wong & Fieger, 2010).

If the local skin heating continues, following a brief nadir (C), SkBF will continue to rise in a longer, slower fashion until maximum vasodilation is reached (D). Unlike the initial axon reflex, which depends primarily on sensory afferents, studies show that this second increase in SkBF (the plateau) is driven primarily by the release of vasodilatory mediators from the endothelium of the blood vessel (Minson, Holowatz, Wong, Kenney, & Wilkins, 2002; Wilkins, Holowatz, Wong, & Minson, 2003; B. J. Wong & Minson, 2011b; B. J. Wong, Wilkins, Holowatz, & Minson, 2003; B. J. Wong, Wilkins, & Minson, 2004; B. J. Wong, Williams, & Minson, 2006). The size of the axon reflex (expressed as a percentage of participant's CVC_{MAX}) can be compared to normative values or to participant's own values to determine if changes in small-fiber nerve function are present.

Axon Flares

A second approach for small-fiber neuropathy in patients using local skin heating is to measure the size of the hyperemic flare that develops around the heat probe during local skin heating (Figure 1-2). This hyperemic area, known as an *axon flare*, typically extends 5-10 cm from the heat source. Like the axon reflex, studies show that the size of the axon flare also depends on small-fiber nerves (P. R. Vas & G. Rayman, 2013b). Because of this, measuring the size of the axon flare that develops in the skin after skin heating can provide another way to screen patients for signs of small-fiber neuropathy.

The size of the axon flare can be determined using several commercially available imaging methods, including Laser Doppler Imaging (LDI) and, more recently, Full-Field Laser Perfusion Imaging (FLPI) (Figure 1-3). As with the size of axon reflex that develops during skin heating, the size of the flare that develops after skin heating can be compared using flares recorded previously from patients with risk factors for neuropathy or can be compared to flares from healthy controls. Research shows that changes in flare size can detect signs of small-fiber neuropathy in otherwise asymptomatic patients, and can successfully distinguish patients with painful versus non-painful neuropathies (Bickel et al., 2002; Green, Krishnan, Finucane, & Rayman, 2010; Krämer, Schmelz, Birklein, & Bickel, 2004; S. T. Krishnan, C.

Quattrini, M. Jeziorska, R. A. Malik, & G. Rayman, 2009; S. T. Krishnan & G. Rayman, 2004; Nouri et al., 2012; P. R. Vas & G. Rayman, 2013a, 2013b; P. R. J. Vas, A. Q. Green, & G. Rayman, 2012; P. R. J. Vas & G. Rayman, 2013a, 2013b).

Studies also show that the size of the axon flare that develops in the skin during local

Figure 1-2. Axon Flare Developing around Heat Probe on Skin during Local Skin Heating

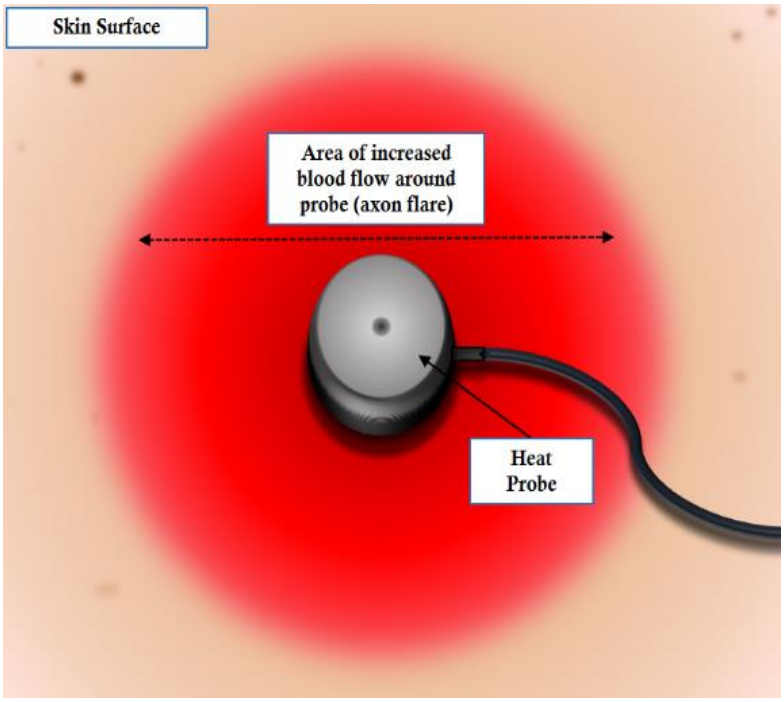

Figure 1-2. Illustration of axon flare (in red) developing around the heat probe (grey) on the surface of the skin during >42 °C local skin heating. Original artwork, copyright Noah Zanville, 2017.

skin heating is positively correlated with the density of small-fiber nerves in the skin (Andreas Bickel et al., 2009). This suggests that axon flares may be an alternative to invasive methods such as skin biopsy for studying small-fiber neuropathy, which can leave scars (Polston, 2018). In addition, results of a recent study, which found that BCS who received Taxol® showed signs of diminished flares nearly a year after treatment (Sharma, Venkitaraman, et al., 2015), suggest that axon flares may be useful in screening BCS for signs of long-term TIPN after treatment. To date, however, neither axon reflexes nor axon flares have been tested as an early detection method for TIPN in BCS receiving Taxol®.

Figure 1-3. Example of Axon Flare in Skin of Right Toe Measured with Full-Field Laser Perfusion Imaging (FLPI)

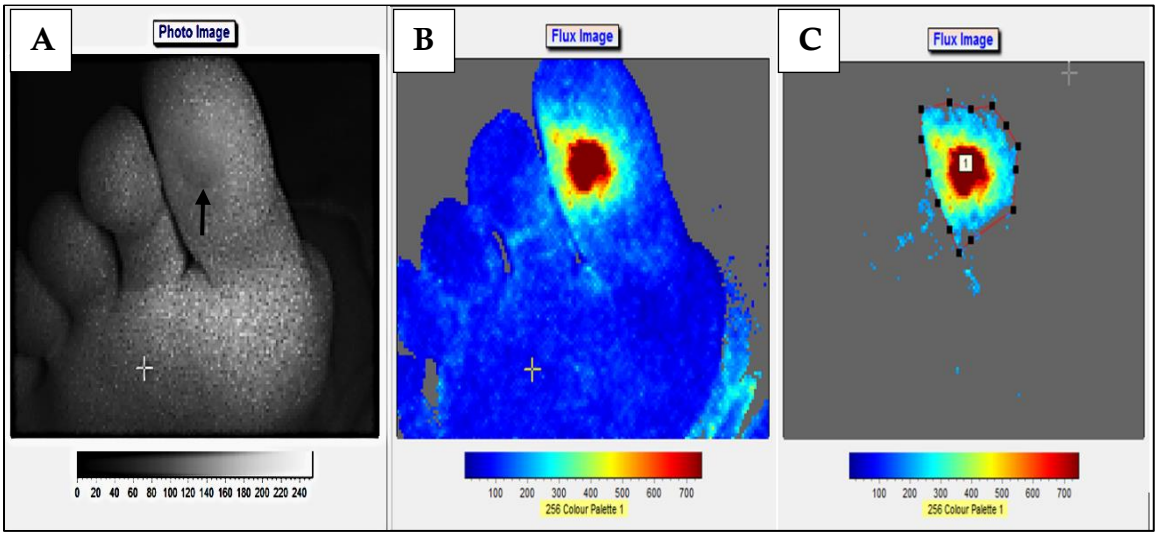

Figure 1-3. Panel A depicts the surface of the toe after 44 °C local skin heating viewed through imager's camera, in which the faint outline of the heat probe is still visible (black arrow). Panel B depicts cutaneous blood flow in surface of the toe visualized through the laser-speckle contrast imager. In the image, blue represent areas of lower blood flow, and red areas of higher blood flow. Panel C illustrates the size of the resulting axon flare, which is calculated by removing blood flow values from the surrounding skin which are lower than a pre-specified threshold. Original images**,** copyright Zanville and Champion, 2014 (unpublished).

Study Purpose and Aims

The purpose of this study was to determine whether the mean size of (a) axon reflexes or (b) axon flares in the right toe could be used to detect early signs of small-fiber TIPN in BCS receiving weekly Taxol®. Participants for the prospective study, which included both BCS and HC, were evaluated at 3 times points (Times 1, 2, and 3) occurring during the first 6 weeks of BCS' weekly Taxol® therapy. Specific aims of the study were:

Aim 1: To compare the mean size of axon reflexes (expressed as a $\%CVC_{MAX}$) in the right great toe of BCS receiving Taxol® during local skin heating to axon reflexes in the right toe of HC.

Hypothesis 1.1: The mean size of axon reflexes will not differ between BCS and HC prior to treatment with Taxol® (Time 1).

Hypothesis 1.2: The mean size of axon reflexes will differ significantly between BCS and HC at Times 2 and 3 (i.e., during Taxol® therapy).

Hypothesis 1.3: The mean size of axon reflexes will differ for BCS receiving Taxol® between Times 1, 2, and 3 (respectively).

Aim 2: To compare the mean size of axon flares (in cm^2) in the right great toe of BCS receiving Taxol® to axon flares in the right toe of HC.

Hypothesis 2.1: The mean size of axon flares will not differ between BCS and HC prior to treatment with Taxol® (Time 1).

Hypothesis 2.2: The mean size of axon flares will differ significantly between BCS and HC at Times 2 and 3 (i.e., during Taxol® therapy).

Hypothesis 2.3: The mean size of axon flares will differ for BCS receiving Taxol® between Times 1, 2, and 3 (respectively).

Aim 3: To determine if (a) the mean size of axon reflexes or (b) the mean size of axon flares are significantly correlated with concurrent measurement of a validated clinical measure for TIPN in BCS receiving Taxol®, the Total Neuropathy Score (Reduced Version, Short Form (TNSr-SF)).

Hypothesis 3.1: The mean size of axon reflexes in the right toe will correlate significantly with (a) the *overall severity* of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and (b) with the *severity of individual signs and symptoms* of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time point.

Hypothesis 3.2: The mean size of axon flares in the right toe will correlate significantly with (a) the *overall severity* of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and (b) with the *severity of individual signs and symptoms* of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time point.

Theoretical Model

This section introduces the theoretical model that guided the design of the study and selection of the study's aims. Information is divided into three parts. Part 1 describes the scientific assumptions of the model and provides evidence for why these assumptions are justified. Part 2 introduces the model and outlines the antecedent, covariate, and outcome

variables included in the model. Part 3 describes the instruments used to collect data on variables included in the model.

Part 1: Scientific Assumptions

The core premise of this study is that early signs of small-fiber TIPN could be detected in BCS receiving weekly Taxol® using either (1) a change in the mean size of axon reflexes that occurred in the right toe during local skin heating, or (2) a change in the mean size of axon flares that occurred in the right toe following local skin heating. Based on current evidence, we hypothesized that signs of small-fiber neuropathy might be visible as a difference in mean axon reflex/flare size between our two cohorts (BCS and HCs) during the first 6 weeks of Taxol® therapy (i.e., Times 2 and 3) (Hypotheses 1.2 and 2.2). We also hypothesized that any damage to small-fiber nerves in the toe would grow worse for BCS as exposure to Taxol® increased (i.e., difference in mean axon reflex/flare size between HC and BCS would be larger at Time 3 than at Time 2).

The premises that small-fiber TIPN can be detected during weekly Taxol® using local skin heating, and that these changes might be a useful early marker for TIPN in BCS is predicated on the following number of scientific assumptions:

1. Taxol® not only affects large-diameter sensory nerves, but affects small-diameter sensory nerves (i.e., A-δ and C-fibers) as well.
2. Taxol® impacts a subset of A-δ and C-fibers specifically involved in heat sensation which also help to regulate the motor tone of blood vessels in the surface of the skin.
3. Changes in temperature-sensitive small-diameter sensory nerves are detectable in the distal extremities, where local skin heating is performed.
4. Damage to small-fiber dependent blood flow is detectable using local skin heating with either LDF, in the case of axon reflexes, or full-field laser perfusion imaging, for heat-evoked axon flares.
5. Signs of damage to thermosensitive small fibers that occur during Taxol® therapy develop before signs/symptoms of TIPN are detectable using current neuropathy scales and testing algorithms in a way that would suggest that skin heating may be a useful detector of early signs of small-fiber TIPN in BCS receiving Taxol®.

6. Exposure to Taxol® does not alter the function of peripheral microvasculature or the vasodilatory peptides needed to complete the axon reflex in a way that would confound the test.
7. As exposure to Taxol® increases, changes in axon reflexes and axon flares will become more pronounced.
8. This trend of changes is detectable during the first 6 weeks of treatment (the time frame in which the study takes place).

Support for these assumptions is summarized in Table 1-2:

Table 1-2

Evidence Supporting Assumption that Local Skin Heating Can Be Used to Detect Early Signs of TIPN in the Right Toe during Weekly Taxol® Therapy

Assumption	Evidence	Model(s)	Reference(s)
Assumption #1: Taxol® affects thermosensitive small-fiber sensory nerves	Evidence Taxol® affects structure of small-fiber nerves		
	• Exposure to Taxol® is associated with degeneration of small-fiber nerves in both the cornea and distal extremities, including shortening of axon length, and accumulation of cellular debris.	In Vivo (Rats) In Vivo (Mice) In Vivo (Humans) In Vitro (ISN)	(Authier, Gillet, Fialip, Eschalier, & Coudore, 2000; V. A. Carozzi et al., 2010; Ferrari, Nalassamy, Downs, Dana, & Oaklander, 2013; Gracias, 2011; Kawashiri et al., 2009; Nakata & Yorifuji, 1999; Nennesmo & Reinholt, 1988; Roytta & Raine, 1986; Chiang Siau, Wenhua Xiao, & Gary J. Bennett, 2006)
	• Exposure to Taxol® is not associated with a loss of small-fibers in proximal nerves (e.g., the saphenous nerve, sciatic nerves).	In Vivo (Rats)	(V. A. Carozzi et al., 2010; Flatters & Bennett, 2006)
	• Exposure to Taxol® is associated with infiltration of macrophages and glial cells in DRG of small-fiber nerves.	In Vitro (Rats)	(Peters, Jimenez-Andrade, Kuskowski, Ghilardi, & Mantyh, 2007)
	• In humans, exposure to Taxol® is associated with deficits in C-fiber-mediated sudomotor function (i.e., sweating).	In Vivo (Humans)	(Saad et al., 2016b)

	• Exposure to Taxol® is associated with a dose-dependent reduction in cornel small-fiber nerves.	In Vivo (Mice)	(Ferrari et al., 2013)
	• Evidence Taxol® affects function of small-fiber nerves		
	• Exposure to Taxol® is associated with thermal hyperalgesia in rats.	In Vivo (Rats)	(Pascual, Goicoechea, Burgos, & Martín, 2010)
Assumption #2: Taxol® affects a population of temperature-sensitive small-fiber nerves that also help to regulate the reactivity of cutaneous blood vessels	• Exposure to Taxol® reduces the amount of small-fiber mediated vasodilation in response to both capsaicin and transcutaneous electrical stimulation.	In Vivo (Rats)	(Gracias, 2011)
	• Exposure to Taxol® decreases the expression of TRPV.1 channels in small-fiber nerves needed to vasodilate surrounding blood vessels.	In Vitro (ISN)	(Gracias, 2011)
Assumption #3: Damage to temperature-sensitive small-fiber nerves during Taxol® therapy occurs in the periphery (where	• Exposure to Taxol® is associated with a loss of small-fiber nerves in the cornea, which correlates with loss of intra-epidermal nerves in the periphery ($r = 0.66$).	In Vivo (Mice)	(Ferrari et al., 2013)
	• Exposure to Taxol® is not associated with a loss of small-fibers in proximal nerves (e.g., the saphenous nerve, sciatic nerves).	In Vivo (Rats)	(V. A. Carozzi et al., 2010; Flatters & Bennett, 2006)

local skin heating is assessed)			
	• Exposure to Taxol® is also associated with a loss of C-fiber-mediated sudomotor function in the periphery.	In Vivo (Humans)	(Saad et al., 2016b)
Assumption #4: Damage to temperature-sensitive small-fiber nerves during Taxol® therapy is detectable with local skin heating using either (a) axon reflexes or (b) axon flares	Evidence for Detectability Using Axon Reflexes:		
	• Exposure to Taxol® decreases the expression of the TRPV.1 channels in sensory nerves.	In Vitro (ISN)	(Gracias, 2011)
	• Exposure to Taxol® decreases the release of CGRP from small-fiber nerves in response to capsaicin.	In Vitro (ISN)	(Gracias, 2011)
	• Exposure to Substance P, a common inflammatory peptide release during pain, reduces the size of axon reflexes.	In Vivo (Human)	(B. J. Wong, Tublitz, & Minson, 2005)
	Evidence for Detectability Using Axon Flares:		
	• Anesthetic cream† abolishes axon flares, but does not affect maximum vasodilation during skin heating, confirming that axon flares are neurogenic in nature.	In Vivo (Humans)	(Krämer et al., 2004; S. T. Krishnan & G. Rayman, 2004; P. R. Vas & G. Rayman, 2013b)

	• Axon flare size is directly related to the severity of neuropathy in patients with diabetes.	In Vivo (Humans)	(S. T. Krishnan & G. Rayman, 2004; P. R. Vas & G. Rayman, 2013b)
	• The size of axon flares is inversely correlated with hemoglobin A1C levels ($r = -0.50$, $p < 0.001$), an important marker for diabetes.	In Vivo (Humans)	(P. R. Vas & G. Rayman, 2013a)
	• Axon flare size can detect signs of persistent small-fiber dysfunction in BCS approximately one-year after Taxol® therapy.	In Vivo (Humans)	(Sharma, Venkitaraman, et al., 2015)
	• Test-Retest reproducibility of axon flares generated in the dorsal foot $r = 0.83$ ($p < .0004$).	In Vivo (Humans)	(Krämer et al., 2004)
Assumption #5: Damage to temperature-sensitive small-fiber nerves during Taxol® therapy develops before signs and symptoms of taxane-induced neuropathy are	• Axon flare size can identify subclinical signs of small-fiber neuropathy in diabetic patients without clinically-detectable neuropathy.	In Vivo (Humans)	(Krämer et al., 2004; P. R. J. Vas et al., 2012)

detectable using available methods			
Assumption #6: Taxol® does not alter the function of blood vessels or vasodilatory peptides in a way that would confound the test.	• Exposure to Taxol® does not reduce the total amount of CGRP available to the nerve from the spinal cord in rats or in isolated sensory neurons.	In Vivo (Rats) In Vitro (ISN)	(Gracias, 2011; Pittman, Gracias, Vasko, & Fehrenbacher, 2013)
	• Exposure to Taxol® does not inhibit the release of CGRP from the spinal cord.	In Vivo (Rats) In Vitro (ISN)	(Gracias, 2011)
	• Taxol® does not interfere with the ability of the smooth muscle in the walls of blood vessels to vasodilate.	In Vivo (Rats)	(Gracias, 2011)
	• Taxol® does alter release of CGRP from nerve terminal in response to capsaicin.	In Vitro (ISN)	(Gracias, 2011)
Assumption #7: As exposure to Taxol® increases, the size of axon-reflexes/flares will decrease.	• Early exposure to Taxol® enhances CGRP-release from small-fiber nerves, while longer, higher-dose exposures to Taxol® decreases capsaicin-evoked CGRP-release from small-fiber nerves.	In Vitro (ISN)	(Pittman et al., 2013)

Assumption #8: That changes in small-fiber nerve function are likely to be detectable during the first six weeks of Taxol® therapy.	• A high percentage of BCS show initial symptoms of TIPN during the first two weeks of Taxol® therapy.	In Vivo (Humans)	(Forsyth et al., 1997; Loprinzi et al., 2011a; E. M. Smith et al., 2013; Takemoto et al., 2012; Tofthagen et al., 2012; Wiernik, Schwartz, Einzig, et al., 1987)

Part 2: Model Introduction

Theory addressing many aspects of TIPN is lacking. This includes theory addressing the role of early detection in managing TIPN. Because of this, a theoretical model describing proposed relationships between Taxol® exposure and the development of small-fiber TIPN had to be developed for this study. The model (presented in Figure 1-4) describes the hypothesized relationship between Taxol® exposure, subclinical changes in nerve function (including subclinical changes to small-fibers involved in the sensation of heat), and the appearance of clinically detectable signs/symptoms of TIPN.

Because of the interdisciplinary nature of the topic, research informing the development of the theoretical model was drawn from several areas. These include (1) pre-clinical research on the effect that Taxol® has on small-fiber nerves involved in the perception of heat and painful stimuli; (2) clinical research evaluating different screening methods for TIPN (including studies describing why early detection methods for TIPN are needed); and (3) research describing the use of axon reflexes and/or axon flares to detect subclinical signs of small-fiber neuropathy in other populations.

Figure 1-4. Theoretical Model Outlining Proposed Relationships among Taxol® Exposure, Subclinical Nerve Damage, and Clinically-Detectable Signs/Symptoms of Taxane-Induced Neuropathy in Breast Cancer Survivors (BCS)

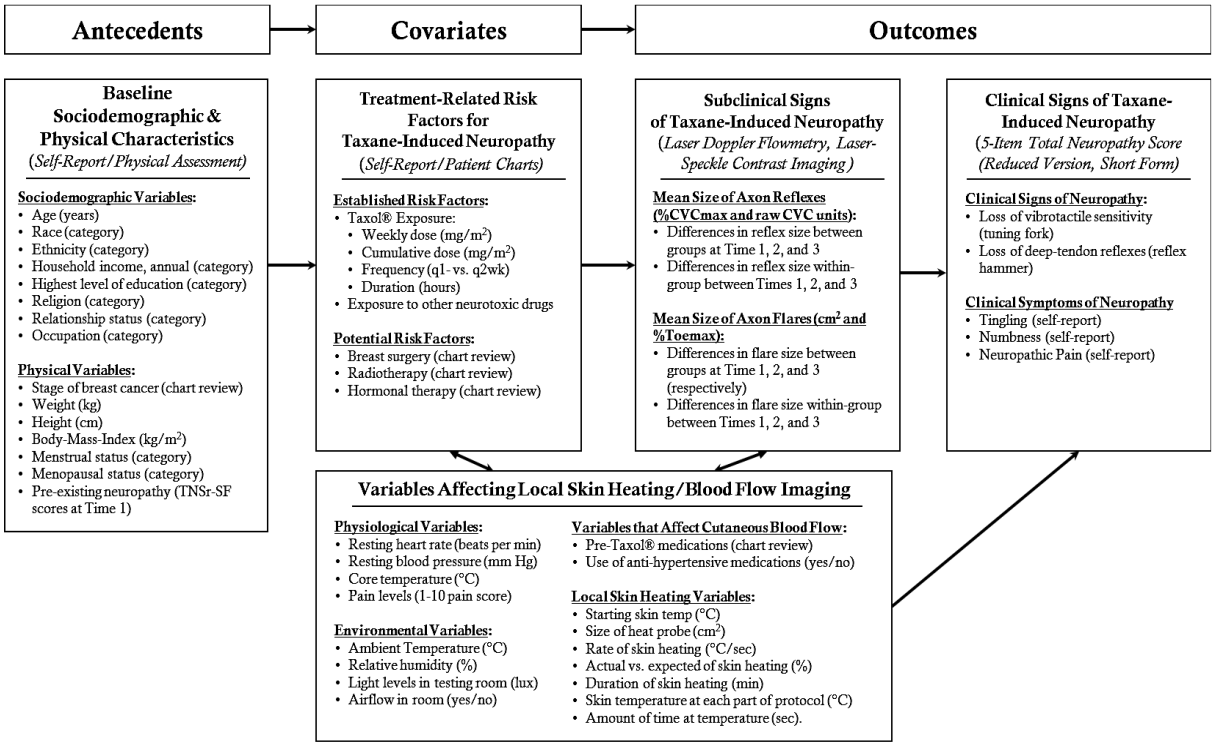

Figure 1-4. The above model illustrates (from left to right) the proposed relationships between BCS' (1) baseline characteristics, (2) exposure Taxol® (and other potential risk-factors for neuropathy) and the development of (3) subclinical changes in small-fiber sensory nerves detectable using local skin heating as either a change in mean size of axon reflexes or axon flares. As illustrated above, the model also proposes that these subclinical changes in small-fiber nerves will precede clinically-detectable signs and symptoms of small-fiber TIPN detectable using validated clinical screen tools for TIPN such as the Total Neuropathy Score (Reduced Version, Short Form; TNSr-SF). Because, theoretically, the ability to detect subclinical signs of small-fiber TIPN using local skin heating depends on a number of factors, the bottom portion of the model also includes variables known to affect both local skin heating and the accuracy of protocols which rely on non-invasive blood flow imaging.

Model Antecedents

The left side of the theoretical model describes characteristics that may predispose BCS to developing TIPN during weekly Taxol® (i.e., model antecedents). These variables include characteristics such as BCS' age at enrollment (in years); race (White, Black/African American, Native Hawaiian/Pacific Islander, Asian, American Indian/Alaskan Native, or other); ethnicity (Hispanic versus Non-Hispanic); estimated annual income (in dollars); level of education (highest attained, in years); relationship status (self-reported); religious/spiritual affiliation (self-reported); occupational category (professional, management/administration/ technical, clerical or service, homemaker, self-employed, unemployed, not employed, disabled; retired, student, or other); and stage of breast cancer (per current National Comprehensive Cancer Network staging guidelines).

The left side of the model also includes physical characteristics needed to interpret blood flow and neuropathy data collected during the study. These include physical characteristics such as height at baseline (in cm), weight (in kg), and body-mass-index (BMI) (in kg/m^2) for BCS at each visit. This list also includes evidence of previously undiagnosed neuropathy identified at the baseline visit.

Model Covariates

Risk Factors for TIPN. The middle portion of the theoretical model describes variables that have been shown to increase BCS' risk for developing TIPN during Taxol® therapy. These include established risk factors such as (1) Taxol® dose, frequency, and total exposure; (2) exposure to other neurotoxic agents, and (3) physical risk factors for TIPN such as participant height (Openshaw et al., 2005), BMI (Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge, et al., 2012), or diabetes/poor glycemic control (Bhatnagar et al., 2014; Johnson et al., 2015; Seretny et al., 2014; Visovsky, Meyer, Roller, & Poppas, 2008a; Wampler, Hamolsky, Hamel, Melisko, & Topp, 2005).

The middle portion of the theoretical model also includes potential risk factors for *TIPN* such as breast surgery and exposure to hormonal therapy. While studies have not shown that these interventions increase BCS' risk of developing TIPN directly, studies have shown that these interventions put BCS at risk for other types of neuropathy or painful symptoms. Radiation to the breast and upper chest wall, another potential cause of

neuropathy in BCS (Bruera, 2013) was not included in the list of potential risk factors for TIPN because having received prior radiotherapy was an exclusion criterion for the current study.

In addition, because the risk for developing TIPN during treament can be influenced by baseline characteristics (e.g., amount of Taxol® exposure being affected by stage of cancer), and vice versa (e.g., the genetics moderating the severity of taxane-induced neuropathy), the relationship between antecedent and covariate in the conceptual model are assumed to be bi-directional (this is depicted with bi-directional arrows).

Variables Influencing Ability to Detect Small-Fiber TIPN Using Local Skin Heating. The middle portion of the theoretical model lists variables that are shown to impact researchers' ability to detect small-fiber neuropathy using LDF and FLPI using local skin heating:

- ***Physiological variables*** including heart rate, mean arterial blood pressure (MAP), pain (Lei & You, 2012; Lei, You, Andersen, Graven-Nielsen, & Arendt-Nielsen, 2008), and medication with vasoactive properties (Dalle-Ave et al., 2004; Debbabi, Bonnin, Ducluzeau, Leftheriotis, & Levy, 2010; Houghton, Meendering, Wong, & Minson, 2006; Pauling et al., 2012; Rousseau et al., 2011; Tew, Klonizakis, Crank, Briers, & Hodges, 2011).
- ***Environmental variables*** such as ambient temperature, humidity, light levels, and air flow in the testing room (Mahe, Durand, Humeau-Heurtier, Leftheriotis, & Abraham, 2012; Roustit & Cracowski, 2012).
- ***Imaging variables*** such as the type of blood flow imager being used (Roustit & Cracowski, 2012), imager settings, and distance between the imager and the skin.
- ***Skin-heating variables*** including the size of the heating element used, area of skin being heated, baseline skin temperature, rate of skin heating, maximum temperature used, and duration of the heat stimulus (Minson, 2010), and time-of-day (TOD) testing is performed (Aoki, Kondo, Shibasaki, Takano, & Katsuura, 1997; L. A. Stephenson & Kolka, 1985).

The middle portion of the theoretical model also includes variables representing women's menopausal status (i.e., pre-, peri-, or post-menopausal) and menstrual status at each visit (actively menstruating versus not). Variations in progesterone and estrogen during

women's menstrual cycle have been shown to affect both thermoregulation and neurogenic responses to skin heating (Charkoudian & Johnson, 1997; Charkoudian, Stephens, Pirkle, Kosiba, & Johnson, 1999).

Model Outcomes

The right side of the model illustrates two outcomes related to small-fiber nerves hypothesized to follow exposure to Taxol®. According to the model, as exposure to Taxol® increases for BCS during the first 6 weeks of their cancer therapy, small-fiber nerves will be affected, leading to proximal outcome, subclinical neuropathy. According to the model, this subclinical small-fiber neuropathy will manifest as a change in the size of axon reflexes (Aim 1) and axon flares (Aim 2) in right toe for BCS during local skin heating at weeks 2 and 6 of weekly Taxol® therapy (Times 2 and 3) compared to their baseline visit (Time 1). The model also hypothesizes that at Times 2 and 3, subclinical signs of small-fiber neuropathy will be detectable as a difference in the size of both axon reflexes and axon flares for BCS compared to HCs.

The model also hypothesizes that as Taxol® therapy continues, BCS will develop a second outcome, developing overt (i.e., clinically-detectable) signs and symptoms of TIPN (the second outcome). According to the model, the *overall severity* of these clinically-detectable signs and symptoms of TIPN (measured by total scores BCS on the TNSr-SF at each time point) and the *severity of individual signs and symptoms* of TIPN (measured by total scores on each of the 5 TNSr-SF items at each time point) will correlate with severity of BCS' subclinical TIPN (meaured by the mean size of axon reflexes and axon flares at the same time point). A summary of instruments used to collect data for antecedent, covariate, and outcome variables appearing in the theoretical model is listed in Table 1-3.

Table 1-3

Instruments Used to Collect Data for Variables Listed in Theoretical Model

Type of Variable	Variable Category/Variable	Instruments
Antecedent Variables	Sociodemographics	
	Sociodemographics (e.g., age, race)	Demographic form
	Stage of breast cancer	Medical chart review
	Physical Variables	
	Height, weight	Stadiometer/scale

	Undiagnosed neuropathy	Total Neuropathy Score
Co-variables	Established Risk-Factors for TIPN	
	Dose, duration, frequency, and total exposure to Taxol®	Medical chart review
	Dose, duration, frequency, and total exposure to other neurotoxic agents	Medical chart review
	Obesity (BMI)	Calculated from height/weight
	Diabetes/Poor glycemic control	Medical chart review
	Potential Risk Factors for TIPN	
	Breast surgery	Medical chart review
	Radiotherapy	n/a
	Exposure to hormone therapy	Medical chart review
	Factors Affecting Blood Flow Response to Local Skin Heating	
	Resting heart rate (HR)/blood pressure (BP)	Automated heart rate/blood pressure monitor
	Current pain level	Numeric Pain Rating Scale
	Exposure to vasoactive drugs	Screening Form (self-report)
	Room temperature/humidity	Temperature/humidity probe
	Ambient light	Hand-held light meter
	Airflow in room	Researcher determination
	Menopausal status	Demographic form
	Factors Affecting Non-Invasive Blood Flow Imaging	
	Imager settings	Imager
	Distance between imager and skin	Standardized pre-experiment
	Size of heating element	Standardized pre-experiment
	Starting skin temperature	Skin heating unit
	Rate of skin heating	Digital watch
	Maximum temperature reached	Skin heating unit
	Duration of skin heating	Digital watch
Outcomes	Subclinical Signs of TIPN	
	Axon reflexes	Laser Doppler Flowmetry (LDF)
	Axon flares	Full-Field Laser Perfusion Imaging (FLPI)
	Clinically-Detectable Signs/Symptoms of TIPN	
	Tingling	5-Item Total Neuropathy Score (Reduced Version, Short Form)
	Numbness	
	Neuropathic Pain	
	Vibration sensitivity	
	Deep tendon-reflexes	

Summary

TIPN is a toxicity to the antineoplastic drug Taxol®, which is used to treat breast cancer. Studies show that BCS receiving weekly Taxol® are at risk for developing neuropathy which can interrupt cancer treatment and lead to chronic symptoms, leading to calls for reliable early detection methods for this toxicity (Binner, Ross, & Browner, 2011). Early detection methods for the small-fiber component of TIPN are needed to establish the prevalence of small-fiber TIPN in BCS receiving Taxol® and to determine whether preventing/repairing damage to small-fiber nerves should be a focus of therapeutic research addressing TIPN. A summary of the literature addressing these topics is reviewed in Chapter Two.

In addition to the contribution this study makes to efforts to identify potential screening tools for TIPN in BCS, this study makes several contributions specific to the field of Clinical Nursing Science. First, the study introduces a method that may allow nurses to detect changes in small-fiber sensory nerves, which is not currently possible. Nurses play a critical role in evaluating chemotherapy-related toxicities (Tofthagen, Visovsky, et al., 2013b). In many practice settings, the majority of TIPN screening is performed by nurses. This puts nurses in an ideal position to detect early signs of peripheral neuropathy during treatment (Binner et al., 2011). To do this, nurses must have access to tools that allow them to (1) detect changes in nerve function, (2) establish which portions of the nervous system have been affected (i.e., central vs. peripheral; sensory vs. motor vs. autonomic), (3) measure the extent of these changes, and (4) determine what risk(s) these changes pose for BCS so that a plan to keep patients safe can be developed. Furthermore, because screening methods for TIPN need to be fast enough for use in clinical environments (Binner et al., 2011), it is imperative that nurses play a role not only in validating potential screening methods but helping to develop them as well.

Second, by using a technique outside of those commonly used by nurse-researchers, this study helps to expand research in this area. Almost by their nature, drug-induced toxicities like TIPN do not fit within a single discipline. The number of areas affected by TIPN has made this toxicity a concern for oncologists, pharmacists, nurses, physical and occupational therapists, neurologists, health economists, healthcare administrators, and pre-clinical researchers. Traditionally, nurse-researchers working in this area have used more discipline-

specific and less technologically-dependent approaches to screen TIPN. While interdisciplinarity and technological dependence are not virtues in and of themselves, the poorly understood nature of the changes that accompany TIPN's onset, coupled with the limitations of self-report measures, argue for a more quantitative, physiologically-based approach to TIPN detection. By utilizing such a method, this study helps expand the range of options considered appropriate for nurses to explore the complex problem of TIPN.

CHAPTER TWO

REVIEW OF THE LITERATURE

The purpose of this chapter is to review the literature surrounding TIPN in BCS, including the need for methods to detect signs of TIPN before symptoms (i.e., early detection methods) begin. The chapter is divided into five sections. Section 1 begins by reviewing the literature on Taxol®, the primary cause of TIPN in BCS. Findings reviewed in this section include Taxol's® origins, mechanism of action, dosing, and neurotoxic properties. Section 1 also reviews current literature describing the incidence, severity, pathobiology, and treatment options for TIPN. In Section 2, literature describing the impact of TIPN on BCS is reviewed. Findings include the impact TIPN has on BCS' adherence to cancer treatment, and ability to function physically, sleep, and work, as well as their reliance on healthcare resources and analgesic medications. Section 3 summarizes what is known about established and potential risk factors for TIPN, while Section 4 explores the role that neuropathy screening, and early detection has in reducing BCS' risk for developing TIPN and TIPN-related outcomes. Section 5 finishes the chapter by describing a potential early detection method for small-fiber TIPN, local skin heating, which nurses cannot currently test for using available screening tools.

Section 1: Review of Literature on Paclitaxel (Taxol®)

History and Development of Paclitaxel (Taxol®)

The molecule that would one day give rise to Taxol® was first discovered in the forests of Washington during the summer of 1962 by Dr. Arthur Barclay, a botanist working for the U.S. Department of Agriculture. Two years earlier, Barclay and his team of graduate students had been enlisted by the National Cancer Institute to help with the search for plants with anticancer properties. In August of that year, Barclay and his team decided to collect bark from a Pacific Yew tree (*taxus brevafolia*), apparently at random, while exploring an area near Washington State's Mount St. Helens. Unbeknownst to them at the time, the bark they collected that day contained *paclitaxel*, a compound that would revolutionize the treatment of breast cancer (Isah, 2016).

Once back at the labs at the National Cancer Institute, initial tests showed that paclitaxel had significant potential as an anticancer therapy (Ginsberg, 2003; F. Stephenson, 2004). Despite displaying significant promise as a treatment for cancer, issues ranging from researcher's difficulty determining Taxol's ® exact chemical structure, to questions about how

to make the highly hydrophobic molecule infusible in blood, which is largely water, significantly slowed efforts to get Taxol® into the clinical setting. Furthermore, during the early years of Taxol® research, the enormous number of trees needed to generate even small quantities of the drug (1,500 trees per lb. of Taxol®, initially) kept Taxol® in the development pipeline for decades (F. Stephenson, 2004; Wall & Wani, 1995).

In 1998, nearly 30 years after its initial discovery, Taxol® was finally approved for use in female breast cancer by the Food and Drug Administration (National Cancer Institute (NCI), 2014). The completed drug could now be derived from the faster-growing English Yew rather than the slower-growing Pacific Yew (Ginsberg, 2003), and could be combined with Cremaphor EL®, a solvent, to make it soluble in the blood stream,[3] resulting in its emergence as a first-line therapy for breast cancer (Carbognin et al., 2015; Gandhi et al., 2015; Sparano et al., 2008).

Antineoplastic Mechanisms

Primary Antineoplastic Mechanism. Taxol's® primary antineoplastic mechanism of action is based on its ability to disrupt microtubules, tiny filaments which play key roles in cell division (known as *mitosis*) (Rowinsky & Donehower, 1991). Like all tissues, for tumors to grow, cells in the tumor must divide, creating new cancer cells. For this to occur, the cells must perform a carefully choreographed exchange of genetic material during the *anaphase* of mitosis (Perez, 2009; Stanton L. Gerson, 2018).

During anaphase, microtubules located on each side of the cell's nucleus must pull strands of genetic material that make up each chromosome (known as chromatids) to each side of the cell so that the new cells formed during mitosis have the correct amount of genetic material. The cell accomplishes this task by adding tiny heterodimeric proteins known as tubulin to one end of the microtubule (the "+" end), and shedding tubulin on the other end of the microtubule (the "-"end) (Figure 2) (Stanton L. Gerson, 2018).

[3] Cremaphor EL® is now known by its newer brand name, Kolliphor® EL (BASF SE, Germany). While this change has been the source of some confusion in the literature surrounding Taxol®, both names refer to the polyoxyethated castor oil used as a solvent for paclitaxel, which is highly hydrophobic.

Figure 2-1. Structure of a Microtubule

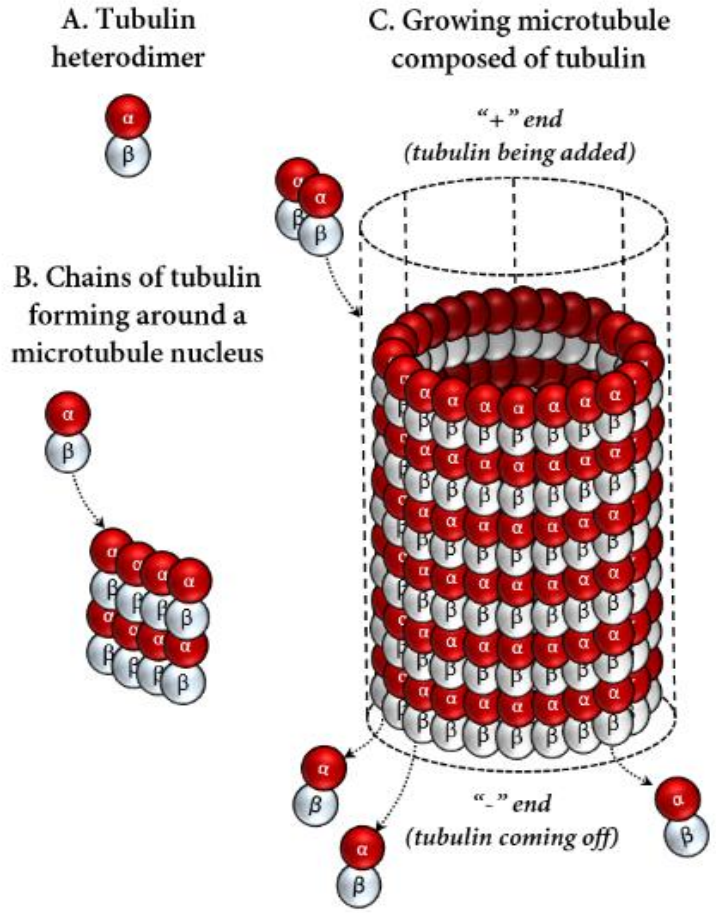

Figure 2-1. Illustration of the structure microtubule used to separate chromosomes during mitosis. Fig 2A illustrates the basic heterodimeric structure of tubulin, the building-block of the microtubule. Fig 2B illustrates how tubulin forms around a core to begin to form the microtubule. Fig 2C illustrates the dynamic process of lengthening and shortening in which tubulin is added to one end (the "+" end) and shed from the other end (the "-"end) of the microtubule. Original artwork, copyright Noah Zanville, 2017.

This ability for microtubules to change their size in response to different stages of mitosis is essential for the smooth exchange of genetic material during cell division (Howard & Hyman, 2003). Studies show that Taxol® binds to microtubules, preventing them from performing the dynamic lengthening and shortening needed to separate chromosomes during mitosis, arresting tumor growth. Studies also show that Taxol® increases the rate at which new microtubules are formed, and increases the probability that tubulin surrounding the microtubule will bind to the "+" end of microtubules (creating longer microtubules) (Grigoriev, Chernobelskaya, & Vorobjev, 1999; Jordan & Wilson, 2004; Kumar, 1981;

Figure 2-2. Illustration of How Taxol® Is Thought to Arrest Tumor Growth during Cell Division

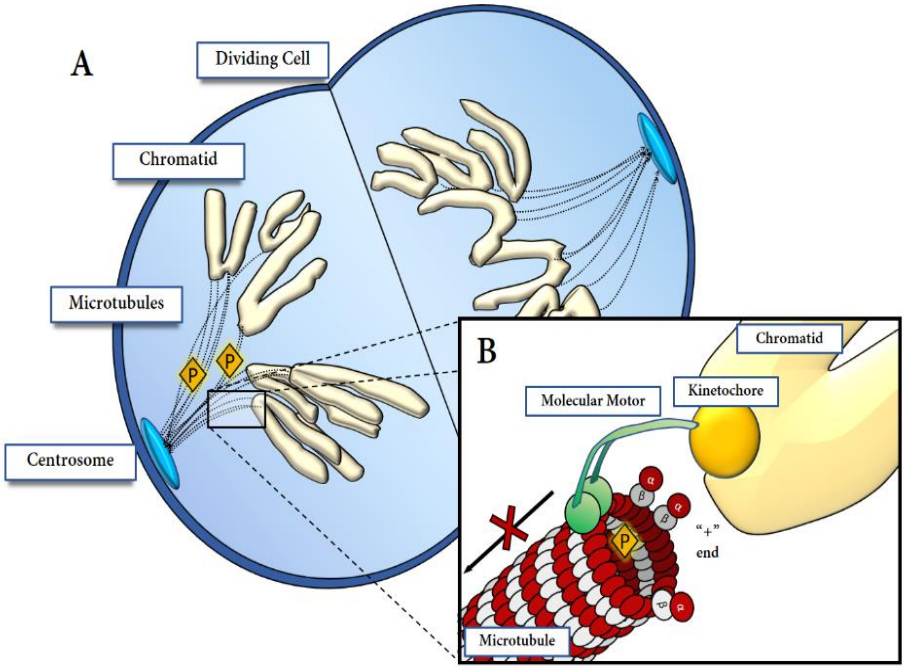

Figure 2-2. Illustration of how Taxol® is thought to interrupt the exchange of genetic material needed for tumor growth. Fig 3A" illustrates a tumor cell (in blue) attempting to pull the strands of genetic material that make up the chromosome (*chromatids*, in tan) to each side of the cell by shortening microtubules in the presence of paclitaxel ("P", in orange). Panel B (inset) shows paclitaxel binding to the β-subunit on the interior of the microtubule, which prevents motor molecules (in green) from binding to the microtubule, preventing chromatids from being pulled to each side of the cell. Original artwork, Noah Zanville, 2017.

Manfredi, Parness, & Horwitz, 1982; Parness & Horwitz, 1981; S. Rao, Horwitz, & Ringel, 1992; Risinger et al., 2014; Schiff, Fant, & Horwitz, 1979; Srivastava et al., 1998; Zasadil et al., 2014). The resulting disruption to cell division, by either triggering cell death directly (Jordan & Wilson, 2004) or allowing non-viable cells to replicate, arrests tumor growth (Figure 2-2).

Additional Antineoplastic Mechanisms. In addition to disrupting microtubules' dynamics needed for tumor growth, Taxol® has several additional properties that contribute to its ability to treat breast cancer. The first has to do with Taxol's ® ability to disrupt the formation of blood vessels needed for tumor growth (known as *antiangiogenesis*) (Jordan & Wilson, 2004). Antiangiogenesis is an effective strategy for interfering with tumor growth because like all tissues, tumors must take in oxygen and dispose of waste to grow. In tumors less than a few centimeters in size, these tasks can be performed through simple diffusion (Spill, Guerrero, Alarcon, Maini, & Byrne, 2015), but tumors larger than a few centimeters

require their own blood vessels to meet the demands imposed by growth (Carmeliet & Jain, 2011).

Research shows that Taxol® has antiangiogenic properties in breast tumors, especially when combined with vascular endothelial growth factor-inhibitors like bevacizumab (Avastin®) (Bocci, Di Paolo, & Danesi, 2013; Pasquier et al., 2005; Schneider et al., 2008; Tonissi et al., 2015). In addition, studies show that Taxol's® microtubule-stabilizing effects can be combined with other systemic therapies such as anthracyclines, platinating agents, vinca alkaloids, and biologic therapies to increase the drug's efficacy and reduce the potential for tumor resistance.

Use in Female Breast Cancer

Current data indicate that of the nearly one quarter-of-a-million cases of female breast cancer projected to occur this year in the U.S., approximately 93% will be detected at the early stage (I or II), and 7% will be diagnosed as late stage (III or IV) cancer (American Cancer Society, 2017). Data from the American College of Surgeons' National Cancer Database on the treatment of breast cancer in the U.S. between 2004-2014 shows that approximately 40% of women with early-stage breast cancer and 60% with late-stage breast cancer received chemotherapy, either by itself, or in combination with other types of therapy during treatment (Figure 2-3) (American College of Surgeons, 2017). Closer inspection of data indicates that use of chemotherapy declines as the age of diagnosis increases (American College of Surgeons, 2017). Taxol® remains one of the most widely-used breast cancer agents in all forms of breast cancer, regardless of age of diagnosis.

Administration Methods and Treatment Schedules

Taxol® is administered as a single intravenous infusion (Food and Drug Administration, 2011). Infusions are typically delivered over 60-180 minutes (i.e., 1-3 hours) based on the frequency of treatment and patient's reaction to Taxol®, which can produce anaphylactic-like hypersensitivity reactions (Quock et al., 2002). Pre-medication with a steroids, H_1-receptor antagonists, and H_2-receptor antagonists 30-60 minutes prior to infusion is used to reduce the incidence and severity of these hypersensitivity reactions, which are a side

Figure 2-3. Use of Chemotherapy (Alone or with Other Systemic Therapies) to Treat Female Breast Cancer in U.S. Hospitals 2004-2014 (*N* = 84,493)

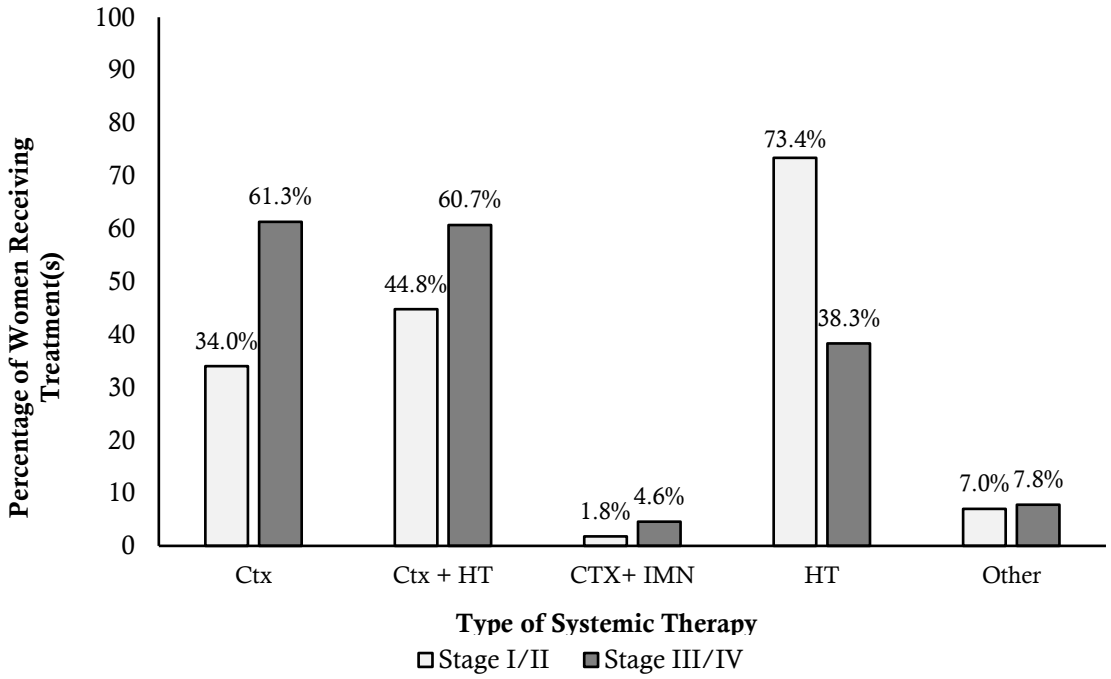

Figure 2-3. Bars in the graph represent percentage of U.S. women in the sample who received the following combinations of cancer treatment for breast cancer between 2004 and 2014: Chemotherapy alone (Ctx); chemotherapy plus hormone therapy (Ctx+HT); chemotherapy plus immunotherapy (CTX+IMN); hormone therapy alone (HT). Bars in light grey represent women with stage I/II breast cancer. Bars in dark grey represent women with stage III/IV breast cancer. Data from the American College of Surgeon's National Cancer Database (https://www.facs.org/quality-programs/cancer/ncdb).

effect of the emulsifier (Cremaphor EL®) used to make the paclitaxel soluble in the blood stream (Feldweg, Lee, Matulonis, & Castells, 2005). Standard treatment schedules for Taxol® include weekly (q1wk) Taxol® (70-90 mg/m^2), bi-weekly (q2wk Taxol® (80-100 mg/m^2), or every 3 weeks (q3wk) Taxol® (175-250 mg/m^2), by itself, or in combination with other therapies (National Comprehensive Cancer Network, 2016).

Efficacy

Results of several meta-analyses and systematic reviews confirm that Taxol® alone or in combination with other therapies is associated with improved tumor response, longer disease-free survival, and greater time-to-progression for BCS with metastatic or non-metastatic breast cancer (Gandhi et al., 2015; Ghersi, Wilcken, Simes, & Donoghue, 2005). Current data indicate that the benefit to BCS receiving the Taxol® appears to be similar

regardless of the formulation being used (i.e., solvent-based (Taxol®) or nanoparticlized (Abraxane®)) (Liu et al., 2017). In addition, meta-analyses comparing Taxol® with other taxanes such as *docetaxel* (Taxotere®) indicate that Taxol® and Taxotere® are nearly identical in terms of disease-free survival (16 trials, N=14,773 women) and overall survival (17 trials, N =16,176 women), but is associated with fewer overall side effects, making it a better choice for many women (Ginés et al., 2011).

Section 2: Introduction to TIPN

Clinical Presentation

Symptoms of TIPN. As noted in Chapter One, TIPN symptoms are characterized by distal neurosensory symptoms that begin following exposure to Taxol® or other drugs in the taxane family. Studies show that exposure to Taxol® is capable of producing a range of neurosensory symptoms including pain, temperature (thermoception), touch (tactile sensation), limb-position (proprioception), and vibration (vibrotactile sense). Symptoms of motor and autonomic neuropathy can be observed at higher doses or in individuals with predisposing factors (Food and Drug Administration, 2011).

***Type*.** While symptom presentations vary, patients with TIPN nearly always report the trifecta of tingling, numbness, and neuropathic pain during Taxol® therapy (Pachman et al., 2016; Sahenk et al., 1994). Additional symptoms of TIPN including "shock-like" or "electric" sensations, burning, skin that feels abnormal, "dead", or "wooden", increased/decreased sensitivity to hot or cold, sensitivity to painless stimuli (hyperalgesia), and pain during normally painless touch (allodynia) are common as well (Lipton et al., 1989; E. L. Smith, Whedon, & Bookbinder, 2002; S. L. Wolf et al., 2012b).

***Onset*.** Initial symptoms of TIPN have been reported as early as 24-48 hours following a single high dose of Taxol® (i.e., $\geq$ 250 mg/m^2). In contrast, at the doses of Taxol® typically used to treat breast cancer today (i.e., 80-175 mg/m^2), TIPN symptoms generally emerge over the first 12 weeks of treatment (Park, Lin, et al., 2011b), growing worse as therapy progresses (Figure 2-5). Patients often display considerable variation in the onset of TIPN, making it difficult to predict when symptoms will develop. However, despite the variation in when symptoms develop, studies show that the appearance of severe TIPN early in treatment is a strong predictor of the severity and duration of TIPN later in treatment (Pachman et al., 2016; Vichaya et al., 2013b).

Figure 2-4. Example of Typical Onset and Severity of Sensory, Motor, and Autonomic Neuropathy Symptoms during Taxol® Therapy

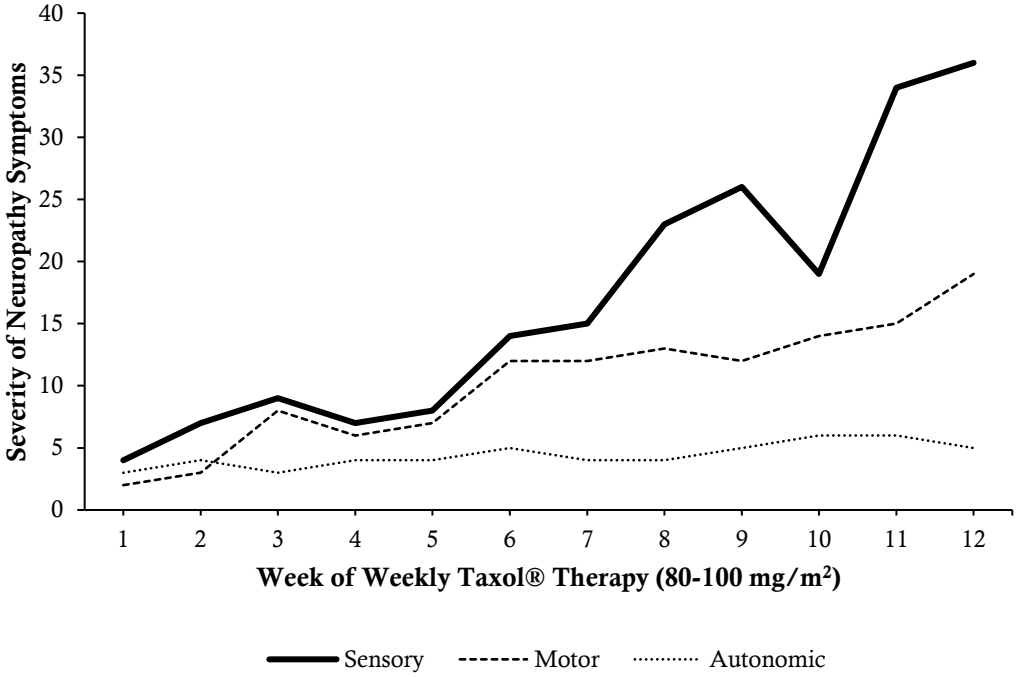

Figure 2-4. The above graph illustrates the onset and severity of sensory, motor, and autonomic neuropathy measured with the EORTC-CIPN20 for patients receiving Taxol®. In the illustration, values on the Y-axis represent patient's deviation from baseline for each subsection of the CIPN-20 (i.e., sensory, motor, and autonomic), reversed to show the growing presence/severity during each week. Higher scores = more symptoms/more severe TIPN. Original illustration developed by Noah Zanville based on data reported in Loprinzi et al., 2011 and Reeves et al., 2012.

Severity. Like other forms of chemotherapy-induced neuropathy, the severity of TIPN symptoms is largely dose-dependent (T. Berger, Malayer, et al., 1997; McGuire et al., 1989; Mielke et al., 2003; Mielke et al., 2005), with severe and debilitating neuropathy symptoms occuring in patients receiving longer and more intense Taxol® therapy (Loprinzi et al., 2011b; Reeves et al., 2012; Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge Jr, et al., 2012; Shimozuma et al., 2012; Simon, Danso, Alberico, Basch, & Bennett, 2017). Also, because the damage to nerves appears to be cumulative for many BCS, TIPN symptoms are often worse for BCS who have received prior treatment with neurotoxic chemotherapy (McGuire et al., 1989).

In addition, current evidence suggests that the severity of TIPN symptoms often waxes-and-wanes between treatments (Pachman et al., 2016), gradually becoming more constant as treatment continues (Lipton et al., 1989). Difficulty feeling vibration is frequently reported during neurological examination (T. Berger, Malayer, et al., 1997; Jessica A. Boyette-Davis et al., 2012b; Jimenez-Andrade et al., 2006; Park, Lin, et al., 2011b; van Gerven et al., 1994; S. L. Wolf et al., 2012a), along with changes in muscle strength (T. Berger, Malayer, et al., 1997). Involvement of cranial nerves is rare (Argyriou et al., 2005; Miltenburg & Boogerd, 2014), but has been reported in some cases (Hausheer, 2008), including changes in vision (Gianni, Munzone, Capri, Villani, et al., 1995), pain, and facial sensation (Lipton et al., 1989). Although some authors have suggested that certain types of symptoms may be more common during specific phases of treatment (e.g., *paresthesia* (altered skin sensation) and pain early, loss of sensation (numbness) later), current evidence suggests that symptoms of all types (sensory, motor, and autonomic) increase in severity based on total exposure to Taxol® (Loprinzi et al., 2011b).

Physical Distribution. TIPN symptoms are length-dependent, affecting the longest nerves of the body first. Because of this, symptoms typically begin in the lower extremities (Cavaletti et al., 1995), although cases that begin in the upper extremity or develop equally are not unusual (Lipton et al., 1989; Miaskowski et al., 2017; Postma, Vermorken, Liefting,

Figure 2-5. "Stocking and Glove" Distribution of Taxane-Induced Neuropathy Symptoms

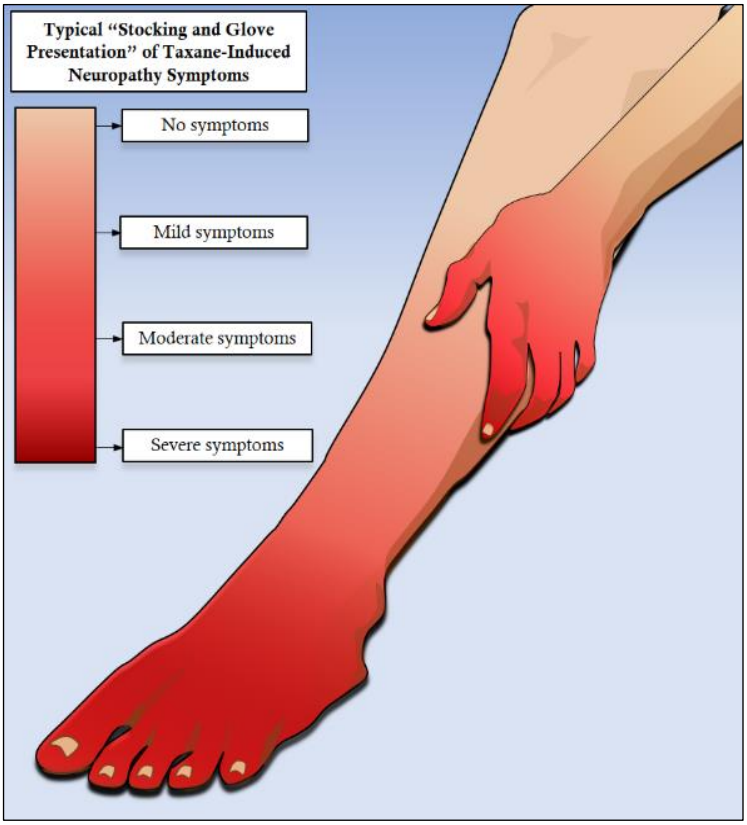

Figure 2-5. Example of length-dependent "stocking and glove" distribution of neuropathy symptoms reported by Breast cancer survivors during Taxol® Therapy. Original artwork, copyright Noah Zanville, 2017.

Pinedo, & Heimans, 1995). As treatment progresses, symptoms move up the limbs, creating a recognizable "stocking and glove" distribution (T. Berger, Malayeri, et al., 1997; Dougherty et al., 2004; Forsyth et al., 1997) (Figure 2-5). Like other distal polyneuropathies, TIPN is generally symmetric, although research evaluating the degree to which TIPN symptoms are symmetric versus asymmetric for BCS during treatment has yet to be performed.

Signs of TIPN. Subjective symptoms of TIPN are nearly always accompanied by objective changes in neurological function (i.e., signs). These changes can include alterations in strength, balance, gait, and motor coordination (Chico et al., 2001). Difficulty buttoning buttons or performing similar fine-motor tasks are often some of the first signs of TIPN (Bridges & Smith, 2014; Holmes et al., 1991b), although the question of whether patient's difficulty performing these tasks is due to true motor deficits or a combination of motor and sensory disturbances has yet to be investigated systematically. Loss of ankle reflexes, another common finding during Taxol® therapy, can make it difficult for BCS to walk or exercise (Cavaletti et al., 1995; Holmes et al., 1991b; Wiernik, Schwartz, Strauman, et al., 1987), and is often considered diagnostic for TIPN in the presence of other signs/symptoms.

Presumed signs of autonomic neuropathy such as paralytic ileus and orthostatic hypotension have also been reported during Taxol® therapy (Lipton et al., 1989), but are thought to be rare (Loprinzi et al., 2011b) although recent data indicates that autonomic neuropathy is highly under-assessed in the context of TIPN (E. M. Smith et al., 2014). Other presumed signs of autonomic disturbance such as cardiac arrhythmias have been observed during Taxol® (Anderson & Sawyer, 2008; Gianni, Munzone, Capri, Fulfaro, et al., 1995; McGuire et al., 1989), although it is unclear whether these disturbances are caused by paclitaxel or Cremaphor EL®, the solvent used to make paclitaxel miscible in the bloodstream.

Additional Signs/Symptoms Associated with Taxol®. In addition to the symptoms classically associated with TIPN, an estimated 40% of BCS who receive Taxol® develop intense muscle tenderness (myalgia), joint pain (arthralgia), and malaise following their infusion (Augusto et al., 2008b; Loprinzi et al., 2011a; Mross et al., 2002; Tofthagen, McAllister, et al., 2013)).[4] This syndrome, known as taxane-induced musculoskeletal pain,

[4] Also, referred to as paclitaxel-induced acute pain syndrome (P-APS) or taxane-induced pain syndrome (TAPS).

often begins within hours of patients' Taxol® first infusion (Pachman et al., 2016), peaking 4-5 days post-Taxol® (Loprinzi et al., 2011b), and fading 5-7 days post-infusion (Chiu et al., 2017; Gianni, Munzone, Capri, Villani, et al., 1995; McGuire et al., 1989). Like TIPN, the severity of taxane-induced musculoskeletal pain appears to be closely linked with dose and frequency of Taxol® being used (Chiu et al., 2017; Davis, Carpenter, & Otte, 2016; Pachman et al., 2016), causing some to question whether taxane-induced musculoskeletal pain is really an early form of TIPN caused by sensitization of peripheral nerves (Davis et al., 2016; Loprinzi et al., 2011b). Research into this question is ongoing.

Pathobiology of TIPN

The cellular and molecular changes responsible for TIPN are complex and poorly understood (Miltenburg & Boogerd, 2014). Current evidence suggests that the sensory neuropathy associated with TIPN is likely to be the result of several overlapping changes to the structure and function of sensory nerves. These changes include:

- Disruptions in microtubule orientation and function, impairing the ability of nerves to transport molecules along the length of their cell bodies (known as *axonal trafficking*) (Komiya & Tashiro, 1988; LaPointe et al., 2013; Nakata & Yorifuji, 1999; Nennesmo & Reinholt, 1988; Shemesh & Spira, 2010; Theiss & Meller, 2000).
- Injury to the mitochondria in nerves which help to buffer oxidative stress, regulate nerve signaling, and produce energy in the form of adenosine triphosphate (ATP) needed to prevent terminal arbors of the nerves from withering and dying back (Bennett, Liu, Xiao, Jin, & Siau, 2011; Zheng, Xiao, & Bennett, 2012).
- Sensitization of axon and body of peripheral nerves at the dorsal root. This includes alterations in the number and sensitivity of receptors involved in nociception such as TRPV.1 channels triggered by the release of inflammatory cytokines, nerve growth factor, reactive oxygen species, immune cells, and exposure to Taxol® directly (Han & Smith, 2013; Pachman, Barton, Watson, & Loprinzi, 2011; Park et al., 2013; Park et al., 2008b; Pittman, Gracias, Vasko, & Fehrenbacher, 2014).

Growing research shows that damage to DNA and DNA-repair mechanisms is likely to play an important role in the development of neuropathies during treatment with platinum-based therapies like oxaliplatin and cisplatin (Avan et al., 2015; Fehrenbacher, 2015; Podratz

et al., 2011; Vasko, 2016). In contrast, evidence suggests that damage to DNA/DNA-repair is not likely to play a central role in the development or worsening of TIPN.

Need to Clarify the Role that Small-Diameter Sensory Nerves Play in Development and Progression of TIPN

The degree to which these cellular and molecular changes affect different populations of sensory nerves has been the source of some controversy in the field. Until recently, it was thought that Taxol® preferentially affected large-diameter sensory nerves (Dougherty et al., 2004; Loprinzi et al., 2011b; Sahenk et al., 1994). However, growing evidence from both clinical and pre-clinical settings suggests that Taxol® can affect the structure and function of smaller-diameter sensory nerves (Argyriou, Koltzenburg, Polychronopoulos, Papapetropoulos, & Kalofonos, 2008; Authier et al., 2000; Bennett, Liu, Xiao, Jin, & Siau, 2011; Boyette-Davis, Xin, Zhang, & Dougherty, 2011; J. A. Boyette-Davis et al., 2012; V. Carozzi et al., 2009; Gilardini et al., 2012; Gracias, 2011; N. Gracias et al., 2011; Kosturakis et al., 2014; Melli, Jack, Lambrinos, Ringkamp, & Hoke, 2006; Park et al., 2008a; Persohn et al., 2005; Pittman et al., 2013; Pittman, Gracias, Vasko, & Fehrenbacher, 2014; Saad et al., 2016a; C. Siau, W. Xiao, & G. J. Bennett, 2006; Vichaya et al., 2013a).

Clarifying the role that small-fiber nerves have in TIPN is important for at least four reasons. First, the majority (>85%) of sensory fibers found in the periphery are unmyelinated (Feldman, Nave, Jensen, & Bennett, 2017) (Table 2-1). Consequently, this creates the potential for broad neurological involvement if Taxol's ® neurotoxic effects are not limited to large-fiber nerves.

Second, small-fiber nerves play an important role in the perception of painful stimuli, raising questions about whether damage to small-fiber nerves may play a role in some of the painful symptoms BCS report during weekly Taxol® therapy. Support for the hypothesis that damage to small-fiber nerves may be involved in painful symptoms can be found in the results of a recent study, which found that small-fiber nerves exposed to Taxol® released a molecule (MCP-1) that appeared to sensitize surrounding large-fiber nerves to pain (Zhang et al., 2013). In addition, the recent success of "scrambler therapy" (which uses low-frequency signals to "scramble" afferent pain signals traveling to the central nervous system) in reducing both painful and non-painful neuropathy symptoms associated with TIPN in several recent human trials suggests not a larger involvement of small-fiber nerves that was once thought, but a

potential role for small-fiber neuropathy in symptoms traditionally associated with large-fiber nerve dysfunction (e.g., numbness, paresthesia).

Third, studies in patients with multiple myeloma receiving *bortezomib* (an anticancer agent also associated with CIPN) found that patients who showed signs of small-fiber dysfunction before the start of treatment were 3-times more likely to develop pain and numbness during treatment (Vichaya et al., 2013a), raising questions about whether alterations in small-fiber function may also be a useful predictor of the progression and severity of TIPN.

Finally, small-fiber nerves are also a vital part of the peripheral portion of the autonomic nervous system (Devigili et al., 2008). The C-fibers help to regulate physiological functions ranging from sweating (sudomotor function) to vascular tone (R. Freeman, 2007).

Table 2-1

Expanded Description of Characteristics of Sensory Nerve Fibers

Fiber Type	Conduction Speed (m/s)	Degree of Myelination	Sensory Receptor(s)	Type of Information Transmitted
A-α	72-120	Full	Muscle spindle	Proprioception
			Golgi tendon organ	Proprioception
A-β	42-72	Full/Mostly	Muscle spindle	Proprioception
			Meissner's corpuscles	Light touch
			Merkel discs	Light touch
			Hair receptors	Light touch/vibration
			Pacinian corpuscles	Pressure, vibration
			Ruffuini endings	Pressure, vibration
A-δ	12-36	None/Light	None (bare)	Cold
			None (bare)	Pain (fast)
C	0.5-2.0	None	None (bare)	Heat/Warmth
			None (bare)	Pain (slow)
			None (bare)	Itch

For all these reasons, research clarifying the role that small-fiber nerve function has in TIPN is needed. However, clinically-feasible methods for detecting early signs of small-fiber neuropathy have been limited, especially in the oncology setting, making it difficult to investigate the problem.

Epidemiology of TIPN

Prevalence of TIPN among BCS.

Prevalence during Taxol® Therapy. The prevalence of TIPN in BCS receiving TIPN is not well understood. Results of a recent meta-analysis, which includes data from 31 studies, estimate the prevalence of TIPN in BCS receiving any form of Taxol® at 70.3% (95% *confidence interval* (CI) [43.5–98.1]) (Seretny et al., 2014). Similar estimates of TIPN's prevalence listed in studies not considered in the meta-analysis by Seretny et al. estimate the prevalence of TIPN in BCS receiving Taxol® to 60-90% (Argyriou et al., 2005; Lam et al., 2016; Osmani, Vignes, Aissi, Wade, Milani, Levy, et al., 2012). The lack of clarity about TIPN's prevalence in BCS can be traced to issues ranging from underreporting (which continues to be an issue in many settings (Markman, 2006b)) to variations in how and when TIPN are measured.

Prevalence after Completing Taxol® Therapy. As with the prevalence of TIPN during treatment, the prevalence of TIPN following treatment is poorly understood. Current evidence suggests that a large portion of the BCS who develop TIPN during Taxol® therapy will experience some degree of TIPN after treatment (Argyriou et al., 2005; Bakitas, 2007; A. Beijers, Mols, Dercksen, Driessen, & Vreugdenhil, 2014; Calhoun et al., 2003; Forsyth et al., 1997; Hershman et al., 2011; Kuroi et al., 2009; Osmani, Vignes, Aissi, Wade, Milani, Lévy, et al., 2012; Park et al., 2013; Park, Lin, Krishnan, Goldstein, et al., 2011c; Pignata et al., 2006; E. M. Smith et al., 2013).

For example, a recent study of 502 BCS receiving taxanes found that more than 80% of BCS reporting TIPN during treatment continued to report symptoms of TIPN 6 months after their cancer treatment (Pereira et al., 2016). Similarly, results of a smaller study by the author in 22 BCS found that more than 50% of BCS in the sample continued to report pain, tingling, muscle weakness, and difficulty feeling the shape of small objects in their hands or feet more than one year following the end of Taxol® (Zanville et al., 2016).

In addition, while the majority of BCS who develop TIPN report an improvement in their symptoms following treatment, current data indicates that for a subset of BCS, the pain and neurological changes associated with TIPN will continue for years, and in some cases, indefinitely following treatment (Tanabe et al., 2013). For example, results of one of the largest prospective studies of TIPN to date, which included data from 1,237 BCS, found that 73% of BCS with TIPN surveyed reported still having some level of TIPN up to 2 years post-treatment (Greenlee et al., 2017). Similarly, Fontes et al. found that more than 20.0% of BCS

in their study reported symptoms of TIPN up to 3 years after the end of treatment (Fontes, Pereira, Castro-Lopes, & Lunet, 2016). Even more troublingly, almost 60% of BCS in a recent study reported TIPN approximately 5-years post-treatment (Bao et al., 2016), while a smaller study found that just 14% of BCS who had received a taxane during treatment had achieved total remission of their TIPN a median of 3-years post-treatment (range: 1-13 years) (Osmani, Vignes, Aissi, Wade, Milani, Levy, et al., 2012). Additional studies are needed to clarify the prevalence of TIPN in BCS (particularly among long-term survivors).

Severity of TIPN among BCS.

Severity during Taxol® Therapy. Current data indicate that the majority of BCS (70%) develop TIPN classified as mild-to-moderate, with approximately 30% developing more severe, long-standing symptoms (Hershman et al., 2011; Simon et al., 2017). However, estimates of how severe the neurological changes BCS with TIPN experience during Taxol® are not fully known. The lack of clarity about the severity of TIPN in BCS can be attributed to a variety of factors related to TIPN measurement. These include variations in: (1) how TIPN severity is measured, (2) which signs/symptoms are evaluated, and (3) how often the severity of TIPN is evaluated (Majithia, Temkin, et al., 2016). In addition, many practice settings rely on questionnaires which use items that combine the incidence and severity of TIPN during evaluation, which also contributes to a lack of clarity about TIPN severity.

The push for shorter, more clinically-useful screening methods for TIPN, while likely to increase overall screening for TIPN, may also inadvertently contribute to a lack of clarity about the severity of TIPN. The reason for this is that shorter instruments, by virtue of their design, must forgo detailed assessment needed to understand the severity of all of the signs and symptoms associated with TIPN. Together, these factors underscore the need for large representative trials using multiple evaluation methods to improve estimates of TIPN's severity in BCS receiving Taxol®.

Severity after Completing Taxol® Therapy. It is estimated that approximately one in five (20%) BCS will develop signs and symptoms of TIPN during their weekly Taxol® therapy that will persist after treatment. Studies show that for many BCS, these neurological changes will be more than a nuisance, interfering with BCS' quality of life, and their ability to work and function (A. Beijers et al., 2014; Greenlee et al., 2017; N. U. Lin et al., 2007; Osmani, Vignes,

Aissi, Wade, Milani, Levy, et al., 2012; Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge Jr, et al., 2012).

While these facts underscore the high risk that BCS who receive Taxol® have for developing TIPN- and TIPN-related issues, the lack of methods for evaluating damage to different types of nerve fibers after treatment has made it difficult to evaluate the severity of TIPN comprehensively following treatment. In addition, the lack of objective data on functional changes in sensory neurons in BCS makes it difficult to correlate the duration of symptoms with biological changes.

Options for Preventing and Treating TIPN

Pharmacotherapy. Options for preventing or treating TIPN are currently limited, creating an "urgent unmet medical need" (R. D. Rao et al., 2007). Decades of research have yet to produce any therapies specifically for TIPN (Hershman et al., 2014), and interventions used to treat other types of pain and neuropathy show little efficacy for TIPN (Hershman et al., 2014; Majithia, Loprinzi, et al., 2016). The need for ways to manage TIPN has created a situation where providers may be required to use therapies for which there is insufficient evidence or risk TIPN worsening. A prime example of this are anticonvulsants such as gabapentin (Neurontin®), pregabalin (Lyrica®), and lamotrigine (Lamictal®). These drugs have been proven to be highly useful in reducing the severity of other forms of neuropathy. However, multiple studies (including a recent phase III, randomized, double-blind, placebo-controlled study) now show that anticonvulsants such as gabapentin are not better than placebo for treating symptoms associated with TIPN (Mitchell et al., 2006; R. D. Rao et al., 2007). Despite this evidence, results of a recent survey found that use of gabapentin for TIPN was extensive, being prescribed as a first-line treatment for TIPN in more than 70.0% of cases (Song et al., 2017).

There is also growing evidence that opioids are used to manage TIPN in many practice settings (Hirayama et al., 2016; R. Wong & Sagar, 2006). While there is some evidence supporting the use of opioids for manage other types of chemotherapy-induced neuropathy (e.g., oxaliplatin-induced peripheral neuropathy) (Nagashima et al., 2014), evidence supporting the use opioids for TIPN is less convincing (Inoue et al., 2013). Recently, the combination opioid and norepinephrine-reuptake inhibitor tapentadol (Nucynta®) was tested for CIPN in a prospective trial of 31 patients unresponsive to gabapentin and other

common therapies (Galie, Villani, Terrenato, & Pace, 2017). While results of the trial showed improvements in pain and sensory symptoms in patients taking tapentadol, a high incidence of opiate-related effects such as dizziness/blurred vision (28.4%) and drowsiness (42.8%) was noted. The high incidence of side effects such as these with opiates, and well-established risk of dependence with drugs of this sort, suggest that other strategies are needed to manage painful TIPN symptoms in the larger population of BCS.

Section 3: Impact of TIPN

Topics reviewed in this section include the literature describing the potential and actual impact TIPN has on BCS' (1) adherence to cancer treatment, (2) balance, strength, and sleep, (3) occupational function, (4) healthcare-resource utilization and cost, and (5) medication use.

Impact of TIPN on Cancer Treatment

One of the most serious risks for BCS receiving Taxol® is the potential to have to stop or alter their cancer treatment because of TIPN. Like other antineoplastic therapies, maintaining the recommended dose and frequency of Taxol® (i.e., dose-intensity) is essential for maximum tumor response (Lyman, Dale, Tomita, Whittaker, & Crawford, 2013; Norton, 1997). Examples of dose-limiting events related to TIPN include (1) delays in the dose of Taxol® being delivered; (2) deviations from the recommended schedule of treatment; and (3) the need to substitute another antineoplastic therapy or abandon treatment altogether because of worsening neuropathy (Speck et al., 2013). Evidence-based guidelines indicating when to deviate from Taxol® have yet to be published, but the severity, duration, impact, and potential for permanent TIPN all must be considered.

Historically, research in this critical area has been limited, although recent work by several groups has begun to fill in these gaps. Results of retrospective analysis by Speck et al found that 36.4% of women receiving taxanes had their dose reduced because of TIPN (Speck et al., 2013). On average, women in this study received 28.4% less Taxol® by the end of treatment than the amount outlined in their treatment regimen (Speck et al., 2013). A year later, Bhatnagar et al found that 40% of the 120 women receiving taxanes in their study had undergone TIPN-related dose-reductions (Bhatnagar et al., 2014). Likewise, just this past year, Lam et al reported nearly identical findings: 46% of women in their sample of 188 BCS had been forced to make TIPN-related dose reductions (Lam et al., 2016).

The type and frequency of taxane therapy are strong risk factors for TIPN severe enough to alter cancer treatment. Speck et al found that BCS receiving Taxol® were dose-reduced almost 7-times more often than women receiving Taxotere® (Taxol® = 16.1% vs. Taxotere® 2.4%; $p < .001$) (Speck et al., 2013). Speck et al also found that the odds of having a TIPN-related dose reduction were twice as high for BCS receiving weekly Taxol® (80 mg/m^2) compared to BCS receiving Taxol® every 3 weeks (175 mg/m^2), after adjusting for age, race, menopausal status, and diabetes (OR: 2.1, 95% CI [0.97, 4.60]; $p < 0.06$) (Speck et al., 2013).

Impact of TIPN on Physical Function

Impact on Dexterity, Strength, Gait and Balance. One of the most well-understood impacts of TIPN in BCS is the effect that taxane-induced neuropathy can have on dexterity, strength, gait, and balance (Bao et al., 2016; Dougherty et al., 2004; Jansen et al., 2011; Miaskowski et al., 2017; Tofthagen et al., 2012). Falling is garnering increasing attention from clinicians as a potential risk of TIPN, as patients and researchers have begun to describe the powerful way that TIPN can alter proprioception, postural control, and sensory input from the periphery.

These changes coupled with loss of strength, flexibility, and hearing that can occur during cancer treatment, can considerably increase women's risk of falling (Kneis et al., 2016; Marshall, Zipp, Battaglia, Moss, & Bryan, 2016; Miaskowski et al., 2017; Tofthagen et al., 2012; Wampler et al., 2007). For example, a regression analysis by Bao et al. found that women with moderate-to-severe TIPN were more than twice as likely to fall as women without TIPN, after controlling for differences in neuropathy severity, age, and BMI (adjusted OR: 2.27, 95% CI [1.24, 4.16]; $p < 0.008$) (Bao et al., 2016). This risk also appears to be specific to taxanes, with a recent study by Tofthagen et al. finding that the risk of falling was 10-times higher for women receiving taxanes than those that received platinum analogues (OR: 10.14 (taxane vs. platinum), 95% CI [0.84, 122.13]; $p < 0.068$) (Tofthagen et al., 2012).

Pilot research suggests that the increased risk of falling associated with taxanes may be able to be mitigated through exercise and balance-training. For example, one group found that visual computer feedback assisted balance training (Cammisuli, Cavazzi, Baldissarro, & Leandri, 2016; Fernandes & Kumar, 2016). Another group found that the dance, Argentine

Tango, was associated with reduced postural sway in BCS with TIPN (Worthen-Chaudhari, Lamantia, Monfort, Chaudhari, & Lustberg, 2016).

Impact on Sleep. Another physical function that can be negatively impacted by TIPN is sleep. Although studies exploring the effect that TIPN has on sleep are limited, early evidence suggests that the pain and increased sensitivity to touch associated with TIPN can significantly decrease the quality and amount of sleep BCS (Hong et al., 2014; Kim et al., 2014; Tian et al., 2015; Tofthagen et al., 2011; Tofthagen, McAllister, et al., 2013).(Hong, Tian, & Wu, 2014; Kim et al., 2014; Tian et al., 2015; Tofthagen, McAllister, & McMillan, 2011). The severity of these symptoms appears to be a strong predictor for sleep disturbance. For example, a recent study evaluating the effect of TIPN on sleep quality in 706 cancer patients by Hong et al found that TIPN severity was a significant predictor of poor sleep after controlling for differences in age, sex, education level, social support, anxiety, or depression ($\beta = 0.649$, $p < .0001$) (Hong et al., 2014). Similarly, more than half (52.6%) of women with cervical cancer in a recent study who reported severe TIPN reported poor sleep during treatment (Tian et al., 2015).

Results of the same analysis found that the odds of reporting poor sleep during treatment rose as the severity of TIPN increased. Patients in this study who reported mild TIPN during treatment were nearly twice as likely (OR: 1.91) as patients without TIPN to report poor sleep (Hong et al., 2014). Even more troubling, results of the study found that women who reported moderate TIPN were approximately three-and-a-half times more likely to report poor sleep (OR: 3.66; $p < .001$), and women who reported moderate/severe TIPN were 7-times (OR: 7.01 $p < .001$) more likely to report poor sleep than those without TIPN (Hong et al., 2014). The odds of reporting poor sleep during treatment were especially high for women reporting severe TIPN during treatment, who were more than 13-times more likely (OR: 13.41; $p < .001$) to report poor sleep during the study compared to women with no TIPN (Hong et al., 2014). Other studies have found significant associations between the severity of TIPN and poor sleep ($p = 0.016$) (Tian et al., 2015) as well as insomnia ($p < .0001$) (Bao et al., 2016).

Qualitative descriptions of the negative impact TIPN can have on sleep from BCS themselves have also been included in several recent investigations (Bakitas, 2007; Kuroi et al., 2009; Speck et al., 2013; Tanay et al., 2016), adding important insight into the lived experience

of BCS who struggle to sleep because of their neuropathy. Studies exploring the impact of TIPN on different dimensions of sleep and sleep-related outcomes in BCS are needed to better understand the role of taxane-induced neuropathies on sleep.

Impact of TIPN on Occupational Function

The signs and symptoms associated with TIPN can interfere with BCS' ability to work, especially when combined with the fatigue, post-surgical pain, distress, and cognitive disruption often reported by BCS following treatment. For example, a recent prospective study by Beijers et al. found that TIPN symptoms in the hands and feet can interfere with BCS' ability to work (A. Beijers et al., 2014). A similar study by the author in 22 BCS found that the incidence, number, and severity of upper-extremity TIPN symptoms BCS reported were all associated with reports of being less able to work 1month after Taxol® therapy (Zanville et al., 2016). Results of this same study also found that sensory symptoms such pain, tingling, and numbness affecting the hands and feet were the strongest predictors of BCS' self-reported ability to work following treatment (Zanville et al., 2016). Qualitative descriptions of the impact that TIPN can have on BCS' ability to work have started to appear in the literature as well (Kuroi et al., 2009; Tanay et al., 2016), adding a much-needed patient perspective on the subject (Forsyth et al., 1997).

Despite the evidence that taxane-induced neuropathy symptoms can negatively impact BCS' ability to perform job-related tasks, research defining the economic impact of TIPN-related work loss in BCS is an important gap in the literature in the field. Results of a 2001 study by Calhoun et al. in women with ovarian cancer with TIPN estimated that the average cost of lost wages and increased use of paid caregivers for women was $4,220 (Calhoun et al., 2001), but studies investigating the topic in BCS receiving Taxol® have yet to be performed.

Impact of TIPN on Healthcare Utilization and Costs

Another area of BCS' lives that TIPN can negatively impact is on women's use of healthcare resources. Studies show that TIPN can significantly increase the financial burden for BCS during and after treatment (E. L. Smith et al., 2002). The impact of TIPN on the financial dimension of BCS' experience is poorly understood, being notably absent from other large cost analyses of impact of chemotherapy-related adverse events in BCS (Hassett, O'Malley, Pakes, Newhouse, & Earle, 2006).

The few studies that have been performed in this area show that TIPN is associated with a significant increase in the use of healthcare services, often at a significant cost to patients. For example, results of a recent case-control study by Pike et al of 908 patients (including 70% who had breast cancer) found that patients in the sample who had CIPN (of which, approximately 30% had received a taxane)[5] were significantly more likely to have been hospitalized ($p < .0001$); to have visited the emergency room ($p = .0037$); and to have seen their oncologist ($p = .0339$), neurologist ($p < .0001$), or another physician ($p = .0030$) over a 12-month period compared to controls without CIPN (Pike et al., 2012). Patients in the study with CIPN also scheduled an average of 12 more visits with their providers over the course of a year than patients without CIPN, including visits to the outpatient oncology clinic ($p < .0001$), neurologist ($p < .0001$), and primary care physician ($p = .0085$) (Pike et al., 2012).

Not surprisingly, the increased use of healthcare resources by patients with CIPN in Pike et al.'s study was associated with additional healthcare-related costs. Patients with CIPN in this study spent an average of $17,344 more in healthcare-related costs annually than patients with CIPN ($p < .0001$); including an average of $7,552.00 more in inpatient costs ($p < .0001$) and $3,745 more in outpatient services ($p = .0064$) (Pike et al., 2012). The financial impact for patients with CIPN who also had diabetes was even greater; on average, diabetic patients with CIPN incurred $21,739 in additional costs, averaged 18 more outpatient visits ($p < .0001$), had 30% higher usage of CIPN-related drugs ($p = .0003$), and 6-times as many visits to the neurologist as diabetic patients without CIPN ($p < .0001$) (Pike et al., 2012). Estimates of the financial impact of TIPN from other studies were smaller than those reported by Pike et al., but troubling nonetheless, ranging from an average of $4,908 in additional costs over a 9-month period (Calhoun et al., 2001) to $8,914 in additional costs over a 3-year period (Hess et al., 2015).

Impact of TIPN on Medication Use

While a relatively large amount of research on the topic of TIPN has been devoted to efforts to test potential therapies for TIPN, surprisingly little research has been devoted to

[5] The exact percentage of patients in receiving a taxane in the study by Pike et al. was as follows: (1) non-diabetic patients with CIPN: 33%; (2) non-diabetic patients without CIPN: 28%; (3) diabetic patients with CIPN: 42%; and (4) diabetic patients without CIPN: 24%.

investigating whether developing TIPN increases patients' reliance on existing therapies for pain and neuropathy. Results of several analyses and anecdotal reports suggest that TIPN is associated with increased reliance on medication, during or after treatment, to help manage painful symptoms (E. L. Smith et al., 2002).

For example, a recent survey of 300 physicians by Hirayama et al. investigating the type of medications physicians prescribed to patients to help them manage CIPN symptoms found that 71.1% of doctors who responded to the survey "routinely or prophylactically" administered NSAIDs to their patients for pain (Hirayama et al., 2016). In addition, 42.6% of physicians reported prescribing their patients antiepileptic drugs to manage CIPN, and nearly half (40.9%) of doctors routinely or prophylactically prescribed opioids to their patients for CIPN (Hirayama et al., 2016). These findings are consistent with findings from Pike et al., who found that patients with CIPN during their cancer treatment were 16% more likely than patients without CIPN to be prescribed amitriptyline, gabapentin, amifostine, glutamine, tricyclic antidepressants, anti-epileptics, NSAIDs, or opioids to manage symptoms of CIPN ($p < .0001$) (Pike et al., 2012).

In addition, recent work suggests that chemotherapy regimens associated with high rates of TIPN such as weekly Taxol® may be associated with higher use of analgesics among patients. Results of two prospective studies involving a total of 179 patients with cancer found that approximately 30% of patient receiving either weekly Taxol® (70-90 mg/m^2) or Taxol® every 2-4 weeks (175 mg/m^2) used non-prescriptions to manage their neuropathy (Loprinzi et al., 2011b; Reeves et al., 2012). These same studies also found that 23-41% of patients receiving weekly Taxol® reported using opiates to manage their TIPN symptoms, compared to 12-20% of patients receiving less-frequent Taxol® (Loprinzi et al., 2011b; Reeves et al., 2012).

Section 4: Risk Factors for TIPN

This section reviews factors which can put BCS at risk for developing TIPN and TIPN-related outcomes. Risk factors are divided into two parts. Part 1 reviews the literature on established risk factors for TIPN such as the intensity and duration of Taxol® therapy. Part 1 also describes trends in how Taxol® is being given in the non-metastatic setting that increases both the incidence and severity of TIPN among BCS.

Part 2 reviews factors such as breast surgery, radiation therapy (radiotherapy), and hormonal therapy that may put BCS at risk for developing TIPN (potential risk factors), but which have not been shown to increase BCS' risk for TIPN directly.

Part 1: Established Risk Factors for TIPN

Treatment-Related Risk Factors for TIPN.

Dose-Intensity and Treatment Duration. The most consistent predictor of the onset and severity of TIPN is the amount of Taxol® to which BCS are exposed in a specific time frame (i.e., dose-intensity) (Augusto et al., 2008b; Pazdur, Kudelka, Kavanagh, Cohen, & Raber, 1993; Wozniak et al., 2016). While reports vary, nearly all BCS in published trials display evidence of TIPN after 700-1,000 mg/m^2 of Taxol® (Argyriou, Kyritsis, Makatsoris, & Kalofonos, 2014). In addition, because damage to peripheral nerves associated with Taxol® is cumulative, the duration of treatment is a strong predictor of TIPN severity (Fontes et al., 2016; Johnson et al., 2015), with higher-dose, more frequent treatments (i.e., more intense therapy) over longer time periods being associated with severe and longstanding neuropathies for BCS.

Trends in Taxol® Administration Increasing BCS' Risk for Developing TIPN.

Growing Reliance on Taxol® as a Treatment for Breast Cancer. The success treating breast cancer with Taxol® over the past few decades has significantly increased the use of the drug as a therapy for breast carcinoma (Gralow et al., 2008; National Comprehensive Cancer Network, 2014b, 2015; Sparano et al., 2008; Zeichner, Terawaki, & Gogineni, 2016). However, studies show that this increasing dependence on Taxol® as a cornerstone of breast cancer treatment is increasing BCS' risk for developing TIPN. Results of a 2015 Cochrane analysis found that adding a taxane to a cancer treatment for BCS with metastatic cancer increased their risk of developing serious (i.e., grade 3 or 4) peripheral neuropathy nearly five-fold (RR: 4.84, 95% CI [3.18, 7.35], 5,783 participants; $p < 0.00001$) (Ghersi et al., 2015), underscoring the high risk that women receiving taxanes like Taxol® have for developing TIPN during treatment.

Growing Reliance on More Frequent Taxol® Infusions. Another factor driving the growing incidence of TIPN in BCS receiving Taxol® is the increasing reliance among providers on more frequent Taxol® dosing. Early clinical trials for Taxol® used high doses of the drug (e.g., 250-350 mg/m^2) to treat women's cancers, but used relatively long intervals

between treatments (typically 3 weeks) (Grem et al., 1987; Holmes et al., 1991a; Kris et al., 1986; Roytta & Raine, 1986; Wiernik, Schwartz, Einzig, et al., 1987; Wiernik, Schwartz, Strauman, et al., 1987). While results of these trials confirmed that giving large doses of Taxol® every 3 weeks was effective against many forms of breast cancer, giving doses of Taxol® this large were associated with profound myelosuppression, which is potentially lethal. In addition, the discovery in the mid-1990s that at any one moment, only a fraction of tumor cells were undergoing cell division (limiting the total number of cancer cells that could be killed with a single dose of antineoplastic therapy) suggested that more frequent Taxol® administration might further increase the efficacy of this already effective therapy (Demicheli, Retsky, Swartzendruber, & Bonadonna, 1997; Mielke et al., 2003; Norton, 1997).

Clinical trials now confirm that more frequent Taxol®, delivered either weekly or every other week, is associated with better response rates, better overall survival, and longer median time-to-progression in many forms of breast cancer than traditional, 3-week dosing (Seidman et al., 2008; Woodward & Twelves, 2010). In addition, more frequent Taxol® administration is associated with lower rates of chemotherapy-resistance between treatments (Woodward & Twelves, 2010), boosting Taxol's® anti-angiogenic properties (Bocci et al., 2013). Administering Taxol® more frequently also has been shown to reduce the incidence and severity of myelosuppression (Carbognin et al., 2015; Gandhi et al., 2015; Sparano et al., 2008; Williams & Bryant, 2011), which continues to be a serious side effect of the drug, particularly for elderly BCS (Fontanella, Bolzonello, Lederer, & Aprile, 2014; Lyman, Abella, & Pettengell, 2014)).

Unfortunately, more frequent Taxol® dosing is also associated with an increased incidence and severity of TIPN (Gandhi et al., 2015; T. C. Huang & Campbell, 2012; Lee & Swain, 2006; Nabholtz, Vannetzel, Llory, & Bouffette, 2003; Seidman et al., 2008; Sparano et al., 2008; Williams & Bryant, 2011; Winer et al., 2004). Results of a recent analysis of data of 4,552 women with axillary node-positive or high-risk node-negative breast cancer by Schneider et al using multivariate regression found that BCS receiving weekly Taxol® (80 mg/m^2) had a 34% higher odds of developing TIPN compared to BCS receiving traditional q3week Taxol® (175mg/m^2) (OR: 1.34, 95% CI [1.09, 1.64]; $p < .006$) (Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge Jr, et al., 2012). Similarly, results of another trial comparing weekly versus q3wk Taxol® in 572 BCS with metastatic

cancer who had already received a taxane found that BCS receiving weekly Taxol® developed severe (grade 3 or 4) sensory and motor neuropathy at twice the rate of BCS receiving q3wk Taxol® (Seidman et al., 2008).

Growing Reliance on Shorter Taxol® Infusions. In addition to the growing reliance on more frequent Taxol® dosing, the growing ability to deliver Taxol® more quickly (sometimes in an hour or less) is contributing to the rise of TIPN among BCS. In the 1980s during early trials for Taxol®, the drug had to be administered over periods of time ranging from 24-96hours to avoid a potentially lethal hypersensitivity reaction associated with Taxol® (Kris et al., 1986; McGuire et al., 1989; Pazdur et al., 1993; Woodward & Twelves, 2010).[6] Research discovered that these hypersensitivity reactions were actually caused by the solvent used to make paclitaxel soluble in the blood stream (Cremaphor EL®) and not paclitaxel itself. The development of premedication regimens for Taxol® in recent years [7] has made it possible to give Taxol® much more quickly, reducing infusion times from 24 hours to an average of 1-3 hours.

In addition, novel formulations of paclitaxel that can be delivered in as short as 20 minutes have recently been approved (Feldweg et al., 2005). While early data from the trials evaluating these novel formations confirm that shorter infusions are associated with greater convenience for patients, lower-costs for infusion centers (Dranitsaris et al., 2016), and lower rates of neutropenia (Williams & Bryant, 2011), because delivering the same dose of paclitaxel in a shorter timeframe increases the concentration of the drug in the blood stream, studies show that the incidence and severity of TIPN is higher with shorter Taxol® infusions than traditional dosing (Tanabe et al., 2013). For example, the results of a 2011 Cochrane meta-analysis comparing outcomes for patients receiving Taxol® over 24 hours vs. 3 hours in 3 trials (total $N = 1{,}469$) found that shorter Taxol® infusions carried a 26% higher risk of sensory TIPN compared with 24-hour infusions (RR = 1.26, 95% CI [1.09, 1.46] (Williams & Bryant, 2011).

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[6] This was such a serious issue during Phase I Clinical trials for Taxol® that researchers had to infuse the drug anywhere from 1-14 days to mitigate this potentially lethal toxicity (Woodward & Twelves, 2010).

[7] Recommended pre-Taxol® drug protocol consists of (1) corticosteroids (e.g., dexamethasone), (2) antihistamines (e.g., diphenhydramine), and (3) H2-receptor antagonists (e.g., famotidine).

Growing Rates of Taxol® Re-exposure as Survivorship Increases. Another factor contributing to the growing incidence and severity of TIPN among BCS is the fact that many BCS will receive Taxol® multiple times over the course of their lives, increasing their cumulative exposure to these neurotoxic agents. Current data indicate that more than half of breast cancers recur at least once (Colleoni et al., 2016). This is problematic in part because cancer can become resistant to previously effective therapies, limiting the pool of potential therapies that can be used to fight the cancer. Research has shown that Taxol® can be used to treat breast cancer that stopped responding to other agents, and in some cases, re-used in women whose tumors have become resistant to Taxol® after a break in treatment (Gralow et al., 2008). While this feature increases Taxol's ® utility, it also increases the number of women being exposed to Taxol® and the total amount of Taxol® to which they are being exposed.

Introduction of New Taxanes with Equal or Higher Rates of Neuropathy. The tremendous success of Taxol® as a treatment for breast cancer has spawned a vigorous search for new types of taxanes with fewer toxicities and even stronger therapeutic profiles. This search has led to the development of several new formulations of paclitaxel, the best known of which is the agent *nab-paclitaxel* (Abraxane®). Unlike Taxol®, which relies on Cremaphor EL® to get the highly-hydrophobic paclitaxel molecules into the bloodstream, Abraxane® uses nanoparticlized albumin to deliver paclitaxel, eliminating the need for premedication (Zeichner et al., 2016) and improving Taxol's® pharmacokinetics (Ueno & Mamounas, 2016). However, because newer formations of Taxol® such as Abraxane® can be delivered more quickly than traditional Taxol®, rates of TIPN (including severe TIPN requiring dose-modification or discontinuation) are equal to or higher than rates of TIPN in patients receiving traditional Cremaphor-based Taxol® (N. U. Lin et al., 2007; Rugo et al., 2015; Ueno & Mamounas, 2016).

Demographic Risk Factors for TIPN.

***Age*.** The incidence of TIPN appears to increase in older women. Results of the ECOG-5103 trial ($N = 3,411$ women) found that for every decade of life women age, their risk of developing TIPN increased by 13% ($p < .000093$) (Schneider et al., 2015). Kanbayashi et al. found similar results in their study of 227 women, showing that BCS 60 or older who received Taxol® developed TIPN at nearly twice the rate of women receiving Taxol® 60 or younger (OR: 1.99, 95% CI [1.4, 2.9], $p < .0004$) (Kanbayashi et al., 2010). Bao et al. also found higher

rates of TIPN in women ≥ 65 (Older: 67.6% vs. Younger: 55.4%; $p = .066$) (Bao et al., 2016). Similar findings have been reported in other analyses (Lam et al., 2016).

Age also may be a risk factor for TIPN that persists after treatment. Results of a recent multivariate analysis of 219 BCS receiving Taxol® by Tananbe et al., for example, found that along with symptom severity, being older than 60 was significantly associated with the duration of women's TIPN ($p = .027$) (Tanabe et al., 2013). However, age was not found to be a risk for TIPN-related dose-reductions in two other retrospective analyses (Bhatnagar et al., 2014; Speck et al., 2013), suggesting that more work needs to be done to clarify the impact that age-related increases in TIPN-severity have on clinical outcomes.

Race. A number of studies have identified race as a risk factor of TIPN in BCS. Schneider et al recently found that African-American women were twice as likely to develop grade 2-4 TIPN as Caucasian women (Hazard Ratio (HR): 2.1, $p = 5.6 \times 10^{-16}$) and more than two-and-a-half times more likely than Caucasian women to develop severe/debilitating TIPN (i.e., grade 3-4) during taxane therapy (HR: 2.6, $p = 1.1 \times 10^{-11}$) (Schneider et al., 2015). Interestingly, in a different arm of the same trial, self-reported race was not linked with an increased risk of TIPN, underscoring the critical need to base TIPN diagnosis on reliable biomarkers rather than race as a social construct.

Studies suggest that race may be an important risk factor for predicting which BCS will have to reduce their dose of chemotherapy because of TIPN. For example, a retrospective analysis of reasons for dose reductions in 123 BCS found that African-American women receiving taxanes had their dose of chemotherapy reduced because of TIPN at more than twice the rate as Caucasian BCS with TIPN (53% vs. 22%; $p < .001$) (Bhatnagar et al., 2014). Results of this study also found that the rate of TIPN-related dose reductions was almost four-times higher in African American women receiving Taxol® than African American women receiving Taxotere® (Taxol®: 78% vs. Taxotere®: 20%; $p = .001$).

Physical Risk Factors for TIPN.

Obesity. Obesity is thought to play an important role in TIPN. An analysis of more than 4,500 women with breast cancer found that women with BMI in the obese range (i.e., ≥ 30 kg/m^2) were 23% more likely to develop TIPN during treatment than non-obese women receiving the identical cancer treatment (Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge, et al., 2012). Bao et al. reported an even larger risk of

developing TIPN in obese women in their study of 296 women receiving taxanes, with women classified as being obese having nearly 2-times the risk of developing TIPN compared to women with BMI classified as normal (adjusted OR: 1.94, 95% CI [1.0, 3.7]). Other studies have linked *body-surface-area* (BSA) to risk of TIPN, showing a 2-3-fold increased risk of TIPN based on BSA (Schneider et al., 2015).

Obesity may also be an important risk factor for developing TIPN that fails to resolve after treatment. Results of the recently completed Pathways Study which used multivariate analyses to study risk factors for TIPN in 1,237 women with breast cancer found that women classified as overweight (i.e., BMI = 25-29 kg/m^2) were more than twice as likely to still have TIPN 24 months after treatment (OR: 2.37, 95% CI: [1.2, 4.9], $p < .02$), compared to women with BMI in the recommended range (i.e., BMI<25) (Greenlee et al., 2017). Results of the same study found that women who were classified as obese per current guidelines (i.e., ≥ 30 kg/m^2) were more than 3-times more likely to report having TIPN 2 years after their treatment had ended (OR: 3.21, 95% CI [1.5, 7.0], $p < .003$). Currently the reason why patients with higher BMI and BSA appear to be at increased risk of TIPN is not known.

Diabetes and Poor Glycemic Control. Poor glycemic control and diabetes are both well-established risk factors for developing sensory and autonomic neuropathy (Balcioglu & Muderrisoglu, 2015). However, evidence demonstrating that diabetes/poor glycemic control is associated with TIPN is mixed; for example, a 2015 study of 950 patients with lung cancer by Johnson et al. found that diabetic patients receiving taxanes had almost two-and-a-half greater odds of developing TIPN than non-diabetic patients receiving the same drug regimen (OR: 2.41, 95% CI [1.5, 3.9], $p = .0002$) (Johnson et al., 2015). Likewise, Schneider et al. found that women displaying grade 2-4 hyperglycemia[8] at any point during their treatment were 47% more likely to develop TIPN than women with normal range blood glucose, after adjusting for age, race, obesity, and menopausal status (adjusted OR: 1.47, 95% CI [1.2, 1.8], $p < .001$) (Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge, et al., 2012). The risk of developing TIPN in this study was even higher for hyperglycemic

[8] According to the guidelines for Version 2 of the National Cancer Institute's Common Toxicity Criteria Adverse Events (NCI-CTC-AE, Ver. 2) (which was used in the study), unless otherwise specified, Grade 2 or greater hyperglycemia is defined as a fasting blood glucose level >160–250 mg/dL or >8.9–13.9 mmol/L.

women receiving Taxol® if they were receiving weekly Taxol®; the odds of developing TIPN during treatment was 98% higher for women with poorly-controlled glucose levels than women with normal range blood glucose levels receiving weekly Taxol® (OR: 1.98, 95% CI [1.3, 3.1], $p < .004$).

These findings are consistent with anecdotal reports from other studies that the risk and severity of TIPN is often higher among patients with diabetes or poor glycemic control (Bhatnagar et al., 2014; Seretny et al., 2014; Visovsky et al., 2008a; Wampler et al., 2005). However, this finding is not universal, with many studies showing little or no association between diabetes/poor glycemic control and taxane-induced neuropathies (Kanbayashi et al., 2010; Mols et al., 2016; Pereira et al., 2016; Tanabe et al., 2013). One possible reason for the lack of agreement in these studies may have to do with the lack of tools for measuring sub-clinical neuropathy. For example, in the discussion of their analysis, Johnson et al pointed out that the increased risk of TIPN in diabetic patients "…could be due to existing asymptomatic diabetic neuropathy since up to 50% of diabetic patients may have asymptomatic neuropathy" (Johnson et al., 2015). More research is needed to resolve this discrepancy.

Menopause. To date, there has been little reason to think that menopausal status is a risk factor for TIPN. However, a 2007 study of a *paclitaxel poliglumex*, a novel formulation of Taxol®, found much higher than expected incidence of neuropathy (77.8%) during the study. Because the sample was largely post-menopausal, during the discussion the authors pointed out that "paclitaxel appears to be cleared more slowly in the absence of estrogen (personal communication, Cell Therapeutics)," which could mean taxane-induced neuropathies could (indirectly) be "…modulated by menopausal status."(N. U. Lin et al., 2007). Similarly, Schneider et al.'s large study of more than 4,500 women found that pre-menopausal women had an approximately 20% lower risk of developing TIPN during taxane therapy compared to post-menopausal women (OR: 0.77, 95% CI [0.6, 1.0], $p < .025$), and that this difference in risk between pre- and post-menopausal women was even larger in women receiving weekly Taxol® (OR: 0.70, 95% CI [0.5, 1.1], $p < .092$).

Genetic Risk Factors for TIPN. One of the most active areas of TIPN research has been research seeking to understand the role genetics plays in taxane-induced neuropathies. To date, more than 300 polymorphisms have been linked to the development of TIPN in both animals (S. B. Smith, Crager, & Mogil, 2004) and humans (Abraham et al., 2014; Hertz et al.,

2012; Leskela et al., 2011; Mir et al., 2009; Rizzo et al., 2010; Schneider et al., 2015). Results of these studies implicate genetically-mediated differences in the size and speed of the inflammatory response, ability to metabolize and excrete Taxol®, maintain optimal cell signaling, and direct mitochondrial behavior in the development of neuropathy during Taxol® therapy (Chen et al., 2015). While these studies have offered important insights into the mechanisms driving TIPN, many of these studies lack statistical power and agreement, slowing efforts to find clinically-useful genetic predictors for TIPN.

To address these shortcomings, several groups have taken advantage of emerging statistical and analytic techniques. For example, Chen et al. recently used a mass spectrometry-based proteomics and Ingenuity Pathway Analysis to identify a network of just 12 proteins that predicted women who would develop at least a 20% increase in TIPN symptom severity during treatment with Taxol® (Chen et al., 2015). Similarly, in an animal model of TIPN, Xu et al. found than Taxol®-induced neuropathic behavior improved when genes involved in neuro-inflammation and glial cell activation were down-regulated with a CB_2-receptor antagonist (Xu et al., 2014).

Psychological Risk Factors for TIPN. Psychological state and traits also appear to play a role in the incidence and severity of TIPN in BCS. Although psychological considerations have been largely absent from studies of TIPN, several recent studies have begun to explore the influence of depression and anxiety on this condition. A prospective study of 296 post-menopausal BCS receiving taxanes by Bao et al. found that the severity of TIPN was strongly associated with both anxiety ($p = .001$) and depression scores ($p = .016$) measured using the Hospital Anxiety and Depression Scale (Bao et al., 2016). Likewise, results of a study of 174 patients (including 100 BCS receiving Taxotere®) found that while TIPN did not predict anxiety and depression scores, anxiety scores were significantly higher for BCS who reported painful TIPN (Ventzel, Jensen, Jensen, Jensen, & Finnerup, 2016), suggesting a connection between TIPN severity and anxiety. More work is needed to clarify the role that psychological risk factors play in TIPN.

Part 2: Potential Risk Factors for TIPN

This section risk factors that may put BCS at risk for developing TIPN (potential risk factors), but which have not been shown to increase BCS' risk for TIPN directly. Potential risk factors for TIPN include breast surgery, radiation therapy (radiotherapy), and hormonal therapy.

Breast Surgery. Little research has been performed exploring the degree to which nerve injuries incurred during breast surgery may increase or decrease the risk women have of developing TIPN during Taxol® therapy. This is somewhat surprising given that the majority of women undergo breast surgery prior to starting Taxol®, and because there is extensive evidence that surgery on or around the breast can result in damage to the nerves that innervate the upper extremity (Abdullah, Iddon, Barr, Baildam, & Bundred, 1998; Amichetti & Caffo, 2003; Andersen, Aasvang, Kroman, & Kehlet, 2014; Andersen et al., 2013; Caffo et al., 2003; Carpenter et al., 1998; Carpenter et al., 1999; Del Bianco et al., 2008; Duale, Ouchchane, Schoeffler, & Dubray, 2014; Fassoulaki, Sarantopoulos, Melemeni, & Hogan, 2000; S. R. Freeman et al., 2003; Gartner et al., 2009; Gottrup, Andersen, Arendt-Nielsen, & Jensen, 2000; Ivens et al., 1992; Kuehn et al., 2000; Langford et al., 2014; Maycock, Dillon, & Dixon, 1998; Mejdahl, Andersen, Gartner, Kroman, & Kehlet, 2013; Miaskowski et al., 2014; Passavanti et al., 2006; Rietman et al., 2003; Rietman et al., 2004; Rietman et al., 2006; Schulze, Mucke, Markwardt, Schlag, & Bembenek, 2006; Tasmuth, von Smitten, & Kalso, 1996; Vilholm, Cold, Rasmussen, & Sindrup, 2009).

The incidence and severity of surgically-induced neuropathy in these studies was primarily related to the location and extent of the surgery. Surgeries that involve less contact with the axilla (e.g., axillary lymph node dissection or lumpectomies) are associated with less frequent, severe, or longstanding neuropathies (Passavanti et al., 2006; Rietman et al., 2003; Rietman et al., 2004; Rietman et al., 2006).[9]

Despite the limited work in this area, some groups have found a potential connection between breast surgery and TIPN. For example, Pereira et al found that the relative risk of developing TIPN for women whose treatment included axillary lymph node dissection was more than 2-times higher (RR: 2.28, 95 % CI [1.2, 4.3]) than women whose surgery did not

[9] This was especially true if the intercostobrachial nerve was damaged, which is common in surgeries that involve sweeping the axilla for potentially cancerous lymph nodes such as axillary lymph node dissections.

have axillary involvement, after adjusting for age, education level, and stage of cancer (Pereira et al., 2016). However, when the authors controlled for the effect of chemotherapy in their analysis, axillary lymph node dissection no longer was a significant predictor of TIPN in their model (RR: 1.24, 95 % CI [0.7, 2.0]), suggesting that chemotherapy-exposure is the dominant risk factor for TIPN, even among women whose treatment includes surgery.

Radiotherapy. Another potential risk factor for TIPN is accidental or intentional irradiation of nerves surrounding the breast or other areas harboring breast tumors (known as radiotherapy). Between 33-47% of women receive radiotherapy during treatment (Brackstone et al., 2015; National Academies of Sciences, 2016; National Comprehensive Cancer Network, 2014a), and between 0.5-5.0% of women will develop radiation-induced peripheral neuropathies (Bruera, 2013).

As noted above, neuropathy symptoms associated with breast surgery are characterized by sensory disturbances that appear shortly after surgery, improve over time, and affect the area(s) immediately surrounding (or distal to) the surgical site. In contrast, radiation-induced neuropathies are slow to develop (Burns, 1978; Wu et al., 2014), grow worse over time (Wu et al., 2014), and can affect both peripheral and central portions of the nervous system (Avila, Goenka, & Fontenla, 2011; Gosk, Rutowski, Urban, Wiecek, & Rabczynski, 2007; Kelly, Dinkin, Drappatz, O'Regan, & Weiss, 2011; Y.-S. Lin, Jen, & Lin, 2002).

While reports of the amount of time it takes for radiation-induced neuropathies to develop vary, time-of-onset appears to be inversely proportional to the amount of radiation women received (P. S. Berger & Bataini, 1977), with high radiation exposures actually taking longer to appear. For instance, a recent study by Wu et al. found at least a 6-month gap between the end of treatment and the development of radiation-induced peripheral neuropathy symptoms, with an average time of onset of 39 months (range: 37–65 months). Several authors have reported cases of radiation-induced peripheral neuropathy in BCS anywhere from 6-20 years after treatment (P. S. Berger & Bataini, 1977; Johansson, Svensson, & Denekamp, 2000; Y.-S. Lin et al., 2002). For example, Gosk et al. reported cases of radiation-induced sensory and motor neuropathy up to 23 years after radiotherapy (Gosk et al., 2007). Similarly, Johansson, Svensson and Denekamp identified cases occurring in BCS up to 34 years after radiotherapy (Johansson et al., 2000), with up to 51% of BCS in their

study reporting severe sensory loss an average of 10 years after treatment (Johansson et al., 2000).

Hormonal Therapy. A third potential risk factor for TIPN is hormonal therapy. Agents in this category (also referred to as endocrine therapies) include *selective estrogen-receptor modulators* such as tamoxifen (Nolvadex®), and *aromatase inhibitors* such as anastrazole (Arimidex®). Hormonal therapies have been indispensable for treating estrogen- and progesterone-sensitive breast cancers (which account for approximately 60% of breast carcinomas) but are known to produce musculoskeletal symptoms such as joint pain (*arthralgia*) and muscle soreness (*myalgia*) in approximately 50% of women who receive these therapies (Sestak et al., 2008).

Traditionally, the musculoskeletal symptoms associated with hormone therapy have been distinguished from TIPN by the quality of the symptoms and the fact that aromatase inhibitors are typically prescribed after Taxol® therapy, when many BCS' neuropathy symptoms have begun to subside). That said, the musculoskeletal symptoms associated with hormonal therapy have a strong resemblance to the muscle pain which affects an estimated 85% of BCS during the first few days of starting Taxol® therapy, raising questions about whether these two sets of symptoms share a common pathological pathway (J. Robarge, 2015; S. Wolf, Barton, Kottschade, Grothey, & Loprinzi, 2008).

Data from clinical trials show that exposure to taxanes are a risk factor for developing aromatase-induced musculoskeletal pain (Henry et al., 2012; Sestak et al., 2008), but studies clarifying whether hormonal therapies increase the incidence, severity, or duration of TIPN during or after Taxol® have yet to be performed. A 1995 study by Tasmuth et al. found hormonal therapy was a significant predictor of chronic pain in BCS after both lumpectomy and modified radical mastectomy (Tasmuth, von Smitten, Hietanen, Kataja, & Kalso, 1995), but a recent systematic review and meta-analysis of 31 studies of CIPN (which included data from 4,179 patients) did not identify hormonal therapy as a risk factor for TIPN (Seretny et al., 2014). The results of this meta-analysis are limited in part by the lack of clinical measures designed to differentiate musculoskeletal symptoms from TIPN. In addition, recent pre-clinical research by Robarge et al. in rats showing that exposure to aromatase inhibitors elicits mechanical sensitivity similar to that observed in TIPN suggests that more research is needed

to clarify whether they increase the incidence or severity of taxane-induced TIPN symptoms on BCS after treatment (J. D. Robarge et al., 2016).

Section 4: The Role of Early Detection in Managing TIPN in BCS during Taxol® Therapy

This section reviews the role that neuropathy screening and early detection has in managing TIPN. Part 1 describes the objectives that screening BCS for TIPN are designed to meet. Part 2 describes the methods used by nurses and other providers to detect early signs of TIPN during treatment. Part 3 describes limitations associated with existing screening methods (including the inability to detect small-fiber TIPN) that would argue for the need for an early detection method for small-fiber TIPN. Part 4 reviews the literature on the two potential early detection methods for small-fiber TIPN evaluated in this study, axon reflexes and axon flares.

Part 1: Purpose of Screening TIPN

Evaluating BCS for neuropathy serves many purposes, which vary by women's stage of cancer treatment (Table 2-2). Prior to starting Taxol®, neuropathy screening is used to establish BCS' baseline sensory, motor, and autonomic function, increasing the accuracy of screening during treatment (Hile, Fitzgerald, & Studenski, 2010). Baseline neuropathy screening also helps identify undiagnosed pain or neuropathy that may increase women's risk of developing TIPN during treatment. These include hereditary forms of neuropathy (e.g., Charcot Marie Tooth syndrome ((Hausheer, 2008)), chronic pain, diabetic neuropathy, or nerve impingement (Backonja & Galer, 1998; Park et al., 2008a; Stubblefield et al., 2009; Tofthagen, Visovsky, et al., 2013a; Visovsky et al., 2008b).

Table 2-2

Goals of Screening Patients for Neuropathy before, during, and after Taxol® Therapy

Stage of Therapy	Type of Assessment	Clinical Objectives
Before Taxol®	Screening	• Identify potential risk-factors for TIPN • Establish baseline neurological function
During Taxol®	Screening Diagnosis	• Identify potential signs/symptoms • Differentiate TIPN from other neurological conditions

	Assessment	• Assess location, severity, frequency, duration, and type of symptoms • Evaluate impact of TIPN on quality of life, work, social life • Develop plan for addressing TIPN related-issues • Monitor for signs of worsening TIPN
After Taxol®	Screening	• Identify undiagnosed signs/symptoms of TIPN
	Assessment	• Evaluate impact of TIPN • Monitor for signs of improvement • Develop plan for addressing TIPN related-issues

Notes. Abbreviations: TIPN= Taxane-Induced Peripheral Neuropathy

Once treatment begins, the focus of assessment shifts to detecting signs and symptoms of TIPN (Table 2-2). This includes screening BCS for possible signs of TIPN, differentiating these signs from conditions that can mimic TIPN (e.g., diabetic neuropathy, pain caused by tumors on or around nerves) and assess the type, severity, and impact TIPN is having. Detecting signs of TIPN as early as possible during treatment is critical so that both BCS and their providers can plan for potential disruptions in cancer treatment that may take place (Bouhassira & Attal, 2011). Being able to identify TIPN before symptoms start to interfere with women's ability to function also gives survivors and their support system time to develop strategies to avoid TIPN-related falls and to plan for potential disruptions to work and daily activities.

After cancer treatment, neuropathy screening is used to identify signs/symptoms of TIPN that may have been overlooked during treatment (Baron et al., 1997) (Table 2-2). The priority at this stage is determining which neurological changes are the most distressing and disabling and developing a clear plan to address them. Because TIPN can affect BCS' comfort, sleep, ability to perform work, and perform routine physical tasks after treatment, developing an effective plan for addressing TIPN is likely to require involvement from a variety of specialists, including neurologists; pain-management experts; physical, occupational, and vocational therapists; sleep specialists, and others.

It is also important to note that even in the absence of interventions in the clinical setting, early detection is vital. Fundamentally, TIPN is no different from other neurological conditions for which we currently have limited treatment options, but which nurses are called to manage effectively (e.g., Parkinson's, Alzheimer's, Guillain-Barre). To do this, nurses need tools that can identify signs of the disorder quickly, establish a diagnosis, determine which

neurological functions are being affected, and develop a plan with patients and other caregivers to manage symptoms and minimize potential negative outcomes.

Part 2: Methods to Screen BCS for TIPN during Taxol®

There are several methods used to detect TIPN in BCS during and after Taxol®: (1) self-report, (2) neuropathy grading scales, (3) structured questionnaires, (4) neurological examination, and (5) neurophysiological testing. A summary of each method is described below.

Self-Report. The simplest way to screen for TIPN is to ask patients to describe their symptoms. Often this is accomplished by simply asking BCS if they are having symptoms of TIPN such as tingling, numbness, or pain. This information is charted in the patient's medical record and can be followed then throughout the treatment period.

Neuropathy Grading Scales. Another method used to screen BCS for TIPN is with a neuropathy grading scale. Scales like the National Cancer Institute's Common Toxicity Criteria Adverse Events (NCI-CTC-AE) and Eastern Cooperative Oncology Group's (ECOG) scale have been cornerstones for TIPN assessment for decades (Argyriou, Zolota, Kyriakopoulou, & Kalofonos, 2010; Cavaletti, Alberti, Frigeni, Piatti, & Susani, 2011; Cavaletti & Marmiroli, 2012). Signs and symptoms are assessed by providers, who assign sensory, motor, and autonomic symptoms a numerical score based on the level of symptoms and degree of functional impairment (Table 2-3).

Questionnaires. A third method used to screen TIPN is to allow patients to rate their own symptoms using a structured questionnaire. Examples of measures in this category include the Functional Assessment of Cancer Treatment Gynecologic Oncology Group – Neurotoxicity Subscale, Neuropathy Pain Scale, and Peripheral Neuropathy Scale. Unlike neuropathy grading scales, which require the nurse to rate patients' sensory or motor changes on a scale, questionnaires allow patients to report different types of sensory and motor symptoms they are experiencing (e.g., tingling, numbness, burning), providing a more detailed picture of TIPN. In addition, the recent development of several *patient-reported outcome* measures for CIPN, which combine questions asking about different types of TIPN symptoms with an evaluation about the impact that these symptoms are having on different activities (e.g., walking, climbing stairs, driving), are helping researchers understand the true impact of TIPN (Table 2-3) (Binda et al., 2013; Cavaletti et al., 2013; Griffith, Dorsey, Renn, Zhu,

Johantgen, Cornblath, Argyriou, Cavaletti, Merkies, Alberti, Postma, Rossi, Frigeni, Bruna, Velasco, Kalofonos, Psimaras, Ricard, Pace, Galie, Briani, Dalla Torre, et al., 2014; Lavoie Smith et al., 2013),

Table 2-3

Types of Symptoms and Functional Impairments Assessed Using Common Screening Tools for Taxane-Induced Neuropathy

	NPS	FACT-Ntx	CIPN-20	PNS	SCIN
Sensory Symptoms					
Pain/discomfort (presence)	•	•		•	•
Pain (intensity)	•				
Pain (depth)	•				
Pain (location)	•				
Dull pain	•				
Sharp pain	•				
Numbness		•	•	•	
Tingling		•	•	•	•
Shooting/burning pain	•		•		
Increased skin sensitivity	•				
Itch	•				
Joint pain/muscle cramps		•	•		
Stiffness/tightness				•	
Difficulty feeling objects		•		•	
Ringing/buzzing in ears		•			•
Difficulty hearing		•	•		•
Difficulty feeling hot/cold?			•		
Motor Symptoms					
Weakness		•			
Clumsy/lack of coordination				•	
Autonomic Signs/Symptoms					
Dizziness upon standing?			•		
Blurred vision?			•		
Difficulty getting/maintaining erection?			•		
Function Impairment					
Difficulty standing/walking		•	•		
Difficulty writing			•		
Difficulty with small objects			•	•	
Difficulty with hand weakness			•		

Difficulty ambulating because of foot drop	•	
Difficulty getting up because of leg weakness	•	
Difficulty using pedals in car when driving	•	

Notes. Abbreviations: NPS = Neuropathy Pain Scale; FACT-GOG-Ntx = Functional Assessment of Cancer Treatment Gynecologic Oncology Group – Neurotoxicity Subscale; EORTC CIPN20 = European Organization for Research and Treatment of Cancer Chemotherapy Induced Peripheral Neuropathy; PNS = Peripheral Neuropathy Scale; SCIN= Scale for Chemotherapy-Induced Long-Term Neurotoxicity.

Neurological Examination. A fourth approach used to screen BCS for TIPN during Taxol® is evaluating patient's neurological function directly. In the clinical setting, this is often accomplished using a *composite measure*, which combines elements of self-report with simple tests for signs of TIPN such as changes in reflexes and vibrotactile sensitivity (E. M. Smith, 2013a; E. M. Smith, Cohen, Pett, & Beck, 2010).

The best-known composite measure for TIPN is the Total Neuropathy Score® (TNS), which combines questions about sensory symptoms such as tingling, numbness, and pain, with manual testing for deficits in the patient's ability to feel vibration and reflex testing with a reflex hammer (Donovan, 2009; E. M. Smith, 2013a; E. M. Smith et al., 2010; Visovsky et al., 2008a). Early versions of the TNS also included electodiagnostic testing (Cavaletti et al., 2006; Cornblath et al., 1999), but were set aside in later versions to make it possible to perform the TNS in the busy clinical setting.

More detailed information about the type and degree of neurological changes that occur during Taxol® therapy can be collected using *quantitative sensory testing*, but the testing procedures and need for specialized equipment required for quantitative sensory testing make this approach a better choice for diagnosis than routine screening (Cruccu et al., 2010; Gruener & Dyck, 1994; Schattschneider, Uphoff, Binder, Wasner, & Baron, 2006; Shipton, 2013; Siao & Cros, 2003; Zochodne, 2007).

Electrodiagnostic Testing. Another approach traditionally used to screen patients for TIPN is *neurophysiological testing* (also known as *electrodiagnostic testing*). As the name implies, neurophysiological testing uses physiological parameters such as the amplitude, speed, shape, and latency of nerve impulses to evaluate nerve structure and function (T. Berger, Malayer, et al., 1997). Examples of common neurophysiological tests include nerve conduction velocities,

needle electromyography, sensory nerve action potentials, and evoked potentials (Atherton et al., 2007; Obermann et al., 2008; Shipton, 2013; Valeriani, Le Pera, Niddam, Chen, & Arendt-Nielsen, 2002). Results are objective, can be obtained quickly, and can be compared to normative values, providing detailed information about nerve physiology (T. Berger, Malayer, et al., 1997; Bromm & Lorenz, 1998; Truini et al., 2010).

Part 3: Limitations of Screening Methods for TIPN

While valuable parts of overall TIPN assessment, current methods for evaluating TIPN have limitations that make them poorly suited for detecting early signs of TIPN in general and detecting early signs of small-fiber TIPN.

Limitations Associated with Self-Report. While participant's subjective experience of TIPN is vital for understanding the severity and impact that different signs and symptoms of TIPN have for BCS (Bridges & Smith, 2014), it is not a reliable method for detecting TIPN. Studies show that some percentage of BCS fail to disclose TIPN when asked. Reasons can include: fear of offending providers; concern that reporting symptoms will result in their cancer treatment being suspended; uncertainty whether the signs or symptoms they are experiencing constitute TIPN; difficulty describing their neuropathy symptoms to providers; forgetting to disclose signs and symptoms of TIPN because of stress; needing to discuss other symptoms associated with their cancer treatment during assessment; and/or being asked about TIPN using terminology that is unfamiliar or confusing (Hershman et al., 2014; Lavoie Smith, 2010; Lavoie Smith et al., 2009; Paice, 2009; E. L. Smith et al., 2002; Stubblefield et al., 2009; Tofthagen, 2010; Tofthagen, Visovsky, et al., 2013a, 2013b; Vadalouca et al., 2012).

Relying on self-report to detect early symptoms of TIPN can also be difficult if symptoms do not fall into a single category or seem paradoxical in nature (e.g., painful numbness) (Dworkin et al., 2003). There is also some indication that relying on self-report to identify early signs of TIPN may be harder for BCS who have higher symptom burden. Research in patients with multiple, co-occurring symptoms has shown that multiple symptoms can create a "blinding" effect which makes it more difficult for BCS to describe individual symptoms in the cluster. Relying on self-report to detect TIPN without an objective marker is also problematic because analgesics being taken by BCS can mask TIPN symptoms, making it difficult to identify early signs of TIPN and begin to predict the toxicity's likely trajectory (Cavaletti et al., 2011; Cavaletti & Marmiroli, 2012; Stubblefield et al., 2009).

Relying on self-report to detect early signs of TIPN is also problematic because, by definition, patients cannot report changes of which they are not aware. Because of this, relying on self-report risks missing signs of TIPN that may be appearing before symptoms. While the introduction of composite measures like the TNS (which includes tests for potential signs of TIPN such as changes in reflexes and vibrotactile thresholds) is certainly a step in the right direction, because the sensory testing performed in the TNS still relies on patient feedback, it is not clear that these approaches are a reliable way of detecting the earliest signs of TIPN.

Limitations Associated with Pain/Neuropathy Tools Not Validated for TIPN. Although useful for evaluating pain and neuropathy from other sources (e.g., diabetes, injury), evidence suggests that screening tools which have not been validated for TIPN specifically by nurses are at risk for missing TIPN. For example, a recent study by Smith et al in 386 patients with cancer found that 50% of patients reported no pain at all on a standard 1-10 numeric pain scale but reported moderate-to-severe neuropathic pain when screened with a scale specifically designed to measure chemotherapy-induced neuropathic pain (Lavoie Smith et al., 2009).

Limitations Associated with Electrodiagnostic Testing. While there is evidence supporting the usefulness of neurophysiological testing to diagnose TIPN and detect abnormalities before treatment (Argyriou et al., 2008; T. Berger, Malayer, et al., 1997; Quasthoff & Hartung, 2002), evidence supporting the use of neurophysiological testing to detect early signs of TIPN is limited (Quasthoff & Hartung, 2002). Testing is expensive, requires specialized staff (Cavaletti et al., 2011; Stubblefield et al., 2009). Studies in this area show poor concordance between electrodiagnostic tests and TIPN symptoms (Argyriou et al., 2005; Pan & Kao, 2007), made worse by the fact that neurophysiological abnormalities often only appear after BCS have had TIPN for some time. This limits the usefulness of this type of testing for early detection.

Additional Limitations. The lack of tools for detecting small-fiber neuropathy is also an impediment to clinical trials evaluating potential therapies for TIPN (Herrmann, 2008). There are several reasons for this. First, to be effective, clinical trials for TIPN must establish whether participants are free from neuropathy at baseline (Umapathi et al., 2007). Otherwise, results of the trial risk rejecting potentially effective therapies.

Second, because it is possible for an intervention to improve neurological function without improving symptoms (e.g., preventing structure changes in nerves without preventing

functional changes such as the onset of tingling or pain), to determine whether therapies are palliative or protective in nature, measures that do not depend on symptoms are needed.

Finally, physiologically-based screening tools for TIPN are needed to clarify whether the mechanism(s) being targeted can be modified without reducing Taxol's ability to fight breast cancer. Being able to answer this question will depend on researchers having access to tools that allow them to understand the mechanisms that give rise to and perpetuate taxane-induced neuropathies and monitor the effect of interventions BCS in vivo. This is especially true as it pertains to the small-fiber component of TIPN, which has been largely unstudied in humans because of the lack of reliable screening tools.

Section 5: Potential Method for Detecting Early Signs of Small-Fiber TIPN in BCS Receiving Taxol®

This section reviews the literature on two potential early detection methods for small-fiber TIPN evaluated in this study, axon reflexes and axon flares.

Local Skin Heating

A potential method for detecting small-fiber neuropathy during the early portion of Taxol® treatment is *local skin heating*. Local skin heating (also known as *local thermal hyperemia*) is a non-invasive test that uses heat, delivered with a small heat probe attached to the skin to stimulate small-fiber nerves in the skin (Roustit & Cracowski, 2012). When these temperature-sensitive small-fiber nerves are exposed to heat, action potentials from the nerve travel antidromically (i.e., backwards) along fiber, triggering the release of neuropeptides such as calcitonin gene-related peptide (CRGP) on surrounding blood vessels, causing them to dilate. This reflexive dilation in response to skin heating is known as *axon reflex-mediated vasodilation*. There are two basic approaches used to screen patients for signs of impaired axon reflex-mediated vasodilation using local skin heating: *axon reflexes* and *axon flares*. A description of each technique is provided below.

Measuring the Size of Axon Reflexes during Local Skin Heating. The most well-known method for evaluating small-fiber nerve function using local skin heating is to track the response with LDF (Blaise, Roustit, Millet, & Cracowski, 2011). LDF takes advantage of the fact that while human skin has adapted to reflect ultraviolet light, it is permeable to light in the infrared spectrum (Oberg, 1990). During LDF monitoring, a beam of near infrared light (~760 nm) is transmitted from a small laser diode through the skin where it strikes red blood cells

Figure 2-6. Basic Theory of Monitoring Axon Reflex during Local Skin Heating Using Single-Point Laser Doppler Flowmetry

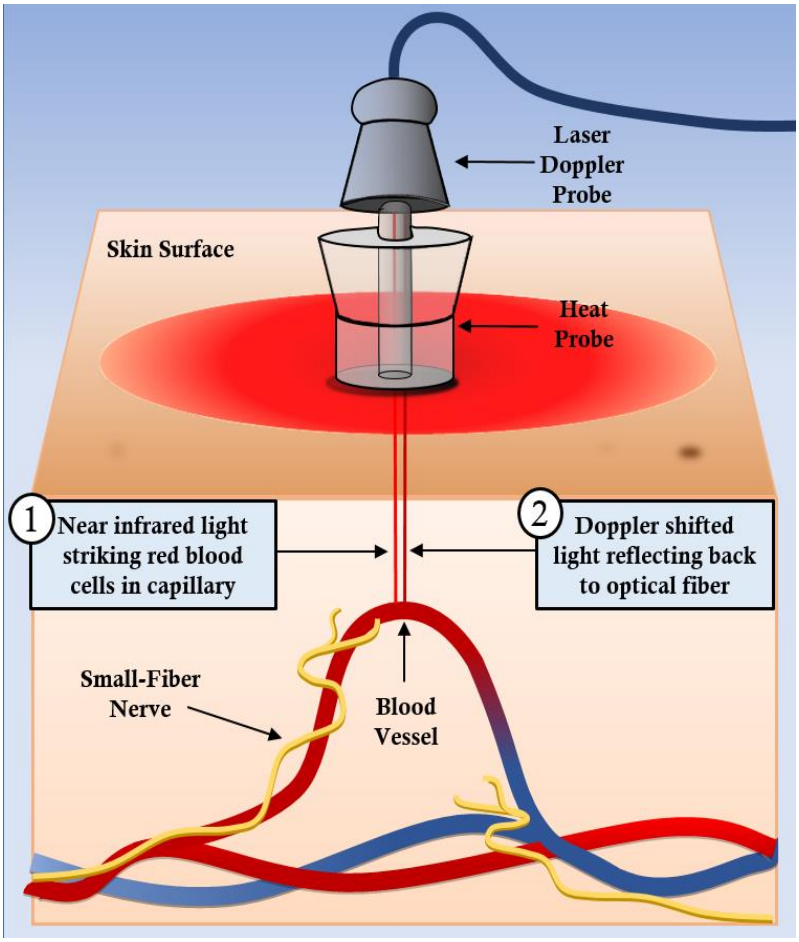


Figure 2-6. The illustration depicts the basic process used to monitor changes in skin blood flow (SkBF), including the axon reflex, during local skin heating. Initially, a heat probe is placed on the skin surface. Next, a single-point laser Doppler probe placed in the center of heat probe, allowing a beam of near-infrared light (~765 nm) to pass through the skin (1). As the light passes through the skin, it strikes red blood cells (RBCs) passing through the superficial blood vessels located in dermis. As the light strikes, these RBCs, it is Doppler shifted (2), allowing the laser Doppler monitor to infer the degree of vasodilation in the vessels caused by the stimulation of surrounding temperature-sensitive, small-fiber afferents. Original artwork by Noah Zanville, copyright, 2017.

passing through the superficial capillaries beneath (Figure 2-6). As the light from the LDF strikes the red blood cells, it undergoes a Doppler shift, with the resulting frequency of the wave being proportional to the velocity of the red blood cell. The Doppler-shifted light is then detected by an optical fiber located next to the laser diode (Cracowski, Minson, Salvat-Melis, & Halliwill, 2006; Ingemar Fredriksson, 2012; Oberg, 1990; B. J. Wong & Fieger, 2010; B. J. Wong & Minson, 2011b).

Once this Doppler-shifted signal has been captured by the LDF, it is converted to an arbitrary unit of measure (tissue perfusion units) and displayed on an attached computer screen as a waveform that moves from left to right as LDF monitoring proceeds (Figure 2-7). As discussed in Chapter One, in individuals with intact small-fiber function, exposure to 42 °C heat results in a predictable rise in cutaneous blood flow characterized by (a) baseline blood flow, followed by a (b) steep rise in blood flow following the increase to 42 °C (the axon

Figure 2-7. Increase in Cutaneous Blood Flow in Response to 40-Minute, 42 °C Local Skin Heating

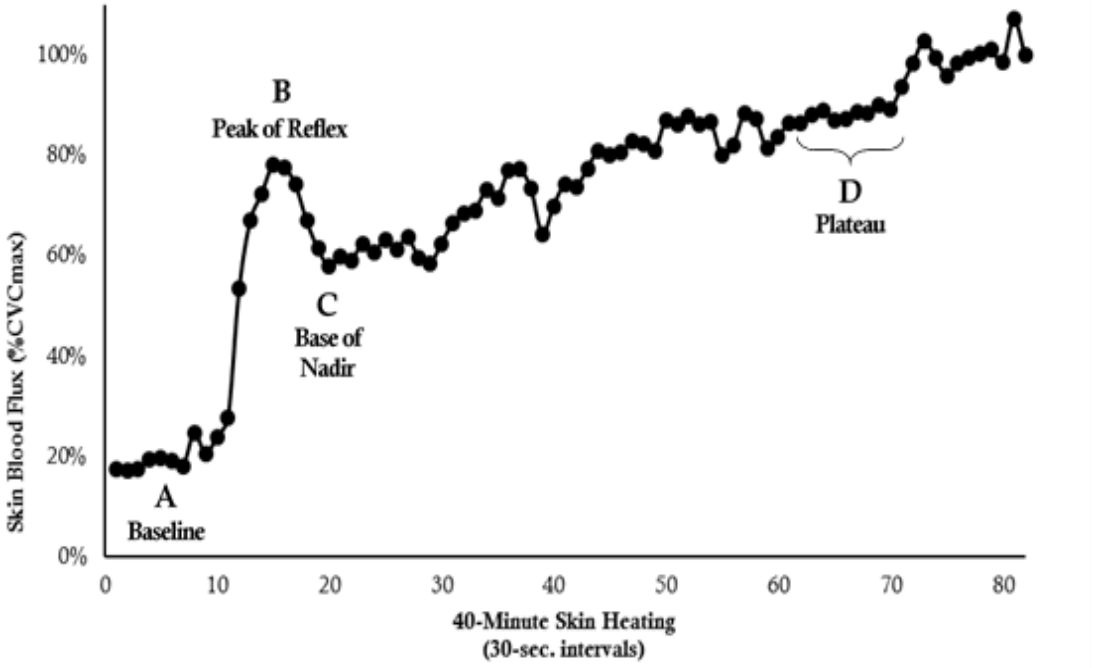


Figure 2-7. As illustrated in Chapter One, graph illustrates the typical, bi-phasic increase in skin blood flow that occurs during 40-minute, 42 °C local skin heating. Points represent 30 seconds of skin blood flow (SkBF) data in the right toe surface during local skin heating. (A) Initial SkBF during 33 °C skin heating (used to establish baseline. (B) Peak of axon reflex after temperature of heat probe turned up to 42 °C (rate: 0.1 °C/1 sec). (C) Brief nadir in SkBF. (D) If 42 °C skin heating continues, SkBF continues to rise in slower fashion, typically plateauing near participant's theoretically maximum SkBF.

reflex), (c) a small return towards baseline (the *nadir*), which is followed by (d) a longer, slower rise in blood flow that plateaus near the maximum range (Roustit & Cracowski, 2012)

Unlike the second rise in blood flow during skin heating, which is driven primarily by the release of nitric-oxide (NO) and other chemical mediators from the interior of the blood vessel during skin heating (Minson et al., 2002; Wilkins et al., 2003; B. J. Wong & Minson, 2011b; B. J. Wong et al., 2003; B. J. Wong et al., 2004; B. J. Wong et al., 2006)), the initial rise in blood flow during skin heating is primarily neurogenic (S. T. Krishnan & G. Rayman, 2004; Mahe, Humeau-Heurtier, et al., 2012; Minson et al., 2001; P. R. Vas & G. Rayman, 2013b).

The non-invasiveness, ease, and sensitivity of evaluating axon reflexes using LDF make it ideal for measuring small-fiber nerve function. Studies using LDF to measure axon reflexes show that when the location of testing is standardized, the coefficient of variability for axon reflexes range between 10-19% (C. S. Huang, Wang, & Tsai, 2012; Roustit, Blaise, Millet, & Cracowski, 2010). In addition, in a study of 124 patients with suspected small-fiber neuropathy, Devigili et al. identified signs of diminished axon-reflexes in more than one-third (38.8%) of patients which correlated well with results of similar neurophysiological testing, quantitative sensory testing, skin biopsy, and clinical assessments (Devigili et al., 2008)

Measuring the Size of Axon Flare after Local Skin Heating. Another approach used to evaluate small-fiber nerve function with local skin heating is to measure the hyperemic area that develops around the surface of the heat probe following skin heating, which is known as an axon flare (Green, Krishnan, & Rayman, 2009; S. T. Krishnan et al., 2009; S. T. Krishnan & G. Rayman, 2004; L. Moor Instruments, 2001; P. R. Vas & G. Rayman, 2013a, 2013b)). Research has shown that the area of increased cutaneous blood flow that develops around the heat probe is not the result of heat from the probe penetrating the surrounding tissue (which is a poor conductor of heat), but because of the network of temperature-sensitive small-fiber nerves embedded in the skin (Figure 2-8). Like the size of axon reflex that develops during local skin heating, research with topical anesthetics have confirmed that the size of flare that develops after skin heating is dependent on temperature-sensitive small-fiber nerves (Green et al., 2009; Krämer et al., 2004; S. T. Krishnan & G. Rayman, 2004; P. R. Vas & G. Rayman, 2013b).

To date, more than a dozen studies have shown that changes in the size of axon flares can detect signs of small-fiber neuropathy in humans, including individuals without clinically-detectable signs such as pain (A. Bickel et al., 2009; Bickel et al., 2002; Green et al., 2010; Green et al., 2009; S. T. Krishnan et al., 2009; S. T. Krishnan & G. Rayman, 2004; Nabavi Nouri et al., 2012; Van der Schueren et al., 2007; P. R. J. Vas et al., 2012; M. C. Wong, 2010). In diabetic patients, changes in size of the axon flares generated on the dorsal foot are 77% sensitive and 90% specific for detecting signs of neuropathy (P. R. J. Vas & G. Rayman, 2013a). Research also has shown that changes in the size of heat-evoked axon flares may be more sensitive than quantitative sensory testing for evaluating neuropathy (Green et al., 2010). Protocols for generating heat-evoked axon flares in as short as 6 minutes have been validated for the non-glabrous skin on the dorsal foot (P. R. Vas & G. Rayman, 2013b; P. R. J. Vas & G. Rayman, 2013a), providing a clinically-feasible test for temperature-sensitive, small-fiber nerve function in the periphery.

Figure 2-8. Illustration of Heat Conductance in Skin during Local Skin Heating

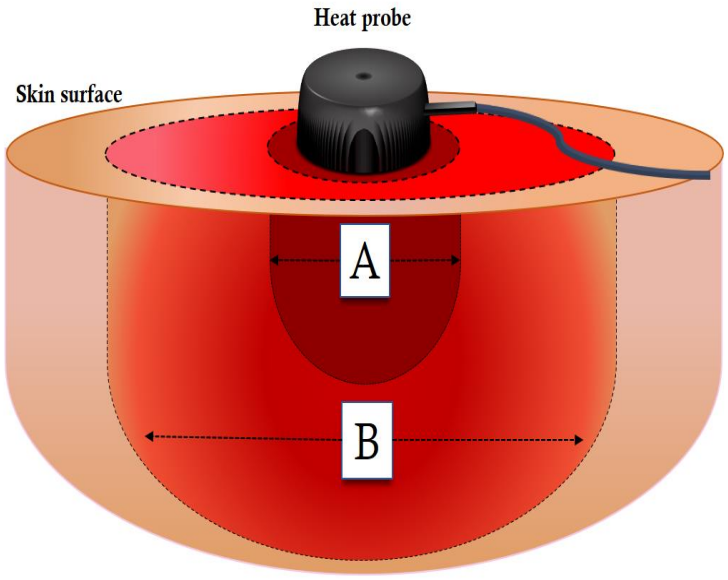


Figure 2-8. Because skin is a relatively poor conductor of heat, heat from the skin heater is transferred primarily to the skin directly underneath the probe (A), with little transfer to the surrounding tissue. The large area of increased skin blood flow that results from local skin heating (axon flare (B)) is primarily due to the activation of cutaneous c-fibers generative axon reflexes to surrounding blood vessels. Original artwork by Noah Zanville, Copyright 2017.

Results of these studies also indicate that there is a strong inverse relationship between the size of axon flares and the severity of neuropathy (S. T. Krishnan & G. Rayman, 2004; Nabavi Nouri et al., 2012; P. R. Vas & G. Rayman, 2013b), providing a potential way to quantify the extent of small-fiber nerve dysfunction. Studies also show that reductions in flare size following skin heating correlate with a loss of small-fiber nerves in the skin (Andreas Bickel et al., 2009), and the corneal measured with skin biopsy and in vivo confocal microscopy (respectively). These findings suggest that axon flares may be a useful companion for these anatomical screening methods. In addition, Sharma et al. were able to detect small-fiber neuropathy in BCS a year following their Taxol® therapy (Sharma, Venkitaraman, et al., 2015). This latter finding suggests that the axon flare approach may be a useful way to screen women for signs of small-fiber neuropathy after treatment.

Potential Benefits of Local Skin Heating for TIPN Research. Local skin heating offers a potentially-useful model for small-fiber TIPN, which is critical to translating findings from the pre-clinical setting to humans. Pre-clinical research with axon reflexes has also shown that Taxol® significantly reduced the size of axon reflexes in the rat hind-paw in response to capsaicin, which activates TRPV.1 receptors in a manner analogous to ~43 °C skin heating). Results of these studies found that Taxol® reduced the size of axon reflexes without changing the amount of calcitonin gene-related peptide being generated in the spinal cord (Gracias, 2011; Pittman et al., 2013), or the ability of the smooth muscle in the surrounding blood vessels to dilate when exposed to a direct vasodilator (Gracias, 2011; N. Gracias et al., 2011; Pittman et al., 2014). These suggests that Taxol® may interfere with the ability of small-fiber nerves to release calcitonin gene-related peptide into surrounding blood vessels. The present study, which stimulates small-fiber nerves using topical heat instead of capsaicin, is, to our knowledge, the first to attempt to replicate the findings from this pre-clinical model in humans during early Taxol® therapy.

Chapter Summary

TIPN is a serious and potentially debilitating side effect of Taxol® therapy for BCS. There is a growing body of literature suggesting that small-fiber sensory nerves may play an important role in TIPN, but methods for screening women for small-fiber neuropathy are limited, especially early in treatment when damage may be difficulty to identify. Results of this review of the literature indicate that an accurate, non-invasive method for evaluating small-

fiber nerve function early in treatment is needed to fill current gaps in clinical assessment and dictate early intervention for TIPN.

CHAPTER THREE

METHODOLOGY

This chapter describes the methods used to investigate whether changes in the mean size of axon reflexes or axon flares during local skin heating could detect early signs of small-fiber TIPN in BCS receiving Taxol®. Information described in this chapter is divided into five sections. Section 1 provides an overview of the study, including the design, sample, study approval, protection of human subjects, funding, eligibility, justification for proposed sample size (power analysis), and methods used to recruit participants for the study. Section 2 describes the instruments used to collect data for the study's three aims, and Section 3 describes the procedures used to recruit participants and collect data from participants using these instruments. In Section 4, the methods used to transfer, store, and prepare data for analysis are described. Section 5 ends the chapter by describing the statistical methods used to analyze data for the study.

Section 1: Study Overview

Design and Sample

A prospective, observational study design was used to collect data from 20 BCS with first-time, non-metastatic breast cancer receiving Taxol®, and 20 HCs. Data was collected at 3 time-points (Times 1, 2, and 3) which took place during the first 6 weeks of BCS' weekly Taxol® therapy. Time 1 took place at week 0, before participants' first weekly Taxol® infusion. Time 2 took place exactly 14 days later, before participants' third weekly Taxol® infusion. Time 3 took place 28 days later, before participants' seventh weekly Taxol® infusion.

Study Approval

Approval for this study was granted by the Institutional Review Board (IRB) at Indiana University in May 2015,[10] as well as by the Scientific Review Committee (SRC)[11] overseeing research at the Indiana University Melvin and Bren Simon Cancer Center. In addition, because the study met 3 of the 4 criteria for a clinical trial outlined by the FDA, in

[10] IRB protocol #1502603664
[11] SRC protocol #0529

accordance with Title VIII of the FDA Amendments Act 801,[12] prior to starting, the study was registered on the ClinicalTrails.gov website (ID#: NCT02549534).

Protection of Human Subjects

All procedures during the study were performed in accordance with policies for the safe and ethical treatment of human subjects outlined in the Declaration of Helsinki (World Medical Association, 2013), and research policies of Indiana University. Verbal and written consent was collected from all subjects prior to participation in the study (Appendices A-1 and A-2). A description of potential risks associated with participating in the study is outlined in Appendix B-1. Steps used to minimize these risks are outlined in Appendix B-2. A summary of stopping rules for the study, which were outlined before visits, is included in Appendix B-3.

Funding

Funding for the study was provided by four sources: (1) a federal training-grant awarded by the National Institutes of Nursing Research (NINR) under the F-31 mechanism (award number: 1F31NR015212-01A1); (2) a doctoral research grant jointly provided by the Midwest Nursing Research Society and the Council of the Advancement of Nursing Science; (3) internal funding from the Indiana University School of Nursing available to doctoral fellows on the National Cancer Institute's R-25 Training in Research for Behavioral Oncology and Cancer Control training program (award number: R25CA117865); and (4) supplemental funding from the Walther Cancer Foundation, Inc.'s Behavior Cooperative Oncology Group (BCOG) Wind Down Fund (#0162.01).

Eligibility Criteria

Inclusion Criteria. Female BCS were eligible for the study if they: (1) were between 18-85 years at the time of enrollment; (2) had histologically-confirmed, first-time, non-

[12] According to Title VIII of the FDA's 2007 "Food and Drug Administration Amendments Act 801" (FDAAA 801), human subjects research in adults with FDA-approved biomedical devices (i.e., devices with a 501(k) approval must register their study on ClinicalTrails.gov if they: (1) involve prospective clinical study of health outcomes that (2) compare an intervention with a device against a control in human subjects with devices that are (3) FDA regulated, and the trial is not (4) a small clinical trial to determine the feasibility of a device or a trial to test a prototype device where the primary outcome measure relates to feasibility and not to health outcomes). During the planning stages of the study, Indiana University-Purdue University Indianapolis's (IUPUI) Office of Regulatory Compliance determined that while this study seeks to determine the feasibility of detecting small-fiber neuropathy in BSC receiving early Taxol® (which would make it exempt from these regulations), that "registering the study with ClinicalTrials.gov would be advised nonetheless."

metastatic breast cancer (i.e., stage I-IIIB breast cancer per breast cancer staging guidelines (National Comprehensive Cancer Network, 2016)); (3) had no prior exposure to neurotoxic chemotherapy or radiotherapy; (4) were scheduled to receive Taxol® on a weekly (80-100 mg/m^2) or bi-monthly (175 mg/m^2)) basis as a part of their cancer treatment; and (5) could read, write, and understood English fluently.

HCs were eligible for the study if they: (1) were between 18-85 years at enrollment; and (2) could read, write, and understand English fluently.

Exclusion Criteria. During screening, potential participants from both arms of the study were excluded if they reported any of the following during their initial screening:

1. History of cardiovascular disease, peripheral arterial, or peripheral vascular disease that could affect peripheral blood flow.
2. History of hypertension or current use of anti-hypertensive medications/supplements (both of which could affect the responsiveness of blood vessels during local skin heating and otherwise blunt the axon reflex response (Bruning, 2013)).
3. Use of statins (which can produce neuropathy-like symptoms in some individuals) (Dalle-Ave et al., 2004; Harris, Nishiyama, Wray, & Richardson, 2010).
4. History of diabetes (with exception of gestational diabetes) (which puts participants at risk for undiagnosed small-fiber neuropathy) (P. R. J. Vas & G. Rayman, 2013c).
5. Diagnosed or suspected neuropathy, neuropathic pain, or nerve injury (which would serve as confounder) (de Jongh et al., 2004; Pauling et al., 2012).
6. Pre-existing pain or arthritis in the toes of either foot (another potential confounder).
7. Current disease or fungal infection of the feet (which theoretically could be made worse by skin heating and shared between participants).
8. Significant damage or deformity to the feet (which could lead to changes in cutaneous blood flux that would make it impossible to measure/interpret findings).

9. Suspected or diagnosed vasospastic disease such as Raynaud's Syndrome (which can alter how participants respond to local skin heating (Bruning, 2013)).
10. Current use of tobacco/tobacco-containing products (which acts as stimulant, increasing sympathetic tone, heart rate, and blood pressure (Valentini & Parati, 2009), and can contribute to hardening of peripheral arteries (Li, Lyu, Ren, An, & Dong, 2017)).
11. Restless Leg Syndrome or other movement disorders (which could make it difficult to interpret data recorded using the LDF or FLPI).

Evidence supporting the use of these exclusion criteria is provided in Table 3-1.

Justification for Proposed Sample Size (Power Analysis)

Prior to starting the study, a power analysis was performed to determine the number of participants needed to address the study's three aims. The proposed sample size ($N = 40$; $n = 20$ per group) was based on the number of participants needed to power comparisons for Aim 1 (differences in mean axon reflex size). Because the studies that use changes in mean axon reflex size to detect TIPN had not been published in 2014 when the current study was being planned, studies evaluating mean axon reflex size in patients with suspected or confirmed diabetic neuropathy and neuropathy-free individuals were used for the power analysis (Bruning, 2013; Houghton, Meendering, Wong, & Minson, 2006).

To ensure that data from these studies was as relevant as possible to this investigation, studies included in the power analysis were limited to: (1) human studies, (2) measuring mean axon reflex size, (3) using heating protocols similar to the one used here, and (4) which had significant representation by female participants (Bruning, 2013; Houghton et al., 2006; Minson et al., 2001; Minson et al., 2002; B. J. Wong & Fieger, 2010; B. J. Wong & Minson, 2011b).

Prior to starting, a 25% attrition rate, five participants per group was assumed to help protect the study against potential losses in statistical power. Using this assumption, results of the power analysis predicted that 15 participants per group ($N = 30$) would give the study >90% power to detect differences in mean axon reflex size as small as 25% CVC_{MAX} (i.e., 75% vs. 50%) between groups using two-tailed independent samples *t*-tests, assuming standard deviations (*SD*) in reflex size no larger than 20% and an alpha of .05 (Aim 1, Hypotheses 1.1 and 1.2). Results of the analysis also predicted that 30 participants would give the study 90%

power to detect changes in mean axon reflex size (%CVC_{MAX}) over time with size as small as 18% using repeated-measures ANOVA ($\alpha = 0.05$) (Aim 1, Hypothesis 1.3), assuming correlations in reflex size between visits of at least 0.5 (i.e., 50%).

Results of the analysis also indicated that 15 subjects per group would provide the study approximately 80.0% power to detect correlations in mean axon reflex size and neuropathy scores of $r = 0.60$ as statistically significant for Aim 3 using a one-sided *t*-test.

Recruitment

BCS for the study were recruited from two breast clinics located in the downtown Indianapolis area -- the Indiana University Melvin and Bren Simon Cancer Center (IUSCC) and the Breast Clinic at the Sidney and Lois Eskenazi Hospital. Clinics were chosen for their (1) proximity to the IU School of Nursing and IU School of Medicine (facilitating transportation of the laser imagers needed for the study to-and-from study visits); (2) availability of a Recruitment Core at each clinic to assist with study accrual; (3) access to rooms with thermostats needed to maintain the specified temperature range for the experiment (25±1 °C); and (4) access to a private room in or near the infusion center, enabling researchers to schedule study visits between Taxol® infusions with minimal disruption to patients or providers. Both breast clinics were also located near the Clinical Research Center used to run study visits for HCs. A detailed description of procedures used to recruit and enroll BCS and HCs for the study is described in Section 3 (Procedures).

Section 2: Methods Used to Collect Data for Study

Methods described in this section are divided into three parts: Part 1 describes methods used to collect data for antecedent variables in the theoretical model listed in Chapter One, including sociodemographics, menopausal status, menstrual status, height, weight, and BMI of study participants. Part 2 describes methods used to collect data for covariates in the theoretical model, including participants' stage of breast cancer; details on their cancer treatment; the temperature, humidity, light levels and airflow in testing rooms; heart rate; blood pressure; and current pain levels. Part 3 describes methods used to collect data for outcome variables listed in the theoretical model, including the mean size of axon reflexes in the right toe (Aim 1); the mean size of axon flares in the right toe (Aim 2); and clinical signs and symptoms of TIPN (Aim 3).

Part 1: Methods Used to Collect Data for Antecedent Variables

Sociodemographics. Sociodemographic information was collected from participants at baseline using a 10-item, multiple-choice questionnaire. Items on the questionnaire have been used to collect sociodemographic information from BCS in previous studies (Champion et al., 2007) (Appendix C). Items 1-7 on the 10-item questionnaire asked participants to identify their race, ethnicity, highest level of education, estimated annual household income, current relationship status, religious/spiritual affiliation, and occupation type from a list of pre-defined options. For each multiple-choice question, participants were given the option to write in their own response if the existing options did not match their experience. Participants' age at enrollment (in years) was collected from their initial eligibility screening form (Appendix D).

Menopausal Status. Items 7-10 on the sociodemographic questionnaire asked participants to categorize their menopausal status (Appendix C). The purpose of collecting this information was to allow researchers to determine if mean axon reflex/flare size differed based on menopausal status during analysis. To evaluate participants' menopausal status, women were first asked to categorize themselves as pre-, peri-, or post-menopausal.

To ensure that this question was understood, women were also asked to select which statement best described when they had last menstruated from a list that included the following choices: "I have not had a menstrual period in the last 12 months," "I have had a menstrual period in the last 12 months but not in the last 3 months," "I have had a menstrual period in the last 3 months, but cycles are less regular," or " I have had a menstrual period in the last 3 months, no change in regularity." Women who reported no longer menstruating were asked to describe why from a list of pre-determined options (e.g., "normal aging," "breast cancer treatment," "medication unrelated to cancer treatment," "surgery (such as hysterectomy or ovaries removed)," "other (please describe)," "don't know/unsure," or "not applicable.")

Stage of Cancer. Information about BCS' stage of breast cancer was collected at enrollment by self-report and verified using participants' medical records available to the researcher.

Height, Weight, and BMI. Participants' height (in cm) was measured at the initial visit using a digital stadiometer (QuickMedical International, Model 235A) or standard tape measure. Participants' weight (in kg) was measured at each visit using a digital scale (Model 5002, Scale-Tronix, Inc.; Wheaton, Illinois). Prior to being weighed, participants were asked to remove their shoes, jacket, and other large items of clothing that could affect their weight. Height and weight values collected at each visit were used to calculate BMI (in kg/m^2) during analysis. Participant's height and weight were recorded on Section 2A of the main data collection form (Appendix I).

Part 2: Methods Used to Collect Data for Covariates

Cancer Treatment. For BCS, the dose (in mg/m^2), duration (in hours), and frequency (weekly vs. bi-monthly) of Taxol® were collected from medical records at each visit. The doses, durations, and frequencies of other cancer treatments BCS received during (e.g., Herceptin®) or prior to entry into the study (e.g., Adriamycin®, Cytoxan®) were also collected from participants' medical records at each visit. Information on the dose, duration, and frequency of medications given before Taxol® to prevent hypersensitivity reactions (e.g., diphenhydramine, famotidine, dexamethasone), as well as medications with the potential to produce symptoms that could be confused with TIPN (e.g., aromatase inhibitors and selective-estrogen receptor modulators) were also collected from participants' medical records. Information on the dose and frequency of medications not permitted in the 24 hours prior to study visits such as NSAIDs were collected by self-report during the pre-study visit communication.

Menstrual Status. As noted above, items 7-10 on the sociodemographic questionnaire asked participants to indicate whether they were pre-, peri-, or post-menopausal. Participants who identified themselves as pre- or peri-menopausal on their initial questionnaire were asked to describe whether they were menstruating (yes/no) at each study visit, and to describe where they were at in their menstrual cycle (i.e., actively menstruating vs. not menstruating actively) (Cracowski et al., 2006).

Physiological Variables.

***Resting Heart Rate*.** During the local skin-heating portion of study visits, resting heart rate (in beats per minute (BPM)) was measured using an automated heart rate monitor placed on the index or middle finger of the hand not fitted with the blood pressure cuff (Dinamap

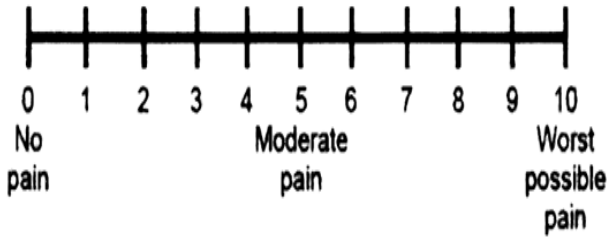
Procare-300 Model®, General Electric Corporation). Participants' heart rate was measured once before the experiment and continuously during the 40-minute skin-heating protocol, but only recorded at 10-minute intervals (i.e., minutes 0, 10, 20, 30, 40). Heart rate values were recorded on the study sheet.

Resting Blood Pressure. Participants' resting systolic and diastolic blood pressure (in mm Hg) was measured during study visits using the same monitor used to measure resting heart rate (Dinamap Procare-300 Model®, General Electric Corporation). As with heart rate, resting blood pressure was measured once prior to starting the 40-minute skin-heating protocol, and at 10-minute intervals thereafter. During analysis, 10-min systolic and diastolic blood pressure values were used to calculate mean arterial blood pressure (MAP) using the following equation: $MAP \cong DP \pm \frac{1}{3}(SP - DP)$ (Rogers & Oosthuyse, 2000). During visits, the maximum cuff pressures for the blood pressure cuff were manually calibrated to 130-145 mm Hg to minimize discomfort during local skin heating.

Pain Levels. Current pain levels were measured at each visit using a Numeric Pain Rating Scale (NPRS) (Figure 3-1). Scores on the 11-point scale ranged from 0 (no pain whatsoever) to 10 (worst pain possible). Participant's pain level was recorded on Section 3 of the main data collection form (Appendix I).

Environmental Variables. Ambient temperature in the testing room (in °C) was measured using a commercially-available temperature probe (Meade Instruments, Model TE256W). Relative humidity in the testing room (%) was measured using the same probe (Meade Instruments, Model TE256W). The light level in the testing room was measured using a hand-held digital light meter (HHLM3 Model, Omega Engineering, Inc.). Finally, because studies have shown that strong airflow can affect the accuracy of laser perfusion imagers like the one used in the study (Mahe, Durand, Humeau-Heurtier, Leftheriotis, & Abraham, 2012; Mahe, Durand, Humeau, et al., 2012), the presence of air from the vents above the testing area (yes/no) was assessed at each visit. Data on the

Figure 3-1. Numeric Pain Rating Scale Used to Measure Pain Levels at Each Study Visit



temperature, humidity, light, and airflow in the testing room was recorded by the research assistant on Section 2B of the main data collection form (Appendix I).

Imaging Variables.

Skin-Heating Variables. Axon reflexes and axon flares were evoked in the palmar surface of the right great toe (i.e., first metatarsal) using a commercially-available skin heater fitted with a 0.33 cm^2 heat probe (Moor SH02 Skin Heating/SHP2 Model Heat Probe; Moor Instruments, Ltd., Axminister, U.K.). For all participants, the heat probe was placed in the middle of the right palmar toe surface.

Part 3: Methods Used to Collect Data for Outcome Variables

Subclinical Signs of TIPN.

Mean Size of Axon Reflexes in Right Toe during Local Skin Heating (Aim 1). Axon reflexes were evoked in the palmar surface of the right great toe (first metatarsal) using a commercially-available skin heater fitted with a 0.33 cm^2 heat probe (Moor SH02 Skin Heating/ SHP2 Model Heat Probe; Moor Instruments, Ltd., Axminister, U.K.). Fluctuations in SkBF in the right toe during local skin heating were measured using a laser Doppler flowmeter equipped with a single-point Doppler probe (Flowmeter: MoorVMS-LDF/Probe: VP12 Model; Moor Instruments). For all participants, the size of the axon reflex response was measured in the middle portion of the toe.

Mean Size of Axon Flares in Right Toe after Local Skin Heating (Aim 2). The size of axon flares in the palmar surface of the right great toe was imaged using a commercially-available full-field laser perfusion imager (FLPI) (MoorFLPI-1, Moor Instruments Ltd.; Axminister, UK). FLPI scans were performed using the following settings: sampling frequency of 25 Hz (i.e., 0.4 frames per second); imager setting: low resolution/high speed setting. Prior to scanning, gain was adjusted manually to improve image contrast. The distance between the head of the imager and palmar surface of the toe was standardized at 15 cm for all participants per current recommendations (Mahe, Durand, Humeau-Heurtier, Leftheriotis, & Abraham, 2012; Mahe, Durand, Humeau, et al., 2012).

Clinical Signs and Symptoms of TIPN (Aim 3). Clinical signs and symptoms of TIPN were measured using the 5-item short form of Total Neuropathy Score (Reduced Version) (TNSr-SF) described in (E. M. Smith, 2013a). The original TNS is a composite assessment tool validated for use in TIPN and other forms of neuropathy (Cavaletti et al.,

2007a; Cavaletti et al., 2006; Cornblath et al., 1999). Like other versions of the TNS, the TNSr-SF combines questions about the presence and location of potential symptoms of TIPN such as pain, numbness, and tingling with testing for objective signs of TIPN such as partial or total loss of deep-tendon reflexes, and changes in vibrotactile sensitivity. However, unlike the Original and Reduced-versions of the TNS (which are longer and require specialized testing equipment (Cavaletti et al., 2006; Cornblath et al., 1999)), the Short Form of the TNSr has just 5 items and was designed specifically for use by busy nurses in the outpatient oncology setting (E. M. Smith, 2013a), making it an ideal choice for the current study.

As with earlier versions of the TNS, items on the TNSr-SF are scored using a 5-point scale, which ranges from 0 (normal) to 4 (severe) based on how far up the limbs each sign or symptom of TIPN extends (Cornblath et al., 1999; E. M. Smith, Beck, & Cohen, 2008; E. M. Smith et al., 2010).[13] Individual scores on each of the 5 items are then summed to create a total neuropathy score which ranges from 0 to a maximum of 20, with higher scores indicating more severe TIPN (Griffith, Merkies, Hill, & Cornblath, 2010; E. M. Smith et al., 2010).

Section 3: Description of Procedures Used to Recruit Participants and Collect Data during Study Visits

This section describes procedures used to recruit participants for the study and collect data using the methods described in the previous section. Procedures are divided into two parts: Part 1 describes the process used to recruit, screen, enroll, and schedule participants, including the steps used to match BCS and HCs by age during enrollment. Part 2 provides a description of procedures used to collect data from participants during each of their three study visits.

Part 1: Recruitment Procedures

Recruitment for Breast Cancer Survivors. As described in Section 1, BCS were recruited from two breast clinics in the Indianapolis area with the help of trained staff from the Recruitment Core who oversee research at each clinic. Approximately once a week, Recruitment Core staff would alert researchers via email or phone messages of names of

[13] Per TNS scoring guidelines, participants receive 0 points if neurological function is normal/intact, 1 point if participants show signs/symptoms in their fingers/toes, 2 points if their signs/symptoms extend up to ankles or wrists, 3 points if their neuropathy signs/symptoms extend above the knees or elbows, and 4 points if their signs/symptoms extend above the knee or ankle.

women in the breast clinics who were going to be receiving Taxol® as part of their treatment regimen. Using this information, researchers contacted these BCS and set a time to explain the study to them. If interested, BCS were screened for eligibility.

To minimize potential disruption to BCS in the days immediately following their cancer diagnosis, before reaching out to potential participants, researchers consulted with staff in the breast clinic to determine when and how best to approach women based on their current level of distress. However, because weekly Taxol® was typically given after 8-12 weeks of induction therapy with Adriamycin® and Cytoxan®, in all but one case, researchers did not need to approach BCS immediately after their diagnosis about the study.

To protect participants' privacy, recruitment-related meetings and phone calls were held in a private location. Also, emails between participants and researchers were encrypted and sent on secure servers provided by the university.

During meetings introducing the study to potential BCS, researchers described the purpose and goals of the study, including potential risks associated with participating. During all discussions, researchers emphasized that participation in the study was voluntary, and that the decision not to participate or to withdraw from the study would in no way affect their cancer treatment.

BCS expressing interest in the study were screened, and if eligible, invited to participate. When possible, eligible BCS were consented on the spot. BCS whose eligibility was determined over the phone were permitted to bring a signed copy of their informed consent to their initial visit or to fax it to researchers beforehand. Per university policy, no study-related procedures were performed until written consent had been collected from participants (consent forms provided in Appendices A-1 and A-2).

Recruitment for Healthy Controls. HCs for the study were recruited from the greater Indianapolis area. Potential HCs were made aware of the study using a combination of word-of-mouth, fliers posted throughout the community (Appendix E), IRB-approved advertising on social media (e.g., Facebook™), and referral from other participants ("snowball-sampling"). Screening and enrollment procedures for HCs were identical to those described for BCS above.

Age-Matching during Recruitment. Studies show that even in individuals with healthy nerve function, mean axon flare size declines by approximately 5% per decade

(Minson, Holowatz, Wong, Kenney, & Wilkins, 2002; Vas & Rayman, 2013a). In addition, studies have shown that neurological endpoints that depend on large-fiber nerves such as tactile discrimination and vibratory sensation decline with age (Decorps, Saumet, Sommer, Sigaudo-Roussel, & Fromy, 2014; Flanigan, Lauria, Griffin, & Kuncl, 1998). Because of this, during enrollment for HCs for the trial, age was used as an additional selection criterion to reduce the chance that differences in mean axon reflex or mean flare size observed during the study would be due to differences in the age of participants rather than Taxol® exposure.

The process of age-matching involved the following steps. First, HCs meeting the other eligibility criteria were asked their age, which was then converted to its corresponding decade (i.e., 20-29, 30-39, 40-49, 50-59, 60-69, 70-79). Next, the number of HCs in each age category was matched to the number of BCS already enrolled in the study using a chart made for the study (Table 3-1). In some cases, otherwise eligible HCs were wait-listed until BCS in their age-category entered the study to try to ensure groups were evenly matched on age. However, because BCS were the focus of this study, all BCS who were eligible for the study were enrolled, regardless of age.

Table 3-1

Schema Used to Match Potential Healthy Female Controls to Already-Enrolled Breast Cancer Survivors by Age, and to Record Race during Recruitment

	Breast Cancer Survivors Race				*Healthy Female Controls* Race				
Age (by Decade)	CA	AA	Other		CA	AA	Other	Sub-Total	Grand Totals
18-19									
20-29									
30-39									
40-49									
50-59									
60-69									
70-79									
80-85									
Subtotal (n)									
Subtotal (%) Target, by category									
Target, by study arm	20				20				40

Notes. Caucasian = CA; African-American = AA; Other = includes women that identify as Hispanic/Latina, Asian/Pacific Islander, Native American/Alaskan Native, others.

In addition, because studies have shown that both breast cancer and TIPN are more prevalent in certain racial groups than others (American Cancer Society, 2017; Bhatnagar et al., 2014; Schneider et al., 2015), race was recorded to improve analysis of the study's findings following completion of the trial. However, race was not used as an eligibility criterion for the study.

Consent, Enrollment, and Scheduling. After determining eligibility, participants in both arms of the trial were consented using IRB-approved consent form (Appendices B-1, B-2). After being consented, signed copies of consent and HIPAA forms were made available to participants upon request. During the initial consent meeting, participants were also given the option to fill out the 10-item sociodemographic questionnaire (Appendix C). Participants who did not wish to fill out the questionnaire at this visit were given the option to email this form back to researchers or to bring the completed questionnaire to their first visit.

After their consent meeting, participants were sent an introductory email using encrypted university servers. The goals of this introductory email were to: (1) welcome participants to the study; (2) reiterate the purpose and goals of the study; (3) identify potential dates/times for each of the three study visits; (4) provide participants with contact information for the members of the research team; (5) remind participants about the importance of avoiding caffeine, alcohol, and NSAIDs prior to each visit; (6) provide the address and instructions for reaching the location of their study visits; and (7) provide information about parking for their visits. An example of the welcome letter is provided in Appendix G.

For women in the BCS group, the timing of study visits was based on the timing of BCS' Taxol® infusions, which generally took place in the mid-morning or early afternoon.[14] Because the protocol used in this study took 60-

Figure 3-2. Typical Structure of Study Visit for Breast Cancer Survivors Based on Oncologist Visit and Taxol® Infusion

Activity	Duration (in minutes)
Arrive at infusion center	11:15 am
Get blood drawn	11:30-11:45 am (15 min.)
Visit with oncologist	11:45-12:15 am (30 min.)
Perform study visit	12:30-01:45 pm (75 min.)
Receive Taxol® pre-medications	01:45-02:15 pm (30 min.)
Receive Taxol® infusion	02:15-03:15 pm (60 min.)

[14] The reason for this is that Taxol® often takes up to 3 hours or longer to administer (including premedication times) depending on patient's tolerance for the drug. Because of this, clinicians in the

75 minutes to complete, in order to make it possible for BCS to fit their study visit between their visit with the oncologist/nurse practitioner and Taxol® infusion, study visits were performed in the mid-morning or early afternoon (Figure 3-2).

For women in the HC group, the dates and times for each of their three scheduled visits were determined by checking the participant's availability against researchers' availability. Visits for HCs were scheduled between 6:00 a.m. and 7:00 p.m. Whenever possible, study visits for HCs were scheduled at the same time during all three visits to minimize variations in blood flow related to time of day.

Prior to visits, BCS and HC were emailed or phoned. The purpose of this pre-visit communication was to: (1) ensure participants felt well enough to attend their visit; (2) remind them to wear loose-fitting clothes (which assisted with reflex testing and testing for vibrotactile thresholds using the tuning fork); (3) remind them to avoid caffeine and alcohol for 12 hours prior to their visit and to avoid NSAIDs for 24 hours prior to their visit; and (4) remind them to avoid eating for at least 1 hour prior to their visit.

In addition, prior to each visit, BCS were asked if they had been prescribed any medications since their last visit (e.g., corticosteroids, anti-emetics). The purpose of asking this question was to identify any medications not listed on participant's original paperwork which could affect the study's outcomes. A copy of the form used to collect data about pre-medications BCS may have been assigned before study visits is available in Appendix H.

Part 2: Description of Study Visits

Overview of Study Visits. Study visits took approximately 60-75 minutes. Procedures performed at each visit were divided into two general categories: those taking place before the start of local skin heating, and those taking place during and after skin heating (Figure 3-3).

Procedures taking place *before* the start of skin heating included: (1) performing the arrival procedures (described below); (2) measuring participants' height and weight and collecting data on the temperature, humidity, light levels and airflow in the testing room; (3) assessing participants' current pain level using the 11-point Numeric Pain Rating scale; and (4) evaluating clinical signs and symptoms of TIPN using the TNSr-SF.

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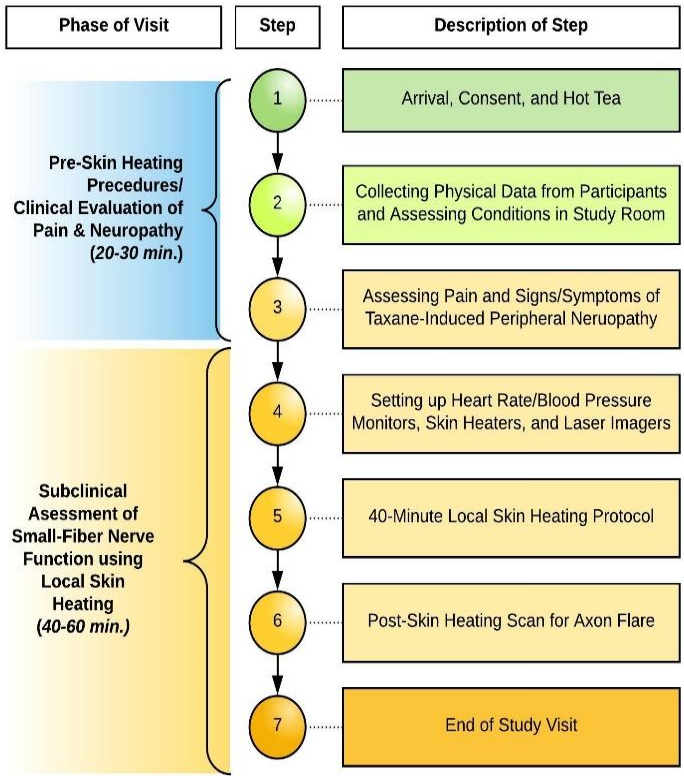
breast clinics (which were open until 5:00 p.m.) often would only start Taxol® infusions between 8:00 a.m. and 2:00 p.m.

Figure 3-3. Overview of Phases, Steps, and Activities Performed at each 60-75 Minute Study Visit



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Procedures taking place *during* and *after* the start of skin heating included: (4) setting up heart rate and blood pressure monitors and performing the pre-skin heating FLPI scan; (5) performing the 40-minute skin-heating protocol and recording the size of the axon reflex; (6) performing the post-heating scan to measure the size of the post-heating axon flare; and (7) completing all post-visit activities. A detailed description of procedures performed during study visits is described below.

Study Procedures Performed before the Start of Local Skin Heating.

Step 1: Arrival, Verification of Consent, and Body-Warming Protocol. Upon arrival, participants were greeted in the lobby of the Breast Clinic (for BCS) or the lobby of Clinical Research Center (for HCs). After confirming that researchers had collected a signed copy of participants' informed consent form, participants were given 150 ml of decaffeinated hot tea or water to drink. As described in the protocol developed by Rayman et al., the purpose of the hot tea was to begin to increase participants' core temperature, which was designed to increase peripheral blood flow by lowering sympathetic tone (Vas & Rayman, 2013b). The tea also was used to create a nurturing environment that would encourage participants to relax, with the goal of improving peripheral blood flow. While participants drank their tea, researchers verified their willingness to participate. Responses were recorded on the main data sheet (Section 1B, Appendix I).

Step 2: Collection of Physical Data on Participant Height and Weight and Environmental Data on Temperature, Humidity, Light Levels, and Airflow in Testing Rooms. Participants were led to a digital scale and weighed. Prior to being weighed, researchers zeroed the scale, and women were instructed to remove their shoes and any heavy garments/accoutrements (e.g., jackets, purses, backpacks). Participants were also asked to hand their tea to the research assistant during weighing to avoid altering weights. If it was the participant's first visit, her height (in cm) was measured using a digital stadiometer or standard tape measure. Participants' weight and height were recorded on Section 2A of the study assessment form (Appendix I).

Next, researchers led participants to the testing room, which was pre-heated to ~25±1 °C (~77° F). Once inside, researchers seated participants in a reclining chair equipped with pillows and asked them to remove their shoes and socks. To help increase participant's core temperature, researchers placed a blanket over her legs and feet, and placed a heated neck-wrap (Kaz HC1475 Wellness Wrap, Softheat® Corp.) around her neck and shoulders. Researchers also placed a memory-foam pillow behind her head/neck to ensure BCS were comfortable.

After situating participants, researchers visually inspected the skin on the palmar toe surface for callouses, bruises, or scars that could interfere with LDF or FLPI imaging (Cordovil et al., 2012). Potential issues with the skin on the toe were recorded on Section 2C on the main data collection form (Appendix I).

Step 3: Assessment of Pain Using Numeric Pain Rating Scale. After assessing the skin on the toe, participants' current pain level was verbally assessed using the 11-point Numeric Pain Rating Scale illustrated in Figure 3-2. Pain scores were recorded on Section 3 of the data sheet (Appendix I). Next, clinically-detectable signs and symptoms of TIPN were assessed using items from the TNSr using the procedure described below.

Step 4: Assessment of Clinical Signs/Symptoms of TIPN using TNSr-SF. Clinical signs/symptoms of TPN were assessed using the TNS-r Short Form (TNSr-SF) (E. M. Smith, 2013a). Clinical symptoms of TIPN (tingling, numbness, neuropathic pain) were assessed by asking participants to indicate if they were experiencing each symptom (yes/no), and if so, how far up the limb(s) the symptom(s) extended. Symptoms were scored using a 5-point scale (0 = none, 1 = symptoms limited to fingers/toes, 2 = symptoms extend to ankle, 3 =

symptoms extend to knee, 4 = symptoms extend past the knees) (E. M. Smith et al., 2010). Per TNS guidelines, symptoms extending into the upper extremity automatically received a 4 (i.e., scored as severe). Similarly, per TNS guidelines, for cases where symptoms extended further up the limb on one side than the other, the side with the more severe score was used.

After symptoms were assessed, deep-tendon reflexes were tested using a 5.7-ounce Tromner-style reflex hammer (MDF Instruments). Prior to testing, participants were instructed to relax their limbs. During testing for ankle reflexes, participants' ankles were gently dorsiflexed before striking the tendon to make it easier to elicit reflexes. For accuracy, reflexes were tested several times in each location, and scored using a similar 5-point scale to the one described above (0 = normal, 1 = ankle reflexes reduced, 2 = ankle reflexes absent, 3 = ankle reflexes absent/others reduced, 4 = all reflexes absent) (E. M. Smith et al., 2010).

Finally, vibrotactile thresholds were tested with a 128-hertz stainless steel tuning fork (American Diagnostic Corporation). For this study, thresholds were tested in only the right and left interphalangeal joints of the great toe, right and left medial malleoli, and right and left tibial tuberosities to save time. Testing was performed in the same order for all participants. The procedure was as follows. At each location, the researcher struck the tuning fork on his knee and placed the tip of the tuning fork on the skin over the site being tested so that the vibration was transferred to the underlying tissue. Prior to starting, participants were instructed to verbally indicate when they felt vibrations from the tuning fork on their skin. This was done to ensure the tuning fork was in place. Participants were then instructed to indicate when they could no longer could feel the vibration coming from the tuning fork.

Participants reporting that vibration had stopped when in fact it was still going (indicating a loss in vibrotactile sensation) were noted and assigned points on the TNSr-SF using the following scale: 0 = normal, 1 = reduced in toe, 2 = reduced up to ankle, 3 = reduced up to knee, 4 = reduced above knee. To ensure that participants did not see the tuning fork stop vibrating, prior to starting, participants were instructed to close their eyes during testing.

Study Procedures Performed during and after Start of Skin Heating.

Step 5: Preparation for Local Skin Heating. After clinical signs/symptoms of TIPN were assessed, researchers attached the instrumentation needed for the 40-minute skin-heating protocol to participants. To begin, researchers placed an external heart rate monitor on the

participant's index or middle finger and a blood pressure cuff around the participant's antebrachium. Next, researchers placed pillows under the participant's arm to ensure that the arm was approximately at heart level. In most cases, the right arm was used to monitor blood pressure because of the position of instrumentation relative to the participant, but in cases where BCS had a chemotherapy port or surgery on the right side, the left arm was used to minimize strain on lymphatics vessels in the affected side.

Before starting blood pressure monitoring, researchers performed an initial blood pressure reading to ensure that tightness of the cuff during inflation was comfortable to minimize movement during blood flow assessment. If necessary, the pressure of the cuff was reduced from the standard 160 mm Hg to 135-145 mm Hg. Changes in cuff pressure were recorded in the study notes.

Once heart rate and blood pressure monitors had been calibrated, the equipment was set to record the participant's heart rate and blood pressure at 10-minute intervals. Initial pilot testing for the study revealed that cutaneous blood flow would sometimes drop during the 60-90 second period that the blood pressure cuff was inflated, because either the blood pressure cuff startled participants or because it was mildly uncomfortable. This posed a potential problem for the study because if the axon reflex had not fully peaked by the time the first blood pressure reading began (i.e., minute 10), the blood pressure reading could reduce the size of the axon reflex. To avoid this, blood pressures were intentionally performed at minutes 2, 12, 22, and 32 rather than 0, 10, 20, and 30 to ensure that changes in peripheral blood flow associated with the start of the blood pressure cuff would not interfere with the axon reflex. The timing of BPs during 40-minute local skin heating is illustrated in Figure 3-4. Heart rate and blood pressure values were recorded in Section 4C of the Main Study Form (Appendix I).

While one member of the research team set up the automated heart rate and blood pressure, the other member of the team placed the participant's right foot in a custom-made orthotic designed to standardize the position of the foot. Memory foam was used to stabilize the sides and head of the foot in the orthotic, and 15 cm plastic "bridge" was used to connect the boot to the head of the laser imager. The purpose of this bridge was to ensure that the laser imager was within the recommended distance of the skin surface (~15-17 cm), and to standardize the distance between the skin and the imager during all experiments.

Figure 3-4. Timing of Blood Pressure Recordings during 40-Minute Local Skin Heating Protocol.

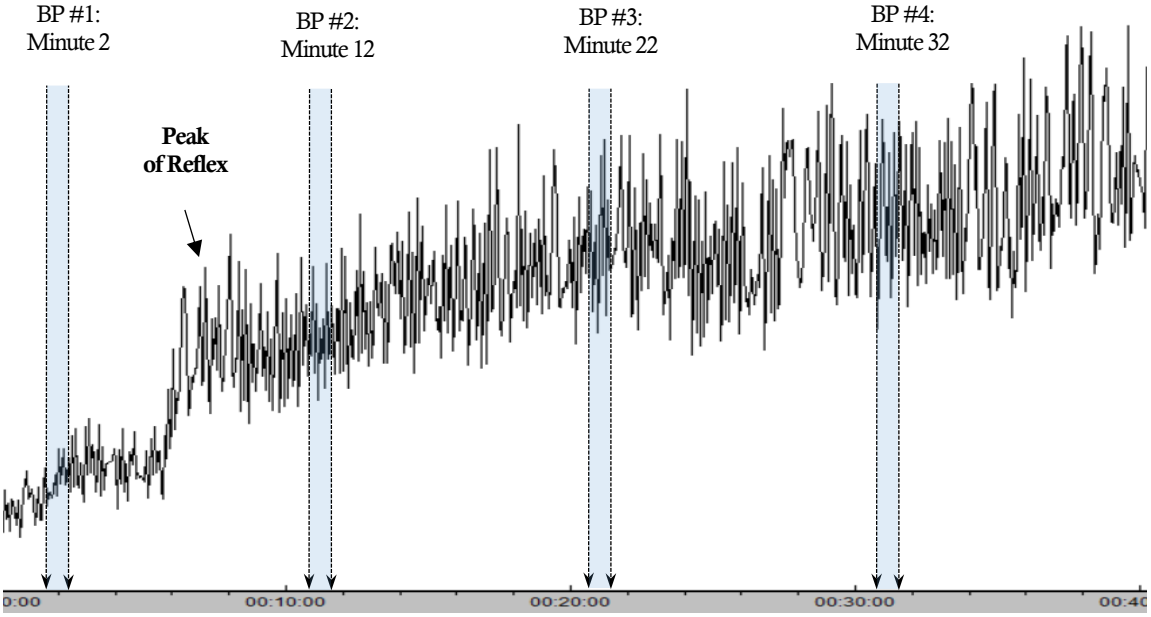


Figure 3-4. The tracing depicts a representative laser Doppler tracing showing the increase in skin blood flow in response to local skin heating. The bars in blue show the timing of the automated blood pressure (BP) recordings at minutes 2, 12, 22, and 32 specifically designed to minimize the potential for routine BP monitoring to interfere with the peak of the axon reflex.

Once the participants' foot had been situated in the orthotic boot, they were encouraged to rest quietly or sleep while researchers began blood flow assessment. Before starting, researchers verified that participants had been given at least 15 minutes to acclimate to the ambient room temperature (25±1 °C). Initially, a 2-minute scan of blood flow in the toe surface was performed using the FLPI. The purpose of this scan was to provide an index of baseline blood flow in the right toe which would be used for comparison during analysis with the post-heating response. Baseline data was saved to an external hard drive attached to a computer laptop.

Next, the heat probe (moorSH02 SHP2 Skin Heating Probe, Moor Instruments, Ltd.) and laser Doppler probe (VP12 model needle probe, Moor Instruments, Ltd.) were attached to the right toe. Custom-made "probe-slips," made from two pieces of hypoallergenic tape put back-to-back so that they would not stick to the skin, were used to hold the heat probe/laser in

place during skin heating. [15] Prior to starting blood flow monitoring, the Doppler signal was evaluated to ensure that the Doppler had been properly positioned. If necessary, the heat probe/laser was adjusted until researchers were confident the signal was adequate for testing.

Step 6: Local Skin Heating Protocol and Post-Heating Scan for Axon Flare Size. Prior to the start of skin heating, 30 seconds of blood flow data in unheated skin (e.g., 29 °C) was collected for reference. Next, the skin was heated to 33 °C at a rate of 0.1 °C/s and clamped for 5 minutes. The goal of this 5-minute starting period was to ensure that participants started from similar levels of vasodilation during the experiment (Bruning, 2013; Huang, Wang, & Tsai, 2012). After 5 minutes, the temperature of the heat probe was increased to 42 °C at a rate of 0.1 °C/s to evoke a neurogenic response (axon reflex) (Bruning, 2013; Minson, Berry, & Joyner, 2001; Wong & Minson, 2011). Once the probe reached 42 °C, the heater was clamped for 30 minutes. After 30 minutes at 42 °C, the heat probe was increased to 44 °C at a rate of 0.1 °C/s to induce near maximum vasodilation, providing the reference point needed to determine the size of the axon reflex as a $\%CVC_{MAX}$ (Wong & Minson, 2011). During each heat ramp, the number of seconds it took the skin heater to reach 33 °C, 42 °C, and 44 °C was recorded to ensure heat stimuli were being delivered similarly for all participants (Appendix K).

After skin heating was complete, data was saved to the external hard drive, and the heat probe/Doppler unit was carefully removed. Skin was visually assessed for signs of damage. Once researchers verified the status of skin under the heat probe, a final 2-minute FLPI scan was performed to capture the size of the post-heating axon flare.

Step 7: Post-Study Visit Activities. After completing the final FLPI scan for the axon flare, participants were informed the visit was concluded, and helped to gather their belongings. Participants were given an opportunity to use the bathroom, drink water, and presented with their 10-dollar gift card for completing the visit. Prior to disbursement, a signature was collected from participants to verify they had received their gift card. If it had not

[15] Pilot testing during the early stages of the study found that while sticky tape could be used to secure the heat probe/laser needed to assess the axon reflex, when it came time to remove the heat probe so that the axon flare could be assessed, the adhesive in the tape would stretch the skin, flooding the skin with additional blood flow, preventing an accurate assessment of the post-heating flare. To address this, we developed "probe-slips" that use tension, not adhesive, to hold the heat probe/laser. This ensured that the heat probe/laser could be held secure during the 40-minute axon reflex protocol and released quickly when it was time to evaluate the post-heating flare.

been collected earlier, information about participants' menstrual status was collected from all pre- and peri-menopausal women and recorded in the study notes. HCs needing validation for their parking were provided with vouchers for the parking garage. BCS who needed assistance finding the location of their chemotherapy infusion were escorted to their destination.

Section 4: Methods Used to Store, Transfer, and Prepare Data for Analysis

This section is divided into two parts: Part 1 describes the procedures used to store, transfer, and prepare non-imaging data; and Part 2 describes the procedures used to store, transfer, and prepare imaging data on the mean size of axon reflexes and axon flares in the right toe.

Part 1: Storage, Transfer and Preparation for Non-Imaging Data

Data Storage for Non-Imaging Data. During study visits, non-imaging data was recorded by hand on study sheets. This included information about participant's height (cm)/weight (kg), conditions in the testing rooms (temperature (°C and °F), relative humidity (%), light levels (lux), airflow in the testing room (yes/no), current pain level (0-10), and TNSr-SF scores (0-20) measuring signs and symptoms of TIPN.

Data Transfer for Non-Imaging Data. Following study visits, participant data recorded on study sheets was reviewed by both members of the research team (i.e., Co-Investigator and Research Assistant) and verified. Once data had been verified, study sheets were signed and sealed in manila envelopes. Sealed envelopes were then transported back to the lab for storage in locked cabinets in rooms with key-card access only.

Data Preparation for Non-Imaging Data. Once back at the lab, data from these envelopes was transferred from study sheets to electronic databases for analysis. Prior to analysis, data that had been entered into the electronic database was checked against original data from the study sheets at least 3 times to ensure accuracy.

At the end of the study, a random audit of 5% of participant data was performed to ensure data had been entered into databases accurately.

Following visits, data on clinical signs and symptoms of TIPN were transferred from data sheets into databases. Total TNSr-SF scores for each participant (range: 020) were entered into databases, along with scores for each of the 5 items included in the TNSr-SF (range: 0-4) (i.e., tingling, numbness, neuropathic pain, vibration, reflexes). To help evaluate the onset and progress of participant's TIPN symptoms, data on the presence (yes/no) of each

of the 5 signs/symptoms were recorded for both the left and right toe, and the ankle, knee, and upper extremity for each participant.

Part 2: Storage, Transfer, and Preparation for Imaging Data

Data Storage for Imaging Data. During study visits, imaging data (including recordings from the laser Doppler on the size of axon reflexes in the toe (Aim 1) and pre- and post-heating FLPI images used to calculate the size of axon flares in the toe (Aim 2) were saved on an external hard drive for temporary storage. During all visits, both members of the research team verified that imaging files collected has been successfully saved on the external hard drive before moving to the next step of the protocol.

Data Transfer for Imaging Data. Following each visit, imaging files saved to the external hard drive were uploaded to encrypted databases for storage. To protect the study against a loss of data if something were to occur to the university databases, copies of each imaging file were saved to two separate encrypted databases provided by the university.

Data Preparation for Imaging Data.

Mean Axon Reflex Size (Aim 1). To calculate the mean size of axon reflexes in the right palmar toe surface, the following steps were performed. First, continuous data on SkBF recorded using the laser Doppler was converted to numeric values using software included with laser Doppler (MoorVMS-PC, ver. 3.1, Moor Instruments, Ltd., Axminister, UK). Numeric data was then divided into 30-second increments, providing an estimate of mean SkBF in the toe over 40-minute skin-heating period. Next, 30-second increments were downloaded into Excel (Microsoft Corporation), and information about the timing of key events during the skin heating (e.g., start 42 °C) and any issues that could affect the interpretation of the data (e.g., participant movement) were added to Excel files manually.

After this, 10-minute blood pressure readings from the study visit were added to Excel files, and used to calculate the circulatory vascular conductance (CVC) (blood flux/mm Hg) for participants at each visit. Participant's maximal blood flow during skin heating (CVC_{MAX}) was then calculated by averaging CVC values during the period between the reach of 44 °C and end of skin heating (~3 minutes). Finally, SkBF during the 30 seconds corresponding to the peak of the axon reflex were divided by participant's CVC_{MAX} value to express the mean size of axon reflexes as a $\%CVC_{MAX}$. Mean SkBF at other points during the 40-minute skin-heating protocol (i.e., Start 33 °C, Reach 33 °C, Start 42 °C, Reach 42 °C, the post-reflex

nadir, Start 44 °C, and Reach 44 °C) was also calculated to provide additional information about the possible impact of Taxol® on the axon reflex-mediated blood flow response in BCS.

Mean Axon Flare Size (Aim 2). During visits, toe height and width were measured (in cm) using a standard 12-inch (30.48 cm) ruler. Toe height was measured from the skin fold at the base of the distal phalanx to the tip of the great toe. Toe width was measured at the widest point of the plantar surface of the great toe. Following each visit, data on the height and width of the great toe was entered into software included with the laser perfusion imager (MoorFLPI Review, Ver. 4.0; Moor Instruments, Ltd.), and used to calculate flare size using the following process.

First, images of mean SkBF in the plantar surface of the toe before and after local skin heating were downloaded from the encrypted servers at the university where they were stored. Data on the size of the image (e.g., 14 cm x 17 cm) were then entered into the software for each participant so that the software could determine the size of flares relative to the size of the surrounding frame. However, during analysis, the size of the image had to be adjusted to account for the use of the zoom during visits.[16] Corrected image sizes were calculated and entered into the software. Images of SkBF in the toe were then smoothed using the imaging software (Moor FLPI (Ver. 4.0); Moor Instruments, Ltd.) and converted from 12- 256 colors.

Next, polygons were drawn around the heads of the first and second toes using the software to demarcate regions of interest telling the imager where to calculate mean blood

[16] During the study, careful attention was paid to measuring the size of the toe accurately and ensuring the distance from imager to tissue was identical for all participants (which was kept at 15 cm for all participants with the use of a specially-designed orthotic boot that connected the participant's foot to the imager). However, during analysis, it became clear that we had failed to take into account the impact that using the zoom feature on the FLPI camera would have on our ability to determine the size of data. Specifically, because the zoom feature makes the focus of the camera (the toe) occupy more space in the frame during analysis, it became clear that because different levels of zoom had been used depending on our needs, we no longer could use standard reference for the size of the toe.

To address this, the following process was used to deduce the estimate frame size from data collected during visits. First, the image of the toe measured using the laser imager was pulled up on a computer screen. The height and width of image was measured (in cm) using a standard ruler. These values were then divided by the actual height and width of the toe measured during the visit to determine the amount (%) the image had been zoomed-in during visits. Next, the heights and width of the frame surrounding the image was measured off of the computer screen. Using the level of zoom calculated before, the actual height and width of the frame during the study visit were calculated by dividing the apparent size of the frame by the conversion factor. This same process was applied to data for each participant.

flow.[17] The size of post-heating axon flares (in cm^2) was calculated for each participant by dividing the size of the regions of interest drawn around the flare by the percentage of pixels in the region of interest above the specified threshold (3 standard deviations (*SD*) above baseline blood flow), and multiplying the resulting value by 100 (L. Moor Instruments, 2001).

In addition, because the size of participant's toes varied somewhat, flare size was also calculated as a percentage of participant's maximum toe size (referred to hereafter as $\%Toe_{MAX}$). Finally, to help evaluate participant's response to skin heating in the toe, participant's maximum hyperemic response (sometimes referred to as the LDI_{MAX} in protocols that use Laser Doppler Imaging (LDI) to evaluate flare size) was calculated by averaging blood flow in the 0.33 cm^2 area directly under the heat probe (S. T. Krishnan & G. Rayman, 2004; P. R. Vas & G. Rayman, 2013b).

Section 5: Methods Used to Analyze Study Data

Statistical Software and Pre-Analysis Methods

Analyses for the study were performed using Statistical Package for the Social Sciences (SPSS) software, Version 24 and 25 (IBM, Corporation; Chicago, Illinois). Figures were generated using SPSS and Excel (Microsoft Corp.). Demographic, cancer treatment, menopausal/menstrual status, height, weight, BMI, pain scores, and environmental conditions were described using descriptive statistics. Normality of distributions was assessed using Q-Q plots and Shapiro Wilks' tests. Continuous variables were compared using two-tailed independent samples *t*-tests. Categorical variables were compared using Pearson's Chi-Squared tests. For comparisons with <5 observations per cell, Fisher's exact test was substituted to avoid overestimating statistical significance using the Exact Test Extension for SPSS (Version 24). For all tests, an alpha ≤ 0.05 was considered statistically significant.

Analyses for Aim 1

[17] To ensure accuracy, polygons were checked for fit prior to analysis. For this analysis, fit $\geq$ 99.0% was arbitrarily chosen as criteria for quality. Polygons not meeting this cut-point initially were adjusted until fit above the threshold. In addition, because the assumption was that size of the axon flare would be at its largest immediately after the heat probe was removed, polygon fit was based on first image in the 2-minute sequence. To ensure that SkBF immediately after taking off the heat probe was representative of SkBF during the entire 2-minute scan, mean SkBF during pre- and post-heating scans was compared at 20-second intervals (i.e., 0 sec, 20-sec., 40-sec, etc.)

Differences in Mean Axon Reflex Size between BCS and HCs (Hypothesis 1.1, 1.2). Two-tailed, independent-samples *t*-tests were used to test the hypothesis that mean axon reflex size would not differ significantly between HCs and BCS prior to Taxol® (Hypothesis 1.1). Two-tailed, independent-samples *t*-tests were also used to test the hypothesis that mean axon reflex size would differ significantly between HCs and BCS as exposure to Taxol® increased (Times 2 and 3) (Hypothesis 1.2). Analyses were performed with data expressed both as a percentage of participants' maximal blood flow during the 40-minute skin-heating protocol (%CVC_{MAX}) and raw CVC units (flux/mm Hg).

Differences in Mean Axon Reflex Size in BCS over Time (Hypothesis 1.3). One-way repeated-measures analysis of variance (RMANOVA) was used to test the hypothesis that mean axon reflex size would differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (Hypothesis 1.3). One-way RMANOVA was used also to test the hypothesis that mean axon reflex size would not differ significantly for HCs over the study. Results are reported as Wilk's Lambda. Estimates of effect size (eta^2) and observed power are reported as well. Prior to testing, Mauchly's test was used to confirm that the assumption of sphericity had not been violated. For $\varepsilon \leq .75$, a Greenhouse-Geisser correction was applied; for $\varepsilon > .75$, a Huynh-Feldt correction was applied.

Analyses for Aim 2

Differences in Mean Axon Flare Size between BCS and HCs (Hypothesis 2.1, 2.2). Two-tailed, independent-samples *t*-tests were used to test the hypothesis that mean axon flares size would not differ significantly between HCs and BCS prior to Taxol® (Hypothesis 2.1). Two-tailed, independent-samples *t*-tests were also used to test the hypothesis that mean axon flare size would differ significantly between HCs and BCS as exposure to Taxol® increased (Times 2 and 3) (Hypothesis 2.2). Analyses were performed with data expressed in actual flare size (in cm^2) and as a percentage of total toe size (%Toe_{MAX}).

Differences in Mean Axon Flare Size in BCS over Time (Hypothesis 2.3). One-way repeated-measures analysis of variance (RMANOVA) was used to test the hypothesis that mean axon flare size would differ for BCS receiving Taxol® between Times 1, 2, and 3 (Hypothesis 2.3). One-way RMANOVA was also used to test the hypothesis that mean axon reflex size would not differ for HCs over the study. As with the analyses for Hypothesis 1.3, results are reported as Wilk's Lambda. Estimates of effect size (eta^2) and observed power are

reported as well. Prior to testing, Mauchly's test was used to confirm that the assumption of sphericity had not been violated. For $\varepsilon \leq .75$, a Greenhouse-Geisser correction was applied; for $\varepsilon > .75$, a Huynh-Feldt correction was applied.

Analyses for Aim 3

For Aim 3, mean and median TNS-r SF scores were used to describe the location and severity of tingling, numbness, and painful neuropathy symptoms at each visit. Boxplots illustrating minimum, maximum, mean, median, and interquartile scores for each group were used to visualize the distribution of scores at each visit. Frequencies were used to describe the percentage of women reporting different symptoms at each visit. Bivariate correlations were used to test the magnitude and direction of correlations between total TNS-r SF scores, mean reflex size, and mean flare size (Aims 3.1, 3.2). Correlations were tested with (a) axon reflex data expressed as both $\%CVC_{MAX}$ and raw CVC units, and (b) axon flare data expressed as both actual flare size (cm^2) and $\%Toe_{MAX}$. Due to limitations in sample size, correlations between scores for individual TNS-r SF items, axon reflexes, and axon flares were tested non-parametrically using two-tailed Spearman's Ranked Tests.

CHAPTER FOUR

RESULTS

The purpose of this study was to determine whether BCS receiving weekly or dose-dense Taxol® would display changes in the mean size of axon reflexes or axon flares during the early portion of their cancer therapy. If evident, then axon reflex-mediated blood flow might be used as an early detection method for the small-fiber component of TIPN.

Results for the study are divided into five sections. Section 1 presents results of screening for participant demographics, physical characteristics, cancer stage, and exposure to cancer treatment. Section 1 also presents results of the analyses comparing environmental conditions for BCS and HCs during study visits. Section 2 presents results for Aim 1 comparing the mean size of axon reflexes in the right great toe of BCS receiving Taxol® during local skin heating to healthy female controls (HCs). Section 3 presents results for Aim 2 comparing the mean size of axon flares in the right great toe of BCS receiving Taxol® during local skin heating to HCs. Finally, Section 4 summarizes findings from Aim 3, which determine whether the mean size of axon reflexes or axon flares were correlated with (a) the overall severity of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and (b) with the severity of individual signs and symptoms of TIPN (measured by total scores on each of the five items (range: 0-4) at each time point.

Section 1: Recruitment, Enrollment, and Sociodemographics

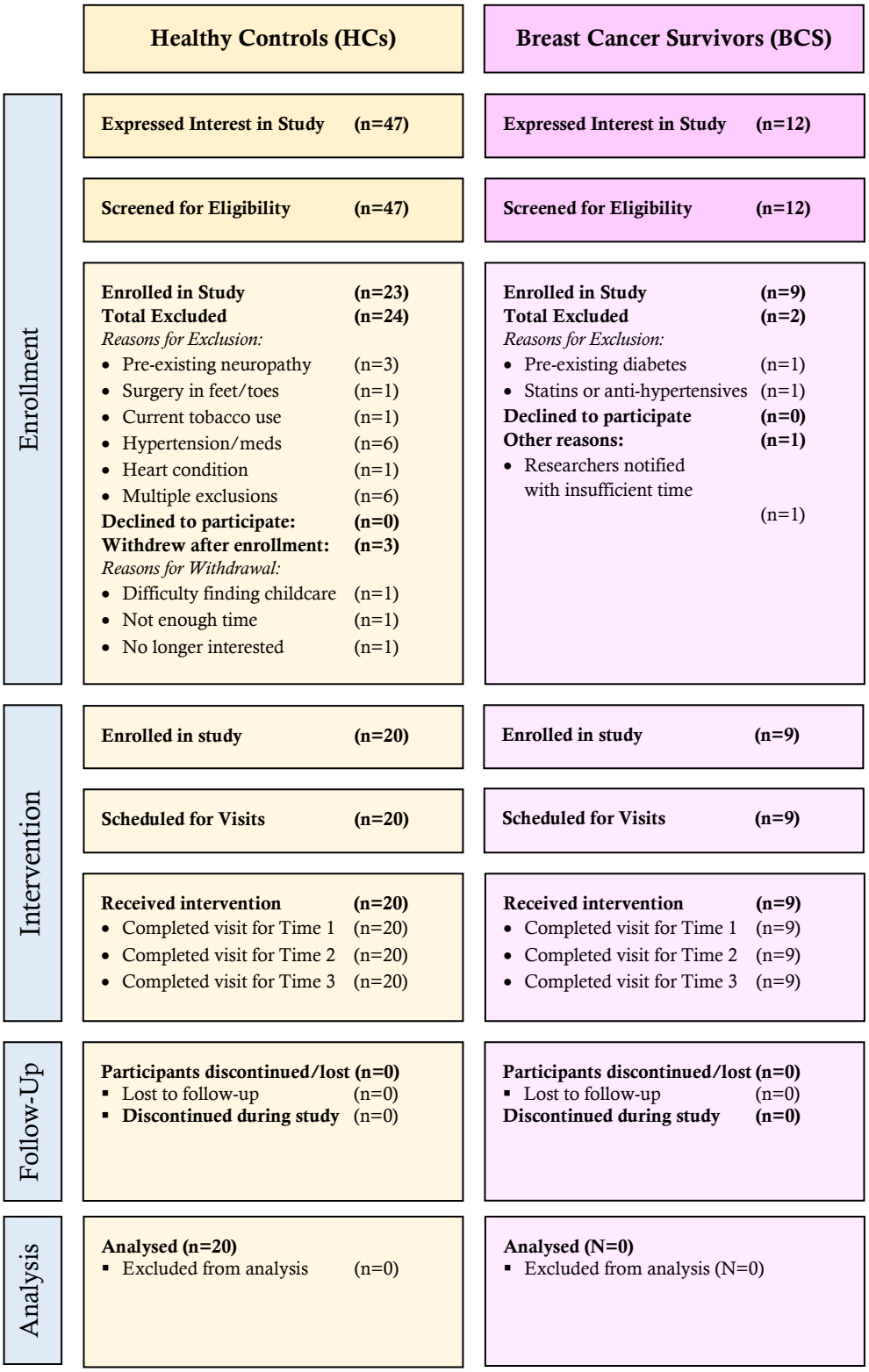
Recruitment, Screening, Enrollment, and Attrition

Healthy Controls. Recruitment, screening, enrollment, and attrition of HCs is depicted in Figure 4-1. Recruitment for HCs began in September of 2015 and finished in May of 2016. Between these two dates, a total of 44 healthy women were screened for the study. Of these, 24 (54.5%) were ineligible to participate. Reasons for ineligibility included: pre-existing neuropathy ($n = 3$); surgery in feet/toes ($n = 1$); current use of tobacco ($n = 1$); pre-existing hypertension or use of anti-hypertensive medications ($n = 6$); heart condition ($n = 1$), or a combination of exclusion criteria ($n = 6$). In addition, during enrollment, three HCs who were invited to participate withdrew prior to their first study visit. Reasons for withdrawal in these women included difficulty finding childcare ($n = 1$), not having enough time to participate ($n = 1$), and no longer wanting to participate ($n = 1$). The three women who had to withdraw

before starting the study were replaced with three women from the waiting list. After the start of the study, there was no attrition, with all 20 enrolled HC completing the study.

Breast Cancer Survivors. Recruitment, screening, enrollment, and attrition for BCS is also depicted in Figure 4-1. Recruitment for BCS began in September of 2015 and ended in June of 2016. Between these two dates, a total 12 BCS with first-time, non-metastatic cancer receiving weekly or bi-monthly Taxol® were screened for eligibility. Of these, two BCS (16.6%) were ineligible to participate. Reasons for ineligibility included pre-existing diabetes (n = 1) and use of statins/anti-hypertensives (n = 1). Of the nine remaining BCS identified during recruitment, all nine were eligible, and agreed to take part in the study. Once the study began, there was no attrition among the nine enrolled BCS.

Figure 4-1. Recruitment, Screening, Enrollment for Healthy Female Controls (n = 20) and Breast Cancer Survivors (n = 9)



Sociodemographics

Age, Race, and Ethnicity. Women in both arms of the trial were primarily middle-aged, with a mean ± *SD* age for BCS of 44.6 ± 9.2 years, and 45.5 ± 11.7 years for HCs. The majority of women in the study identified themselves as White (BCS =77.8%; HCs = 65.0%) and Non-Hispanic (BCS = 88.9%; HCs = 100.0%) on their sociodemographic form. Approximately fifteen percent of HCs and of BCS identified themselves as non-white on their intake form. Ethnicities reported by these women included Armenian-American, Indian-American, Lebanese-American, and Hispanic/Latina.

Differences in categorical demographic variables between the two groups were compared with two-tailed Chi-squared tests or Fisher's exact tests, as appropriate. For both tests, an alpha of 0.05 was used as the threshold for determining statistical significance. Differences in continuous demographic variables were compared using two-tailed *t*-tests. Findings are summarized in Table 4-1. Results did not find a statistically-significant difference in mean age between BCS and HC, or the percentage of BCS and HC in each category of age, race, or ethnicity.

Table 4-1

Age, Race, and Ethnicity of Sample

Variables	Breast Cancer ($n = 9$)	Healthy Controls ($n = 20$)	Stat (df)†	p-value
Age at enrollment, years (mean ± *SD*)	44.6 ± 9.2	45.5 ± 11.7	.21 (27)	.832
Age, by decade (% (*n*))				
18-19	0.0 (0)	0.0 (0)		
20-29	0.0 (0)	15.0 (3)		
30-39	44.0 (4)	20.0 (4)		
40-49	22.2 (2)	20.0 (4)	2.97	.605†
50-59	33.3 (3)	35.0 (7)		
60-69	0.0 (0)	10.0 (2)		
70-79	0.0 (0)	0.0 (0)		
80-85	0.0 (0)	0.0 (0)		
Race, by category (% (*n*))				
White	77.8 (7)	65.0 (13)		
Black/African American	0.0 (0)	20.0 (4)		
Native Hawaiian/Pacific Islander	0.0 (0)	0.0 (0)	1.90	.481†
Asian	0.0 (0)	0.0 (0)		
American Indian/Alaskan Native	0.0 (0)	0.0 (0)		
Other:	22.2 (2)	15.0 (3)		

Armenian-American	0.0 (0)	5.0 (1)		
Indian-American	11.1 (1)	5.0 (1)		
Lebanese-American	0.0 (0)	5.0 (1)		
Hispanic/Latina	11.1 (1)	0.0 (0)		
Ethnicity (% (*n*))				
Non-Hispanic/Latino	88.9 (1)	100.0 (20)	2.42	.310†

Notes. Differences between continuous variables were compared using two-tailed independent samples *t*-tests. Differences in categorical variables were compared using two-tailed Pearson's Chi-Squared tests. For comparisons with fewer than 5 observations per cell, Fisher's exact test was used instead (denoted by † symbol). For all analyses, an alpha of 0.05 was used as the threshold for statistical significance.

Education, Income, and Occupation Type. Education, income, and occupation type for the sample are listed in Table 4-2. Most women in the sample were well-educated, with 77.8% of BCS and 80.0% of HCs reporting completion of a bachelor's or graduate degree on their sociodemographic form. More than 70.0% of the women in both groups reported working in occupations classified as "professional" or "managerial" in nature, and more than two-thirds (68.9%) reported estimated annual incomes >$75,000.

Comparison of education, income, and occupational category between the two groups using two-tailed Chi-squared tests did not reveal statistically-significant differences in income or occupational type between BCS and HC. However, results found a significantly higher percentage of HC (70.0%) who reported having completed a graduate or professional degree than BCS in the sample (22.0%) (χ^2=11.03 (4), p = .026).

Table 4-2

Education, Income, and Occupation Type for Sample

	Breast Cancer (n = 9)	Healthy Controls (n = 20)	Stat (df)†	p-value
Education, Highest Level Attained (% (*n*))				
Graduate or professional degree	22.2 (2)	70.0 (14)		
Some graduate school	0.0 (0)	5.0 (1)		
Bachelor degree or four-year college	55.6 (4)	10.0 (2)		
Associate degree or two-year college	0.0 (0)	10.0 (2)		
Some college	0.0 (0)	0.0 (0)	10.05	.010†
Technical or trade school	0.0 (0)	0.0 (0)		
High school graduate/GED	22.2 (2)	5.0 (1)		
Some high school	0.0 (0)	0.0 (0)		
Elementary school or less	0.0 (0)	0.0 (0)		

Annual Income (% (n))				
$<15,000	0.0 (0)	5.0 (1)		
$15,001-$30,000	0.0 (0)	0.0 (0)		
$30,001-$50,000	33.3 (3)	20.0 (4)		
$50,000-$75,000	0.0 (0)	5.0 (1)	2.86 (6)	.755†
$75,001-$100,000	22.2 (2)	10.0 (2)		
$100,001-$150,000	11.1 (1)	35.0 (7)		
$150,001-$200,000	11.1 (1)	15.0 (3)		
$>200,000	22.2 (2)	10.0 (2)		
Type of Occupation (% (*n*))				
Professional	66.7 (6)	65.0 (13)		
Management/Administration Technical	11.1 (1)	5.0 (1)		
Clerical or Service	0.0 (0)	15.0 (3)		
Homemaker	11.1 (1)	0.0 (0)		
Self-employed	0.0 (0)	0.0 (0)	7.27 (7)	.315†
Unemployed	0.0 (0)	0.0 (0)		
Not employed, disabled	11.1 (1)	0.0 (0)		
Retired	0.0 (0)	5.0 (1)		
Student	0.0 (0)	5.0 (1)		
Other:	0.0 (0)	5.0 (1)		

Notes. Differences between continuous variables were compared using two-tailed independent samples *t*-tests. Differences in categorical variables were compared using two-tailed Pearson's Chi-Squared. For comparisons with fewer than 5 observations per cell, Fisher's exact test was used instead (denoted by † symbol). For all analyses, an alpha of 0.05 was used as threshold for statistical significance.

Menopausal and Menstrual Status.

Menopausal Status. Menopausal status for the sample is summarized in Table 4-3. At the start of the study, 77.7% of BCS identified as pre-menopausal and 22.2% identified as peri- or post-menopausal. However, during cancer treatment, three BCS in the pre-menstrual group stopped menstruating due to their treatment, making the percentage of women in each arm of the trial who identified as pre- vs. post-menopausal nearly identical (BCS: 44.4% vs. HC: 50.0%; $\chi^2 = 1.97$, $p = .197$).

Table 4-3

Menopausal Status of Participants

Variable	Breast Cancer ($n = 9$)	Healthy Controls ($n = 20$)	*p*-value
Menopausal Status (% (n))			

Pre-menopausal	44.4 (4)	50.0 (10)	
Peri-menopausal	0.0 (0)	15.0 (3)	.439†
Post-menopausal	55.5 (5)	35.0 (7)	
Menstrual Status (% (n))			
Had no period in last 12 mo.	33.3 (3)	35.0 (7)	
Had period in last 12 mo., but not in the last 3 mo.	0.0 (0)	20.0 (4)	
Had period in last 3 mo., but cycles are less regular	22.2 (2)	10.0 (2)	.532†
Had period in last 3 mo., but no change in regularity	33.3 (3)	35.0 (7)	
Not reported	11.1 (1)	0.0 (0)	
Reasons for Peri-/Post-Menopausal Status (% (n))			
Normal aging	22.2 (2)	30.0 (6)	
Breast cancer treatment	11.1 (1)	0.0 (0)	
Medication not related to breast cancer	11.1 (1)	0.0 (0)	
Surgery (e.g., hysterectomy, ovariectomy)	11.1 (1)	10.0 (2)	.688†
Don't know/unsure	0.0 (0)	15.0 (3)	
Not applicable	22.2 (2)	45.0 (9)	
Not reported	22.2 (2)	0.0 (0)	

Notes. Differences between continuous variables were compared using independent samples *t*-tests. Differences in categorical variables were compared using Fisher's exact test was substituted for Pearson's Chi-Squared due to sample size. For all analyses, an alpha of 0.05 was used as threshold for statistical significance.

Menstrual Status. Between 10-40% of HCs were menstruating during study visits (depending on the visit). By contrast, none of the BCS were menstruating during visits. However, results of Fisher's exact tests comparing the percentage of women menstruating at each visit found that these differences were not statistically significant at any time point (Fisher's exact test: Time 1 = .147; Time 2 = .185; Time 3 = 1.00).

Physical Characteristics

Height, Weight, and BMI. Mean height, weight, and BMI for the sample at each visit are summarized in Table 4-4. Mean heights for both groups were within the expected range for women in the U.S. (Fryar, Gu, & Ogden, 2012). At baseline, more than a third of participants were classified as either overweight or obese according to current BMI guidelines (i.e., BMI >30) (World Health Organization, 2006). Overall, both body weight and BMIs were slightly lower for BCS than for HC during the study, although these differences were not statistically significant.

Table 4-4

Mean Height, Weight, and Body-Mass-Index (BMI) during Study

Variable	Time	Breast Cancer ($n = 9$)	Healthy Controls ($n = 20$)	t	df	p-value
		Mean (*SD*)	Mean (*SD*)			
Height (cm)	Time 1	160.1 (10.7)	164.4 (8.6)	1.16	27	.256
Weight (kg)						
	Time 1	67.2 (15.3)	70.1 (15.4)	.481	26	.635
	Time 2	67.2 (17.7)	71.0 (15.3)	.549	25	.588
	Time 3	66.5 (17.7)	70.8 (15.5)	.636	26	.530
BMI (kg/m^2)						
	Time 1	26.5 (6.6)	25.9 (5.5)	-.251	26	.804
	Time 2	26.6 (6.1)	26.2 (5.6)	-.144	25	.887
	Time 3	26.2 (6.3)	26.3 (5.6)	.031	26	.975

Notes. Values are presented as mean (*SD*). Between-group differences were compared using independent samples *t*-tests. Within-group differences across study visits were compared using repeated-measures analysis of variance (RMANOVA). For all tests, $\alpha = 0.05$ was considered statistically significant.

Participants' BMI category (i.e., underweight, normal, overweight, obese) is listed in Table 4-5. Comparison of the percentage of BCS and HCs in each BMI category during the three visits found that the percentage of BCS in the obese and overweight categories decreased over the 6-week study (Time 1: 33.0%, Time 2: 28.6%, Time 3: 14.3%) while percentage of HCs listed as overweight or obese increased slightly (Time 1: 42.1%, Time 2: 47.4%, Time 3: 52.7%). However, analysis of BMI values for each group over the 6 weeks with RMANOVA found that changes in BMI during the study were not significant for either HCs ($F(2, 17) = .060$, $p = .942$, $\eta^2 = .007$, observed power = 5.8%) or BCS ($F(2, 5) = 1.11$, $p = .399$, $\eta^2 = .308$, observed power = 15.6%).

Table 4-5

Comparison of World Health Organization Body-Mass-Index (BMI) Categories for Breast Cancer Survivors ($n = 9$) and Healthy Female Controls ($n = 20$), at Each Time Point (Time 1, 2, and 3)

Time Point	Classification	BMI Range	Breast Cancer ($n = 9$) %	Healthy Controls ($n = 20$) %	*(df)*	p-value

Time 1 (Week 0 of Taxol®)	Underweight	<18.5	0.0	5.3	4.62 (4)	.318†
	Normal	18.5-24.9	66.7	52.6		
	Overweight	25.0-29.9	11.1	10.5		
	Obese, Class I	30.0-34.9	0.0	26.3		
	Obese, Class II	35.0-39.9	22.2	5.3		
	Obese, Class III	>40.0	0.0	0.0		
Time 2 (Week 2 of Taxol®)	Underweight	<18.5	0.0	5.3	3.31 (4)	.539†
	Normal	18.5-24.9	71.4	47.4		
	Overweight	25.0-29.9	0.0	15.8		
	Obese, Class I	30.0-34.9	14.3	26.3		
	Obese, Class II	35.0-39.9	14.3	5.3		
	Obese, Class III	>40.0	0.0	0.0		
Time 3 (Week 6 of Taxol®)	Underweight	<18.5	0.0	5.3	6.30 (4)	.299†
	Normal	18.5-24.9	71.4	42.1		
	Overweight	25.0-29.9	14.3	21.1		
	Obese, Class I	30.0-34.9	0.0	26.3		
	Obese, Class II	35.0-39.9	0.0	5.3		
	Obese, Class III	>40.0	14.3	0.0		

Notes. † = Differences in the distribution of women in each group falling into each BMI classification were tested with Fisher's exact test, $\alpha = .05$.

Cancer Stage and Treatment. Mean exposure to cancer treatment, including Taxol®, during the study is summarized in Table 4-6. Eight of the nine BCS enrolled in the study (88.9%) received weekly Taxol® at 80-100 mg/m^2 for 6-12 weeks (average number of cycles given was 10). One of the nine BCS (11.1%) received dose-dense (i.e., bi-monthly) Taxol® at 175 mg/m^2 dosing range with Herceptin® (trastuzamab) for 8 weeks. Prior to starting Taxol®, seven of the nine BCS (77.8%) received four cycles of Adriamycin® and Cytoxan® (AC) therapy. Two of the seven participants receiving AC therapy received lower doses or fewer cycles of AC therapy due to nausea/vomiting. In addition, three of the nine BCS in the study (33.3%) received leuprolide acetate (Lupron®), a gonadotrophin-releasing hormone analogue, during treatment to protect their ability to conceive a child following treatment.

Table 4-6

Cumulative Exposure to Taxol® and Other Anticancer Agents during Study for Breast Cancer Survivors (BCS) (n = 9)

Treatment	Time Point			
	Prior to Starting Taxol®	Time 1 (Week 0)	Time 2 (Week 2)	Time 3 (Week 6)
Taxol® exposure, mg/m^2	--	0 ± 0	162 ± 5	485 ± 15
Adriamycin® exposure, mg/m^2	210 ± 33	--	--	--
Cytoxan® exposure, mg/m^2	2,121 ± 141	--	--	--
Herceptin® exposure, mg/kg	38 ± 0	--	--	--

Notes. Data represent cumulative exposure to each agent BCS received by each point during the study. Values expressed as mean ± *SD*.

Cumulative exposure to cancer treatment during the study is summarized in Table 4-6. Prior to their first visit, seven of the nine BCS received AC therapy. Cumulative exposure to Adriamycin® for these participants was 210 ± 33 mg/m^2 and 2,121 ± 140.5 mg/m^2 for Cytoxan®. At Times 1 and 2 of the study, all nine BCS received Taxol®. Cumulative exposure to Taxol® for BCS at Time 2 was 162 ± 5 mg/m^2. Four weeks later, at the final study visit (Time 3), cumulative exposure to Taxol® for BCS was 485 ± 15 mg/m^2. Cumulative exposure to Taxol® for the eight BCS receiving weekly Taxol® and the one BCS receiving dose-dense Taxol® found that exposures were nearly identical during the study (Weekly Taxol® = 160 mg/m^2 vs. Dose-Dense (Bi-Weekly) Taxol® =175 mg/m^2; Time 3: Weekly Taxol® = 480 vs. Dose-Dense (Bi-Weekly) Taxol® = 525 mg/m^2).

Summary

Demographic data indicated BCS and HCs were well-matched on age, race, ethnicity, occupation type, income, height, body-weight, and menopausal status. Minor differences in education level and menstrual status were noted. For BCS, cumulative Taxol® exposure was nearly identical at each point during the study, providing a rational basis for further comparison.

Section 2: Physiological and Environmental Factors Affecting Local Skin Heating

Resting Heart Rate and Blood Pressure

Overall, heart rate and blood pressures remained stable during study visits, providing the basis for interpretation of SkBF data from LDF and FLPI monitoring. Automated blood pressure testing identified several individuals with systolic blood pressures in the pre-hypertensive range. However, women in both groups were more likely to display an elevated blood pressure reading during their first visit, suggesting that uncertainty about the testing

procedures may have played a role. Heart rates for women in both groups during the study were in the 60-70 BPM range, consistent with a restful state.

Comparison of heart rates between the two groups found that heart rates were approximately 10 beats per minute faster for BCS than HC throughout the study. Analysis of heart rates and blood pressures during the experiment found that both parameters fell by approximately 5.0% over the 40-minute skin heating, suggesting that women were comfortable and that differences in axon reflex or flare size identified during the study were not a function of increased vascular tone or heart rate during the peak of skin heating.

Pain Levels

Results of data from the Numeric Pain Rating Scale administered at the start of each visit found that both groups were largely pain free during the study. Only 8.7% of pain scores reported by HCs across the three visits were higher than a 0 ("no pain whatsoever"). Reasons given by HCs for pain at the start of their visit included headache ($n = 2$) and neck pain ($n = 2$). Data on the cause of pain for one HC at her visit was not available. Of the few HCs that reported any pain, none did so at more than one visit. Likewise, only 11.1% of pain scores for BCS were greater than a 0. Reasons given by BCS for their pain included headache ($n = 1$), pain due to swelling in right arm ($n = 1$), and pain following placement of the chemotherapy port ($n = 1$). All instances of pain in the BCS occurred at baseline, before the start of Taxol®.

Temperature, Humidity, Light, and Airflow in Testing Rooms

An analysis of environmental conditions including the ambient temperature (°C), relative humidity (%), light levels (lux), and presence of airflow in the testing rooms that could potentially interfere with the laser imager (yes/no) is provided in Appendix L. Briefly, results of the analysis showed that environmental conditions were well-controlled during study visits. Ambient temperatures in the testing rooms varied less than a degree, on average, from the target of 25±1 °C. Relative humidity levels varied more during the study but were similar between groups and time points. Light levels in the testing rooms varied the most, with evidence of significantly brighter light for HCs at Times 1 and 2 compared to BCS. Analysis of light levels for HCs over time found that brightness in the test room decreased by approximately 300 lux over the 6-week study. These differences can be explained by the decision to move some HCs to a room with better temperature controls but dimmer lights as the study proceeded (discussed in Chapter Five).

Section 3: Results of Analyses for Aims 1, 2 and 3

Preliminary Findings: Differential Response to Local Skin Heating in the Right Toe

During data collection, two distinct blood flow responses were observed in the right toe during local skin heating. The first response (the expected response) was characterized by five events: (a) baseline SkBF at 10-20% of the participant's CVC_{MAX}, followed by (b) a rapid 3- to 4-fold increase in cutaneous blood flow that occurred with minutes of the increase to 42 °C (the axon reflex); (c) a brief return towards baseline (the nadir); which was followed by (d) a longer, slower increase in cutaneous blood flow that eventually plateaued; and (e) a final increase in blood flow following the increase to 44 °C which was 100% of the participants' CVC_{MAX}. This expected response to local skin heating in the toe is illustrated in Figure 4-2 using data collected during the study.

Figure 4-2. Expected Response to 40-Minute Local Skin Heating between 33-44 °C

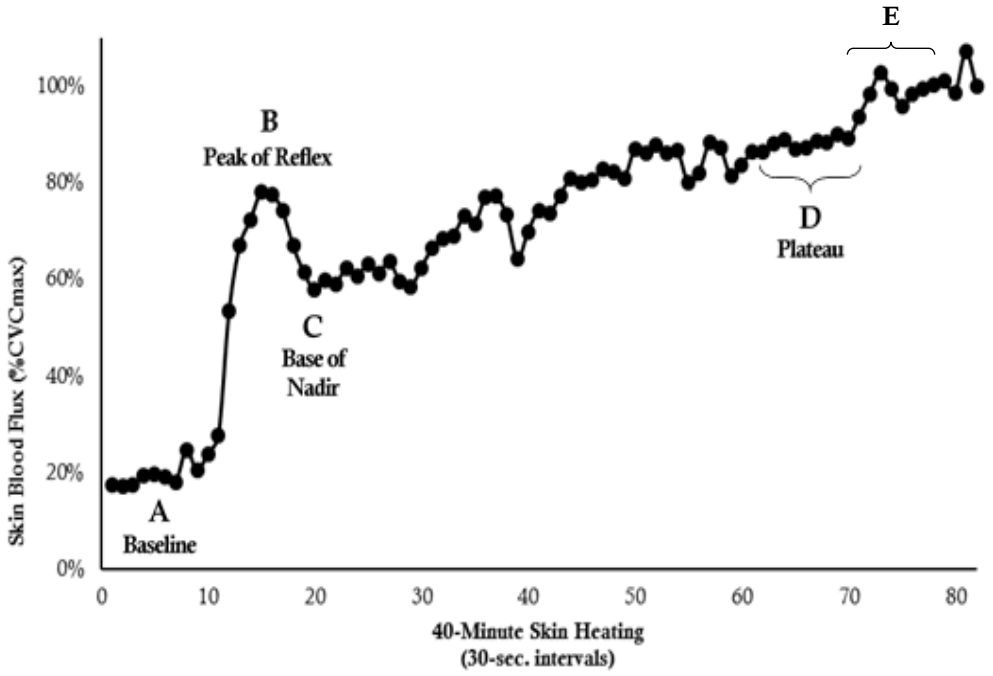


Figure 4-2. Points for skin blood flow (SkBF) in the right toe averaged over 30 seconds. (A) Under normal conditions, 33 °C skin heating is used to establish baseline blood flux, after which the heat probe is turned up to 42 °C at a rate of 0.1 °C/s. In individuals with healthy neurovascular function, this increase in topical heat elicits a rapid, 3- to 4-fold increase in SkBF, known as an axon reflex (B). Following a brief nadir (C), if 42 °C skin heating continues, SkBF continues to rise, plateauing at some point (D). Increasing the heat to 44 °C causes blood vessels to dilate to their near maximum (E).

While the magnitude and duration of these events can vary based on temperatures being used, duration of skin heating, and other factors, variations on this basic response would be expected during local skin-heating protocols such as the one used here. In addition, this response should also be apparent regardless of how the data is expressed. This includes whether blood flow data is expressed in arbitrary tissue perfusion units (the basic unit of the Laser Doppler), as circulatory vascular conductance (CVC) which normalizes the data to participant's blood pressure, or as a percentage of the participant's maximal blood flow ($\%CVC_{MAX}$) which often provides a more practical way to evaluate the increase in cutaneous blood flow that occurs during skin heating.

Figure 4-3. Attenuated Response to 40-Minute Local Skin Heating Observed during Study

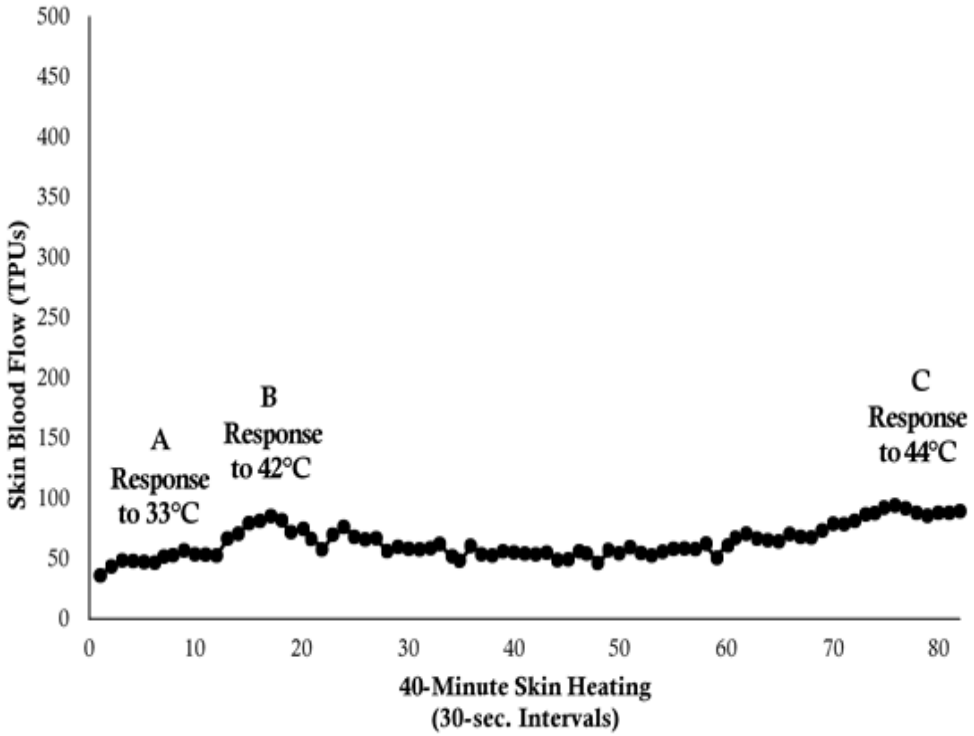


Figure 4-3. Points on the graph represent skin blood flow (SkBF) in the right palmar toe surface averaged over 30 seconds. In contrast the expected response to local skin heating (Figure 4-2), the attenuated response was characterized by (A) SkBF in the expected-to-low range during 33 °C skin heating. Increasing skin heating to 42 °C creates a perceptible but highly-diminished increase in baseline SkBF similar to the axon reflex. No clear nadir is evident, and blood flow returns to near basal rate. Increasing skin temperature to 44 °C also increases SkBF to or slightly above the level seen at 44 °C.

During data collection, however, approximately half of BCS and more than 15% of HCs displayed a diminished response to skin heating in the right toe. In contrast to the

expected response, the *attenuated response* (Figure 4-3) was characterized by: (1) baseline blood flow at or below the expected range; (2) a noticeable but extremely muted response to 42 °C skin heating; (3) a small return toward baseline; (4) a long period of low blood flow; ending with (5) a second, blunted rise of blood flow following the increase to 44 °C.

At Time 1, an attenuated response to skin heating in the right toe was observed in more than half (55.6%) of BCS, compared to 15.0% of HCs (Panel A). At Time 2, nearly half (44.5%) of BCS displayed an attenuated response to skin heating compared to 20.0% of HCs. At Time 3, the percentage of BCS displaying an attenuated response to skin heating decreased to 37.5%, compared to 20.0% of HCs. Testing with Fisher's exact test found that the percentage of BCS who displayed attenuated responses was significantly higher than HCs at Time 1 ($p = .024$), but not at Time 2 ($p = .335$) or Time 3 ($p = .337$).

Figure 4-4. Comparison of Frequency of Attenuated Response to Local Skin Heating at Each Visit between Breast Cancer Survivors (BCS) and Healthy Controls (HCs)

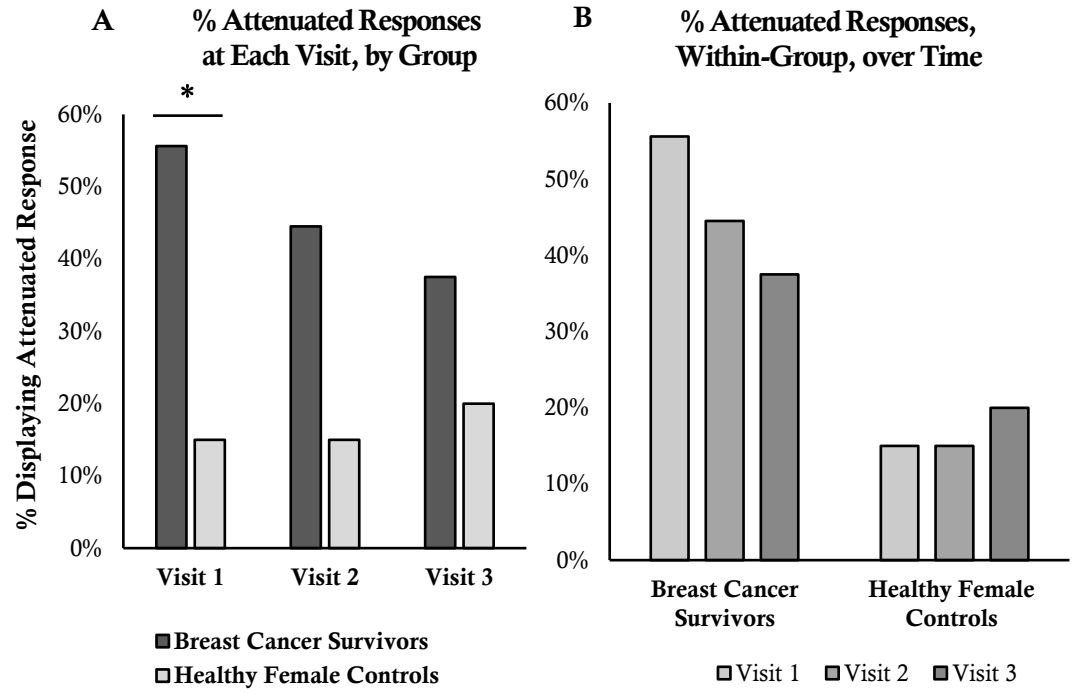


Figure 4-4. Columns represent the percentage of breast cancer survivors (BCS) and healthy female controls (HCs) displaying an attenuated response to skin heating in the palmar right toe. Panel A illustrates differences in percentage of attenuated response between groups, at each time point. Panel B illustrates trends for each group over time. Within-group differences over time were compared non-parametircallyusing Friedman's Test. Between-group differences at each time point were compared using Fisher's exact test ($\alpha = 0.05$).

Impact of Attenuated Response on Analysis for Mean Axon Reflex Size

The appearance of two different responses to local skin heating during the study presented a challenge during analysis. In particular, because SkBF values from the attenuated response were so much lower than the expected response, the plan for analyzing results from this data had to be modified. The reason for this has to do with how the size of the axon reflex that occurs during skin heating is calculated. In microvascular research, the size of the axon reflex that occurs during local skin heating is often expressed as a percentage of participants' maximal cutaneous vascular conductance (CVC) (which is simply the blood in the skin normalized to the participant's blood pressure (TPUs/mm Hg) (Bruning, 2013). This ratio, known as the $\%CVC_{MAX}$ (Minson, 2010), provides a useful shorthand for interpreting how large the axon reflex was relative to the participant's (theoretical) max (Minson, 2010).

Sub-Maximal Response. In addition to the attenuated response, prior to analysis data from five additional HCs visits were set-aside because blood flow at the peak of the axon reflex was larger than blood flow during 44 °C skin heating (maximal blood flow) (Figure 4-8). Both the timing and nature of these response (which included the same 3- to 4-fold increase in baseline blood flow described earlier) strongly suggested that axon reflexes were elicited during study visits. However, because expressing axon reflex data as $\%CVC_{MAX}$ involves dividing the size of the reflex by maximum blood flow, reflexes that are larger than max blood flow appear extremely large by comparison (e.g., 118%). Consequently, including this data in the final analysis would have been problematic both interpretively (values this large overestimate the neurogenic response to skin heating) and practically (values this large distort any calculation that involves measures of central tendency). No sub-maximal responses were observed in the BCS group.

However, like all ratios, any change that reduces the size of the denominator will cause a corresponding increase in the size of the numerator (the size of the axon reflex, in this case). For example, if mean blood flow during the axon reflex (the numerator) is 3.90 CVC units, and mean blood flow value at the peak of skin heating (44 °C) is 4.99 CVC units, then the size of the axon reflex would be $3.99 \div 4.99 = 78.2\ \%CVC_{MAX}$. However, if the estimate of the participant's maximal blood flow is reduced by 10% from 4.99 CVC to 4.41 CVC, the estimate of axon reflex size will also increase by 10%: $3.99 \div 4.41 = 88.5\%CVC_{MAX}$.

During analysis, it became clear that adding the low blood flow values to those from the expected response could compromise the study's ability to address its aims because of how the data was expressed. Because of this, even though blood flow data from the attenuated group had the same general appearance as blood flow from the expected group when it was presented as a $\%CVC_{MAX}$, results of the analyses with the two phenotypes together and apart confirmed that data from the attenuated group was likely to mask the true effect of Taxol® on the size of axon reflexes being studied. Finally, given the unexpected nature of this phenomena, after discussion with experts in the field, the decision was made to run side-by-side analyses on the data to ensure that data from individuals with differences in underlying anatomy or physiology were not being performed. While this came at a significant cost in statistical power for the study, the study is based on many assumptions about how blood flow will respond to local skin heating and the types of deviations from that response that can be

Figure 4-5. Example of Sub-Maximal Blood Flow Response to Local Skin Heating in Right Palmar Toe Surface

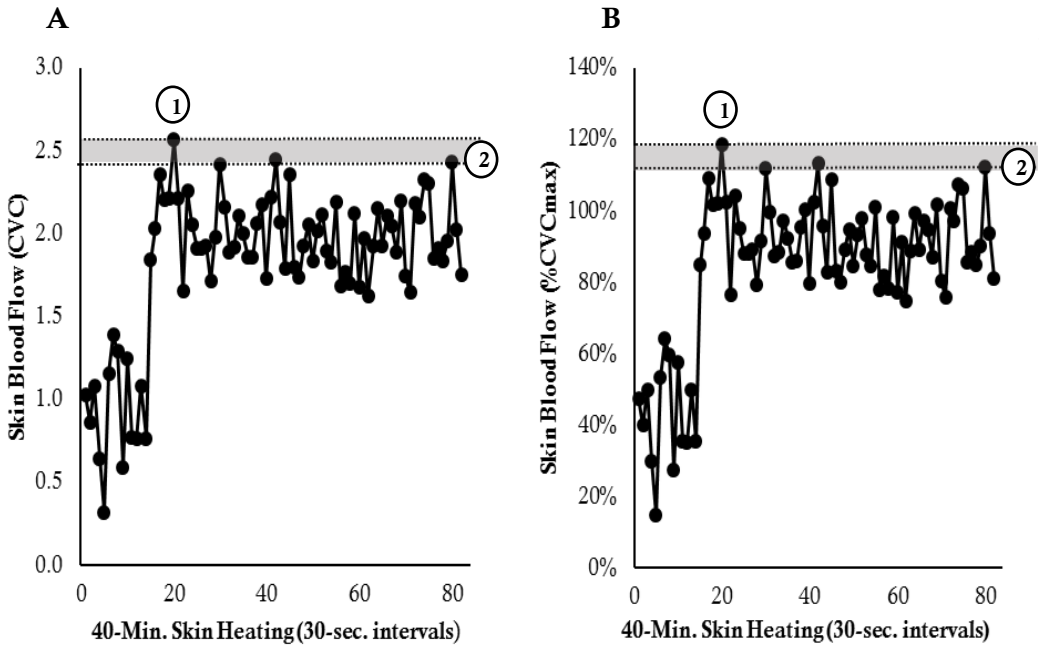


Figure 4-5. Graphs above illustrate skin blood flow (SkBF) in the right palmar toe, measured using laser Doppler Flowmetry during 40-minute local skin heating at 42 °C. Points on each graph represent mean SkBF over a 30-second period. Panel A illustrates an example of a sub-maximal response to local skin heating, expressed in raw CVC units (blood flow/mm Hg). Panel B illustrates a sub-maximal response as a percentage of participant's maximum blood flow ($\%CVC_{MAX}$). Dotted lines indicate blood flow at (1) the peak of the axon reflex and (2) end of the skin-heating protocol.

interpreted within those assumptions. For these reasons, separate analyses were performed for Aim 1 using data from both the expected and attenuated groups, which are presented below.

After removal of both attenuated and sub-maximal responses, the final sample of axon reflexes contained data from 55 visits in the HC group (Time 1: n =14; Time 2: n =14, Time 3: n =14) and 27 visits in the BCS group (Time 1: n =3; Time 2: n =4; Time 3: n =5). Results of analyses for Aim 1 using this data are presented below.

Section 4: Main Findings for Aim 1

Hypothesis 1.1: The mean size of axon reflexes will not differ significantly between HCs and BCS prior to treatment with Taxol® (Time 1)

To test the hypothesis that mean axon reflex size would not differ significantly between HCs and BCS before Taxol® (Time 1), two-tailed independent-samples t-tests (α = 0.05) were used to compare mean SkBF at the 30-seconds corresponding to the peak of the axon reflex between HCs and BCS. To ensure that any differences in mean SkBF (or lack thereof) would not be affected by how SkBF data was expressed, t-tests were performed with mean axon reflex size expressed as both a percentage of participants' maximal blood flow (%CVC_{MAX}) and in raw CVC units (TPUs/mm Hg).

Results of the analyses are summarized in Table 4-7 and illustrated in Panel A of Figures 4-6 and 4-7, respectively. Results did not find a significant difference in mean axon reflex size between HCs and BCS at Time 1, regardless of whether SkBF data was expressed in %CVC_{MAX} (p = .729) or in raw CVC units (p = .703). Thus, results of the analyses confirm Hypothesis 1.1.

Additional Analyses for Hypothesis 1.1

Theoretically, other portions of the skin-heating response besides the axon reflex could have differed between HCs and BCS receiving Taxol®. Because of this, two-tailed independent-samples t-tests (α = 0.05) were also used to compare mean SkBF at Time 1 between the two groups during other parts of the 40-minute skin-heating protocol (i.e., start 33 °C, reach 33 °C, start 42 °C, reach 42 °C, nadir, start 44 °C, and reach 44 °C). As with the main analyses for hypothesis 1.1, comparisons were performed with SkBF expressed in both %CVC_{MAX} and raw CVC units.

Results of the additional analyses for Hypothesis 1.1 are illustrated in Panel A of Figures 4-6 and 4-7 (respectively). Results found that mean SkBF was significantly higher for

HCs at the Time 1 visit during the first 5 minutes of skin heating (i.e., Start 33 °C, Reach 33 °C) than for BCS. However, at Time 1 mean SkBF was not significantly different between HCs and BCS for any other portion of the 40-minute skin-heating response, suggesting that both groups started the experiment with similar small-fiber nerve function in the palmar toe surface.

Hypothesis 1.2: The mean size of axon reflexes will differ significantly between HCs and BCS at Times 2 and 3 (i.e., during Taxol® therapy).

To test the hypothesis that mean axon reflex size (%CVC_{MAX}) would differ between HCs and BCS during weekly Taxol® (i.e., Times 2 and 3), two-tailed independent-samples *t*-tests were used ($\alpha = .05$). Results are summarized in Table 4-7 and illustrated in Panel B of Figures 4-6 and 4-7 (respectively).

Contrary to our original hypothesis, results did find not a statistically significant difference in mean axon reflex size between HCs and BCS at Time 2 or Time 3 when blood flow was expressed as a %CVC_{MAX} (Time 2: $p = .615$; Time 3: $p = .394$). However, when the mean size of axon reflexes in the right toe was expressed in raw CVC units, results found a significant difference in mean axon reflex size between HCs and BCS at Time 3 ($p = .043$), with BCS showing large axon reflexes than HCs. Thus, while results of the analysis did not confirm the original hypothesis for Aim 1.2 as originally stated (i.e., as %CVC_{MAX}), the difference in mean axon reflex size between HCs and BCS at Time 3 with data expressed in raw CVC units suggests an increase in small-fiber nerve function in BCS after 6 weeks of weekly Taxol® therapy relative to healthy counterparts.

Additional Analyses for Hypothesis 1.2

As with analysis for Time 1, two-tailed independent-samples *t*-tests ($\alpha = 0.05$) were used to compare mean SkBF between HCs and BCS at Times 2 and 3 to determine whether other portions of the response to 40-minute skin-heating would differ between HCs and BCS during Taxol® therapy.

Results of the additional analyses for hypothesis 1.2 are illustrated in Panel B of Figures 4-6 and 4-7 (respectively). Results did not find a significant difference in mean SkBF between HCs and BCS at any portion of the 40-minute skin-heating protocol when data was expressed as a %CVC_{MAX}. However, when data was expressed in raw CVC units, results found that mean SkBF was significantly higher for BCS after 6 weeks of Taxol® (i.e., Time 3)

in the portions of the response corresponding to the increase in local heat in the toe (i.e., start 42 °C (p = .023), reach 42 °C (p = .022), and reach 44 °C (p = .005)). Mean SkBF was also higher for BCS than HCs at Time 3 at the start of 44 °C skin heating when blood flow data was expressed in raw CVC units, but differences just missed the cut-off for statistical significance at $\alpha = .05$ (p = .058).

In addition, just like Time 1, results of the additional analysis showed that mean SkBF was significantly higher at Times 2 and 3 for HCs during the first 5 minutes of skin heating (i.e., start 33 °C, reach 33 °C) than for BCS.

Hypothesis 1.3: The mean size of axon reflexes will differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively)

Next, to test the hypothesis that mean axon reflex size would differ significantly for BCS between Times 1, 2, and 3 (i.e., as exposure to Taxol® increased), repeated-measures analysis of variance (RMANOVA) was used, with mean axon reflex size as the continuous dependent variable, and time point as the categorical independent variable.

Results of the RMANOVA for hypothesis 1.3 are summarized in Table 4-8 and illustrated in Figure 4-8. Results did not find a significant effect of time on mean axon reflex size for BCS when data was expressed as $\%CVC_{MAX}$ ($F(2, 1) = 3.29$, p = .363, η^2 = .868, observed power = 11.0%). However, there was a trend toward larger mean axon reflex size for BCS at Time 3 when mean axon reflex size was expressed in raw CVC units ($F(2, 1)$ = 106.78, p = .068, η^2 = .995, observed power = 53.6%), although results missed the cut-off for statistical significance of $\alpha = 0.05$. Thus, these results do not confirm initial hypothesis for Aim 1.3.

Additional Analysis for Hypothesis 1.3

While not stated as explicitly as a hypothesis, a core assumption of the study was that mean axon reflex size would not differ significantly for HCs during the study (which if true, might suggest issues with the reproducibility of the method used to generate axon reflexes in the study). To test the hypothesis that mean axon reflex size would not differ significantly for HCs between Times 1, 2, and 3, RMANOVA was used.

Results are summarized in Table 4-7. As hypothesized, results did not find a significant effect of time on mean axon reflex size for HCs, regardless of whether data was expressed as a $\%CVC_{MAX}$ ($F(2, 9) = 1.54$, p = .266, η^2 = .255, observed power = 24.6%) or in

raw CVC units ($F(2, 8) = 1.16$, $p = .892$, $\eta^2 = .028$, observed power = 6.2%). These results suggest that the differences in mean axon reflex size between BCS and HCs in our study were not the result of issues with the reproducibility of the test.

Summary of Findings for Aim 1

- At Time 1 (pre-Taxol®) and Time 2 (week 2 of Taxol®), there was no difference in mean axon reflex size between HCs and BCS regardless of how data was expressed.
- At Time 3 (week 6 of Taxol®), there was no difference in mean axon reflex size between BCS and HCs when data was expressed as $\%CVC_{MAX}$. However, when data was expressed in raw CVC units, between-group comparisons showed that axon reflexes were significantly larger for BCS than HCs ($p = 0.43$).
- Further analysis at Time 3 showed that SkBF in the toe was also significantly higher for BCS during the period immediately surrounding the axon reflex (start 42 °C ($p = .023$); reach 42 °C ($p = .022$). BCS also showed an increased response to skin heating at Time 3 during the maximum temperature used during skin-heating (start 44 °C ($p = .058$); reach 44 °C ($p = .005$).
- Within-group comparison for BCS showed no difference in mean axon reflex size between Times 1, 2 and 3 when data was expressed as a $\%CVC_{MAX}$ ($p = .363$). However, results showed a trend towards larger reflexes at Time 3 when axon reflex size was expressed in CVC units ($p = .068$). Within-group comparison for HCs confirmed that axon reflex size did not differ significantly between Times 1, 2, and 3.
- Although preliminary in nature, results from aim suggest that early signs of small-fiber TIPN may be detectable in BCS receiving weekly or bi-weekly Taxol® using 40-minute local skin heating in the palmar toe surface.

Table 4-7

Differences in Mean Axon Reflex Size (expressed both as a %CVC_{MAX} and in raw CVC units) between Healthy Female Controls (HCs) and Breast Cancer Survivors (BCS) during 40-Minute, Local Skin Heating in the Right Palmar Toe

	Differences across Visits						
Size of Axon Reflex (%CVC_{MAX})	Time 1 (Week 0) (Pre-Taxol®)	Time 2 (Week 2 of Taxol®)	Time 3 (Week 6 of Taxol®)	$F(df1, df2)$	p-value	η^2	Power
	Mean (*SD*)	Mean (*SD*)	Mean (*SD*)				
Healthy Controls	72.1 (24.3)	70.3 (10.8)	66.0 (9.6)	1.54 (2, 9)	.266	.255	24.6%
Breast Cancer Survivors	76.7 (17.3)	73.0 (10.7)	71.4 (18.3)	3.29 (2, 1)	.366	.868	11.0%
Differences between groups: *t*	-.352	-.511	-.873				
df	17	18	18				
Sig.	.729	.615	.394				
Size of Axon Reflex (raw CVC units)							
Healthy Controls	3.12 (1.45)	2.87 (1.13)	2.75 (.582)	1.16 (2, 8)	.892	.028	6.2%
Breast Cancer Survivors	3.44 (1.31)	3.27 (.650)	3.53 (.987)	106.78 (2, 1)	.068	.995	53.6%
Differences between groups: *t*	-.388	-.799	-2.174				
df	16	18	18				
Sig.	.703	.435	**.043**				

Notes. Values presented as mean (*SD*) percentage of the maximum circulatory vascular conductance (%CVC_{MAX}) value that was recorded in the right toe during the participant's skin-heating procedure. Differences in the size of axon reflexes between groups at each visit were compared using separate independent measures *t*-tests. Mean differences in axon reflex size for HCs over the course of the 6-week study were evaluated using repeated-measured analysis of variance (RM-ANOVA). *F*-values for this group are reported as Wilk's Lambda. Due to limited sample size, mean axon reflex size for BCS at Times 1, 2, and 3 was compared non-parametrically using Friedman's Test. For all tests, $\alpha = .05$.

Figure 4-6. When expressed as %CVC_{MAX}, Mean Size of Axon Reflexes in Right Toe Are Not Significantly Different between Female Breast Cancer Survivors (BCS) and Healthy Female Controls (HCs)

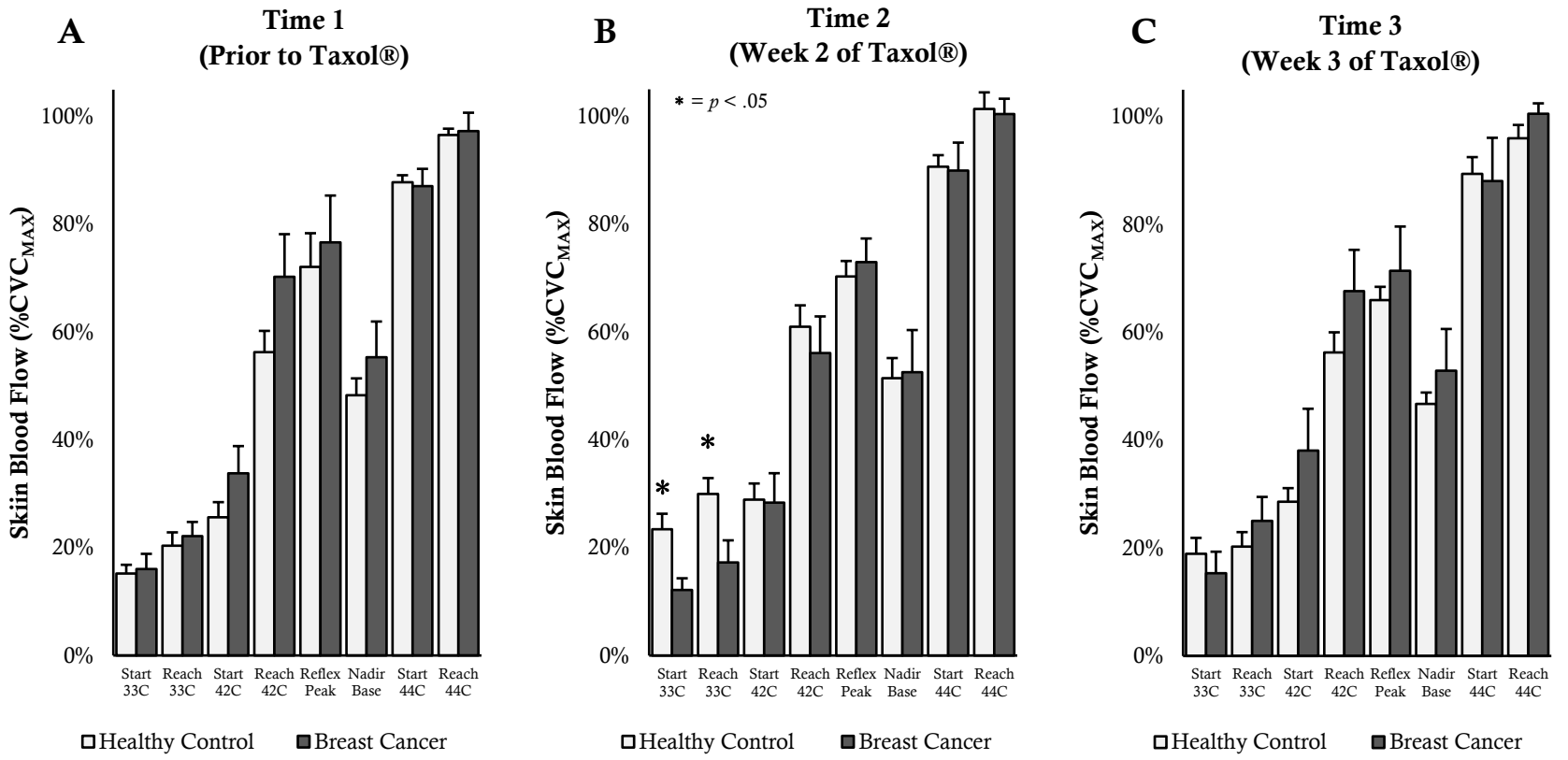


Figure 4-6. Data is expressed as a percentage of participants' maximal blood flow during 40-minute local skin heating (%CVC_{MAX}). Columns represent mean ± SD blood flow in the palmar surface of the right toe averaged over a 30-second period. Panel A represents response to skin heating at Time 1 (prior to starting Taxol®), Panel B responses at Time 2 after 2 weekly Taxol® infusions, and Panel C after 6 weekly Taxol® infusions. Columns in light grey represent blood flow response for HCs. Columns in dark grey represent blood flow for BCS receiving weekly or bi-weekly Taxol ®. Differences in blood flow between groups were tested with two-tailed, independent samples *t*-tests, $\alpha = 0.05$.

Figure 4-7. When Expressed in Raw CVC Units, Mean Axon Reflex Size in Right Palmar Toe Is Augmented during Local Skin Heating for Breast Cancer Survivors (BCS) after 6 Weeks of Taxol®

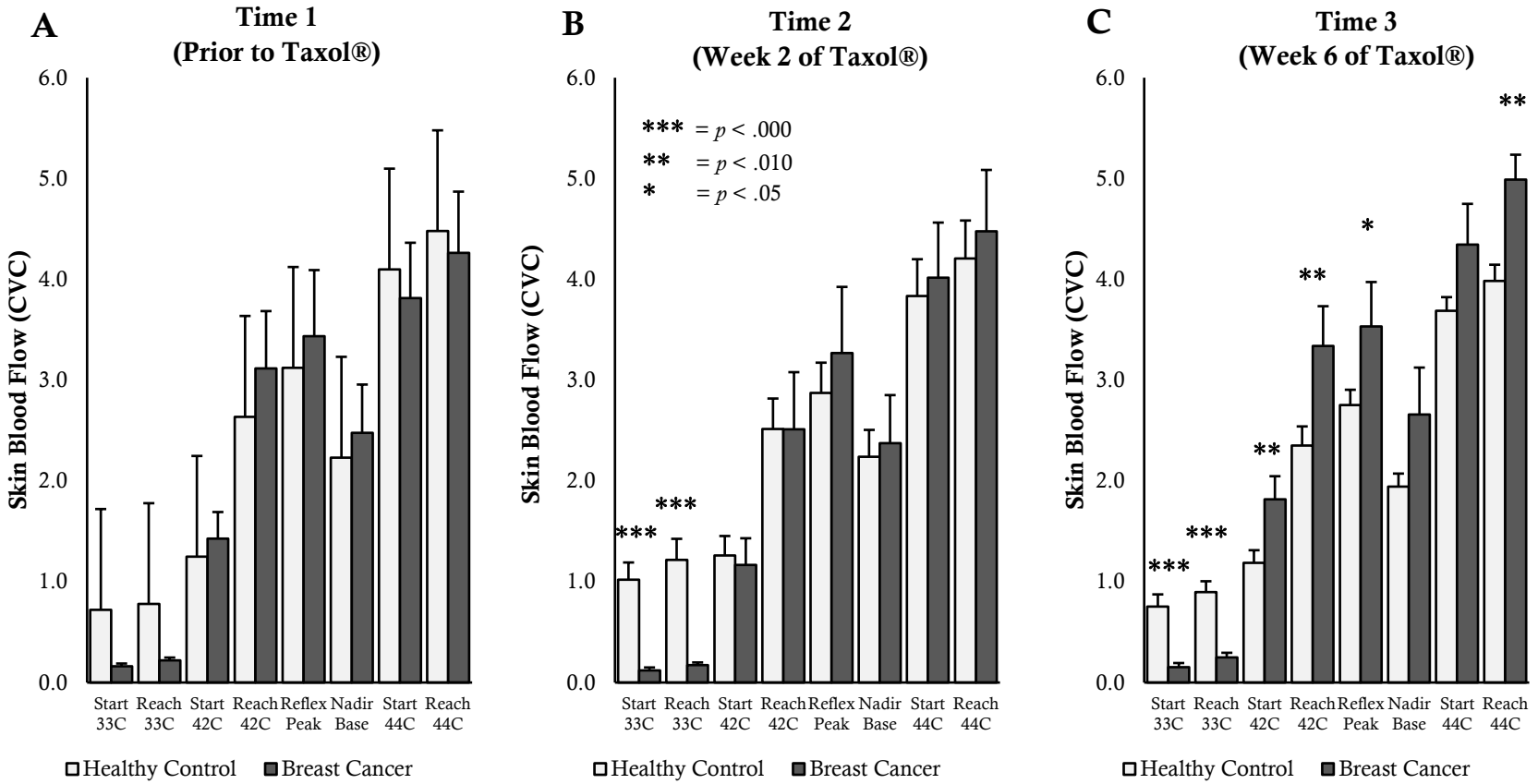


Figure 4-7. Data is expressed in mean ± SEM CVC (blood flux/mm Hg). Columns represent skin blood flow (SkBF) averaged over 30-second period during 40-minute skin heating in palmar right toe. Columns in light grey represent the response to skin heating for healthy female controls (HCs). Columns in dark grey represent the response to skin heating for BCS receiving weekly or bi-weekly Taxol®. Panel A illustrates differences between groups at Time 1 (prior to starting Taxol®); Panel B differences at Time 2 after 2 weekly Taxol® infusions, and Panel C after 6 Taxol® infusions. Differences in mean blood flow response groups were tested with two-tailed, independent samples *t*-tests, $\alpha = 0.05$.

Figure 4-8. Differences in Mean Axon Reflex Size in Right Palmar Toe Surface for Breast Cancer Survivors (BCS) (n = 9) before Taxol® (Time 1), after 2 Weeks of Weekly Taxol® (Time 2) and after 6 Weeks of Weekly Taxol® (Time 3).

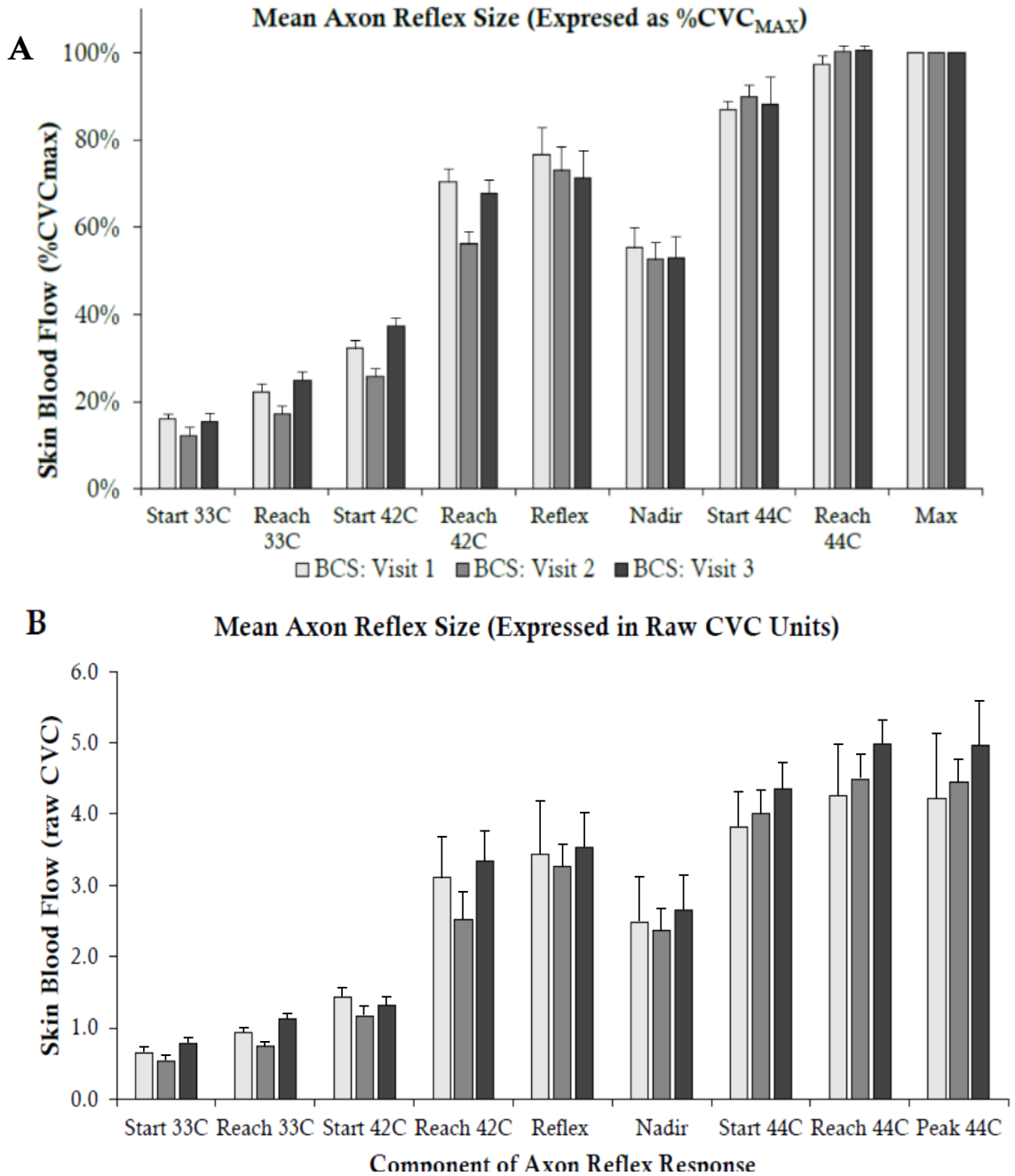


Figure 4-8. Data are expressed in mean ± SEM. Columns represent mean skin blood flow (SkBF) averaged over 30-second period during 40-minute skin heating in palmar right toe. Columns in light grey represent the response to skin heating at Time 1 (prior to starting Taxol®); mid-grey, the response at Time 2 (after 2 weekly Taxol® infusions), and dark grey (after 6 Taxol® infusions). Within-group differences in mean SkBF between time points were tested with one-way, RM-ANOVA models, $\alpha = 0.05$.

Section 5: Results for Aim 2

Data on the size of post-heating axon flares was available for 96.2% (n = 26) visits for BCS and 98.3% (n = 59) visits for HCs. In one BCS, data on the size of flares was missing because the participant was removed from the skin-heating portion of the study at Time 3 because she developed a sensitivity to the heat probe (discussed in Appendix N). Missing data on flare size from the HC group was due to the unavailability of the imager on the day of the visit. During analysis, data from an additional nine visits for HCs (including data from all 3-time points for two HCs) had to be discarded because the size of the post-heating flare could not be distinguished from the surrounding blood flow. A detailed discussion of this is included in Chapter Five. No data from the BCS group had to be discarded for this reason. The final analysis included data on the size of post-heating axon flares from 96.2% of visits for BCS (n = 9) and 84.8% visits for HCs (n = 18).

Preliminary Observations

The mean size of axon flares in the palmar surface of the right toe at each visit for HCs and BCS is summarized in Table 4-7. Mean axon flare size ranged from 2.3 ± .61 to 3.5 ± 2.8 cm^2, covering between 36-44% of the surface area of the toe (%Toe_{MAX}). On average, flares were approximately 7-10 larger than the heat probe (0.33 cm^2). Mean flare size varied widely between individuals and time points, with *SD*s in flare size ranging from 12-17% for HCs and 17-21% of BCS (Table 4-7). Flares often displayed a "crescent" or "horseshoe" shape, with areas of hyperemia following the borders of toe on one or both sides (Figure 4-9)

Figure 4-9. Example of Shape of Axon Flare in Right Palmar Toe Elicited Using 40-Minute Local Skin Heating

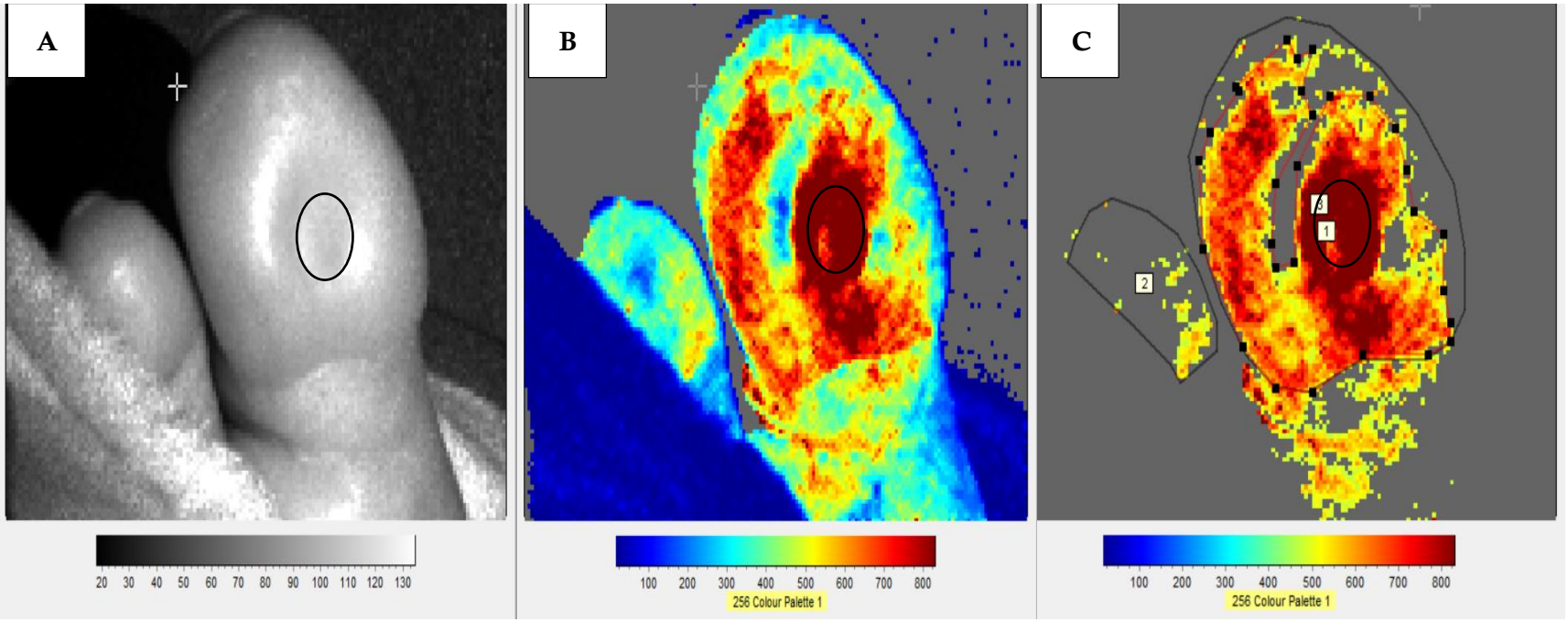


Figure 4-9. Images show heat-evoked axon flare generated in palmar surface of the right toe in a female breast cancer survivor (BCS) before the start of Taxol®. The axon flare was captured with a full-field laser perfusion imager (FLPI). Red areas in the image indicate areas of high perfusion, while regions in blue indicate areas of low perfusion. Panel A shows the toe visualized through a camera. Panel B shows the full perfusion read-out from the FLPI, while Panel C shows the final axon flare with surrounding areas of low perfusion subtracted. The 3.3 cm^2 flare pictured above was typical of those sampled in the study, with an area of intense hyperemia under/around the probe (outlined in black), crescent-shaped border extending around the edge of the toe.

Main Findings for Aim 2

Hypothesis 2.1: The mean size of axon flares will not differ significantly between BCS and HCs prior to treatment with Taxol® (Time 1).

To test the hypothesis that mean axon flare size would not differ significantly between HCs and BCS before Taxol® (Time 1), two-tailed independent-samples *t*-tests ($\alpha = 0.05$) were used to compare the mean size of axon flare in the right toe immediately after removal of the heat probe between HCs and BCS at Time 1. To minimize the chance that differences in mean flare size between groups would be due to how data was expressed, analyses were performed with flare size expressed in cm^2 and as a percentage of the total surface area of the toe ($\%Toe_{MAX}$).

Results of the analyses for hypothesis 2.1 are summarized in Table 4-8. Results found no difference in mean flare size between HCs and BCS at Time 1, regardless of whether mean flare size was expressed in cm^2 or $\%Toe_{MAX}$. Thus, results of the analyses confirm hypothesis 2.1.

Hypothesis 2.2: The mean size of axon flares will differ significantly between BCS and HCs at Times 2 and 3 (i.e., during Taxol® therapy)

To test the hypothesis that mean axon flare size ($\%CVC_{MAX}$) would differ between HCs and BCS during weekly Taxol® (i.e., Times 2 and 3), two-tailed independent-samples *t*-tests were used ($\alpha = .05$).

Results of the analyses for hypothesis 2.2 are summarized in Table 4-8. Contrary to our hypothesis, results found no difference in mean flare size between HCs and BCS at Time 2 or Time 3, regardless of whether mean flare size was expressed either in cm^2 or as a $\%Toe_{MAX}$ (Table 4-8). Thus, results of the analysis did not confirm hypothesis 2.2.

Hypothesis 2.3: The mean size of axon flares will differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively)

To test the hypothesis that mean axon flares size would differ significantly for BCS between Times 1, 2, and 3 (i.e., as exposure to Taxol® increased over the 6-week study), RMANOVA was used, with mean flare size as the continuous dependent variable, and time point as the categorical independent variable. As with the analyses for hypotheses 2.1 and 2.2, analyses for hypothesis 2.3 were performed with mean flare size expressed in cm^2 and

%Toe_{MAX} to ensure that differences between groups were consistent regardless of how data was expressed.

Results for hypothesis 2.3 are summarized in Table 4-8. Results did not find a significant effect of time on mean flare size for BCS between Times 1, 2, 3, regardless of whether mean flare size expressed in cm^2 ($F(2, 8) = .946$, $p = .439$, $\eta^2 = .240$, observed power = 14.8%), or as a percentage of participant's Toe_{MAX} ($F(2, 6) = 2.40$, $p = .173$, $\eta^2 = .444$, observed power = 31.4%) (Table 4-8). Thus, results of the analysis for Aim 2.3 did not confirm the hypothesis that mean size of axon flares would differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively).

Additional Analysis for Hypothesis 2.3

As with hypothesis 1.3. (within-group differences in mean axon reflex size over time), another assumption during the study was that mean flare size would also not differ significantly for HCs during the study. With this in mind, RMANOVA was used to test the hypothesis that mean axon flare size would not differ significantly for HCs between Times 1, 2, and 3.

Results are summarized in Table 4-8. Results did not find a significant effect of time on mean flare size when data was expressed in cm^2 ($F(2, 11) = 1.309$, $p = .309$, $\eta^2 = .719$, observed power = .988). However, when mean flare size was expressed as a %Toe_{MAX} for HCs, results found a significant effect of time on flare size ($F(2, 40) = 5.33$, $p = .009$, $\eta^2 = .210$, observed power = 81.1%). Post-hoc testing with Tukey's test revealed that flares were approximately 1 cm^2 smaller, on average, for HCs at Time 2 compared to Time 1 or Time 3 ($p = .014$) (Table 4-8). Thus, results did not confirm our hypothesis that mean size of axon flares would not differ significantly for HCs between Times 1, 2, and 3 (respectively).

Summary of Findings for Aim 2

- Results confirmed the presence of axon flares in the palmar toe surface for both BCS and HCs following 40-minute local skin heating. Flares size varied widely between participants and time points, but displayed a crescent shape matching the arrangement of capillaries and nerves in the toe.
- Between-group comparisons found no difference in mean axon reflex size between HCs and BCS at Time 1, 2 or 3. Within-group comparisons for BCS also did not find a

significant difference in mean flare size between Times 1, 2, and 3, regardless of how data was expressed in cm^2 ($p = .439$) or as a $\%Toe_{MAX}$ ($p = .173$).

- However, within-group comparisons for HCs found a significant difference in mean flare size between Times 1, 2, and 3 when flare size was expressed as a $\%Toe_{MAX}$ ($p = .009$). Post-hoc testing showed that flares were approximately 1 cm^2 smaller for HCs at Time 2 than at Time 1 or Time 3 ($p = .014$).

Table 4-8

Comparison of Mean Axon Flare Size in Right Toe following Local Skin Heating for Healthy Female Controls (n = 20) and Breast Cancer Survivors Receiving Weekly Taxol® (n = 9) at Times 1, 2 and 3

	Differences between Visits							
	Visit 1 (Week 0 (Pre-Taxol®))	Visit 2 (Week 2 of Taxol®)	Visit 3 (Week 6 of Taxol®)	$F(df1, df2)$	p	Partial η^2	Power	Post-Hoc Results
Actual Flare Size (in cm^2)								
Healthy Controls	2.82 (.73)	2.27 (.61)	3.04 (1.1)	1.309 (2, 11)	.309	.719	.988	
Breast Cancer	3.41 (1.6)	2.52 (1.1)	3.48 (2.8)	.946 (2, 8)	.439	.240	.148	
Differences between Groups: t	-1.225	-.634	-.520					
df	22	10.6	8.69					
Sig.	.234	.540	.616					
Size of Flare Relative to Total Surface Area of Toe ($\%Toe_{MAX}$)								
Healthy Controls	43.8 (16.9)	37.9 (12.0)	39.0 (16.3)	14.08 (2, 11)	.001	.192	.226	T2 vs. T3; **$p < .003$**
Breast Cancer	41.9 (17.1)	35.8 (20.5)	36.1 (18.5)	2.40 (2, 6)	.173	.444	.314	
Differences between groups: t	.271	.335	.384					
df	22	25	22					
Sig.	.789	.740	.705					

Notes. Differences between groups at each visit were compared using separate independent measures *t*-tests. Mean differences in axon reflex size for each group over the course of the 6-week study period were evaluated using repeated-measured analysis of variance (RM-ANOVA). *F*-values are reported as Wilk's Lambda. For all tests, $\alpha = 0.05$ was used as the threshold for statistical significance. † = values were corrected because of unequal variances being detected during analysis using Levene's Test.

Section 6: Results for Aim 3

The objective of Aim 3 was to explore whether changes in small-fiber nerve function that occurred during Taxol® therapy (measured by the mean size of axon reflexes or axon flares in the palmar surface of the right toe) would be significantly correlated with (a) the overall severity of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point), and (b) the severity of individual signs and symptoms of TIPN (measured by scores on each of the 5 items (range: 0-4) at each time point).

As discussed in Chapter Three, the short form of the Total Neuropathy Score, Reduced Version) (TNSr-SF) measures 5 common signs and symptoms of TIPN: tingling, numbness, neuropathic pain, loss of tendon reflexes, and diminished vibratory sensation. Potential signs and symptoms of TIPN are tested in both the lower- and upper-extremities and are scored from 0 (normal) to 4 (severe) based on how far up the limbs the sign or symptom extend. However, because TIPN is a length-dependent neuropathy (meaning that more severe signs/symptoms are associated with farther extension), higher scores on individual items (range: 0-4) and overall TNSr-SF score for the instrument (range: 0-20; the sum of the 5 items' scores) are often interpreted as severity scores (Cavaletti et al., 2007b; Vasquez et al., 2014). Finally, in cases where a sign/symptom is worse on one side vs. another, the higher of the 2 scores is used as the score for that item.

Following a summary of the percentage of TNSr-SF data that was available for analysis, results of a preliminary analysis TNSr-SF scores describing the frequency, onset, pattern, severity, and physical distribution of each of the 5 signs/symptoms for BCS during the first 6 weeks of Taxol® are presented. After this, results of the main analyses for Aim 3 are presented, including correlations between the mean size of axon reflexes/flares (respectively) and overall and item TNSr-SF scores for BCS at each time point (Hypotheses 3.1 and 3.2).

TNSr-SF scores were available for 96.7% of HC visits ($n = 58$) and 100% of BCS visits ($n = 27$). As expected, no women in the HC group reported any tingling, numbness, or neuropathic pain during the study. Baseline testing identified five HCs with signs of mildly diminished reflexes in their ankles and/or knees at baseline. Two HCs displayed mild hyperreflexia at their baseline visit during reflex testing. In addition, five BCS displayed signs of mildly diminished vibrotactile thresholds at baseline, with total TNSr-SF scores ≤ 3 out of 20 (which is considered within normal limits).

Preliminary Analysis of Total and Individual TNSr-SF Scores: Frequency, Onset, Pattern, Severity, and Physical Distribution of TIPN for BCS during Early Taxol® Therapy

Frequency of TIPN. In this study, we defined the frequency of TIPN as the percentage of BCS who reported *any* abnormal finding on their TNSr-SF, regardless of severity (i.e., any non-zero score on the 0-4 scale) for each of the five signs or symptoms of TIPN, at each time point. The frequency of tingling, numbness, neuropathic pain, diminished reflexes and vibratory thresholds for BCS at each visit is illustrated in Figure 4-10.

Results found that two-thirds (66.7%) of BCS who received weekly or bi-weekly Taxol® during the study developed some degree of TIPN. Overall, results showed that as exposure to Taxol® increased, so did the frequency of TIPN for BCS. However, the percentage of BCS who showed a diminished ability to feel vibration coming from the tuning fork during vibratory testing decreased during the study (discussed in greater detail in "Onset of TIPN").

Figure 4-10. The Frequency of Tingling, Numbness, Neuropathic Pain, Diminished Reflexes, and Diminished Vibratory Sensation for Breast Cancer Survivors (BCS) Receiving Weekly or Bi-Weekly Taxol® Therapy (n = 9) at Time 1, 2, and 3

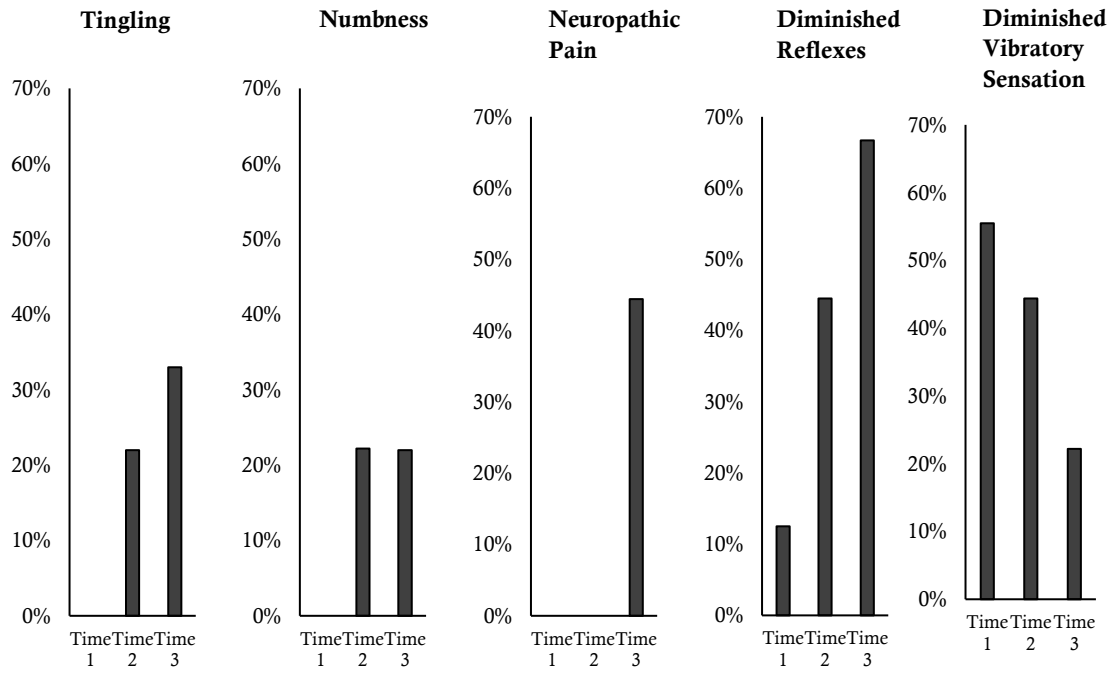


Figure 4-10. Bars represent the frequency (%) of breast cancer survivors (BCS) who reported tingling, numbness, neuropathic pain, or showed diminished reflexes or diminished vibratory thresholds. Time 1= pre-Taxol®, Time 2 = week two of Taxol®, and Time 3 = week 6 of Taxol®. Data indicate only frequency of symptoms, regardless of severity or location.

Onset and Pattern of TIPN. To determine when different signs and symptoms of TIPN began for BCS in the study, we calculated the percentage of BCS who reported a non-zero score for each sign/symptom at each time point, regardless of the location being tested (i.e., toes, ankles, legs, upper-extremities). Results found that for most BCS, TIPN developed quickly, starting after just 2 weeks of Taxol® therapy (mean Taxol® exposure: 161.5 ± 5.0 mg/m^2). At Time Two, 22.2% of BCS reported tingling and numbness in their hands or feet. In addition, 44.4% showed signs of diminished reflexes in their ankles and knees, and 44.5% of BCS showed a diminished ability to feel vibration coming from the tuning fork during vibratory testing. After six Taxol® infusions (mean Taxol® exposure: 484.6 ± 15 mg/m^2), the percentage of BCS reporting these signs/symptoms had risen for most, with 33.3% of BCS reporting tingling and 44.4% of BCS reporting neuropathic pain in their extremities. In one case, a BCS who reported no TIPN symptoms at Time 2 developed new onset tingling, numbness, and neuropathic pain in her upper and lower-extremities by Time 3. In another case, a BCS who had reported only tingling and numbness in her hands and feet at Time 2 developed new onset neuropathic pain in her feet at Time 3. One BCS who reported numbness in her fingertips at Time 2 reported that her symptoms had resolved by Time 3.

Figure 4-11. Pattern of Taxane-Induced Peripheral Neuropathy (TIPN) Symptoms during First 6 Weeks of Taxol® (*n* = 6)

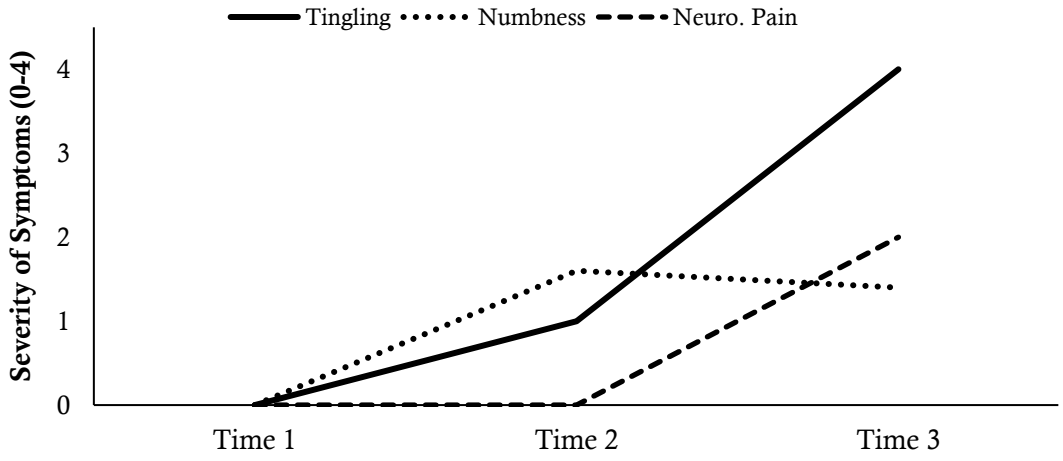


Figure 4-11. Lines represent mean item scores (0-4) for tingling, numbness, and neuropathic pain for the six BCS who reported symptoms at Time 1 (week 0 of Taxol®), Time 2 (2 weeks of Taxol®) and Time 3 (6 weeks of Taxol®).

A total of six BCS (66.7% of the sample) showed signs of diminished reflexes at Time 3 as well. However, at Time 3, the percentage of BCS (22.2%) showing signs of decreased ability to feel vibration was considerably lower than the percentage of BCS showing signs of decreased vibrotactile thresholds at Time 2 (44.4%).

Severity of TIPN. As noted earlier, strictly speaking, the TNSr-SF measures *extension* (i.e., how far the up limb(s) different signs and symptoms of TIPN extend), not *severity*. However, because TIPN is a length-dependent neuropathy (meaning that mild neuropathy symptoms would be highly unlikely in patients showing a high degree of extenson), higher scores on both the invididual TNSr-SF items (0-4) and total score (0-20) are often described as severity scores (Cavaletti et al., 2007b).

Figure 4-12. As Exposure to Taxol® Increases, So Does Severity of Taxane-Induced Peripheral Neuropathy (TIPN) for Female Breast Cancer Survivors (BCS) (n = 6)

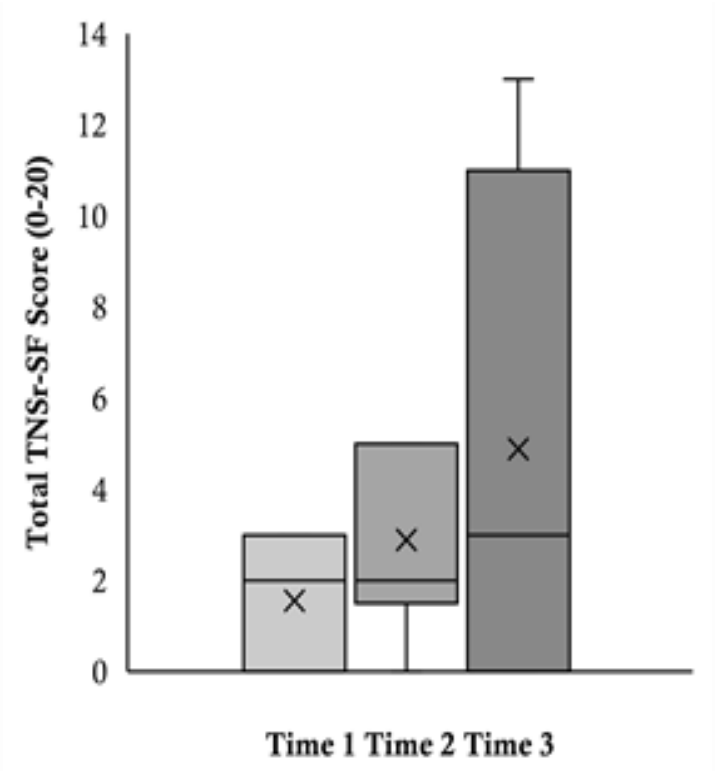


Figure 4-12. Boxes represent mean ("X"), median (dark line) and interquartile range (25%, 75%) for total TNSr-SF scores for BCS receiving weekly Taxol®. TNSr-SF scores range from 0 (no TIPN whatsoever) to 20 (most severe TPN). Light grey box = TNSr-SF scores for BCS before Taxol® (Time 1). Mid-grey box= TNSr-SF scores for BCS after 2 weeks of weekly Taxol® (Time 2). Dark grey = TNSr-SF scores for BCS after 6 weeks of weekly Taxol® (Time 3).

To determine the severity of TIPN for BCS in the sample, boxplots representing mean and median total TNSr-SF scores (0-20) for BCS at each time point were generated (Figure 4-12). Results showed that one third (n = 3) of BCS who received weekly Taxol® during the 6-week study developed no TIPN at all. Of the six remaining BCS who developed TIPN during the study, half (n = 3) developed TIPN that would be classified as "mild" per current scoring guidelines (scores ≤ 7 out of 20 possible points) (Cavaletti et al., 2007b; Vasquez et al., 2014). The other half (n = 3) displayed TNSr-SF scores between 10-13 during the study, indicating "moderately severe" TIPN (i.e., scores of 7-14). Two of the BCS with more severe TIPN had their dose of Taxol® reduced after the study because of these symptoms.

Physical Distribution of TIPN. Finally, to help determine whether signs/symptoms of TIPN developed symmetrically for BCS during the study (which has important implications for whether axon reflexes/flares should be tested on one or both sides during Taxol® therapy), data from the TNSr-SF was evaluated to determine whether BCS reported sign/symptoms symmetrically (i.e., on both sides) *at each visit.*

To do this, in addition to scoring the severity of each sign/symptom (from 0-4) at each location (e.g., toes, ankles, lower-extremities, upper-extremities), we also recorded data on the severity of each sign or symptom (0-4) in each limb individually (i.e., right foot, left foot). The reason we did this is that even though during TNSr-SF testing, both limbs are tested for neuropathy, as noted earlier, in cases where a sign or symptom is worse (i.e., numerically higher) in one limb vs. another, scoring instructions for the TNSr-SF are to only record the score for the worse of the two limbs. This is problematic, in part, because it contributes to the impression that all presentations of TIPN are uniformly symmetrical (Hausheer et al., 2006), which may or may not be true.

Using the data on the severity of each sign/symptom of TIPN in a limb, we calculated the percentage of BCS who developed tingling, numbness, neuropathic pain, diminished reflexes or diminished vibratory thresholds on both sides (i.e., bilaterally) vs. one side only (unilaterally) in each location we tested (toes, ankles, knees, upper-extremities) at each visit. The percentage of BCS who reported bilateral signs/symptoms of TIPN at each visit were then averaged to create an overall value representing the percentage of BCS that displayed bilateral signs/symptoms of TIPN across all body areas. Because BCS were neuropathy-free

at baseline (Time 1), only TNSr-SF scores during Taxol® therapy (i.e., at Time 2 and Time 3) were used for this analysis.

Results of the analysis are illustrated in Figure 4-13. Results found that 100.0% of the tingling, numbness, and diminished reflexes observed in BCS at Time 2 affected both limbs equally. However, at Time 3, only 50.0% of tingling and 16.7% was symmetric. Neuropathic pain which was present for nearly half (44.5%) of the sample at Time 3 (see Frequency above), was non-symmetric for more than half of BCS tested. Loss of reflexes was relatively symmetric for BCS in our sample. In addition, while the percentage of BCS that showed signs of vibratory deficits at Time 3 was low (just 22.2%), 100.0% of instances where vibratory deficits were observed at Time 3 were symmetrical compared to just 33.3% of instances at Time 2.

Figure 4-13. Percentage of Signs and Symptoms of Taxane-Induced Peripheral Neuropathy (TIPN) that Were Present in Both Limbs Equally during Neuropathy Testing for Breast Cancer Survivors (BCS) during Weekly Taxol® Therapy (n = 9)

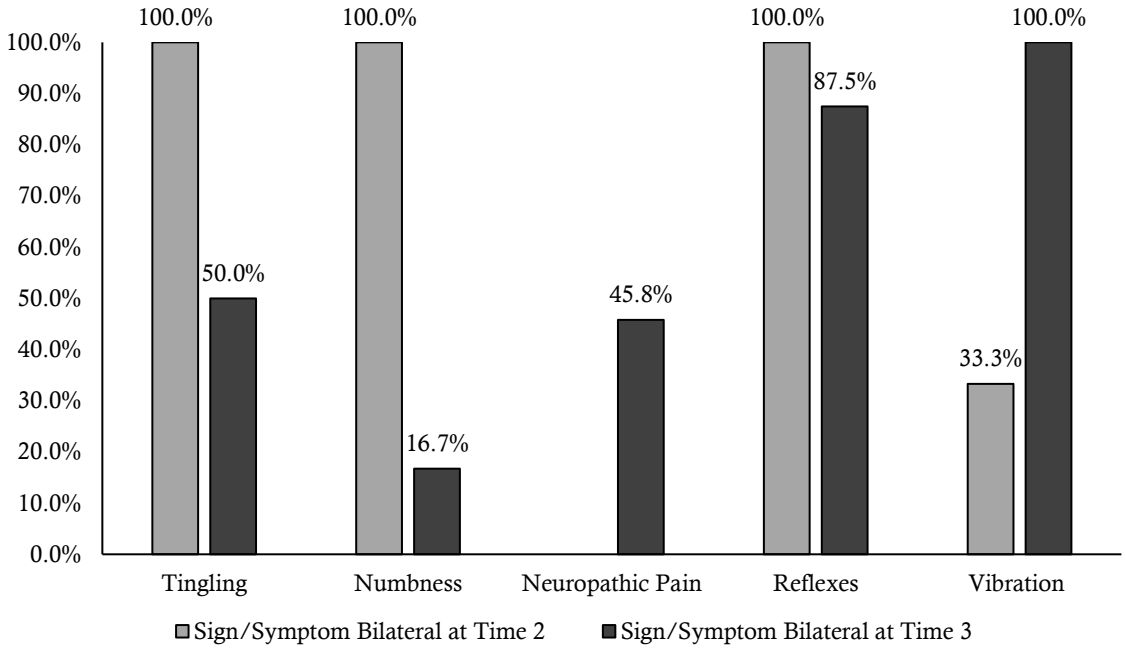


Figure 4-13. Bars indicate the percentage of neuropathy signs/symptoms that were reported on both sides of the body (i.e., bilaterally) during neuropathy testing for female BCS at Times 2 (2 weeks of weekly Taxol®) and Time 3 (6 weeks of weekly Taxol®). Bar represent the percentage of neuropathy signs/symptoms that were reported on both sides of the body (bilaterally) averaged across all testing locations (i.e., toes, medial metatarsal, patella, upper extremity). Bars in light grey represent reports of bilateral sign/symptoms at Time 2; bars in dark grey represent reports of bilateral signs/symptoms at Time 3.

Main Findings for Aim 3

<u>Hypothesis 3.1:</u> The mean size of axon reflexes in the right toe will correlate with (a) the overall severity of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and (b) with the severity of individual signs and symptoms of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time point).

To test the first part of hypothesis 3.1 – namely, that the *overall severity* of TIPN during Taxol® <u>would correlate</u> with the mean size of axon reflexes (%CVC_{MAX} and raw CVC units) at each time point -- two-tailed Spearman Rank-Ordered tests (r_s) were used ($\alpha = .05$).

Results of the analyses for the first part of hypothesis 3.1 are summarized in Table 4-9. Results did not find a statistically significant correlation between total TNSr-SF scores at Times 1, 2, or 3 and mean axon reflex size at the same time point. *P*-values for correlations were highly non-significant at $\alpha = .05$, ranging from .600 and .935 (data not shown). Correlations showed both positive and negative associations between TIPN severity and mean axon reflex size, depending on the time point and way data was expressed. The finding that axon reflexes and TNSr-SF scores were correlated at Time 1 reflect the fact that despite being free from tingling, numbness, or neuropathic pain at baseline, several BCS showed mild signs of diminished vibrotactile sensation at Time 1, giving them total TNSr-SF scores of 3 or less (which is considered within normal limits).

Table 4-9

Correlation between Overall Severity of Taxane-Induced Peripheral Neuropathy (TIPN) Symptoms and Mean Size of Axon Reflexes in Right Toe for Breast Cancer Survivors (BCS) at Times 1, 2 and 3 ($n = 9$)

Overall Severity of Signs/Symptoms of TIPN	Mean Axon Reflex Size, (%CVC_{MAX})			Mean Axon Reflex Size, (Raw CVC units)		
	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
Time 1	-.211			.316		
Time 2		.273			.273	
Time 3			-.051			-.205

Notes. Values in the table represent correlations between total TNSr-SF scores (range: 0-20) and mean axon reflex size (as a %CVC_{MAX} and raw CVC units (flux/ mm Hg). Due to the limited sample size, correlations were tested using Spearman Rank-Ordered correlations (r_s). Correlations between mean axon reflex size and TNSr-SF scores at Time 1 reflect the fact that despite being free from tingling, numbness, or neuropathic pain at baseline, several BCS

showed mild signs of diminished vibrotactile sensation at Time 1, with total TNSr-SF scores ≤ 3. * ≤ 0.05; ** = ≤ 0.01

To test the second part of hypothesis 3.1 (which stated the *severity of individual signs and symptoms of TIPN* would correlate with the size of axon reflexes (in both $\%CVC_{MAX}$ and raw CVC units)), two-tailed Spearman Rank-Ordered tests (r_s) were used ($\alpha = .05$). Results did not find any statistically significant correlations between TNSr-SF scores for individual neuropathy signs/symptoms and mean size of axon reflexes for BCS at Times 1, 2, or 3 (Table 4-10). As expected, correlation between axon reflex size and the severity of tingling, numbness, or neuropathic pain at Time 1 (baseline) were not possible because BCS' were free from these symptoms (i.e., had a TNSr-SF score of "0," making correlations impossible. However, moderate to large, non-statistically significant correlations between mean axon reflex size, loss of reflexes, and decreased vibratory sensation were identified at Time 1 because (as discussed above) several BCS showed mild signs of diminished vibrotactile sensation during their baseline testing.

Table 4-10

Correlation between Severity of Individual Signs and Symptoms of Taxane-Induced Peripheral Neuropathy (TIPN) Symptoms and Mean Size of Axon Reflexes in Right Toe for Breast Cancer Survivors (BCS) at Times 1, 2 and 3 ($n = 9$)

Severity of Individual Signs/Symptoms of TIPN		Mean Axon Reflex Size, ($\%CVC_{MAX}$)			Mean Axon Reflex Size, (Raw CVC units)		
Paresthesia (Tingling)		Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
	Time 1	--			--		
	Time 2		-.131			-.131	
	Time 3			.354			-.354
Numbness							
	Time 1	--			--		
	Time 2		.000			.000	
	Time 3			--			--
Neuropathic Pain							
	Time 1	--			--		
	Time 2		--			--	
	Time 3			.354			-.354
Loss of Reflexes							
	Time 1	.866			.866		

	Time 2		.207		.207	
	Time 3			-.474		-.474
Decreased Vibratory Sensation						
	Time 1	-.474			.000	
	Time 2		-.068			-.068
	Time 3			-.354		-.354

Notes. Values in the table represent correlations between item TNSr-SF scores (range: 0-4) and mean axon reflex size (%CVC_{MAX} and raw CVC units (flux/ mm Hg)). Due to the limited sample size and range of scores, correlations were tested using Spearman Rank-Ordered correlations (r_s). Cells marked with "--" indicate correlations could not be generated because mean TNSr-SF score for BCS at that time point were 0. * ≤ 0.05; ** = ≤ 0.01

<u>Hypothesis 3.2:</u> The mean size of axon flares in the right toe will correlate with (a) the overall severity of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and (b) with the severity of individual signs and symptoms of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time point).

Like hypothesis 3.1, hypothesis 3.2 had two parts; to test the first part of hypothesis 3.1 (which stated that the overall severity of TIPN during Taxol® would correlate with the mean size of axon reflexes (%CVC_{MAX} and raw CVC units) at each time point -- two-tailed Spearman Rank-Ordered tests (r_s) were used ($\alpha = .05$).

Results of the analyses for the first part of hypothesis 3.2 are summarized in Table 4-11. Testing did not find a statistically significant correlation between total TNSr-SF scores at Times 1, 2, or 3 and mean flare size at that same time point. *P*-values for correlations were highly non-significant, ranging between .138 and .994 (data not shown). In all but one case (Time 3, with flare size expressed in cm^2), flare size displayed a positive association with total TNSr-SF scores. As with correlations for hypothesis 3.1, correlations between mean axon flare size and TNSr-SF scores at Time 1 were due to the fact that several BCS showed mild signs of diminished vibrotactile sensation at Time 1.

Table 4-11

*Correlation between Overall Severity of Taxane-induced Peripheral Neuropathy (TIPN) Symptoms and Mean Size of Axon Flares in Right Toe for Breast Cancer Survivors (BCS) at Times 1, 2 and 3 (*n *= 9)*

	Mean Axon Flare Size,	Mean Axon Flare Size,

Overall Severity of Signs/Symptoms of TIPN		(cm^2)			($\%Toe_{MAX}$)		
		Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
	Time 1	.365			.228		
	Time 2		.208			.138	
	Time 3			-.049			.323

Notes. Values in the table represent correlations between total TNSr-SF scores (range: 0-20) and mean axon flare size (in cm^2 and $\%Toe_{MAX}$). Due to the limited sample size, correlations were tested using Spearman Rank-Ordered correlations (r_s). Correlations between mean axon flare size and TNSr-SF scores at Time 1 reflect the fact that despite being free from tingling, numbness, or neuropathic pain at baseline, several BCS showed mild signs of diminished vibrotactile sensation at Time 1, with total TNSr-SF scores ≤ 3. * ≤ 0.05; ** = ≤ 0.01

To test the second part of hypothesis 3.2 (which stated the *severity of individual signs and symptoms of TIPN* (measured by total scores on each of the 5 items (range: 0-4) would correlate with the mean size of axon flares in the right toe at each time point (in cm^2 and $\%Toe_{MAX}$), two-tailed Spearman Rank-Ordered tests (r_s) were used ($\alpha = .05$).

Results are summarized in Table 4-12. Testing did not find a statistically significant correlation between TNSr-SF scores for individual signs/symptoms of TIPN at Times 1, 2, and 3 and mean size of axon flares at the same time point. *P*-values were highly non-significant at $\alpha = .05$, ranging between .134 and 1.00 (data not shown). As with the correlations between overall TNSr-SF scores and flares, in most cases described above, flares showed a positive association with scores for the severity of individual signs/symptoms of TIPN.

Table 4-12

Correlation between Severity of Individual Signs and Symptoms of Taxane-Induced Peripheral Neuropathy (TIPN) Symptoms and Mean Size of Axon Flares in Right Toe for Breast Cancer Survivors (BCS) at Times 1, 2 and 3 ($n = 9$)

Severity of Individual Signs/Symptoms of TIPN		Axon Flare Size, (cm^2)			Axon Flare Size, ($\%Toe_{MAX}$)		
		Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
Paresthesia (Tingling)							
	Time 1	-			-		
	Time 2		.104			.207	
	Time 3			.247			.378
Numbness		Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
	Time 1	-			-		

	Time 2		.000			-.104	
	Time 3			.000			.412
Neuropathic Pain		Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
	Time 1	-			-		
	Time 2		-			-	
	Time 3			.247			.343
Loss of Reflexes		Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
	Time 1	.412			.577		
	Time 2		.327			.186	
	Time 3			.078			.540
Decreased Vibratory Sensation		Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
	Time 1	.276			.080		
	Time 2		-.111			.037	
	Time 3			.265			.234

Notes. Values in the table represent correlations between item TNSr-SF scores (range: 0-4) and mean axon flare size (in cm^2 and $\%Toe_{MAX}$). Due to the limited sample size and range of scores, correlations were tested using Spearman Rank-Ordered correlations (r_s). Cells marked with "--" indicate correlations could not be generated because mean TNSr-SF score for BCS at that time point were 0. * ≤ 0.05; ** = ≤ 0.01

CHAPTER FIVE
DISCUSSION AND RECOMMENDATIONS

Chapter Organization

This chapter discusses findings from the current study and their implications for efforts to develop viable early-detection methods for TIPN in BCS receiving Taxol®. The discussion is organized into seven sections. Sections 1-3 discuss findings and implications for Aims 1-3. In addition, a brief list of recommendations for research and policy related to that aim are discussed at the end of each section. Section 4 summarizes findings, implications, and recommendations for the study (by aim and hypothesis), while Section 5 discussed strengths and weakness of the study. Section 6 provides recommendations for future research related to early detection of TIPN, divided by aim (i.e., 1-3). Section 7 provides a final summary of findings and closing remarks about the study.

Purpose

The purpose of this study was to determine if a technique used to detect signs of small-fiber neuropathy in other populations of patients (local skin heating) could detect early signs of small-fiber TIPN in BCS receiving the antineoplastic agent paclitaxel (Taxol®). The aims and hypotheses for this study were:

1. To compare the mean size of axon reflexes (expressed as a $\%CVC_{MAX}$) in the right great toe of BCS receiving Taxol® during local skin heating to the mean size of axon reflexes in the right toe of HCs (**Aim 1**).
    - Hypothesis 1.1: The mean size of axon reflexes will not differ significantly between HCs and BCS prior to treatment with Taxol® (Time 1).
    - Hypothesis 1.2: The mean size of axon reflexes will differ significantly between BCS receiving Taxol® between Times 1, 2, and 3 (respectively).
    - Hypothesis 1.3: The mean size of axon reflexes will differ significantly between BCS and HCs at Times 2 and 3 (i.e., during Taxol® therapy).
2. To compare the mean size of axon flares (in cm^2) in the right great toe of BCS receiving Taxol® during local skin heating to the size of axon flares in the right toe of HCs (**Aim 2**).
    - Hypothesis 2.1: The mean size of axon flares will not differ significantly between BCS and HCs prior to treatment with Taxol® (Time 1).

- Hypothesis 2.2: The mean size of axon flares will differ significantly between BCS and HCs at Times 2 and 3 (i.e., during Taxol® therapy).
- Hypothesis 2.3: The mean size of axon flares will differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively).

3. To determine if (a) the mean size of axon reflexes or (b) the mean size of axon flares will correlate significantly with scores on the validated clinical measure for TIPN in BCS receiving Taxol®, the Total Neuropathy Score (Reduced Version, Short Form (TNSr-SF)) (**Aim 3**).
    - Hypothesis 3.1: The mean size of axon reflexes in the right toe will correlate with (a) the *overall severity of TIPN* (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and (b) with *the severity of individual signs and symptoms of TIPN* (measured by total scores on each of the 5 items (range: 0-4) at each time point.
    - Hypothesis 3.2: The mean size of axon flares in the right toe will correlate significantly with (a) the *overall severity of TIPN* (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and (b) with *the severity of individual signs and symptoms of TIPN* (measured by total scores on each of the 5 items (range: 0-4) at each time point.

Section 1: Discussion for Aim 1

Hypothesis 1.1: The mean size of axon reflexes in the right toe will not differ between HCs and BCS prior to treatment with Taxol® (Time 1).

Experiments for hypothesis 1.1 compared the mean size of axon reflexes in the right toe between BCS and HCs before the start of Taxol® (i.e., Time 1). As discussed in Chapters One and Two, studies show that the initial rise in SkBF that occurs during local skin heating (known as the axon reflex) can be used as a marker for small-fiber nerve function in the periphery (Cracowski et al., 2006). We hypothesized that the mean size of axon reflexes in the right toe would not differ significantly between BCS and HCs at Time 1, indicating that BCS entered the study with intact small-fiber nerve function. Results found no difference in mean axon reflex size between HCs and BCS at $\alpha = 0.05$.

This finding is important for two reasons. First, results provide evidence that BCS in the current sample entered the study with small-fiber nerve function equivalent to HCs. As

with other studies that use a control group to identify changes in an intervention group in a prospective design, a core assumption of this strategy was that both groups *begin from the same position on the outcome(s) of interest*. Results of the analyses for hypothesis 1.1 suggest that this assumption was met. At Time 1, axon reflexes were nearly identical in size for both groups (HCs: 72 ± 24% CVC_{MAX} vs. BCS: 77 ± 17%). Furthermore, the mean axon reflex size for BCS in our study (77 ± 17%) was nearly identical to the size of axon reflexes from healthy women in other studies (75 ± 10%), indicating that small-fiber nerve function was similar for BCS and HCs at baseline (Time 1) (Hodges & Sparks, 2013; McGarr, Hodges, & Cheung, 2017; B. J. Wong & Minson, 2011a; B. J. Wong et al., 2006).

The second reason why findings from Hypothesis 1.1 are important is that they provide insight into the function of small-fiber nerves before chemotherapy, which has been a growing topic of interest for neuropathy researchers. Recently, several groups identified possible signs of neuropathy (including small-fiber neuropathy) in patients before cancer treatment (Jessica A. Boyette-Davis et al., 2012a; Cata, Weng, Lee, Reuben, & Dougherty, 2006; Kosturakis et al., 2014). Of relevance to this investigation, researchers in these studies found that patients with cancer were less able to detect heat or warmth in their hands than HCs (suggesting alterations in small-fiber nerves, which play key roles in the perception of heat and cold). The findings were unexpected, not only because patients in these studies had no obvious risk factors for neuropathy (e.g., diabetes), but because none of the patients had received chemotherapy yet (which is one of the primary risk factors for neuropathy in patients with cancer (Stubblefield et al., 2009)). These findings raised the question of whether cancer itself could directly (or indirectly) increase patient's risk for developing neuropathies such as TIPN during treatment (Jessica A. Boyette-Davis et al., 2012a; Cata et al., 2006).

In contrast to the findings from these studies, results of this study did not find a difference in mean axon reflex size prior to treatment that would suggest underlying issues with small-fiber nerve function. There may be several reasons why we did not observe alterations in small-fiber nerve function like those suggested by Boyette-Davis et al. and others. First, the temperatures used to stimulate small-fiber nerves in the previous studies (44-47 °C) were somewhat higher than those used in our study (42-44 °C). Second, the heat probes used in the previous studies were approximately 10-times larger than the heat probe used in this

study (3.2-3.6 cm^2 vs 0.33 cm^2). Third, the rate at which the skin was heated in these studies was faster than the rate used to heat the skin in the current study ((0.3 °C/s vs 0.5 °C/s).

This combination of higher temperatures, larger heating area, and faster heating rate is likely to have stimulated nerve endings more strongly than nerves in the current study. This may partially explain why Boyette et al and others may have observed deficits in small-fiber nerve function not observed in the present study. Furthermore, in the studies by Boyette et al. and others, temperature sensitivity was tested in the hands, not the skin of the palmar foot, which is thicker than skin in the hands and therefore, may be less sensitive. Finally, the amount of time researchers in these studies heated the skin (≤ 1 min) was vastly shorter than the duration used in our study (~40 min); this is relevant because during prolonged skin heating, sensory nerves begin to accommodate to the heat stress, potentially influencing the results. Additional studies combining both self-reported and physiological methods for evaluating small-fiber nerve function are needed to determine whether cancer itself put BCS at risk for developing TIPN.

Another reason why findings from Hypothesis 1.1 are important is that these results provide the first description of the axon reflex response in BCS. Experts caution that the response to local skin heating (including axon reflex size and shape) can vary in patients with different disease processes, making it important to characterize axon reflexes in each population (Cracowski et al., 2006). Previous work indicates that the axon reflex response is poorly characterized in patients with cancer, making it difficult to perform research using local skin heating in oncology populations. Results from the analysis for hypothesis 1.1, while preliminary in nature, provide a starting place for larger studies characterizing the response in BCS and other populations with cancer.

Hypothesis 1.2: The mean size of axon reflexes will differ significantly between BCS and HCs at Times 2 and 3 (i.e., during Taxol® therapy)

The purpose of studies testing hypothesis 1.2 were to determine whether the size of axon reflexes (a marker for small-fiber nerve function in the periphery) would differ significantly between HCs and BCS during the early portion of BCS' weekly Taxol® therapy. Specifically, the analyses for hypothesis 1.2 sought to determine whether the size of axon reflexes' size would differ between HCs and BCS after 2 weeks and 6 weeks of weekly Taxol® (Times 2 and 3, respectively). To test this hypothesis, axon reflex size was averaged for both

groups at Time 2 and 3 (respectively) and compared using two-sided *t*-tests. As is common in analyses using SkBF data, statistical comparisons were performed with blood flow data expressed as both a percentage of participant's maximal SkBF (%CVC_{MAX}) and in raw SkBF divided by participant's mean arterial blood pressure (CVC units).

Results of the analysis found that when mean axon reflex size was expressed as a %CVC_{MAX}, axon reflex size did not differ significantly between HCs and BCS at Time 2 (p = .615) or at Time 3 (p = .394). However, when mean axon reflex size was expressed in CVC units, mean axon reflex size at Time 3 differed significantly between HCs and BCS, with BCS showing significantly larger axon reflexes after 6 weeks of Taxol® compared to HCs. Results also found that at Time 3, mean SkBF in the toe was significantly higher for BCS at other portions of the 40-minute skin heating that involved temperatures >42 °C (e.g., start 42 °C (p = .023), reach 42 °C (p = .022), and reach 44 °C (p = .005)).

These findings are important for three key reasons.

First, the finding that mean axon reflex size differed between BCS and HCs at Time 3 provides evidence supporting the conclusion that Taxol® may affect small-fiber nerve function in humans. The role that damage to small-fiber nerves play in the signs/symptoms of TIPN that BCS develop during Taxol® therapy is controversial. Until recently, researchers thought that small-diameter nerves were unaffected during Taxol®, with the bulk of the neuropathy that patients reported during treatment being caused by disruptions to large-fiber, myelinated nerves (Dougherty et al., 2004; Loprinzi et al., 2011b; Sahenk et al., 1994).

Results of recent pre-clinical studies in animals and neuronal model systems have called this view into question, showing that exposure to Taxol® is associated with a host of changes to small-fiber nerves with the potential to produce or worsen neuropathy. These changes include alterations in neurotransmitter release, reductions in the number of nerve fibers in the skin, and changes in nerve-mediated vasodilation, among others (Ferrari et al., 2013; N. G. Gracias et al., 2011; Pittman et al., 2013; Zhang et al., 2013). While these findings suggest a broader involvement of small-fiber nerves in TIPN than previously suspected, the lack of viable methods for measuring changes to small-fiber nerves in vivo in humans (including methods suitable for routine use in the practice) have hampered efforts to validate this hypothesis in the clinical setting.

While preliminary in nature, results from the analyses we performed for hypothesis 1.2 suggest that Taxol® does affect small-fiber nerve function in humans. At Time 3, there was a statistically-significant difference in mean axon reflex size between HCs and BCS when data was expressed in raw CVC units (HCs: 2.8 ± .60 vs. 3.5 ± .99, p = .043). However, this difference in axon reflex size between HCs and BCS at Time 3 was not statistically significant when data was expressed as $\%CVC_{MAX}$ (HCs: 66 ± 10% vs. BCS: 72 ± 18%, p = .394).

The fact that differences in axon reflex size were only significant when data was expressed as $\%CVC_{MAX}$ was likely due to both the size of the sample (which was smaller than intended), and the fact that like other types of data, differences in mean axon reflex size can appear larger or smaller depending on how the data is expressed. For example, when expressed as the mean size of axon reflexes for BCS in raw CVC units (raw blood flow normalized to the participant's blood pressure), we found that, on average, axon reflexes were 128% larger for BCS at Time 3 than they were for HCs at the same time point. When we expressed mean axon reflex size as a $\%CVC_{MAX}$ (CVC divided by the maximum level of vasodilation that the participant displayed during the 40-minute skin heating), we still found that at Time 3, mean axon reflex size was larger for BCS than HCs. However, the size of this difference was considerably smaller when we expressed data in raw CVC units (108% larger for BCS when data was expressed in CVC compared to 128% when expressed as $\%CVC_{MAX}$). Together, this suggests that in a more statistically-powerful sample, the differences in mean axon reflex size we observed between BCS and HCs at Time 3 would have still been statistically significant, regardless of how the data was expressed.

Several studies published since the start of this dissertation support the hypothesis that Taxol® can affect small-fiber nerve function in humans (Saad et al., 2016a; Sharma, Venkitaraman, et al., 2015). Sharma et al. recently identified signs of small-fiber neuropathy in a cohort of BCS approximately 1-year post-Taxol® therapy. Similarly, Saad et al. recently identify changes in autonomic C-fibers attached to sweat glands in BCS during Taxol® therapy, although it should be noted that BCS in this study had previously received Taxol®. Pre-clinical studies in mice also support the conclusion that exposure to Taxol® is associated with a loss of small-fiber nerves in the skin, suggesting that the effects of Taxol® include both functional and structural changes to small-fiber nerves (Ferrari et al., 2013). Together, these

findings imply the need for diagnostic tools capable of detecting and monitoring these changes during cancer treatment.

A second reason why the finding that mean axon reflex size was significantly different between BCS and HCs at Time 3 is important is that it provides evidence that small-fiber TIPN may have been detectable using local skin heating. This is important because it suggests that local skin heating, a non-invasive technique, may be a potential way to detect signs of small-fiber TIPN in BCS. In addition, the finding that differences in small-fiber TIPN may have been detectable after only 6 weeks of weekly Taxol® therapy suggests that local skin heating may be useful as an early detection method for small-fiber TIPN in BCS. A recent report by the National Comprehensive Cancer Network concluded that underassessment of TIPN "…is a significant problem" (Stubblefield et al., 2009). The lack of routine assessment for TIPN in the clinical setting by nurses, oncologists, and ancillary staff can be attributed to a variety of factors, but one critical issue is the need for simple, accurate tools to evaluate nerve function in patients at the bedside (Kiernan, 2012; Markman, 2006a)).

Findings from hypothesis 1.2 suggest that measuring the size of axon reflexes during local skin heating in the toe may be worthy of consideration as an early detection method for small-fiber TIPN in BCS receiving Taxol®. However, the length of the protocol (40 minutes), the need for a quiet testing room at or near 25±1 °C, and the need for equipment such as LDF and skin heaters suggest this technique may be better suited to detecting early signs of TIPN in the research setting, where the environment can be more easily controlled. In addition, since the start of the dissertation, two new methods for evaluating small-fiber nerve function have been introduced. One uses changes in electrical skin conductance to evaluate autonomic C-fibers (Saad et al., 2016a), whereas the second uses a hand-held device to evaluate small-fiber nerve condition (Matsuoka et al., 2016)). The new methods may provide nurses with other options for screening small-fiber TIPN in BCS than the method tested here. Further studies are warranted to determine which method is the best for evaluating small-fiber TIPN in various settings.

Interestingly, results of the analysis did not find evidence of small-fiber TIPN in BCS after 14 days of weekly Taxol®, even though approximately one-third of BCS in the sample started to display signs/symptoms of TIPN such as tingling, numbness, a loss of reflex, and changes in vibrotactile sensitivity by this point in the study (see: Discussion for Aim 3). At

Time 2, no difference was found in the size of axon reflexes in the right toe between HCs and BCS, regardless of whether data was expressed as a $\%CVC_{MAX}$ (HCs: 70 ± 11% vs. BCS: 73 ± 11%, p = -.615), or in raw CVC units (HCs: 2.9 ± 1.1 vs. BCS: 3.3 ± 0.7, p = .435).

The fact that BCS in the sample who had started to show symptoms of TIPN by Time 2 did not show corresponding signs of small-fiber TIPN by Time 2 was surprising given the findings that, in other populations with neuropathy (e.g., diabetic patients), signs of small-fiber neuropathy often precede symptoms (Green et al., 2010). However, because damage to small-fiber nerves during diabetes often evolves over years and decades rather than weeks or months (as in the case of TIPN), it is possible that 2 weeks was simply too early for signs of small-fiber TIPN to be detectable in BCS receiving Taxol®. It is also possible that during Taxol® therapy, changes in small-fiber nerves occur only *after* changes in larger myelinated nerves. More than one-third of BCS tested reported symptoms consistent with large-fiber involvement (e.g., tingling, numbness) at Time 2; however, at Time 2, none of them reported symptoms that would classically be associated with damage to small-fiber nerves such as burning or pain. It is also possible that the method used to measure small-fiber nerve function (local skin heating) was not sensitive enough to detect early signs of small-fiber TIPN in BCS after 2 weeks of weekly Taxol® (especially in a sample this size).

Third, the finding that the mean axon reflex size was *larger* in BCS at Time 3 than HCs is important because it mirrors findings from pre-clinical models of TIPN showing an increased response in sensory neurons following short-term exposure to Taxol®. Traditionally, animal studies have been divided on the question of whether Taxol® increases or decreases behaviors associated with small-fiber nerves (e.g., paw-withdrawal or tail-flick responses in response to heat). Data from pre-clinical studies disagree on the effect that Taxol® has on heat sensitivity (which would imply changes in the sensitivity of small-fiber nerves), with studies arguing both for and against a possible role for Taxol® (Dina, Chen, Reichling, & Levine, 2001; Mo, Erdelyi, Szigeti-Buck, Benbow, & Ehrlich, 2012).

Similarly, clinical studies in humans are undecided on the question of whether Taxol® increases or decreases patients' sensitivity to heat. Multiple studies in BCS have reported increases in heat sensitivity during Taxol® therapy (Authier et al., 2000; Nahman-Averbuch et al., 2011; Park, Lin, et al., 2011a; Polomano, Mannes, Clark, & Bennett, 2001). Studies showing that Taxol® has no impact on heat sensitivity (Noda, Akita, Ogata, & Saji, 2014), or

may actually reduce BCS' sensitivity to heat also have been reported (Augusto et al., 2008a; Forsyth et al., 1997), making it difficult to conclude that Taxol® alters small-fiber nerve function.

Recent pre-clinical work in isolated sensory neurons by Pittman et al. may shed some light on the seemingly paradoxical effect that Taxol® has on outcomes that measure small-fiber nerves. Using sensory neurons isolated from rats, Pittman et al. exposed neurons to different concentrations of Taxol® for different lengths of time (Pittman et al., 2014). Next, to evaluate the effect that different concentrations and durations of Taxol® had, researchers measured neurotransmitter release from the isolated neurons in response to capsaicin, a naturally-occurring agonist for the TRPV.1 receptor. As noted in Chapter Two, the TRPV.1 receptor, which is widely expressed on small-fiber nerves, is activated by painful stimuli and heat ~42 °C (Dubin & Patapoutian, 2010), which is what makes local skin heating between 42-44 °C selective for small-fiber nerve function. Results of Pittman et al.'s experiment found that the effect of Taxol® on TRPV.1-expressing sensory nerves was both dose- and duration-dependent, with shorter exposures and lower concentrations of Taxol® *increasing* transmitter release, and longer exposures and higher concentrations of Taxol® *decreasing* transmitter release from sensory neurons (Darby, Meng, & Fehrenbacher, 2017; Pittman et al., 2014). This pattern showing an amplified neuronal response to TRPV.1 stimulation in conditions mimicking early Taxol® exposure is consistent with our finding that mean axon reflex size was *larger*, not smaller, for BCS at Time 3 compared to HCs.

Findings from the additional analyses from hypothesis 1.2 showing that blood flow was higher for BCS in other portions of the 40-minute experiment also support the idea that early Taxol® exposure may be associated with a gain in small-fiber nerve function. At Time 3, SkBF in the toe was significantly higher for BCS during nearly all portions of the protocol; mean SkBF at Time 3 also was significantly higher for BCS at the start 42 °C skin heating (p = .023), when heating reached 42 °C (p = .022), and when heating reached 44 °C (p = .005). Mean blood flow at the start of 44 °C skin heating also nearly met the cut-off for statistical significance (p = .058). Furthermore, when mean SkBF between BCS and HCs was compared during the first 5 minutes of skin heating (33 °C), results showed that SkBF was lower for BCS than HCs. This suggests that the increase in SkBF observed during the

TRPV.1-stimualting sections of the protocol was not due to BCS starting the protocol with higher overall levels SkBF compared to their healthy counterparts.

Recent studies support the idea that Taxol® affects small-fiber nerves but that the effect may depend, in part, on the stage of Taxol® therapy. For example, Sharma et al. recently reported signs of reduced small-fiber nerve function in BCS approximately 1-year post-Taxol® therapy (Sharma, Venkitaraman, et al., 2015). Likewise, Saad et al. reported reductions in small-fiber nerve function in BCS previously exposed to Taxol® (Saad et al., 2016a). While these studies suggest that longer, more chronic exposures to Taxol® may be associated with reductions in small-fiber nerve function, studies directly investigating this question are needed. The answer to this question has implications for neuropathy researchers who may need to target TIPN at specific stages to be effective, as well as nurses screening BCS for TIPN in the clinic who need to know how to interpret the results of diagnostic tests for small-fiber neuropathy.

Hypothesis 1.3: The mean size of axon reflexes will differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively).

The purpose of experiments testing hypothesis 1.3 was to determine whether the mean size of axon reflexes in the right toe would differ significantly for BCS between Times 1, 2, and 3. As discussed in Chapter One in the "Theoretical Model," the argument that local skin heating could be used to detect early signs of small-fiber TIPN in BCS receiving Taxol® was based on several assumptions. These include the assumptions that (1) Taxol® alters small-fiber nerve function in humans in a similar way to that observed in animals exposed to Taxol®, and (2) changes in small-fiber nerve function during Taxol® would lead to changes in the size of axon reflexes that would be detectable using local skin heating.

Furthermore, because TIPN is typically dose-dependent, a fundamental assumption of this study was that as exposure to Taxol® increased over the 6-week study, that differences in mean axon reflex size between BCS and HCs would become more apparent. Consequently, the hypothesis for 1.3 was that the mean size of axon reflexes would differ significantly for BCS between Times 1, 2 and 3. To test this, we compared the mean size of axon reflexes in the right toe of BCS at Times 1, 2, and 3 using RMANOVA. Contrary to the hypothesis, results of the analysis did not find a significant difference in mean axon reflex size for BCS across the 6-week study when data was expressed as a $\%CVC_{MAX}$.

There are several explanations why this may have occurred. First, the fact that mean axon reflex size was not significantly different for BCS between Times 1, 2 and 3 could indicate that our expectations about how much of a change in axon reflex size would occur between Times 1, 2 and 3 were unrealistic. Studies using axon reflexes to measure TIPN had not been published when planning for this study was taking place. Therefore, the original estimates about the degree to which axon reflex size would change for BCS during the first 6 weeks of Taxol® had to be inferred from studies that used axon reflexes to evaluate neuropathy in other populations (primarily diabetic patients) over time (Metzler-Wilson et al., 2012; Minson et al., 2001; B. J. Wong & Fieger, 2010).

Based on these studies, it was estimated that mean axon reflex size might differ approximately 25% for BCS between our study's 3 time-points. Results of the power analysis indicated that 15 BCS would provide the RMANOVA for Hypothesis 1.3 ~90% power to detect changes in mean axon reflex size as small as 18% for BCS between Times 1, 2 and 3. This assumes a Type I error rate ≤ 0.05 and a correlation in axon reflex size of at least 50% between visits. However, results of the analysis for Hypothesis 1.3 showed that mean axon reflex size varied by less than 10% for BCS between Times 1, 2, and 3 (Time 1 = 77 ± 17%; Time 2 = 73 ± 11%; Time 3 = 71 ± 18%), making it clear that the expectations about how much of a change in axon reflex size would occur were not accurate.

Another potential explanation for why mean axon reflex size did not differ significantly for BCS in the sample between Times 1, 2 and 3 is (discussed under Aim 1.2 above), is that 6 weeks was not long enough to detect changes in small-fiber nerve function using this method. The length of this study was determined by factoring in the earliest point at which BCS were likely to show signs of TIPN (2 weeks) and the need for a study feasible for a dissertation. While the data from Aim 3 (discussed below) indicated that the rate at which BCS in the study developed clinical symptoms of TIPN was nearly identical to that observed in other studies, mean axon reflex size did not differ significantly for BCS between Times 1, 2 and 3. This may indicate that 6 weeks was not enough time to identify changes in small-fiber nerve function using this method, or in a sample this size.

Interestingly, a trend was seen towards larger axon reflexes for BCS at Time 3 at (p = .068) when mean axon reflex size was expressed in raw CVC units, but the finding missed the cut-off for statistical significance at α =.05). This finding mimics the outcomes we observed in

hypothesis 1.2, where axon reflexes were larger for BCS than HCs at Time 3, but the differences were only statistically significant in our sample when data was expressed in raw CVC units. As noted above, this could suggest that in a larger sample, this trend towards larger axon reflexes in BCS after 6 weeks of Taxol® would have been significant regardless of how the data was expressed. In addition to increasing the sample size, studies using longer time points will be needed to establish the rate at which small-fiber nerve function changes for BCS with TIPN, and the earliest reliable point that small-fiber TIPN can be detected for BCS during Taxol® therapy.

Finally, while not an explicit aim of the study, a second objective of hypothesis 1.3 was to determine whether the mean size of axon reflexes in the right toe would differ significantly for HCs between Times 1, 2, and 3. Previous studies have established the reproducibility of axon reflexes in HCs using similar skin heating (C. S. Huang, Wang, & Tsai, 2013; Nieuwenhoff et al., 2016). However, we shortened the length of skin heating during the first 5 minutes of the protocol and chose to generate axon reflexes in the glabrous skin of the palmar toe (which has higher and more variable blood flow than non-glabrous skin). Using the protocol, we tested the hypothesis that mean axon reflex size would not differ for HCs across the 3 time-points.

Results confirmed that mean axon reflex size did not differ significantly for HCs between Times 1, 2 or 3 regardless of whether data was expressed as a $\%CVC_{MAX}$ ($p = .266$) or raw CVC units ($p = .892$)., providing evidence that axon reflexes were reproducible for HCs from time-point to time-point. In addition, the size of axon reflexes for HCs in the study was similar to the size of axon reflexes ($75 \pm 10\%$)) generated in non-glabrous skin in longer local skin-heating protocols (typically in the ventral arm over 60-90 min) (Hodges & Sparks, 2013; McGarr et al., 2017; B. J. Wong & Minson, 2011a; B. J. Wong et al., 2006). This suggests that the modified skin-heating protocol we used in this study produced similarly-sized axon reflexes to longer skin-heating protocols.

Section 2: Discussion for Aim 2

The purpose of Aim 2 was to compare the mean size of axon flares (expressed in cm^2) in the right great toe of BCS receiving Taxol® following local skin heating to HCs. As discussed in Chapter Two, research has shown that the size of the hyperemic flare that develops around the heat probe during local skin heating is based on the function of

temperature-sensitive nerves in the skin such as C-fibers (Green et al., 2009; Krämer et al., 2004; S. T. Krishnan & G. Rayman, 2004; P. R. Vas & G. Rayman, 2013b). Consequently, the size of the post-heating flare is a selective test for small-fiber nerve function.

Studies for Aim 2 were designed to test three hypotheses. The first was that the mean size of axon flares will not differ significantly between BCS and HCs prior to treatment with Taxol® (Time 1). The second was that the mean size of axon flares will differ significantly between BCS and HCs at Times 2 and 3 (i.e., during Taxol® therapy). The third was that the mean size of axon flares will differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively).

Results of the analysis did not find a significant difference in mean flare size between HCs and BCS at Time 1 (Hypothesis 2.1) or at Times 2 and 3 (Hypothesis 2.2). In addition, results of the within-group analyses performed for Hypothesis 2.3 did not find a significant difference in mean flare size for BCS or HCs at Times 1, 2, or 3. However, during post-analysis period, two issues were identified that caused the investigator to question the validity of the initial findings. First. There was a tendency for SkBF to increase in the participants' feet over the 40-minute study visit, independent of the action of the heat probe. Second, we had difficulty determining a reliable threshold for what constituted evidence of a true axon flare rather than just high resting perfusion during the analysis for Aim 2.

As discussed below, these issues had a strong impact on the interpretation of the data, suggesting that the lack of significant differences in flare size observed in the main analyses performed for Aim 2 may have been due to factors other than differences in the function of small-fiber nerves in the toe. Because these issues affect *all* data collected for Aim 2, rather than discuss the findings for Aim 2 by each hypothesis, these issues will be discussed together.

Issue #1: Tendency for Skin Blood Flow in the Feet to Increase over Time, Independent of the Heat Probe

The first issue that caused us to question the lack of differences in mean flare size between BCS and HCs at Times 1, 2 or 3 and lack of difference in flare size for BCS between Time 1, 2, and 3 was the discovery that SkBF in the foot increased significantly over the 40-minute experiment. This occurred in areas heated by the probe and in areas of the foot that had not been heated.

Like other tests that use the response to a stimulus to identify signs of a disease, a basic assumption of the axon-flare test is that the increase in SkBF that appears around the heat probe (the response) is the result of the local skin heating (the stimulus). However, a review of images of SkBF in the toe from the experiment for a separate analysis suggested that SkBF may have increased in *other* areas of the foot which had not been heated using the skin heater. This discovery was problematic, in part, because SkBF should only have increased in the portions of the skin that were heated (i.e., right toe). This assumption is what makes it possible to compare pre- and post-heating blood flow to determine the size of the axon flare.

To determine whether these suspicions were correct, we used data we had collected during the experiment and chose an area of skin we had not heated with the same tissue characteristics (i.e., glabrous). For convenience, blood flow in the right 2nd toe was used, which was visible for all participants in their images. Next, we compared mean SkBF in the 2nd toe which had not been heated, before and after the 40-minute local skin-heating protocol and averaged the results for each group at each time-point to determine if mean SkBF increased significantly for BCS in the study in areas of the foot that had not been exposed to the heat stimulus.

Results of the analysis *confirmed* that mean SkBF in the head of the 2nd toe had increased during the 40-minute period, despite not being heated. Mean SkBF in the head of the 2nd toe increased by an average of 43.1% for HCs over the study, and by 48.4% for BCS. Analyses of other regions of the foot such as the other toes and ball of the foot showed a similar trend, indicating that the size of post-heating flare we used as our endpoint for Aim 2 could have been influenced by factors other than stimulation of small-fiber nerves with the heat probe.

The tendency for SkBF in the foot to "drift" upwards over the 40-minute skin-heating period may have been caused by the amount of time participants in the study spent in the heated room, which was longer than participants in other axon-flare protocols (typically, 6-20 min). The tendency for SkBF in the foot to increase during the 40-minute skin-heating period could also have been influenced by factors such as the type of skin being tested in this study (glabrous skin), use of blankets to help raise participants' core temperature, and/or encouragement to relax by the research team.

Regardless of the cause(s), the possibility that the size of post-heating axon flares reported was influenced by variables other than local skin heating meant that any plan that relied on the original pre- vs. post-comparison of SkBF to determine mean axon flare size would be flawed. Therefore, another approach for estimating mean flare size had to be devised. The approach involved comparing SkBF in a toe that had been heated (right great toe) to SkBF in a toe that had not been heated.

Fortunately (as noted above), data on SkBF in the other toes of the foot had already been collected in the process of collecting data on SkBF in the right great toe. Therefore, it was possible to use data on the mean SkBF in the great toe after the 40-minute skin heating and compare it with SkBF in a toe that had not been heated (the 2nd toe) after the 40-minute experiment. Although not optimal, comparing SkBF in two (identical) regions of skin after the 40-minute experiment provided the best possibility to determine flare size accurately while controlling for the unplanned increased in SkBF, which in theory affected all toes equally during the 40-minute experiment. Furthermore, comparing SkBF in the right great toe to another skin region from the same participant enabled the research team to use participants as their own controls, which was important since SkBF varied widely among participants during analyses for the current study.

However, because it was not possible to determine what influence that this unintentional increase in SkBF had on the final size of axon flares reported in our study, it is possible that results from Aim 2 showing no difference in mean axon flare size between HCs and BCS at any time-point may not be accurate.

Issue # 2: Difficulty Applying Traditional Methods for Determining Post-Heating Flare Size in Glabrous Skin of the Toe

A second issue that caused us to question the validity of the results from Aim 2 was the difficulty we had applying traditional methods for determining post-heating flare size to the data we collected in the glabrous skin of the toe. Traditional methods for determining flare size use a pre-defined threshold to determine whether the change in SkBF from pre- to post-skin heating constitutes evidence of a "true" axon flare. In most axon-flare protocols, this threshold is set at 300 tissue perfusion units (TPUs) (S. T. Krishnan & G. Rayman, 2004). In skin that has lower levels of perfusion such as the dorsal foot (where SkBF typically ranges between 50-

100 TPUs), studies show that 300 TPUs is generally a reliable cut-off for determining flare size.

However, results of a pilot study we performed before the start of the dissertation study found that in glabrous skin (which routinely has SkBF in the range of 500-1,000 TPUs), a threshold of 300 TPUs was often too weak to determine the size of post-heating flare consistently. Furthermore, because SkBF in glabrous skin is often higher (and more variable) than non-glabrous skin, results of our pilot work suggested that in glabrous skin, approaches that rely on a "one-size-fits-all" threshold for determining the flare's size risk could over- or under-estimate post-heating flare size.

For both these reasons, we utilized a more-tailored approach for determining flare size. The approach involved measuring mean and standard deviation (*SD*) SkBF in the toe before skin heating, only using a value that was least 3 SDs above the mean as the cut-off for determining flare size (i.e., mean + 3*SD*). Other laboratories have used this and similar methods (e.g., mean + 2*SD*s) to successfully determine flare size (Andreas Bickel et al., 2009).

During analysis it became clear that even this more-tailored approach to determining flare size would not work well in glabrous skin. There were two reasons for this. First, a number of participants in our study had very high SkBF in the toe, which in some cases, exceeded 1,000 TPUs. During image analyses, these high levels of SkBF acted as "background noise," making it impossible to determine which areas of increased SkBF on the image had been triggered by the heat probe, and which were simply due to high levels of perfusion in the toe. On the other hand, because the variance associated with SkBF is tightly correlated with the mean (Cracowski et al., 2006), for participants with low SkBF (e.g., 100-200 TPU), we found that even a cut-off of 3 *SD*s above participant's baseline was often too weak to determine a flare size accurately.[18]

To address these issues, we tried using less stringent cut-offs for determining flare size (e.g., 1 *SD* or 2 *SD* above participant's baseline). However, because of overall variability in SkBF in the right toe in the sample (which varied considerably between participants) and the tendency for SkBF to increase in the toe independently of the heat probe, no solution that involved using a single threshold could be identified. Because of this, the final estimates of

[18] For example, if we estimate that SkBF (mean ± SD) in the right toe before skin heating was 100 ± 30 TPUs, the 3*SD* threshold would only be 190 TPUs (i.e., 100 + 30 + 30 + 30).

mean axon flare size for this study had to be generated on a case-by-case basis based on the range of SkBF participants displayed during their visit. For example, for participants with SkBF in the *low range* (at risk for generating cut-offs that excluded almost none of the surrounding background noise), a more stringent cut-off of mean +3 *SD*s was used. Conversely, for participants with SkBF in the *high range* (at risk for excluding too much blood flow), a more lenient cut-off of mean +1 *SD*s was used.

Despite these attempts, the high levels of SkBF in the toe made it impossible to determine the size of the axon flare for some participants. Furthermore, the variability in SkBF in the glabrous skin of the toe and lack of a reliable threshold for determining flare size in this study suggests that the lack of difference in mean axon flare size between HCs and BCS reported in Aim 2 may not be accurate.

Section 3: Discussion for Aim 3

The purpose of Aim 3 was to determine whether the mean sizes of axon reflexes or axon flares (two physiologic markers for small-fiber nerve function) were significantly correlated with scores on the 5-item Short Form of the Total Neuropathy Score (Reduced Version; TNSr-SF), a clinical screening tool validated for TIPN (E. M. Smith, 2013a).

As described in Chapter Three, the TNSr-SF is a *composite measure* which combines questions about the presence and location of neuropathy symptoms such as tingling, numbness, and neuropathic pain with sensory tests for potential signs of TIPN such as loss of deep-tendon reflexes and changes in vibrotactile sensitivity (Cavaletti et al., 2007a; Cavaletti et al., 2006; Cornblath et al., 1999). However, unlike the original TNS (which is longer and requires access to specialized equipment and personnel (Cavaletti et al., 2006; Cornblath et al., 1999)), the TNSr-SF has just 5 items and is designed specifically for use by nurses in the busy oncology setting (E. M. Smith, 2013a).

Items on the TNSr-SF are scored using a 5-point scale which ranges from 0 (normal) to 4 (severe) based on how far up the limbs each sign or symptom of TIPN extends (Cornblath et al., 1999; E. M. Smith et al., 2008; E. M. Smith et al., 2010).[19] Individual scores on each of

[19] As established in the scoring guidelines for the TNS, participants receive 0 points if neurological function is normal/intact, 1 point if participants show signs/symptoms in their fingers/toes, 2 points if their signs/symptoms extend up to ankles or wrists, 3 points if their neuropathy signs/symptoms extend above the knees or elbows, and 4 points if their signs/symptoms extend above the knee or ankle or are in the upper extremities.

the 5 items (tingling, numbness, neuropathic pain, reflexes, vibration sensibility) are summed to create a total score that ranges from 0 to 20, with higher scores indicating more severity of TIPN (Griffith et al., 2010; E. M. Smith et al., 2010). Studies show that scores on TNSr-SF correlate well with the original TNS (r = 0.937- 0.944, $p < 0.001$) (E. M. Smith et al., 2008), which is considered a gold-standard in the assessment of TIPN (Lavoie Smith, Cohen, Pett, & Beck, 2011). Total scores on the TNSr-SF also show acceptable correlation (r_s = 0.51–0.63) with sensory scores from the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTC-AE) scale (Lavoie Smith et al., 2011).

Aim 3 had two hypotheses. The first was that the mean size of axon reflexes in the right toe will correlate with the *overall severity* of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and with the *severity of individual signs and symptoms* of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time point. The second hypothesis was that the mean size of axon flares in the right toe will correlate significantly with the *overall severity* of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time point, and with the *severity of individual signs and symptoms* of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time-point.

Findings discussed in this section are divided into three parts. Part 1 discusses findings from the preliminary analysis of TNSr-SF scores we presented in Chapter Four, which show the frequency, onset, severity, type, and physical distribution of TIPN BCS reported at each of their three visits. Part 2 discusses results of correlations between mean axon reflex size and TNSr-SF scores we performed for Aim 3.1. Part 3 discusses correlations between mean axon flares size and TNSr-SF scores performed for Aim 3.2.

Part 1: Discussion of Preliminary Analysis of Total Neuropathy Scores on the Frequency, Type, Onset, Severity, and Physical Distribution of TIPN Reported by BCS Receiving Weekly Taxol®

Frequency of TIPN. The need for early detection methods for TIPN is predicated, in large part, on the prevalence of the problem. Results of this study found that two-thirds (66.7%) of BCS developed some degree of TIPN during their first 6 weeks of Taxol® therapy. This finding is consistent with results from other studies, including a recent meta-analysis of 31 studies, which estimate that 70.3% of BCS receiving Taxol® develop TIPN (Seretny et al., 2014). The frequency of TIPN observed in this sample mirrors the frequency of TIPN

observed in BCS in other studies receiving weekly Taxol®, which is equal or higher than rates of TIPN observed in women receiving less frequent Taxol® infusions (Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge Jr, et al., 2012; Seidman et al., 2008). Together, these findings underscore the high risk BCS have for developing TIPN during Taxol® treatment.

Type of TIPN. After 2 weeks of Taxol® (Time 2), 22.2% of BCS in the current study reported tingling and numbness in their fingers and toes. In addition, by Time 2 nearly half (44.4%) of BCS had started to show signs of diminished reflexes in their lower extremities. By week 6 (Time 3), the percentage of BCS displaying diminished reflexes in their ankles and/or knees had increased to 66.7%. In addition, by Time 3 nearly half (44.4%) of BCS reported symptoms in their arms and/or legs consistent with neuropathic pain (i.e., pain with a burning, lancinating, electric, or shock-like quality).

Overall, these are representative of the types of signs/symptoms observed in BCS receiving Taxol® in other studies, which include both painful and non-painful sensory symptoms, loss of deep-tendon reflexes, and changes to vibrotactile function (Pachman et al., 2016; Sahenk et al., 1994). The tool we used to evaluate TIPN in this study (the Total Neuropathy Score) only assessed five potential signs/symptoms of TIPN. Consequently, other common signs/symptoms of TIPN such as aching, cramping, and sensitivity to hot or cold, changes in motor function, and signs of autonomic symptoms (orthostatic hypotension, issues with bowel motility) (Loprinzi et al., 2011a) may have gone undetected. As a result, we cannot conclusively say whether the type of symptoms BCS reported in this study were identical to those reported by other cohorts (Lipton et al., 1989; E. L. Smith et al., 2002; S. L. Wolf et al., 2012b).

Onset of TIPN. Results of this study found that, for two-thirds of BCS who developed TIPN during the study, TIPN began quickly, with approximately 30% reporting tingling and numbness in their fingers and toes after 2 weeks of Taxol®. By week 6 of Taxol® (Time 3), these non-painful symptoms had begun to spread into the feet, lower legs, and for some BCS, included a range of painful neuropathy symptoms.

Overall, the onset of TIPN in this study is consistent with the onset of TIPN reported by others, which show that the majority of BCS who develop TIPN do so during the first 12-week cycle of Taxol® (Park, Lin, et al., 2011b). Observations also show that onset of

symptoms can occur as early as week 2 of therapy for some individuals (Forsyth et al., 1997; Loprinzi et al., 2011a; E. M. Smith et al., 2013; Takemoto et al., 2012; Tofthagen et al., 2012; Wiernik, Schwartz, Einzig, et al., 1987). However, observations regarding the point at which TIPN begins for BCS during Taxol® therapy vary widely. For example, a recent study by Park et al. found that just 30% of BCS developed TIPN by week 6 into their Taxol® infusion treatment, compared to 66.7% of BCS who developed TIPN by week 6 in another study (Park, Lin, et al., 2011a).

Some of this heterogeneity may be due to differences in age, comorbidities, and genetics of participants in studies, which can influence the rate at which TIPN develops (Chen et al., 2015; Johnson et al., 2015; Schneider et al., 2015; Schneider, Zhao, Wang, Stearns, Martino, Jones, Perez, Saphner, Wolff, Sledge, et al., 2012). Patient-level factors notwithstanding, the lack of data in the literature describing the typical onset of neurological signs/symptoms associated with TIPN is problematic for two key reasons.

First, the lack of clarity about the rate at which TIPN develops during Taxol® therapy makes it difficult to define *how early* early-detection methods would need to detect TIPN to be useful. Results of this analysis suggest that to be effective, early detection methods need to be able to detect signs of TIPN within the first few weeks of treatment.

Second, the lack of data on when *specific* signs/symptoms of TIPN tend to occur (and in *what order* these signs/symptoms tend to occur) makes it difficult to determine the type(s) of changes to nerve function and structure that are giving rise to these symptoms. Results of this study offer a glimpse into the rate at which change in small-fiber nerve function and self-reported signs/symptoms of TIPN develop during the first 6 weeks of weekly Taxol® therapy.

Results of the current study also point to a potential relationship between the onset of neuropathic pain and an increase in mean axon reflex size in small-fiber nerves in the right toe, which both occurred after 6-weeks of Taxol® therapy (Time 3). The fact that both of these phenomena developed at the same point during treatment could indicate that the painful neuropathy symptoms BCS reported in their hands and feet at Time 3 may have been caused by an increase in the sensitivity of small-fiber nociceptors (Zhang et al., 2013). It is also possible that the painful symptoms BCS reported at Time 3 were due to changes in large-fiber nerves, as has been suggested by other researchers (Dougherty et al., 2004; Loprinzi et al., 2011b), and/or were caused by a set of factors yet to be recognized. Carefully-designed

prospective studies in humans combining both self-reported symptoms and physiological endpoints are needed to test these theories.

Severity of TIPN. Another important component of early detection for TIPN is the severity of the neuropathy that BCS develop. In the current study, two-thirds (66.7%) of BCS displayed TNSr-SF scores between 0 and 5 on the 20-point scale during the study, developing either no TIPN (33.3%) or TIPN classified as "mild" (33.3%) per current scoring guidelines (scores ≤ 7) (Cavaletti et al., 2007b; Vasquez et al., 2014). By contrast, one-third (33.3%) of BCS in the current sample displayed TNSr-SF scores between 10-13 during the study, indicating "moderately severe" TIPN (i.e., scores of 7-14).

Overall, these results are consistent with other studies showing that approximately 70% of BCS develop mild-to-moderate TIPN during treatment, with approximately 30% developing a more severe range of neurological impairments (Hershman et al., 2011; Simon et al., 2017). The finding that 30% of BCS in this study developed more severe TIPN was particularly striking because the BCS who developed these more severe symptoms did so halfway through their 12-20-week Taxol® therapy. This finding underscores the speed with which some BCS develop severe TIPN during breast cancer treatment.

Qualitative notes from the study also revealed that the severity of BSC neuropathy symptoms waxed and waned between treatments. Symptoms grew worse in the 2-3 days following their Taxol® infusion and grew somewhat better 4-7 days following their weekly treatments. This finding is consistent with reports from other studies (Pachman et al., 2016), although variations in the TIPN severity between Taxol® infusions have been largely unstudied, with two notable exceptions (Loprinzi et al., 2011a; Reeves et al., 2012).

Finally, a review of medical records for BCS in the current study revealed that 22.2% (n = 2) of BCS had to have their dose of Taxol® reduced because of the severity of their TIPN. This figure was actually lower than findings from similar studies, which show that 28.4%-46.0% of BCS receiving Taxol® have to have their dose of Taxol® reduced because of the severity of their TIPN (Bhatnagar et al., 2014; Lam et al., 2016; Park, Lin, et al., 2011a; Speck et al., 2013). The potential to disrupt (or in extreme cases, stop) breast cancer treatment due to the severity of TIPN is a major concern for providers, and according to some sources, may be a reason why some BCS choose to underreport their symptoms during treatment (Paice, 2009; Stubblefield et al., 2009; Vadalouca et al., 2012).

Physical Distribution of TIPN. Although virtually undiscussed in the TIPN research literature, the degree to which signs and symptoms of TIPN develop on *one* or *both* sides of the body (unilaterally or bilaterally) during Taxol® therapy is an important practical consideration for nurses and clinicians responsible for evaluating TIPN. TIPN could be overlooked if only one side of the body is tested.

While results of this analysis found that 100% of signs and symptoms of TIPN at Time 2 were bilateral, at Time 3 more than half (55.5%) of BCS displayed signs or symptoms of TIPN on only one side of their body (i.e., right foot, right ankle, right leg). In an extreme example of this, one participant (39-year old with stage IIA breast cancer) developed almost completely unilateral TIPN, with tingling, numbness, and shooting pain in her left face, back, arm, leg, and foot except for a single area of numbness in the fifth metatarsal of her right foot. Results of the current analysis did not find any location or type of TIPN symptom that was more likely to be unilateral, with participants often reporting a *mixture* of unilateral and bilateral symptoms in different locations (e.g., bilateral pain in the legs and arms, but tingling only in the left finger tips and toes) at Time 3.

To our knowledge, this is the first time the "geography" of TIPN has been examined. The question of whether the neurological changes that accompany Taxol® develop symmetrically has important practical implications for the accuracy of early detection methods like those tested in this study, which may be able to evaluate nerve function in only a single location because of cost or limited time. Results of this analysis also have important implications for screening tools for TIPN like the TNSr-SF, which instructs users to handle discrepancies in neuropathy severity between limbs by using the *most symptomatic* limb to generate the final score. While this strategy makes sense for the purposes of scoring, because the final score does not communicate any information about the side of the body in which the neuropathy symptom(s) was observed, the narrative of TIPN as a symmetrical neuropathy persists, despite evidence that symptoms do not affect both sides of the body equally for some patients.

Part 2: Discussion of Main Findings for Aim 3

Hypothesis 3.1. Correlations between Mean Axon Reflex Size and TNSr-SF Scores

The first part of hypothesis 3.1 stated that the mean size of axon reflexes in the right toe would be significantly correlated with the *overall severity* of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time-point. The purpose of testing this relationship was to determine whether the overall severity of TIPN for BCS during weekly Taxol® would correlate with axon reflexes, a marker for small-fiber nerve function. Non-parametric Spearman Rank coefficients were used to test the strength and direction of bivariate associations. Results of the current analysis did not find significant correlations between mean axon reflex size and total TNSr-SF scores at the same time-point.

The lack of correlation between axon reflex size and TIPN severity at the same time-point in this study was somewhat surprising. This was especially true at Time 3, when severity of TIPN and size of axon reflexes were at their peak for BCS in the study. One reason why total TNSr-SF scores and mean axon reflex size may not have significantly correlated during Taxol® treatment was that the size of this sample was smaller than intended. Because of this, it would have been less likely that we would have been able to detect significant correlations between axon reflexes and total TNSr-SF (even using Spearman's Rank Order Tests instead of Pearson's R), unless associations between these two variables would have been extremely strong.

It is also likely that the lack of correlation observed between overall TNSr-SF scores and axon reflexes was influenced by variation in TNSr-SF scores, especially over the duration of the study. At Time 3, total TNSr-SF scores for the BCS in the study ranged from 0-13 on the 20-point scale. When closely examined, it became clear that total TNSr-SF scores were made up of two distinct groups: (1) scores from the two-thirds of BCS who had very mild TIPN (which ranged from 0-4 on the 20-point scale), and (2) scores from one-third of BCS who developed more severe TIPN (ranged from 10-13 on the 20-point scale). While this distribution of two-thirds "mild" and one-third "severe" matched the severity of TIPN reported in other studies of BCS receiving weekly Taxol® (Hershman et al., 2011; Simon et al., 2017), the wide distribution of TNSr-SF scores in the current smaller sample likely weakened our ability to detect statistically-significant correlations during bivariate testing especially in a sample this size (Motulsky, 1995).

The second part of hypothesis 3.1 stated that the mean size of axon reflexes in the right toe will correlate with "…the *severity of individual signs and symptoms* of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time-point." The purpose of testing this relationship was to determine whether changes in the size of the axon reflex would correlate with scores for individual items on the TNSr-SF (tingling, numbness, neuropathic pain, reflexes, vibrotactile sensation), which are primarily associated with changes in large-fiber myelinated nerves (Dougherty et al., 2004). Non-parametric Spearman Rank coefficients were used to test the strength and direction of bivariate associations between total scores on individual items on the TNSr-SF (range: 0-4) and the mean size of axon reflexes in the right toe.

Results of the current analysis did not find a significant correlation between scores for any of the five individual signs and symptoms listed on the TNSr-SF (range: 0-4) and mean axon reflex size at the same time-point. Given that we did not identify significant correlations between mean axon reflex size and overall TNSr-SF scores (range: 0-20) at the same time-point, the lack of correlation between axon reflexes and individual items was not particularly surprising. Furthermore, given that only one-third of BCS surveyed displayed scores in the high range, it would have been unlikely that we would have found a significant correlation between the size of axon reflexes and scores describing the severity of individual signs/symptoms of TIPN. Studies in samples large enough to allow for sub-group analysis are needed to determine whether the mean size of axon reflexes correlate with the severity of individual signs/symptoms of TIPN.

Hypothesis 3.2. Correlation between Mean Axon Flare Size and TNSr-SF Scores. The first part of Hypothesis 3.2 proposed that the mean size of axon flares in the right toe would correlate with the *overall severity* of TIPN (measured by total scores for BCS on the TNSr-SF (range: 0-20) at each time-point. Results did not find a statistically-significant correlation between mean axon flare size and the overall severity of TIPN at the same time-point. The lack of correlation between mean axon flares size and overall TIPN severity was somewhat surprising since studies consistently show a relationship between the size of the flare that develops in the skin following skin heating and the severity of participants' neuropathy (Breiner, Lovblom, Perkins, & Bril, 2014; Farooqi et al., 2016; Green et al., 2010; S. T. M. Krishnan, C. Quattrini, M. Jeziorska, R. A. Malik, & G. Rayman, 2009; Lysy et al., 2014;

Nabavi Nouri et al., 2012; Sharma, Vas, & Rayman, 2015; Sivaskandarajah et al., 2013; P. R. J. Vas & G. Rayman, 2013a).

There are two potential explanations for this discrepancy in findings.

First, most of studies involving axon flares have been performed in populations whose neuropathy has developed over years, not weeks (e.g., diabetes, impaired glucose tolerance, hyperlipidemia). This may indicate that axon flares are a better measure of small-fiber nerve damage in established neuropathies rather than more acute-onset neuropathies such as TIPN.

Second (as discussed above), the lack of correlation between mean axon flare size and total TNSr-SF scores at the same point we observed may have been affected by our sample size, which was smaller than originally planned. In addition, the issues we had calculating the size of post-heating flares may have reduced the likelihood of detecting significant correlations. In light of these concerns, results of the analysis for Aim 3.2 need to be verified independently.

The second part of hypothesis 3.2 stated that: "The mean size of axon flares in the right toe will correlate with "…the *severity of individual signs and symptoms* of TIPN (measured by total scores on each of the 5 items (range: 0-4) at each time-point." Results of the tests did not find any significant correlations between mean flare size and the severity of individual signs and symptoms of TIPN at same time-point. This finding was somewhat unexpected; studies consistently show *smaller* flares in diabetic patients with more severe neuropathies, which have similar presentation TIPN (S. T. Krishnan & G. Rayman, 2004; Nabavi Nouri et al., 2012; Sharma, Venkitaraman, et al., 2015; P. R. Vas & G. Rayman, 2013b). In addition, many of the studies that show this relationship between smaller flares and more severe neuropathy (i.e., higher scores on their respective neuropathy measure) use questionnaires which assess many of the same signs and symptoms of neuropathy as the TNSr-SF does (e.g., ankle reflexes, vibration thresholds) (Sharma, Vas, et al., 2015). This suggests that smaller flares are not only associated with scores indicating more severe overall neuropathy, but should be associated with scores for many of the *same signs and symptoms* that we tested on the TNSr-SF.

There are several possible explanations for why we did not observe a stronger correlation between the size of axon flares and the severity of individual signs and symptoms of TIPN in the current sample. First, the size of our sample may have been too small to detect relationships between flare size and the severity of individual symptoms. Second (and closely

related), only one-third of BCS in the current sample developed TIPN classified as "moderately severe" during the study. As a result, there were very few scores on the high end of the 0-4 scale needed to create a strong correlation.

Section 4: Summary and Implications of Key Findings

A summary of key findings and their implication(s) for each aim and hypothesis is listed below in Table 5-1.

Table 5.1

Summary of Findings, Implications, and Recommendations for Future Research for Study Hypotheses

#	Hypothesis	Finding(s)	Implication of Finding(s)
Aim 1: Differences in Mean Axon Flare Size between BCS and HCs during Weekly Taxol®			
1.1	Mean size of axon reflexes will not differ significantly between HCs and BCS prior to treatment with Taxol® (Time 1).	• At Time 1, no difference in mean axon reflex size between BCS and HCs	Provides evidence that BCS and HCs started with equivalent small-fiber nerve function • Suggests BCS did not have subclinical small-fiber neuropathy prior to starting chemotherapy
		• At Time 1, no difference in mean axon reflex size between BCS and HCs in other studies	• Suggests BCS started study with intact small-fiber nerve function
		• At Time 1, mean axon reflex size for BCS was 77 ± 17% (CVC_{MAX})	• Provides first characterization of mean axon reflex size in BCS
1.2	Mean size of axon reflexes will differ significantly between BCS receiving Taxol® between Times 1, 2, and 3 (respectively).	• At Time 2, no difference in mean axon reflex size between BCS and HCs	• Two weeks of (weekly) Taxol® may not be sufficient to see changes in small-fiber nerve function • Local skin heating may not be sensitive enough to detect changes in small-fiber nerve function after two weeks of Taxol®
		• At Time 3, when data expressed in $\%CVC_{MAX}$, mean axon reflex size not	• Provides evidence Taxol® may affect small-fiber nerve function in humans

		significantly different between BCS and HCs at α = .05. However, at Time 3, when data expressed in CVC units, mean axon reflex size significantly different between BCS and HCs (p = 0.43)	• Suggests local skin heating may can detect small-fiber TIPN in BCS early in Taxol® therapy • Implies need for diagnostic tests to detect small-fiber TIPN in BCS during Taxol® • Supports findings from pre-clinical studies showing effect of Taxol® on small-fiber nerve function
		• At Time 3, mean axon reflex size approximately 128% for BCS than HCs. • Mean SkBF in the toe higher for BCS at start 42 °C (p = .023), reach 42 °C (p = .022), and reach 44 °C (p = .005)	• Suggests that findings from pre-clinical models showing increase in sensory nerve function during shorter, lower exposures to Taxol® may be correct
		• SkBF was lower for BCS than HCs during first 5 minutes of skin heating (33 °C)	• Suggests increase in SkBF observed during protocol not due to BCS starting with higher overall SkBF compared to HCs
1.3	Mean size of axon reflexes will differ significantly between BCS and HCs at Time 2 and 3	• BCS showed no difference in mean axon reflex size between Times 1, 2, and 3 when data expressed as a $\%CVC_{MAX}$ (p = .363). • Results showed trend towards larger reflexes at Time 3 when axon reflex size expressed in CVC units (p = .068). • Within-group comparison for HCs confirmed axon reflex size did not differ significantly between Times 1, 2, 3 (p = .266)	
Aim 2: Differences in Mean Axon Flare Size between BCS and HCs during Weekly Taxol®			

2.1	Mean size of axon flares will not differ significantly between BCS and HCs prior to treatment with Taxol® (Time 1)	• At Time 1, no difference in mean axon reflex size between BCS and HCs	• None; (1) SkBF in toes increased independent of heat probe; (2) consistent threshold for determining axon flares in palmar toe surface could not be identified (see: Section 2: Discussion for Aim 2)
2.2	Mean size of axon flares will differ significantly between BCS and HCs at Times 2 and 3 (i.e., during Taxol® therapy)	• At Times 2 and 3, no difference in mean axon flare size between HCs and BCS	• Same as implications for hypothesis 2.1
2.3	Mean size of axon flares will differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively)	• Mean axon flare size did not differ significantly between Times 1, 2, and 3, regardless of how data was expressed in cm^2 ($p = .439$) or as a $\%Toe_{MAX}$ ($p = .173$).	• Same as implications for hypothesis 2.1
Aim 3: Correlations between TNSr-SF Scores and Mean Size of Axon Reflexes/Flares for BCS Receiving Weekly Taxol®			
3.1	Mean size of axon reflexes in right toe will correlate with (a) overall severity of TIPN (measured by total scores for BCS on TNSr-SF (range: 0-20) at each time point, and (b) with severity of individual signs/symptoms of TIPN (measured by total scores on each of 5 items (range: 0-4) at each time-point)	• No correlation between mean axon reflex size and overall severity of TIPN or severity of individual signs/ symptoms of TIPN at same time point	• Lack of correlation may have been due to (a) size of sample and (b) heterogeneity of TNSr-SF scores in sample
3.2	Mean size of axon flares in right toe will correlate with (a) overall severity of TIPN (measured by total scores for BCS	• No correlation between mean axon flare size and overall severity of TIPN or severity of individual signs/ symptoms of TIPN at same time point	• Lack of correlation may have been due to (a) size of sample, (b) heterogeneity of TNSr-SF scores in sample, and (c) issues

on TNSr-SF (range: 0-20) at each time point, and (b) severity of individual signs/ symptoms of TIPN (measured by total scores on each of 5 items (range: 0-4) at each time-point)	determining size of axon flares (described above)

Section 5: Strengths and Limitations

Strengths

This study had several strengths. First, the study targeted a population of cancer survivors at high risk for developing TIPN during treatment (BCS), using one of the treatment regimens most associated with this toxicity, weekly Taxol®. Second, the study used longitudinal study design, which was appropriate for research that followed BSC and HCs over a 6-week period. Third, the study included a cohort of HCs and tested outcomes for both groups at baseline, strengthening our ability to draw conclusions about neuropathy during the study. Fourth, the study measured TIPN using physiologic end-points that have been successfully used to detect early signs of small-fiber neuropathy in other populations (axon reflexes and axon flares), and attempted to determine the degree to which these physiologic end-points correlated with clinical signs/symptoms of TIPN measured using the TNSr-SF.

Results of the study provide novel data on the effect of Taxol® on small-fiber nerve function in BCS during early Taxol® therapy and suggest that signs of nerve damage may be detectable as early as 6 weeks using this method. Results of the study also suggest that axon reflex-mediated vasodilation is a non-invasive way to model the evolution of small-fiber nerve damage during TIPN, which remains poorly understood due to the lack of suitable testing methods.

Limitations

The findings from this study must also be considered in light of several limitations. These include (1) small sample size; (2) partial loss of data from Aims 1 and 2; (3) inability to control for variables such as age, body-mass, and menstrual status during the study; and (4) inability to control for variables such as participants' diet and exercise during the study. A discussion of each limitation and its potential impact on the study are summarized below.

The primary limitation of this research is that the number of women in the BCS arm of the trial was smaller than originally planned ($n = 20$) because of issues with recruitment. While post-hoc testing found that reduction from $n = 20$ to $n = 9$ only reduced the statistical power for the between-group comparisons of axon reflex size for Aim 1, Hypothesis 1.2 (the primary aim of the study) from 90.0% to 84.0%, a larger sample would have led to stronger comparisons.

Prior to starting the study, attempts were made to predict the number of BCS that would be available at both recruitment breast cancer sites using recent (2012-2014) recruitment data. Based on this data, we anticipated that approximately 30 BCS meeting our criteria (non-metastatic, first-time female breast cancer patients receiving weekly Taxol®) would present to the oncology clinics. Of these, we estimated that half would be willing to participate.

While data for the study showed that all eligible BCS who were approached about the study were willing to participate, during the study fewer BCS meeting our criteria were available. In some cases, we identified BCS who were set to receive Taxol®, but for a *recurrence* of cancer, which made them ineligible. In other cases, we identified BCS that met the eligibility criteria for the study but were scheduled to receive bi-monthly (i.e., dose-dense) Taxol®, which was not originally the focus of the study. Because of this, midway through the study, we amended the protocol to allow BCS receiving dose-dense Taxol® to participate.

While allowing BCS receiving dose-dense Taxol® made it possible to recruit another participant for the study, the difficulty we had finding eligible BCS for the study suggests that our original estimates of how many BCS would be available for the study were not conservative enough. During the study, we also considered expanding the recruitment sites for the study to include BCS receiving weekly Taxol® at infusion centers in the surrounding community. However, the logistics involved in transporting and setting-up the imaging equipment for the study at each clinic, and difficulty controlling the temperature in each location, would have made this non-feasible.

A second limitation of the study was that we had to set aside a portion of data from participants that displayed the attenuated response during Aim 1 (axon reflexes). We also had to set aside data from Aim 2 (axon flares) in cases where we could not determine the size of the post-heating flares accurately (an investigation into the factors that may have caused the attenuated response is presented in Appendix M). The loss of data associated with both

circumstances substantially reduced the statistical power for the study. Testing after the end of the study showed that the loss of data for Aim 1 reduced the power for the between-group comparisons of mean axon reflex size at Times 2 and 3 (Hypothesis 1.2) from ~ 90.0% to ~54.0%. Because of this, we performed the comparisons with axon reflex size expressed several ways to make sure that the conclusions derived from this data were sound. In addition, after we had completed the analyses, we consulted experts in axon reflexes and local skin heating to ensure that our methods and interpretations drawn from the data were scientifically sound and consistent with current thinking in the field (B. Wong, personal communication, August 4, 2017). While the feedback confirmed that we had handled the data appropriately and drew proper conclusions given the limited size of the sample, results of this study will need to be validated in larger samples.

A third limitation of this study was that we were not able to control for individual variations in menstrual status, heart rate, and blood pressure, which all can vary by time-of-day, week, and/or month (Aoki et al., 1997; Charkoudian et al., 1999; L. A. Stephenson & Kolka, 1985). The reason for this, in addition to the size of our sample, is that the timing of our study visits was built around BCS' Taxol® schedules over which we had no control. Because of this, we cannot be certain whether our results could have been influenced by circadian variations in heart rate/blood pressure, or by differences in menstrual phase (often controlled for in studies of peripheral blood flow).

A final limitation was that we were not able to account for the potential effects of exercise or the diets of participants, both of which could have affected axon reflex-mediated vasodilation (Yim, Petrofsky, Berk, Daher, & Lohman, 2012; Yim, Petrofsky, Berk, Daher, Lohman, et al., 2012). Future studies with samples large enough to allow for analysis of subgroups may be able to address these limitations.

Section 6: Recommendations for Future Research

Due to the number and diversity of findings emerging from this study, recommendations for future research are divided by aim (Aims 1-3). The section also includes an additional set of recommendations addressing the topic of early detection of TIPN during Taxol® therapy. The section concludes with a table (Table 5-1) that summarizes findings, implications, and recommendations (by hypothesis) to validate findings from the study and

continue to advance the field of early detection of small-fiber TIPN in BCS receiving weekly Taxol®.

Recommendations Based on Findings for Aim 1

Results of Aim 1 support the growing hypothesis that Taxol® affects both larger- and small-fiber nerves. Results also suggest that these changes may be detectable as early as 6 weeks into treatment. Together, these findings provide evidence that local skin heating in the distal extremities may be a useful way to detect early signs of small-fiber TIPN, which cannot be identified using currently available methods. Additional studies using statistically-powerful samples are needed to validate these findings. Future studies should evaluate axon reflexes throughout the entirety of BCS' 12-20-week Taxol® treatment, and if possible, after the conclusion of treatment. Doing this will not only provide insight into the *rate* at which axon-reflex size changes during treatment but help resolve whether the amplified response to skin heating observed in this study was related to the early Taxol® exposure, as we suspect. Extended studies would also help to clarify whether continuous exposure to Taxol® results in steady loss in axon reflex size like that observed in patients with diabetes (Sharma, Vas, et al., 2015).

Studies also are needed to address the methodological questions raised by Aim 1. Specifically, studies clarifying whether the way the heat probe was affixed to the toe was the cause of the attenuated response observed during the study are needed. In addition, studies clarifying whether signs of small-fiber TIPN can be detected more easily in non-glabrous regions of the foot are recommended to determine the best location for detecting early signs of TIPN using this method.

Future studies using axon reflexes to evaluate small-fiber TIPN in BCS also should consider including patients with hypertension, which is common in the U.S. (Centers for Disease Control and Prevention, 2017), but had to be excluded from this study because a change to the vessel's ability to dilate unrelated to Taxol® could have confounded the study (Bruning, 2013). Studies in this area should consider focusing on African American women, who are highly at risk for breast cancer (American Cancer Society, 2015), and 2-times more likely to develop severe TIPN during treatment than Caucasian women (Bhatnagar et al., 2014; Schneider et al., 2015). Indeed, this population is likely to be underrepresented in studies

of this sort because of the high incidence of hypertension in this population (Ferdinand & Armani, 2007).

Recommendations Based on Findings for Aim 2

Based on the difficulties we had adapting the axon-flare protocol for use in the glabrous skin of the toe, additional studies testing the technique in this location are not recommended. Although data from Aim 1 showed that the glabrous skin of the toe appears to be suitable for measuring the axon reflexes using a LDF, the vascularity of glabrous skin and potential for high levels of resting perfusion has the potential to mask the post-heating flares. However, results of the study by Sharma et al. showing that damage to small-fiber nerves can be detected in BCS in the dorsal foot almost a year after Taxol® using the Modified LDI_{FLARE} technique strongly suggests that it is the testing location not the method that should be modified in future studies (Sharma, Venkitaraman, et al., 2015). Studies testing this hypothesis directly are needed, including which of the two tests (i.e., axon reflexes or axon flares) is most useful for this purpose.

Given the difference in time it takes to perform the two tests (40-60 min for axon reflexes and 6-20 min for axon flares), research evaluating whether the two tests are comparable in all other respects (e.g., reliability, sensitivity, specificity) will be important. In addition, given the finding one participant described of skin heating resulting in temporary pain in the heating site, studies are recommended to determine whether the Modified LDI_{FLARE} technique, which requires higher skin-heating temperatures, poses a problem for patients receiving Taxol® and other agents known to produce sensory neuropathy.

Recommendations Based on Findings for Aim 3

The results of the preliminary analysis of TNSr-SF scores from Aim 3 showing the frequency of TIPN in the current sample add to the research showing how common TIPN can be for BCS receiving weekly Taxol®. Results from Aim 3 also shed light on how TIPN evolves during the early stages of the disorder, which is an area of TIPN research that remains largely undescribed. Studies documenting the genesis of TIPN using both physiologic and self-reported endpoints are needed. Wherever possible, future studies should use endpoints that not only can detect the clinical signs of TIPN but develop a coherent theory about the cellular and molecular changes that give rise to the different signs and symptoms associated with

TIPN, which continue to be the reason that faster progress has not been made in developing treatments for TIPN.

The present study used two endpoints for small-fiber neuropathy (axon reflexes and axon flares). These endpoints were chosen not only to improve our ability to detect small-fiber TIPN, but because these tests, which can also be performed in animals, allow some inferences to be drawn about the types of changes that may occur in small-fiber nerves (N. G. Gracias et al., 2011; Pittman et al., 2014; B. J. Wong et al., 2005). Studies using a similar strategy are needed to pinpoint the specific changes that give rise to TIPN so that preventative therapies can be identified. Furthermore, because currently there is no evidence-based way to determine when BCS who are showing signs of TIPN should consider altering their cancer therapy, studies using physiological endpoints to determine when the early changes associated with Taxol® give way to other potentially-permanent changes in neuronal sensitivity are urgently needed.

Based on the results of the main analysis for Aim 3, it is recommended that future studies in this area have enough statistical power to accommodate the variation in TIPN severity that occurs during Taxol® therapy and the potential loss of data that may occur because of outliers. Larger samples also allow for multivariate analyses that can control for the effect of variables, strengthening conclusions derived from these studies. In addition, the relative success of axon reflexes in Aim 1 compared to axon flares performed in Aim 2 suggests that to be successful, careful attention will need to be paid to which physiological endpoints should be performed.

Finally, because a number of other tools for screening TIPN are used in clinical practice, studies determining the degree to which different tools correlate with physiological markers for TIPN are recommended. Research should focus on large prospective studies establishing (1) scoring ranges and cut-points for physiological and self-reported tests, (2) correlation between tests, (3) predictive value, and (4) measuring the feasibility of testing in terms of equipment, training needs, time to perform, and cost. In this way, the question of which test(s) should be used to screen BCS for TIPN can be answered definitively.

Additional Recommendations

The finding that physiological signs of TIPN may be detectable within the first 6 weeks of Taxol® raises a larger question of how to use this information to improve outcomes

for BCS showing signs of toxicity. While results of this study are promising on many fronts, having access to earlier information about TIPN is of no value in-and-of-itself if the findings do not affect clinical decision-making about the risks BCS have for developing chronic symptoms or which interventions to use. In addition, because diagnostic testing costs both time and money, introducing another test could add to the already high burden nurses caring for BCS face unless the data can improve their ability to care for their patients.

Because of this, research to determine whether detecting signs of small-fiber TIPN that is associated with outcomes that matter is urgently needed. This includes research to determine if detecting small-fiber TIPN earlier in cancer treatment can lower BCS' risk for developing permanent TIPN, for falling or having accidents because of TIPN, for losing time at work or becoming disabled as a result of TIPN, for experiencing a decline in mental health as a result of TIPN, for having to rely more on medications (including opiates) to manage neuropathy symptoms, and/or for incurring additional healthcare-related costs. In addition, a vital question for the field is whether diagnosing the types of nerve fibers involved in TIPN (i.e., larger fiber vs. small fiber) can lead to more tailored prescription of existing pharmacotherapy, as well as the development of new pharmacotherapies capable of preventing/managing symptoms more effectively. The availability of centers in many hospitals and cancer outreach centers across the U.S. collecting information from millions of patients has made this type of outcomes-based research possible; but studies determining which markers for TIPN can predict which BCS are at risk for the most serious outcomes using this data remain yet a destination on the horizon.

A list of recommendations, by aim and hypothesis, is listed in Table 5-2.

Table 5.2

Summary of Recommendations for Future Research Based on Study Findings

Hypothesis	Finding(s)	Recommendation(s) based on Finding(s)
Aim 1: Differences in Mean Axon Flare Size between BCS and HCs during Weekly Taxol®		
1.1 Mean size of axon reflexes will not differ significantly between HCs and BCS prior to treatment with Taxol® (Time 1).	• At Time 1, no difference in mean axon reflex size between BCS and HCs	• Verify finding in larger samples • Perform studies combining self-reported and physiological methods for evaluating small-fiber nerve function to determine if cancer is risk factor for small-fiber TIPN
	• At Time 1, no difference in mean axon reflex size between BCS and HCs in other studies	• Verify finding in larger samples
	• At Time 1, mean axon reflex size for BCS was 77 ± 17% (CVC_{MAX})	• Verify finding in larger samples • Establish norms for axon reflex size in different populations
1.2 Mean size of axon reflexes will differ significantly between BCS receiving Taxol® between Times 1, 2, and 3 (respectively).	• At Time 2, no difference in mean axon reflex size between BCS and HCs	• Verify finding prospectively in statistically-powerful human studies • Determine earliest point small-fiber TIPN can be detected using local skin heating
	• At Time 3, when data expressed in $\%CVC_{MAX}$, mean axon reflex size not	• Verify finding prospectively in statistically-powerful human studies

		significantly different between BCS and HCs at α = .05. However, at Time 3, when data expressed in CVC units, mean axon reflex size significantly different between BCS and HCs (p = 0.43)	• Determine if diagnosing small-fiber TIPN in BCS early during Taxol® therapy is associated with favorable outcome
			• Perform correlative studies to determine whether early Taxol® associated with increase in small-fiber nerve function and long-term Taxol® associated with decrease in small-fiber nerve function.
		• At Time 3, mean axon reflex size approximately 128% for BCS than HCs. • Mean SkBF in the toe higher for BCS at start 42 °C (p = .023), reach 42 °C (p = .022), and reach 44 °C (p = .005)	• Verify finding prospectively in statistically-powerful human studies
		• SkBF was lower for BCS than HCs during first 5 minutes of skin heating (33 °C)	• Verify finding prospectively in statistically-powerful human studies
1.3	Mean size of axon reflexes will differ significantly between BCS and HCs at Time 2 and 3	• BCS showed no difference in mean axon reflex size between Times 1, 2, and 3 when data expressed as a $\%CVC_{MAX}$ (p = .363). • Results showed trend towards larger reflexes at Time 3 when axon reflex size expressed in CVC units (p = .068). • Within-group comparison for HCs confirmed axon reflex size did not differ significantly between Times 1, 2, 3 (p = .266)	

2.1	Mean size of axon flares will not differ significantly between BCS and HCs prior to treatment with Taxol® (Time 1)	• At Time 1, no difference in mean axon reflex size between BCS and HCs	• None: based on difficulties had adapting axon flare protocol for use in the glabrous skin, additional studies testing technique in this location are not recommended
2.2	Mean size of axon flares will differ significantly between BCS and HCs at Times 2 and 3 (i.e., during Taxol® therapy)	• At Times 2 and 3, no difference in mean axon flare size between HCs and BCS	• Same as recommendation for hypothesis 2.1
2.3	Mean size of axon flares will differ significantly for BCS receiving Taxol® between Times 1, 2, and 3 (respectively)	• Mean axon flare size did not differ significantly between Times 1, 2, and 3, regardless of how data was expressed in cm^2 (p = .439) or as a $\%Toe_{MAX}$ (p = .173)	• Same as recommendation for hypothesis 2.1
3.1	Mean size of axon reflexes in right toe will correlate with (a) overall severity of TIPN (measured by total scores for BCS on TNSr-SF (range: 0-20) at each time point, and (b) with severity of individual signs/symptoms of TIPN (measured by total scores on each of 5 items (range: 0-4) at each time-point)	• No correlation between mean axon reflex size and overall severity of TIPN or severity of individual signs/ symptoms of TIPN at same time-point	• Verify finding samples large enough to allow subgroup analysis
3.2	Mean size of axon flares in right toe will correlate with (a) overall severity of TIPN (measured by total scores for BCS on TNSr-SF	• No correlation between mean axon flare size and overall severity of TIPN or severity of individual signs/ symptoms of TIPN at same time-point	• None: based on difficulties had adapting axon flare protocol for use in the glabrous skin, additional studies testing technique in this location are not recommended

	(range: 0-20) at each time point, and (b) severity of individual signs/ symptoms of TIPN (measured by total scores on each of 5 items (range: 0-4) at each time-point)

Section 7: Chapter Summary and Final Thoughts

The current study used a prospective, two-arm observational design to test whether a non-invasive technique successfully used to detect signs of small-fiber neuropathy in patients with diabetic and inherited neuropathies (local skin heating) could be used to detect small-fiber neuropathy in BCS receiving Taxol®.

Results of this research add new findings to the growing body of evidence that Taxol® affects small-fiber nerves and suggest that studying axon reflexes may be a potential early detection method for small-fiber TIPN in BCS. In addition, the finding that BCS receiving Taxol® displayed an apparent increase in small-fiber nerve function during early Taxol® therapy like the gain of function observed in pre-clinical models of TIPN suggests that the response to local skin heating may vary by stage of Taxol® therapy. Additional studies testing this protocol in larger samples are needed to determine if more accurate data can be collected in less-glabrous regions of the foot and determine the effect that Taxol® has on axon reflex-mediated vasodilation across treatment.

The growing success in treating many forms of breast cancer in the U.S. using Taxol® suggests that clinicians and researchers, including nurses, will have a major role to play in developing and testing the diagnostic tools needed to monitor toxicities like TIPN. It is our hope that findings from this study will contribute to a greater understanding of early detection methods in this high-risk population for TIPN. In addition, because Taxol® is widely used tin the treatment other cancers (where is a leading cause of treatment-related neuropathy), it is our hope that contributions from this study also will contribute to earlier detection and better management of TIPN in other populations of cancer survivors as well.

APPENDIX A-1
INFORMED CONSENT FOR HEALTHY FEMALE CONTROLS (HCs)

INDIANA UNIVERSITY SCHOOL OF NURSING

INFORMED CONSENT STATEMENT FOR STUDY:
"EARLY DETECTION OF
TAXANE-INDUCED NEUROPATHY IN WOMEN WITH BREAST CANCER"

We invite you to take part in a research study for women with breast cancer who are going to be receiving chemotherapy. Please take a few minutes to read through this form carefully before deciding if you would like to join the study. We would be happy to answer any questions you may have before making your decision. The study is being performed by Dr. Victoria Champion (Ph.D., R.N., F.A.A.N.) and Noah Zanville (Ph.D. Candidate, B.S.N., B.A.), a doctoral student at Indiana University School of Nursing. The study is being funded by grants provided by the National Institutes of Nursing Research (N.I.N.R.), the Midwest Nursing Research Society (M.N.R.S.), and Council for the Advancement of Nursing Scholarship (C.A.N.S.).

What is the purpose of study?

The purpose of the study is to test a new way of measuring nerve damage in women receiving chemotherapy. One of the side-effects of chemotherapy can be nerve damage (also known as *neuropathy*).

Measuring neuropathy can be difficult. A new approach for measuring neuropathy is to heat the skin with a small heat probe and to measure the change in blood flow. The reason why this works is because healthy nerves sense the heat from the probe and send signals to the blood vessels in your skin to open. When this same technique is used in women with nerve damage though, these blood vessels won't open because the nerves that send them the signal are damaged. This technique has shown promise as a measure for neuropathy in patients with other types of neuropathy but has never been tried in women with breast cancer.

This study will measure blood flow in your right big toe, because it has the best blood flow and is the place where neuropathy from chemotherapy first starts. If you injure your right toe during the study, we would measure blood flow in your left toe instead. In addition, it is possible that we could ask to measure blood flow in both your right and left toes to compare them, but we would ask your permission ahead of time to do this.

Why was I approached about taking part in the study?

You were approached about participating in the study because you are a healthy woman in the same age group as the women with breast cancer we will be enrolling. We need healthy women in our study so that we can determine if our measure can tell women without neuropathy apart from women who develop neuropathy during chemotherapy treatment.

How many other women will be taking part in the study?

The entire study will recruit forty (40) women. This will include 20 women with breast cancer and 20 healthy women. You will be one of the 20 healthy women taking part in the study.

How long would the study last?

The study will last for six (6) weeks and would involve three (3) study visits.

What does taking part in the study involve?

Here is a short description of the study so that you can know what to expect if you decide to participate:

- After reading this form discussing the purpose, study procedures and potential risks of being in our study and asking and questions, you would sign the form agreeing to participate.

- You will be given a packet with information about the study, and a short questionnaire asking about age, income, and other basic information about you.

- Next, we would schedule you for your three study visits. All of the visits will take place at our testing center located at the *Indiana Clinical Research Center* (I-CRC), located on fifth floor of the IU Healthy University Hospital, in downtown Indianapolis.

- At each of your study visits, you will be greeted by members of the research team. After checking you in, we will weigh you. If it's your first, visit, we will also measure how tall you are and collect your demographic form. We would record all of this on a study sheet.

- Next, we will lead you to a room with a comfortable reclining chair and ask you to sit down. The room will intentionally be heated to 77°F. The reason why the room will be warm is to help improve your blood flow to your skin. When you sit down, we will look at the skin on your right big toe surface for any callouses, bruising, or scarring that could interfere with imaging and make some notes. We will put pillows under your arms, legs, and neck to make sure you are comfortable and improve blood flow.

- Although we don't expect that you will experience any pain, you will be asked about pain using a 1-10 scale. We will also have you complete a short questionnaire about neuropathy and measure your reflexes. The test includes three questions you answer verbally, and two manual tests like those you might get at a doctor's office (for example, testing your reflexes with a reflex hammer). This should only take 3-4 minutes.

- Next, will ask you to lay comfortably for 15 minutes and relax. The goal here is just to be as calm as possible so we can measure your resting blood flow. While you are relaxing, members of our research would use a thermometer to take your temperature,

attach a monitor to your finger that measures your pulse, and attach a blood pressure cuff to your arm to measure your blood pressure. The purpose of measuring your temperature, heart rate, and blood pressure before we start is so that we can know how to interpret any changes in blood flow we see during the experiment. At the same time, we would also attach a small heat probe to your right toe so that we know what temperature we're starting from.

- Because we need your blood flow to your skin to be very strong for the technique to work, while you are getting settled we will put a heating blanket on your abdomen and warm neck pillow around your neck. We will also put a foot warmer on your right foot, and ask you drink some hot tea. Doing this will raise your core temperature.

- As soon as the skin on your toe is the right temperature, we will take a two (2) minute-scan of the circulation in your right toe with a special laser imager that measures blood flow. The imager is FDA-approved and safe. The laser uses infrared light which is painless and invisible to the human eye, so while it's imaging you won't feel or see anything. You will hear some clicking when we turn it on and off, which is just the normal sound of it working.

- After we take this 2-minute scan, we will turn on the heat probe and start heating the skin on your right toe to 107.6°F (42 °C) is achieved. This is about the temperature of warm bath water and shouldn't hurt at all. We will check in with you as we're heating your skin to see how you are doing, and if for any reason the probe felt too hot, you could tell us and we would stop. We will keep heating your skin for about 30 minutes, and then turn the heat probe up a bit to 111.2°F (44 °C) for an additional 5 minutes.

- After heating the skin on your toe for 35 minutes, we will take another scan of your right toe with the LSCI to see how much blood flow increased after the heat probe was on.

- After this, the study visit will be complete. You will be helped to get up and given an opportunity to use the restroom, get some food, water, or coffee if you would like it. We will give you a list of contact information so that you can contact us if you have any questions or concerns before your next study visit. We will also validate your parking if you need us to do so. Each study visit should take approximately 75 minutes to complete.

What are the possible risks of taking part in the study?

All research involves some risks, however minimal. If you choose to take part in the study, it is possible that you could feel some discomfort, pain, or be injured. The risk of being injured is very small. It is possible that during the skin heating, you could feel physically uncomfortable, or that being tested in this way could make you feel anxious. In addition, while we will go to great lengths to protect both your privacy and confidentiality, it is possible that your data could be exposed. Finally, although we do not know about other potential risks of participating, it is possible that there are other side-effects that we cannot predict. During the study, if you feel

uncomfortable for any reason, you can tell us and we will stop, at any time with no repercussions.

What are the potential benefits of taking part in the study?

It is important to remember that you may not benefit directly from participating in this research study. However, one possible benefit of taking part in the study is the knowledge that you will be helping scientists to understand more about nerve-damage in women with breast cancer.

What are my alternatives to taking part in the study?

Taking part in the study is completely voluntary. You can choose not to take part in the study and there will be no penalty.

Will my involvement in the study be confidential?

Although we will make every effort to make sure that only people you have given permission to access your personal information will, we cannot guarantee absolute confidentiality. There are situations where we could have to disclose your confidential records. For example, if we were required by law, we would have to share your personal information. When we publish the results of the study, we pledge to keep your identity private. When we make a database to store the results of the study, we will keep your identity private by assigning you a number, and only using that number to keep track of that data.

In addition, certain groups might view and/or copy your records to ensure quality or to analyze the data. These could include: the study investigator and his/her research associates, the Indiana University Institutional Review Board or its designees, the Indiana Clinical Research Center (I-CRC), and (as allowed by law) state or federal agencies, specifically the Office for Human Research Protections (OHRP) who may need to access your medical and/or research records.

Will taking part in the study cost me anything?

No. Taking part in the study will not cost you anything. If needed, we will validate your parking so that parking does not cost you anything. Because we are asking you not to eat for an hour before your study visit, we will provide you with breakfast at no cost to you.

Will I receive any payment for taking part in the study?

Yes. You will receive payment for taking part in this study. The Clinical Research Center will validate your parking. If you complete all portions of the study, you will receive a $10 gift card for each study visit that you complete, for a total of $30 at the end of the study. In addition, in the case of a technical failure (e.g., laser probe slips, computer will not start), you will still be given a gift card to ensure that your time is compensated.

In the unlikely event that I am injured during the study, how will I be compensated?

If you are injured by taking part in this study, medical treatment will be provided to you. The bill for this will be added to your medical expenses. The amount of the bill that isn't covered by your health insurance will be your responsibility. Also, it is your responsibility to determine how much your health care covers before agreeing to take part in the study. There is no program in place to provide money for any injuries that may take place. However, you are not giving up any legal rights or benefits by participating.

Who do I contact if I have questions or concerns during the study?

If you have any questions about the study or a research-related injury, you can contact Dr. Victoria Champion (Ph.D., R.N., F.A.A.N.; Principal Investigator, School of Nursing, Indiana University) or Noah Zanville (B.S.N., R.N., B.A.; Co-Principal Investigator, School of Nursing, Indiana University) at [redacted]. If you cannot reach the researchers during regular business hours (i.e., 8:00 am-5:00 pm), please call the IU Human Subjects Office at [redacted]. If you need to reach the research team for any reason after regular business hours, please call Noah Zanville at [redacted]. If you have questions about your rights as a research participant or to discuss problems, complaints, or concerns about a research study, or to obtain information or offer input, contact the IU Human Subjects Office at [redacted].

Important statement about the voluntary nature of the study

Like all ethical research, participation in our study is voluntary. You can choose not to take part in the study and can leave the study at any time. If you chose to leave the study, there will be no penalty. For example, if you decide not to participate in this study, it won't affect any current or future relations with Indiana University Hospital or the Indiana University School of Nursing.

As researchers, we can choose to terminate you from the study without your permission under certain circumstances. For example, if the research team notices any changes in your physical or mental health that would make it unsafe for you to participate, we can terminate you from the study for your own safety. In addition, if you do not complete all agreed upon portions of the research study, we can choose to terminate your involvement in the study.

SUBJECT CONSENT

"After having read the above and having been given an opportunity to ask all relevant questions, I hereby give my informed consent to participate in this research study. I will be given a copy of this informed consent document to keep for my records."
Subject's Printed Name: _____
Subject's Signature: _____
Date: _____ (must be dated by the subject)

Printed Name of Person Obtaining Consent: _____
Signature of Person Obtaining Consent: _____
Date: _____

APPENDIX A-2
INFORMED CONSENT FOR BREAST CANCER SURVIVORS (BCS)

INDIANA UNIVERSITY SCHOOL OF NURSING

INFORMED CONSENT STATEMENT FOR STUDY:
"EARLY DETECTION OF
TAXANE-INDUCED NEUROPATHY IN WOMEN WITH BREAST CANCER"

We invite you to take part in a research study for women with breast cancer who are going to be receiving chemotherapy. Please take a few minutes to read through this form carefully before deciding if you would like to join the study. We would be happy to answer any questions you may have before making your decision. The study is being performed by Dr. Victoria Champion (Ph.D., R.N., F.A.A.N.) and Noah Zanville (Ph.D. Candidate, B.S.N., B.A.), a doctoral student at Indiana University School of Nursing. The study is being funded by grants provided by the National Institutes of Nursing Research (N.I.N.R.), the Midwest Nursing Research Society (M.N.R.S.), and Council for the Advancement of Nursing Scholarship (C.A.N.S.).

What is the purpose of study?

The purpose of the study is to test a new way of measuring nerve damage in women receiving chemotherapy. One of the side effects of chemotherapy can be nerve damage (also known as neuropathy). Measuring neuropathy accurately can be difficult.

The new approach that is being tested for measuring neuropathy is to heat the skin with a small heat probe and to measure the resulting change in blood flow. The reason why this works is because healthy nerves sense the heat from the probe and send signals to the blood vessels in your skin to open. When this same technique is used in women with nerve damage though, these blood vessels won't open because the nerves that send them the signal are damaged. This technique has shown promise as a measure for neuropathy in patients with other types of neuropathy but has never been tried in women with breast cancer.

This study will measure blood flow in your right big toe because it has the best blood flow and is the place where neuropathy from chemotherapy first starts. If you injure your right toe during the study, or only develop neuropathy in your left side, we would measure blood flow in your left toe instead. In addition, it is possible that we could ask to measure blood flow in both your right and left toes to compare them, but we would ask your permission ahead of time to do this.

Why was I approached about taking part in the study?

You were approached about taking part because you are a woman who was just diagnosed with breast cancer and will be receiving drugs that have the potential to cause neuropathy.

How many other women will be taking part in the study?

The entire study will include approximately forty (40) women. This includes twenty (20) women with breast cancer receiving Taxol® weekly or every other week (often called "dose-dense"), and twenty (20) healthy women. You will be one of the 20 women with breast cancer taking part in the study.

Because not all women who get weekly or dose-dense Taxol® start with anthracycline and cyclophosphamide (A+C) therapy (e.g., some women get a biologic agent such as *trastuzumab* (Herceptin®)), and not all women that start with AC therapy get weekly Taxol® (e.g., some get a different drug like *docetaxel* (Taxotere®)), we will recruit at least 13 women that will be receiving both AC therapy and weekly or dose-dense Taxol® to make sure we have a statistically powerful sample that can address all our aims.

How long would the study last?

The study will last approximately 6-18 weeks in length, depending on the specific chemotherapy regimen you will be receiving. If you will be receiving A+C therapy followed by weekly or dose-dense Taxol® (i.e., AC+T), we would ask you to participate in four study visits over the course of 12-18 weeks, each lasting just over an hour in length. Your first visit would take place during the 7-14 days before you start AC chemotherapy. Your second visit would take place on the day you start your first Taxol® infusion, which is usually about 12 weeks after your diagnosis. Your third visit would take place on the morning before your third weekly Taxol® or second dose-dense treatment. Your fourth and final visit would take place on the morning just before your seventh weekly Taxol® treatment, or your third dose-dense Taxol® treatment.

If you start the study and for any reason your doctors decide to switch you to a different type of treatment that does not include weekly or dose-dense Taxol®, your time in the study could include just a single visit before you start your AC therapy. Similarly, if your doctors decide to switch you to a different Taxol regimen (e.g., getting Taxol® every three weeks instead of weekly or every two weeks), we would also cancel your second, third, and fourth visits because for this study, we're only studying neuropathy in women receiving weekly or dose-dense Taxol®. You would still be eligible for the study if you will be receiving Taxol and a different drug such as trastuzumab (Herceptin®), even if you're not receiving AC therapy.

What does taking part in the study involve?

Here is a short description of the study so that you can know what to expect if you decide to participate:

- After reading this form and discussing the purpose, procedures, and potential risks of being in our study and asking any questions, you would sign the form agreeing to participate.

- You will be given a packet with information about the study, and a short questionnaire asking about age, income, and other basic information about you.

- Next, we would schedule you for your four study visits. All of the visits will take place in a room at the Cancer Center near the place where you will be receiving chemotherapy.

- At each of your study visits, you will be greeted by members of the research team. After checking you in, we will weigh you. If it's your first, visit, we will also measure how tall you are and collect your demographic form. We would record all of this on a study sheet.

- Next, we will lead you to a room with a comfortable reclining chair and ask you to sit down. The room will intentionally be heated to 77° F. The reason the room will be warm is to help improve your circulation to your skin. When you sit down, we will look at the skin on your right big toe surface for any callouses, bruising, or scarring that could interfere with imaging and make some notes. We will put pillows under your arms, legs, and neck to make sure you are comfortable and to improve blood flow.

- Although we don't expect that you will experience any pain, you will be asked about pain using a 0-10 scale. We will also have you complete a short questionnaire about neuropathy and measure your reflexes. The test includes three questions you answer verbally, and two manual tests like those you might get at a doctor's office (for example, testing your reflexes with a reflex hammer). This should only take 3-4 minutes.

- Next, we will ask you to lay comfortably for 15 minutes and relax. The goal here is just to be as calm as possible so we can measure your resting blood flow. While you are relaxing, members of our research team would use a thermometer to take your temperature, attach a monitor to your finger that measures your pulse, and attach a blood pressure cuff to your arm to measure your blood pressure. The purpose of measuring your temperature, heart rate, and blood pressure before we start is so that we can know how to interpret any changes in blood flow. At the same time, we would also attach a small heat probe to your right toe so that we know what temperature we're starting from.

- Because we need your blood flow to your skin to be very strong for the technique to work, while you are getting settled we will put a warm blanket on your abdomen and heated neck pillow around your neck. We will also put a foot warmer on your right foot and ask you to drink hot tea. Doing this will raise your core temperature.

- As soon as the skin on your toe is the right temperature, we will take a 2-minute scan of the circulation in your right toe with a special laser imager that measures blood flow. The imager is FDA-approved and safe. The laser uses infrared light which is painless and invisible to the human eye, so while it's imaging, you won't feel or see anything. You will hear some clicking when we turn it on and off, which is just the normal sound of it working.

- After we take this 2-minute scan, we will turn on the heat probe and start heating the skin on your right toe to 107.6 °F (42 °C). This is about the temperature of warm bath

water and shouldn't hurt at all. We will check in with you as we're heating your skin to see how you are doing, and if for any reason the probe felt too hot, you could tell us, and we would stop. We will keep heating your skin for about 30 minutes, and then turn the heat probe up a bit to 111.2° F (44 °C) for an additional 5 minutes.

- After heating the skin on your toe for 35 minutes, we will take another scan of your right toe with the LSCI to see how much blood flow increased after the heat probe was on.

- After this, the study visit will be complete. We will help you up and give you an opportunity to use the restroom, get some food, water, or coffee if you would like it. We will give you a list of contact information so that you can contact us if you have any questions or concerns before your next study visit. We will also validate your parking if you need us to do so. Each study visit should take approximately 75 minutes to complete.

Will taking part in the study cost me anything?

If your Taxol® infusion is being performed at IU Simon Cancer Center, your parking will be validated, and it won't cost you anything. If your Taxol® infusion is being performed at Eskenazi, your parking will be validated for a dollar. Because we are asking you not to eat for an hour before your study visit, we will provide you with breakfast at no cost to you.

Will I receive any payment for taking part in the study?

Yes. You will receive payment for taking part in this study. If you complete all portions of the study, you will receive a $10 gift card for each study visit that you complete, for a total of $30 at the end of the study. In addition, in the case of a technical failure (e.g., laser probe slips, the computer will not start), you will still be given a gift card to ensure that your time is compensated.

In the unlikely event that I am injured during the study, how will I be compensated?

If you are injured by taking part in this study, medical treatment will be provided to you. The bill for this will be added to your medical expenses. The amount of the bill that isn't covered by your health insurance will be your responsibility. Also, it is your responsibility to determine how much your health care covers before agreeing to take part in the study. There is no program in place to provide money for any injuries that may take place. However, you are not giving up any legal rights or benefits by participating.

Who do I contact if I have questions or concerns during the study?

If you have any questions about the study or a research-related injury, you can contact Dr. Victoria Champion (Ph.D., R.N., F.A.A.N.; Principal Investigator, School of Nursing, Indiana University) or Noah Zanville (B.S.N., R.N., B.A.; Co-Principal Investigator, School of Nursing, Indiana University) at [redacted]. If you cannot reach the researchers during regular business hours (8:00 a.m.-5:00 p.m.), please call the IU Human Subjects Office at [redacted]

[redacted]. If you need to reach the research team for any reason after regular business hours, please call Noah Zanville at [redacted]. If you have questions about your rights as a research participant or to discuss problems, complaints, or concerns about a research study, or to obtain information or offer input, contact the IU Human Subjects Office at [redacted].

Important Statement about the Voluntary Nature of the Study

Like all ethical research, participation in this study is voluntary. You can choose not to take part in the study and can stop the study at any time. If you choose to stop the study, there will be no penalty. For example, choosing to not participate in this study would not affect any current or future relations with Indiana University Hospital or the Indiana University School of Nursing.

As researchers, we can choose to stop the study without your permission under certain circumstance. For example, if the research team notices any changes in your physical or mental health that would make it unsafe for you to participate, we can terminate you from the study for your safety. In addition, if you do not complete all-agreed upon portions of the research study, we can choose to terminate your involvement in the study.

SUBJECT CONSENT

"After having read the above and having been given an opportunity to ask all relevant questions, I hereby give my informed consent to participate in this research study. I will be given a copy of this informed consent document to keep for my records."
Subject's Printed Name: _____
Subject's Signature: _____
Date: _____ (must be dated by the subject)

Printed Name of Person Obtaining Consent: _____
Signature of Person Obtaining Consent: _____
Date: _____

APPENDIX B-1
POTENTIAL RISKS OF PARTICIPATING IN STUDY

Potential Risks

All research carries some risk. Potential risks of participating in the study include (1) risk of physical discomfort during skin heating (seriousness: moderate/ likelihood: very unlikely); (2) risk of tissue injury during local skin heating (seriousness: very serious/ likelihood: extremely unlikely); (3) risk of psychological or emotional distress during local skin heating (seriousness: moderate/ likelihood: very unlikely); and (4) risk of loss of confidentiality and/or (5) privacy (seriousness: very serious/likelihood: unlikely). A brief description of each of these risks and rationale for our reasoning about the likelihood of each of these happening is provided below:

Potential Risk #1: Risk of Physical Discomfort during Skin Heating

Results of more than a dozen peer-reviewed studies using 44 °C, 20-minute skin heating suggest that it is highly unlikely that participants will find the skin heating uncomfortable. These studies (which included 1,228 total subjects and 461 women) all found this temperature to be highly tolerable on the top of the foot (Arora et al., 1998; Baker, Green, Krishnan, & Rayman, 2007; Boignard et al., 2005; Dusch, Schley, Rukwied, & Schmelz, 2007; Green et al., 2009; Herman et al., 2007; C. S. Huang et al., 2012; Kramer, Rolke, Bickel, & Birklein, 2004; Krishnan, Baker, Carrington, & Rayman, 2004; Krishnan, Quattrini, Jeziorska, Malik, & Rayman, 2007; Nabavi Nouri et al., 2012; Sivaskandarajah et al., 2013; P. R. Vas & G. Rayman, 2013b; P. R. J. Vas et al., 2012; Veves et al., 1998).

In addition, results of our own pilot research testing the feasibility and tolerability of 44 °C, 20 minutes) on the palmar skin of the right toes of healthy women mirrored these findings and suggest that skin heating in the toes may be even better tolerated in this location than in the dorsal foot. The mean pain score for women who were asked to rate any pain they associated with the heat probe on a 0-10 scale every 5 minutes during the skin heating was 0 (range: 0-4) at the right toe, suggesting that heating at this temperature and duration is painless.

Finally, although studies have validated protocols for generating axon-mediated vasodilation in participants with diabetic neuropathy (including painful neuropathy) in just 6 minutes, these protocols involve using hotter probe temperatures (47 °C), which may not be as

well-tolerated (P. R. Vas & G. Rayman, 2013b). Because of our strong desire to test a method that is very well tolerated for identifying chemotherapy-induced peripheral neuropathy early and monitoring it accurately, we intentionally chose the more conservative protocol to ensure that the heat stimulus would be tolerable and pose minimal risks to study participants.

Potential Risk #2: Risk of Tissue Damage Skin Heating

Based on the same literature using skin heating to assess axon reflex-mediated blood flow and our own pilot study, we assess the likelihood of tissue damage or burns during the 20-minute 44 °C skin heating as extremely unlikely. Forty-four degrees Celsius (44 °C; 111.2 °F) is the temperature used in hot tubs and baths. In individuals with intact blood flow and sensory function, skin heating at this temperature is very safe. Forty-four degree skin heating for up to an hour has been used for up to an hour in neonatal infants to assess microvascular function (Moor Instruments, 2014). Findings from our own pilot study discussed above found no signs of tissue damage in healthy adult women during 20-minute 44 °C skin heating of the toe. Based on these results, we assess the risk to participants to be extremely low. In addition, skin heating at 42 °C for 30-40 minutes followed by a brief increase to 43-44 °C has been used extensively in humans to generate axon reflexes without incident (Choi, Brunt, Fujii, & Minson, 2014; Houghton et al., 2006; Iredahl, Lofberg, Sjoberg, Farnebo, & Tesselaar, 2015; Minson et al., 2001; Van Duijnhoven et al., 2009; B. J. Wong & Minson, 2011b).

Potential Risk #3: Risk of Psychological Distress during Skin Heating

A risk to participants is the potential for the skin heating to produce anxiety or distress in participants. Results of our recent pilot study suggest this is unlikely. After asking five healthy women if they "would be willing to undergo 20-minute, 44 °C skin heating in the future" in their toe, all five "strongly agreed" that they would. Anecdotally, many of the participants found the heating soothing, and many reported that the heat probe had a mild analgesic effect after approximately 5-10 minutes. All participants will be monitored closely for signs of distress during the skin-heating protocol. Participants will be reminded at each visit that they can stop at any time and that doing so will in no way affect their cancer treatment or their ability to participate in future research.

Potential Risk #4: Risk of Loss of Confidentiality/Privacy

A risk of participating in the study is a loss of confidentiality. The likelihood of this is small, but as in any study, this is a risk of participating. A detailed plan for ensuring that the

confidentiality of our participants is not compromised is detailed below in "Plan for Minimizing Risks to Confidentiality/Privacy."

Potential Risk #5: Risk of Loss of Privacy

A final risk of participating in the study is a loss of privacy. A detailed plan for ensuring that the privacy of our participants is not compromised is detailed below in "Plan for Minimizing Risks to Confidentiality/Privacy."

APPENDIX B-2
PLAN FOR ADDRESSING POTENTIAL RISKS OF PARTICIPATING IN STUDY

Plan for Minimizing Physical Risks

To minimize physical risks of participating in the study, investigators will take multiple steps. These include (1) checking all equipment to ensure that it is in proper working order prior to each visit; (2) thoroughly cleaning all equipment between uses with approved cleaning agents; (3) assessing the integrity of the skin at the big toe carefully before skin heating; and (4) asking participants if they are having any difficulty feeling heat in their toes prior to skin heating. In the extremely unlikely event that a participant does experience some discomfort from the heat probe, investigators will stop the protocol and assess the skin at the site. If necessary, ice will be used to address discomfort, and aloe vera will be used to care for the skin at the site.

Plan for Minimizing Psychological Risks

In order to minimize the risk of psychological distress during local skin heating, participants will be screened carefully for signs of distress at each visit. If a participant becomes distressed, the investigator will offer to stop the study visit, the needs of the participant will be evaluated carefully, and she will be referred to appropriate members of the healthcare team if necessary.

Plan for Minimizing Risks to Privacy/Confidentiality

To minimize risks to privacy, whenever possible, study-related activities will be performed in a nondescript way away from non-authorized individuals. Participants will be asked about their preferred method for receiving communications about the study (e.g., phone, email), and how to address them to best protect their privacy. In order to protect the confidentiality of participants, all written study materials will be numbered with participant ID numbers. Only IRB-approved individuals will have access to the reference list for these. All study materials will be stored in a locked cabinet located in the investigator applicant's office, which is located in the basement of IUSON. Only IRB-approved study staff will have keys to this cabinet. Only the investigator will have access to data from the participant's medical records, which will only be accessed with the help of HIPAA-approved clinicians caring for the participant. Data from the participant's medical record will be manually entered into the

participant's de-identified study database and never stored in its original form, ensuring that the data cannot be traced back to the actual participant in the event of a data breach. Data will be stored on the University's approved, encrypted back-up servers. De-identified blood flow data will be uploaded on the University's approved, encrypted back-up servers.

APPENDIX B-3
STOPPING RULES FOR STUDY

Prior to starting the study protocol, participants were instructed to let the researchers know the conditions under which they should ask the researchers to stop the study visit (known as the "stopping rules"). These conditions included:

1. During skin heating, if participants ever experienced pain or discomfort greater than a 5 on 0-10 pain scale (with 0 being defined as "no pain whatsoever," 5 representing "moderate pain/discomfort," and 10 being representing "the worst pain possible").
2. If at any point during the visit, participants felt faint, dizzy, physically or psychologically distressed, or felt like it was time to stop for any reason whatsoever.

Prior to each visit, participants were reminded to communicate their status with the researchers so that we could ensure that they were comfortable and make adjustments where possible. Participants were also reminded that they could stop the study at any point, for any reason, no questions asked.

APPENDIX C
SOCIODEMOGRAPHIC QUESTIONNAIRE

Date: _____ Study ID (For staff use only): _____

Please respond to the following questions by filling in the blanks or checking the best answer. All of your responses will be kept confidential.

Ethnicity
Are you of Spanish/Hispanic/Latino origin (CHECK ONE)?

- ☐ Yes
- ☐ No

Race
With which race or races do you identify yourself? (CHECK AS MANY AS APPLY or describe below):

- ☐ White
- ☐ Black or African American
- ☐ Native Hawaiian or Pacific Islander
- ☐ Asian
- ☐ American Indian or Alaskan native
- ☐ Bi- or Multi-Racial (please describe): _____
- ☐ Other (please describe): _____

Education
Please mark the highest level of education you have completed (CHECK ONE):

- ☐ Graduate or professional degree
- ☐ Some graduate school
- ☐ Four-year College graduate (Bachelor's Degree)
- ☐ Two-year College graduate (Associates Degree)
- ☐ Some college Technical or Trade School
- ☐ High school graduate/GED
- ☐ Some high school
- ☐ Elementary School or less

Relationship Status
Please describe your current relationship status (CHECK ONE):

- ☐ Married
- ☐ In a long-term committed relationship
- ☐ Divorced
- ☐ Widowed
- ☐ Single

Religious/Spiritual Affiliation
Please describe your current religious/spiritual affiliation (CHECK ONE):

- ☐ Christian, Catholic
- ☐ Christian, not Catholic
- ☐ Jewish
- ☐ Muslim
- ☐ Buddhist
- ☐ Spiritual but not religious
- ☐ No religious affiliation
- ☐ Other (please specify): _____

Household Income
Please choose the income category that matches your total household income for the last year, before taxes. This information is confidential.

- ☐ < $15,000
- ☐ $15,001 - $30,000
- ☐ $30,001 - $50,000
- ☐ $50,001 - $75,000
- ☐ $75,001 - $100,000
- ☐ $100,001 - $150,000
- ☐ $150,001 - $200,000
- ☐ $200,000
- ☐ Don't know

Occupational Information
How would you categorize your current or most recent occupation? (Please CHECK ONE):

- ☐ Professional (law, medicine, teacher, social work, engineer, professor)
- ☐ Management/Administration Technical (repairman, computer skills)
- ☐ Clerical (secretary) or Service (waiter/waitress, sales)
- ☐ Homemaker
- ☐ Self-employed
- ☐ Not employed
- ☐ Not employed - disabled
- ☐ Retired
- ☐ Student
- ☐ Other (please describe): _____

Menopausal Status
How would you categorize your current menopausal status? (Please CHECK ONE):

- ☐ Pre-menopausal
- ☐ Peri-menopausal
- ☐ Post-menopausal
- ☐ Other (please describe): _____

Please check the ONE statement that best describes you:

- ☐ I have not had a menstrual period in the last 12 months.
- ☐ I have had a menstrual period in the last 12 months, but not in the last 3 months.
- ☐ I have had a menstrual period in the last 3 months, but cycles are less regular.
- ☐ I have had a menstrual period in the last 3 months, no change in regularity.

If you're no longer have periods, please share why your periods stopped?

- ☐ Normal aging
- ☐ Breast cancer treatment
- ☐ Medicine not related to breast cancer
- ☐ Surgery (such as hysterectomy or ovaries removed)
- ☐ Other (please describe): _____
- ☐ Don't know/Unsure
- ☐ Not applicable

APPENDIX D
SCREENING FORM FOR ELIGIBILITY

Part I: Basic Information		
Study ID:	Data Recorded By (initials):	
Date & Time of Screening Date (MM/DD/YYYY) :___/____/_____ *BCS_scr_date* Time (HH:MM, AM/PM):___/____,_____ *BCS_scr_time*	Format of Screen (check one): In Person (1):_____ Phone (2):____ Email (3):____ Other (4)_____	
Participant Name First Name : _____ *BCS_scr_first* Middle Name : _____ *BCS_scr_mid* Last Name : _____ *BCS_scr_last*		
Part II: Inclusion Criteria	**Answer (circle /fill in)**	**Variable**
Current age (years)?	_____	*BCS_scr_age*
Has first-time, histologically-confirmed breast cancer (stage I-IIIb)?	Yes No	*BCS_scr_BC*
No prior neurotoxic chemotherapy or radiation treatment?	Yes No	*BCS_scr_CR*
Will be receiving weekly *paclitaxel*?	Yes No	*BCS_scr_Pax*
Can read, write, and understand English?	Yes No	*BCS_scr_Eng*
Willing to participate in the three study visits?	Yes No	*BCS_scr_three*
*If potential subject answers "No" to any of these, thank them and inform them that they are not eligible for the study		
Part III: Exclusion Criteria: Does the potential participant…		
…have a history of cardiovascular disease, hypertension or peripheral arterial/vascular disease?	Yes No	*BCS_scr_CV*
…use medications/supplements to control blood pressure (e.g., beta blockers, nitrates, calcium channel blockers, statins)?	Yes No	*BCS_scr_Med*
…have diabetes (with the exception of gestational)?	Yes No	*BCS_scr_DM*
…have neuropathy, neuropathic pain, or nerve injury in the legs or feet?	Yes No	*BCS_scr_NP*
…have pain or arthritis in the right toe?	Yes No	*BCS_scr_AR*
…have dermatologic disease or fungal infection affecting the feet?	Yes No	*BCS_scr_DD*
…have suspected or diagnosed vasospastic disease such as Raynaud's syndrome?	Yes No	*BCS_scr_Ray*
…use tobacco/tobacco-containing products?	Yes No	*BCS_scr_Tob*
Based on these responses, is potential subject eligible for study?	Yes No	*BCS_scr_Elig*

Section IV: Permission to Contact/Preferred Method for Contacting		
Can we contact you before study visits with study reminders?	Yes No	
Main Phone: _____	Yes No	
Alternate Phone: _____	Yes No	
E-mail: _____	Yes No	
Fax: _____	Yes No	
Video chat: _____	Yes No	
Other (please specify): _____	Yes No	
Permission to leave a message on voicemail with a reminder about your study visit?	Yes No	
Permission to leave a message with someone else about your upcoming study visit?	Yes No	
Section V: Recruitment Methods: How did participants learn about study?		
☐ Recruitment Core Staff (1)	_____	*BCS_scr_RC*
☐ Directly from PI/Co-I (2)	_____	*BCS_scr_PI*
☐ Posted Flyer (3)	_____	*BCS_scr_PF*
☐ Online Advertisement (4)	_____	*BCS_scr_OA*
☐ Snowball Sampling (5)	_____	*BCS_scr_SS*
☐ Other (6) (Please specify:_____)		*BCS_scr_Oth*
Stage 1: Initial Data Entry: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Entered By (initials)_____		
Stage 2: Data Verification: Date: (MM/DD/YYYY) _____Time: (HH:MM, AM/PM) _____Data Verified By (initials) _____		
Stage 3: Final Data Audit: Date: (MM/DD/YYYY) _____Time: (HH:MM, AM/PM) _____Data Verified By (initials) _____		

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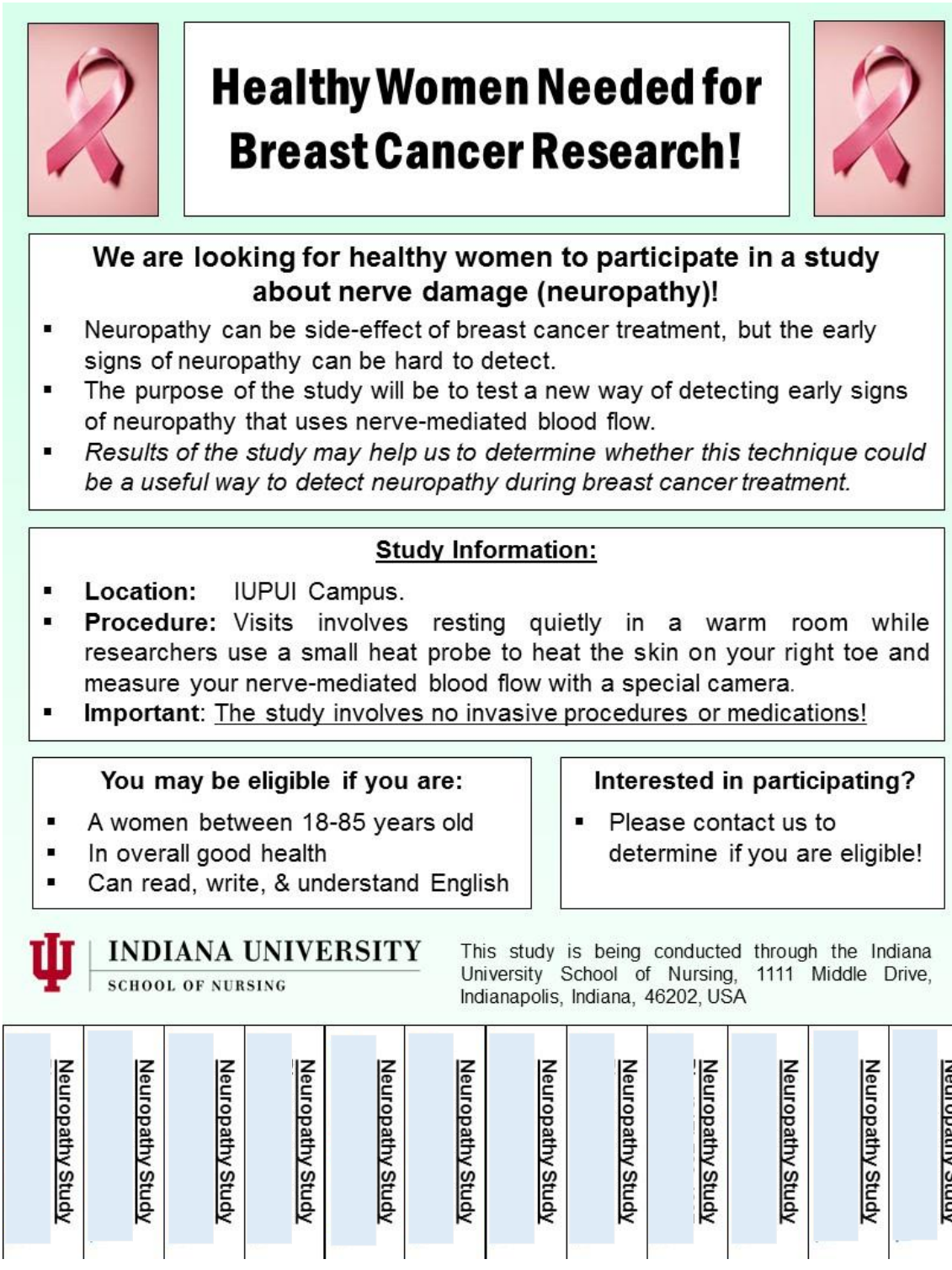
APPENDIX E
FLYER USED TO ADVERTISE STUDY



[20] Note: Portions of flyer shaded in light-blue at bottom of flyer show email for study (redacted) were listed.

APPENDIX F
STUDY BROCHURE

Will I be compensated for my time?

- Yes; if you are eligible for the study, you will receive a $10 gift card for each study visit you successfully complete!

Who will be conducting the study?

- The study is being performed by Victoria Champion (PhD, RN, FAAN) and Noah Zanville (RN, BSN, BA).
- Dr. Champion is an internationally-recognized cancer researcher.
- Noah Zanville is a PhD candidate and fourth year pre-doctoral student at the Indiana University School of Nursing. (IUSON).

INDIANA UNIVERSITY
SCHOOL OF NURSING

Indiana University School of Nursing
Department of Community &
Health Systems
1111 Middle Drive
Indianapolis, Indiana 46202
Phone: [redacted]
Email: nrzanvil@umail.iu.edu

Who is sponsoring the study?

This study is being funded by the:

- National Institute of Nursing Research (NINR)
- Midwest Nursing Research Society (MNRS)/The Council for the Advancement of Nursing Science (CANS)
- Indiana University School of Nursing (IUSON).

Who approved the study?

The safety and scientific merit of the study has been approved by the:

- IU Simon Cancer Center (IUSCC)
- Scientific Review Committee (SRC).
- Indiana University Institutional Review Board.

How can I find out if I'm eligible for the study?

- Ask you study coordinator, physician, or nurse to put you in touch with us and a member of our staff would be happy to give you details about the study and screen you to see if you are eligible!
- You can also contact us directly at [redacted] or nrzanvil@umail.iu.edu

Research Study-IUSC-0529:

Early Detection of Taxane–Induced Neuropathy In Women with Breast Cancer

INDIANA UNIVERSITY
SCHOOL OF NURSING

IRB Protocol # 1502603644

What is 'neuropathy'?

- *'Neuropathy'* is a medical term for nerve damage. This includes both painful and non-painful changes in nerve function.

What does neuropathy have to do with breast cancer?

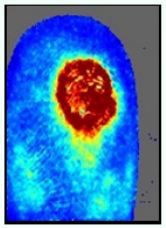
- Neuropathy can be a side effect of chemotherapy. This is called *chemo-therapy-induced peripheral neuropathy* or 'CIPN'.
- CIPN can be a side-effect of the chem-otherapy drug paclitaxel (*Taxol)*, which you will be receiving.

What is the purpose of the study?

- CIPN can be difficult to detect, which can make it difficult for you providers to diagnose and monitor during treat-ment.
- The purpose of our study is to test a new way to detect and monitor CIPN.
- We want to see how this new approach compares to questionnaires and physical checkups that your oncologist and nurses might use.
- We also are interested in if the chang-es in nerve function we see correlate with any CIPN symptoms you may develop.

Can you tell me more about this new approach for measuring CIPN?

- The new approach involves measuring something that *depends* upon healthy nerve function. Something that depends on healthy nerve function is the ability of blood vessels in your skin dilate when exposed to heat.
- To test this, we can put a small heat probe on your skin and see if your blood vessels dilate. While we're heating your skin, we can measure this response using FDA-approved blood flow imager. This is called an *axon reflex*.
- After we heat the skin for about 30 minutes, we can also take the heat probe off your skin and take a picture of your blood flow with a special FDA-approved blood flow camera. This is called an *axon flare (pictured below).*



Has anyone tried this before?

- Yes; researchers have tested this method before patients with other types of neuropathy.
- But, this is the first study to test this method for measuring CIPN in women with receiving Taxol.

Am I eligible to participate ?

- Only a trained member of our team can tell you if you are eligible to for the study. You may be eligible to participate in our study if you:
  - Are being treated for first-time, breast cancer (stage I-IIIB)
  - Will be receiving Taxol either weekly or every other week (known as "dose-dense")

What else should I know before deciding to participate?

Before deciding whether the study is right for you, we want you to know:

- Participation in the study is voluntary.
- The study does not involve any invasive procedures.
- If you don't participate, it will not affect your cancer treatment in any way.

APPENDIX G
WELCOME LETTER FOR STUDY

[*Insert Participant's Name Here*],

It is my pleasure to welcome you to the study 'Early Detection of Taxane-Induced Neuropathy in Women with Breast Cancer' (IRB Study # 1502603664)! We are excited to have you aboard!

Attached below are copies of your (1) Informed Consent and (2) HIPAA Authorization forms (which explain what medical information we will ask for and why this information is necessary). Please take a few minutes to read through both documents carefully. If you have any questions, don't hesitate to contact me. When you're ready, sign both documents. As we discussed, you can scan and send these back to me, arrange a time for me to pick them up in person, or bring them to your first visit.

I have also attached a questionnaire that asks you for basic information needed for the study. Once you have signed your informed consent, please take a few minutes to fill this out and send it back to me using the methods discussed above.

Important Study Information:

- **Schedule of Visits:** Because the timing of your visits is designed to mirror key time points during women's chemotherapy treatments, it is very important that we stick as closely as possible to your visit schedule. We do have some flexibility in the times on these days, and from time-to-time, we may inquire if another time would work to accommodate the needs of other participants.
- **Location of Your Visits**: All your visits will take place in [*location of visit*]. We will plan to meet you in the lobby [*insert location of visit*].
- **Arrival Time**: Although we are having an especially warm winter, in case we have inclement weather, please leave early enough so that you have at least 15 minutes before your study visit. This will give your time to get checked in, change clothes (if needed), and use the bathroom before we start. If you are not sure how much time to provide yourself before your visit, feel free to ask, and we can help you plan.

Other Reminders:

- **What to Wear**: Because this is a study of blood flow, we are intentionally going to keep the room a bit warm (approximately 77° F). Because all of your visits are going to be taking place during the fall/winter months, please plan to bring shorts that you can change into before your visit. This will help you to be comfortable during your visit and make it easier for us to test your reflexes during the first portion of the visit.
- **What to Avoid before the Study:** The day before your visit, I will call to confirm the appointment, see how you're feeling, and remind you to:
    - Avoid eating or drinking anything with caffeine or alcohol for 12 hours before your study visit.
    - Avoid taking non-steroidal anti-inflammatory drugs (NSAIDs) for 24 hours before your study visit, unless directed to do so by your physician (if your physician does tell you to use these, please keep track of how much you took as we'll want to know this information).
    - Avoid eating anything for 1 hour before your visit.
- **What to Expect during Study Visits**: During study visits, we want you to be relaxed and comfortable as possible; besides helping to ensure that you have the very best experience possible from research, our data is influenced by things like heart rate, blood pressure, excess movement, stress, etc. .Because of this, we will take a lot of steps to help you relax (e.g., provide you with warm tea, pillows, a warm neck pillow, etc.), and highly encourage napping or deep relaxation during our blood flow record. For this same reason, laptops, tablets, music, etc. are not allowed during the visit to promote total relaxation and avoid distractions. We certainly understand the need to bring a cell phone, but we will ask you to switch it to silent before the study starts. During study visits, don't hesitate to tell us if there is something we can do to help you be more comfortable - we're happy to help!

[*Participant Name*], again, thank you for agreeing to be a part of our study! We're excited to have you on board. I will email you in a few minutes to schedule you for your three study visits.

Thanks!

[*Researcher' Name, Affiliation, Contact Information*]

APPENDIX H
PRE-VISIT MEDICATION INFORMATION FORM

Pre-Medication Data Collection Form ID #: _____ Visit #:_____ Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Recorded By (initials)_____		
Questions	Answer (circle one)	Variable(s)
Are you feeling well enough to attend tomorrow's study visit?	Yes No	PM_W
1a. If no, reason:_____		
Did your oncologist direct you to take any pre-medications in preparation for tomorrow's chemotherapy infusion?	Yes No	PM_PM
If you were directed to take pre-medication(s), can you please tell us the medication(s) you were directed to take?		
Drug 1: (Name/Dose/Freq/Route)_____		PM_1N, PM_1D
Drug 2: (Name/Dose/Freq/Route)_____		PM_2N, PM_2D
Drug 3: (Name/Dose/Freq/Route)_____		PM_3N, PM_3D
Drug 4: (Name/Dose/Freq/Route)_____		PM_4N, PM_4D
Drug 5: (Name/Dose/Freq/Route)_____		PM_5N, PM_5D
Menstrual Status (if applicable): Are you menstruating currently? Yes No If so, when did you start (date):_____ If not, how many days has it been since your last period?_____		
Stage 1: Data Entry: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____		
Stage 2: Data Verification: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____		
Stage 3: Final Data Audit: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____		

APPENDIX I
MAIN DATA COLLECTION FORM

Data Recorded By: _____					
Step 1: Participant Check-In					
1A: Participant/Visit Info.		1B: Arrival/Consent Verification			
ID Number: _____ Visit Number: _____ Date (MM-DD-YY): _____ Time (HH:MM): _____		Participant arrived?	Yes No		
		Desire to participate verified?	Yes No		
		Brought copy of her Informed Consent?	Yes No		
		IC has IRB stamp at bottom?	Yes No		
		Participant given opportunity to ask questions?	Yes No		
		Given copy of signed IC for records?	Yes No		
		Given a signed copy of HIPAA authorization?	Yes No		
Step 2: Physical and Environment Assessment					
2A: Height & Weight	2B: Environment Characteristics		2C: Skin Assessment		2D: Toe Measurements
Height? _____.____cm	Temp: _____.____°C		Callouses? Yes No		Height? _____.____cm
	Humidity _____.____%		Bruising? Yes No		
Weight? _____.____kg	Light: _____lux		Scar Tissue? Yes No		Width? _____.____kg
	Airflow? Yes No		Other: _____		
Step 3: Pain and Neuropathy Assessment					
Pain Score (1-10):_____			Total TNS Score (0-20):_____		
Step 4: Baseline LSCI Scan/Body Warming Protocol					
4A: Baseline LSCI Scan/Transition			4B: Body Warming Protocol		
Distance from Toe: _____			Heating blanket placed on core?	Yes No	
Time Started: _____			Neck pillow placed around neck?	Yes No	
Time Finished: _____			Participant given warm beverage?	Yes No	
LDF/Skin Probe on? Yes No	Gain:_____		Foot warmer on right foot?	Yes No	

APPENDIX I, CONTINUED

Section 4C: Hemodynamic Monitoring									
Heart Rate monitor attached? Yes No				Timer set for q-10 minute BPs? Yes No					
Blood Pressure cuff attached? Yes No				Baseline temp: _____.____°C					
Time									
HR									
BP									

Section 5: LDF monitoring during skin heating									
Step	1	1A	2	3	3a	4	4a	5a	5b
Activity	Start of LDF Monitoring ?	Start of 33 °C?	Toe at 33 °C for ≥ 5 min?	Start of 42 °C?	Reached 42 °C?	Increased to 44 °C?	Reached 44 °C?	44 °C? for 5 min?	End of Skin Heating?
Time:									
Running Total	0								

Section 6: Post-Skin Heating/Visit Wrap-Up	
6A: Post-Skin Heating	6B: Visit Wrap-Up
Time Heat Probe Removed:_____	Participant helped to gather belongings? (SE_B)
Describe skin condition: _____	Participant offered cold water/restroom? (SE_WR)
Time LSCI Started: _____	Participant given gift card? (SE_CG)
Time LSCI Finished: _____	Last 8 digits of card number:
	Last 6 digits of access number:
Stage 1: Data Entry: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____	
Stage 2: Data Verification: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____	
Stage 3: Final Data Audit: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____	

APPENDIX J
MODIFIED FORM FOR COLLECTING TOTAL NEUROPATHY SCORE DATA

Modified 5-Item TNS Scoring Sheet

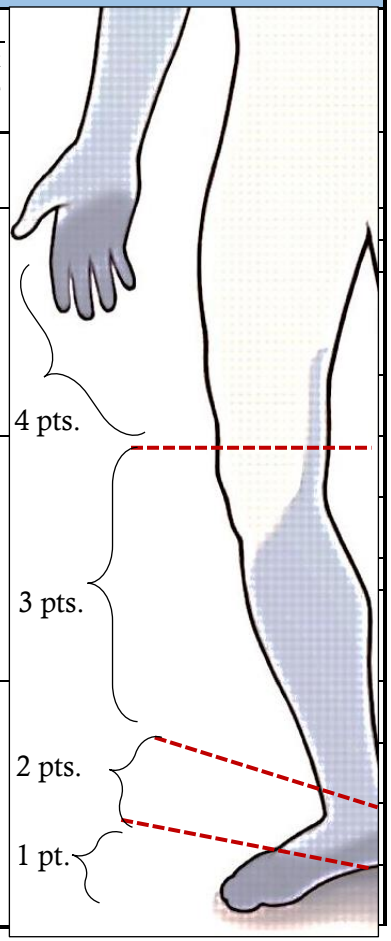
ID #: _____ Visit #:_____

Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Recorded By (initials)_____

Part I: Assessment of Subjective Sensory Symptoms (Proximal Extension)

Instructions: Assess each of the following 3 symptoms, starting at the great toe, on both lower limbs. If participant has a symptom at the toe, continue to test each location until you find an area that the patient does not have symptoms, or scores a 4. If symptoms are asymmetrical, the worse of the 2 sides will be used for grading.

Section/ Neuropathy Symptom	Prompt	Body Area	Normal Sensation? (circle one)	Ind. Score (0-4)	Item Score (0-4)	Notes
1A: Paresthesia (tingling)	'Do you have any *sensations of tingling (pins & needles)* in your arms, legs, hands or feet?	Right Toes	Yes No			
		Right Ankle	Yes No			
		Right Knee	Yes No			
		Right Upper	Yes No			
		Left Toes	Yes No			
		Left Ankle	Yes No			
		Left Knee	Yes No			
		Left Upper	Yes No			
1B: Numbness	'Do you have any *numbness* in your arms, legs, hands or feet?'	Right Toes	Yes No			
		Right Ankle	Yes No			
		Right Knee	Yes No			
		Right Upper	Yes No			
		Left Toes	Yes No			
		Left Ankle	Yes No			
		Left Knee	Yes No			
		Left Upper	Yes No			
1C: Neuropathic pain	'Do you have any *neuropathic pain* in your arms, legs, hands or feet? Examples: burning, shooting, aching, stabbing pain'	Right Toes	Yes No			
		Right Ankle	Yes No			
		Right Knee	Yes No			
		Right Upper	Yes No			
		Left Toes	Yes No			
		Left Ankle	Yes No			
		Left Knee	Yes No			
		Left Upper	Yes No			



Total Score, Part I:	
Grading Scale: 0 = no symptoms; 1 = symptoms extend from toes to mid-foot (not including the heel); 2 = symptoms from mid-foot to ankle; 3 = symptoms extend above ankle to the knee without upper extremity symptoms; 4 = symptoms above the knee or concurrent lower and upper extremity symptoms	

APPENDIX J, CONTINUED

Part 2A: Physical Examination – Vibration Sensibility

Instructions: Starting at a bony prominence on the dorsal part of the great toe, test each lower limb using a 128 Hz tuning fork. Score the participant's vibration sensibility in each limb using the following grading scale: 0 = normal vibration sensation; 1 = absent/decreased from toes to mid-foot (not including the heel); 2 = absent/decreased from mid-foot to ankle; 3 = absent/decreased above ankle to the knee; 4 = absent/decreased above the knee or in lower and upper extremities concurrently.

Neuropathy Symptom	Prompt	Body Area	Normal Sensation? (circle one)	Ind. Score (0-4)	Item Score (0-4)	Notes
2A: Vibration Sensibility	'Next, I am going to test your ability to feel the vibration from this tuning fork. First, I am going to put the tuning fork on your skin; I want you tell if you can feel it. Next, I want you to let me know as soon as you feel the vibration *stop*, okay?'	R. Toe	Yes No	____,____		
		L. Toe	Yes No	____,____		
		R. Ankle	Yes No	____,____		
		L. Ankle	Yes No	____,____		
		R. Knee	Yes No	____,____		
		L. Knee	Yes No	____,____		
		R. Upper	Yes No	____,____		
		L. Upper	Yes No	____,____		
				Total Score, Part 2A:		

Part 2B: Physical Examination – Deep Tendon Reflexes

Instructions: Test each individual reflex (ankle, knee, supinator/brachioradialis, biceps, triceps), starting with the ankle and moving proximally. Score the participant's reflexes using the following grading scale: 0 = All reflexes tested are normal; 1 = Ankle reflex reduce; 2 = Ankle reflex absent; 3 = All reduced; 4 = All reflexes absent.

Neuropathy Symptom	Prompt	Body Area	Normal Reflexes? (circle one)	Ind. Score	Item Score (0-4)	Notes
2A: Tendon Reflexes	'Finally, I am going to check your reflexes using this reflex hammer'	R. Ankle	Yes No			
		L. Ankle	Yes No			
		R. Knee	Yes No			
		L. Knee	Yes No			
		R. Brach.	Yes No			
		L. Brach.	Yes No			
		R. Tricep	Yes No			
		L. Tricep	Yes No			
		R. Bicep	Yes No			

		L. Bicep	Yes No			
				Total Score, Part 2B:		
				Grand Total (1+2A+2B):		
Stage 1: Data Entry: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____						
Stage 2: Data Verification: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____						
Stage 3: Final Data Audit: Date: (MM/DD/YYYY)_____Time: (HH:MM, AM/PM)_____Data Verified By (initials)_____						

APPENDIX K
ANALYSIS OF CONSISTENCY OF HEAT STIMULUS USED TO STIMULATE SMALL-FIBER SENSORY NERVES DURING STUDY

Introduction

To generate axon reflexes reliably, the skin must be heated at a specific rate and duration. The process of increasing the temperature in the heat probe from one temperature to the next is called a *heat ramp*. During our 40-minute skin-heating protocol, three heat ramps were utilized: (1) heating the skin from its starting temperature to a baseline of 33 °C; (2) heating the skin from 33 °C to 42 °C (the heat ramp designed to elicit the axon reflex); and (3) a final heat ramp from 42 °C to 44 °C (that was designed to induced maximum vasodilation), which was then used to determine the size of the axon reflex).

Theoretically, the heat ramp for the experiment can be programmed into the skin-heating system. Protocols for generating axon reflexes have shown that skin heating at a rate of 1.0 °C/10 seconds reliably elicits axon reflexes and is well tolerated by participants. Variations in the rate or duration of heat ramps represent an unseen but critical experimental variable that may explain differences in axon-reflex data observed during the study.

Purpose

The purpose of this additional analysis was to describe the amount of time (in mean and median sec) it took for the skin heater to increase the skin temperature from (a) baseline to 33 °C, (b) from 33 °C to 42 °C, and (c) from 42 °C to 44 °C (maximum vasodilation). In addition, because the interpretation of findings around the mean size of axon reflexes and flares presented in this study depends, in part, on the degree to which the stimuli that elicited these responses were delivered as planned, we compared *actual times* for each of these three events (in sec) against *expected times* determined prior to starting the study to ensure that any difference in mean axon reflex size between participants in our study was not due to differences in how the heat stimulus was delivered.

Methods

During each study visit, we recorded the times that different skin-heating events occurred. Times corresponding to the six skin-heating events that occurred during each visit -- (1) time 33 °C skin heating was started, (2) time 33 °C was reached, (3) time 42 °C skin heating was started, (4) time 42 °C was reached, (5) time 44 °C skin heating was started, and (6) time 44 °C was reached -- were recorded using software included with the laser Doppler (Moor VMS, 3.1; Moor Instruments, Ltd; Administer, UK). As an added precaution, when possible, times for these six skin-heating events were manually recorded by the research assistant to protect against possible data loss.

Analyses

Raw times corresponding to each of the six skin-heating events were recorded by manually reviewing the 40-min skin-heating data for each participant. Times were transferred to an Excel spreadsheet (Excel, 2016 Version, Microsoft Corp.). Data were analyzed in Excel and SPSS, Version 23 (IBM Corporation, Location). Descriptive statistics were used to characterize mean, median, minimum, maximum, range and standard deviations (*SD*) of skin-heating times from (a) baseline to 33 °C, (b) 33 °C to 42 °C, and (c) 42 °C to 44 °C. Repeated-measures analysis of variance (RMANOVA) was used to compare mean skin-heating times within-groups over time.

Results

Complete data on the amount of time (in sec) for the heat probe to increase skin temperature from its stating point to 33 °C (Heat Ramp 1) was available for 98.3% ($n = 59$) of HC visits. During 10% of these visits ($n = 6$), skin in the right toe was already at 33 °C at the start of the experiment, so the time 33 °C was reached was not recorded. Therefore, data from the remaining 88.3% visits ($n = 53$) was used for the analysis of Heat Ramp 1. Complete data on the amount of time it took for the heat probe to increase skin temperature from 33 °C to 42 °C (Heat Ramp 2) and from 42 °C to 44 °C (Heat Ramp 3) was available for 100% of study visits for HCs ($n = 60$). Findings are summarized in Table K-1.

Table K-1

*Mean Duration (in Seconds) of Heat Ramps Performed during 40-Minute Local Skin Heating for Healthy Female Controls at Times 1, 2 and 3 (*n = *20)*

Heat Ramp	*N*	Mean Time (sec)	Median Time (sec)	*SD* (sec)	Range (sec)
Ramp 1 (Baseline to 33°C)					
Time 1	20	52.7	62.0	22.6	66
Time 2	15	43.8	38.0	17.5	70
Time 3	18	44.7	34.5	20.0	67
Ramp 2 (33 °C to 42°C)					
Time 1	20	95.3	91.5	17.3	91
Time 2	20	94.4	90.5	10.4	37
Time 3	20	96.4	92.5	10.4	39
Ramp 3 (42 °C to 44 °C)					
Time 1	20	65.7	67.0	15.3	64
Time 2	20	61.9	63.0	8.3	39
Time 3	20	63.6	66.5	15.1	72

Notes. N = Number of study visits for which complete data on skin-heating times was available; *SD* = standard deviation. Mean and median values refer to the time (in seconds) it took heat probe to raise skin temperature on the toe for each range (i.e., baseline to 33 °C, 33 °C to 42 °C, and 42 °C to 44 °C).

Mean and Median Time it Took to Raise Skin Temperature with Skin Heater

Heat Ramp 1 (Baseline to 33 °C). At Time 1, it took an average of 53 ± 22.6 sec (median: 62 sec., range: 20-86 sec.) to raise the temperature of the skin on the right toe from baseline to 33 °C for HCs. However, at Time 2, it only took an average of 44 ± 18 sec (median: 38 sec; range: 19-89 sec) to raise the skin temperature from baseline to 33 °C. Likewise, at Time 3, mean time to heat the skin to 33 °C was 45 ± 20 sec (median: 35 sec; range: 15-82 sec), meaning that the amount of time it took for the skin heating to heat the skin temperature to 33 °C was approximately 17% faster at Times 2 and 3 than at Time 1.

Heat Ramp 2 (33 °C to 42 °C). Based on our initial calculations from previous literature (Bruning, 2013) and information supplied by the manufacturer, we anticipated it would take approximately 90 seconds to heat the skin on the toe from 33 °C to 42 °C (i.e., increasing local skin temperature 9 °C at a rate of 1.0 °C/10 sec = ~ 90 sec). Results of our

analyses showed that this estimate was relatively accurate, with heating times in our study taking an average of just 5.4 seconds (~6.0%) longer than anticipated to reach 42 °C from 33 °C.

Heat Ramp 3 (42 °C to 44 °C). As with the second heat ramp, analysis of the variability of heating times revealed highly consistent skin-heating times for the third heat ramp, varying approximately 3% between visits (Table 1). Despite the consistency in heating times between participants, data from our experiments showed that heating the skin from 42 °C to 44 °C took significantly longer than expected. Based on our initial calculations, we predicted it would take approximately 20 seconds to raise the superficial skin temperature from 42 °C to 44 °C at a rate of 1.0 °C/10 seconds (i.e., increasing local skin temperature 92 °C at a rate of 1.0 °C/10 sec = ~ 20 sec). Results of our analysis found that, on average, heat ramp 3 took approximately 3-times longer than anticipated, averaging over 60 seconds per visit (Table 1). The result of this delay was that, on average, the skin on each participant's toe received 20% less time at peak temperature (44 °C).

Discussion

Results of the supplemental analysis show that, overall, the actual amount of time it took to heat the skin to the specified temperature during the first two heat ramps was very similar to the amount of time calculated ahead of time. In particular, our finding that on average it took just 6.0% longer than anticipated (approximately 5 seconds) to heat the skin from 33 °C to 42 °C is important, because it suggests that any differences in mean axon reflex size observed for HCs in our study were not likely to be the result of differences in the delivery of the heat stimulus.

As in other studies that use local skin heating, before eliciting axon reflexes we pre-heated the skin on participants' toes to 33 °C and held them there for approximately 5 minutes. The goal of doing this was to ensure that participants started from relatively similar levels of vasodilation prior to eliciting the axon reflex. It was somewhat surprising to note that the amount of time it took to heat participants' skin from baseline to 33 °C during the second and third visits (Times 2 and 3, respectively) were approximately 17% faster than at the Time 1 visit. One explanation for this is that the participants' toes at Times 2 and 3 were colder than

at Time 1. Because study visits for the current study spanned the winter months, there is some precedent for thinking this may have occurred. It is also possible that the faster heat ramp at Times 2 and 3 compared to Time 1 could be evidence of a "learning effect" (i.e., the research team became more skilled at warming the participants through the use of blankets, hot tea, etc).

We were also surprised to find that the amount of time it took to heat the skin in the toe from 42 °C to 44 °C took approximately 3-times longer than anticipated, averaging over 60 seconds per visit. This finding is potentially important because it indicates that participants spent ~20% less time (approximately 1 minute) at peak temperature (44 °C) than anticipated. Theoretically, this could have lead participants to have lower CVC_{MAX}'s, although it is unclear what effect the loss of 60 seconds at peak temperature had on the sample. In addition, while the amount of time it took to heat the skin during this portion of the protocol was longer than expected, this trend was consistent between all visits, indicating CVC_{MAX}'s for participants in the study were based on similar heat stimuli.

Summary

Consistent delivery of heat stimuli during experiments is critical to valid interpretation of studies using heat-dependent endpoints. Analysis of heat ramps for the current study showed that, overall, the rate at which the skin was heated was relatively consistent between time points. Faster-than-expected heating times at Times 2 and 3 compared to Time 1 for the initial heat ramp (baseline to 33 °C) could have been caused by differences in seasons' temperatures (affecting participants' starting skin temperature) or may indicate a learning effect on the part of researchers. Analysis of heating times for heat ramp 2 showed that heating times used to elicit the axon reflex (33 °C to 42 °C) were very similar between visits and achieved the specified target (~90 sec), suggesting that any differences in mean axon reflex size observed for HCs in our study were not likely the result of differences in the delivery of the heat stimulus. However, longer-than-anticipated heating times for heat ramp 3 (42 °C to 44 °C) could not be readily explained. Additional research will be needed to determine the reasons why skin heating took so much longer during this portion of the protocol than anticipated.

APPENDIX L
ANALYSIS OF ENVIRONMENTAL CONDITIONS DURING VISITS

Introduction

Local skin-heating protocols can be highly influenced by the testing environment. Studies show that fluctuations in the temperature, humidity, light levels, and even air flow in the testing room can affect the results of studies using local skin heating (Mahe, Durand, Humeau-Heurtier, et al., 2012; Mahe, Durand, Humeau, et al., 2012; Roustit & Cracowski, 2012). To help ensure that the outcomes observed during the study were not the result of conditions in the testing rooms, during visits these factors were carefully monitored for analysis. Results of these analyses are reported below.

Ambient Temperature in Testing Rooms

Information on the ambient temperature in the testing room at the start of each visit was available for 98.3% of visits involving HCs and 100% of visits involving BCS. Means, standard deviations, and the range of temperatures during visits for both BCS and HCs are listed in Table L-1. Results found that for BCS, temperatures inside the testing rooms were well-controlled, varying less than one degree Celsius at each visit from the target goal of 25±1°C.

Table L-1

Ambient Temperature in Testing Rooms at Start of Visit for Female Breast Cancer Survivors (n = 9) and Healthy Female Controls (n = 20) at Time 1 (Week 0), Time 2 (Week 2 of Taxol®) and Time 3 (Week 6 of Taxol®)

	Between-Group Differences						*Within-Group Differences*	
	Breast Cancer (n = 9)	Healthy Female (n = 20)	t	df	Sig.		Breast Cancer (n = 9)	Healthy Female (n = 20)
Time 1	25.3 (.87)	25.2 (1.0)	0.34	27	.737	f	1.90†	2.31††
Time 2	25.3 (.60)	25.6 (.32)	-1.40	27	.174	df	1.2, 9.2†	1.6, 29.7††
Time 3	25.7 (.51)	25.5 (.26)	0.84	26	.407	Sig.	.202†	.125††

Notes. Temperature values (°C) are expressed as mean (SD). Between-group differences were compared using two-tailed independent samples t-tests. Within-group differences

across study visits were compared using repeated-measures analysis of variance (RM-ANOVA). † = indicates that the assumption of sphericity was violated and a Greenhouse-Geisser correction was applied. †† = indicates that the assumption of sphericity was violated and a Huynh-Feldt correction was applied. For all tests, $\alpha = 0.05$ was considered statistically significant.

Comparison of ambient temperatures between groups at each visit with two-tailed *t*-tests found no difference between groups at Times 1, 2 or 3. Comparison of room temperatures for BCS across the three visits using RM-ANOVA found that temperatures did not differ significantly between visits for BCS ($F(1.2, 9.2) = 1.90$, $p = .202$; partial $\eta^2 = .192$; observed power = 24.7%) or HCs ($F(1.6, 29.7) = 2.31$, $p = .125$; partial $\eta^2 = .114$; observed power = 63.6%).

Relative Humidity in Testing Rooms

Data on relative humidity (%) in testing rooms was available for 100% of visits with BCS and HCs. Data is summarized in Table L-2. Comparison of humidity levels between groups at each visit with two-tailed *t*-tests found no difference between groups at Times 1, 2 or 3. Comparison of humidity levels for BCS at Times 1, 2, and 3 using RM-ANOVA found that humidity did not differ significantly between visits ($F(1.2, 9.2) = 1.90$, $p = .202$; partial $\eta^2 = .192$; observed power = 24.7%). However, testing found a significant main effect of time on humidity levels for HCs ($F(2, 36) = 3.61$, $p = .037$; partial $\eta^2 = .167$; observed power = 63.1%). Post-hoc testing with a Bonferroni correction found that on average, humidity in the testing room was 5.6% higher at Time 3 than Time 2, but this difference missed the cut-off for statistical significance at $\alpha = 0.05$ ($p < .089$).

Table L-2

Relative Humidity in Testing Rooms at Start of Visit for Female Breast Cancer Survivors (n = 9) and Healthy Female Controls (n = 20) at Time 1 (Week 0), Time 2 (Week 2 of Taxol®) and Time 3 (Week 6 of Taxol®)

	Between-Group Differences						*Within-Group Differences*	
	Breast Cancer (*n* = 9)	Healthy Female (*n* = 20)	*t*	*df*	Sig.		Breast Cancer (*n* = 9)	Healthy Female (*n* = 20)
Time 1	25.4 (7.2)	27.9 (9.3)	0.70	27	.491	*f*	1.48†	3.61

Time 2	29.1 (8.8)	29.9 (11.0)	0.91	27	.851	*df*	1.2, 9.8†	2, 36
Time 3	24.2 (8.5)	25.1 (6.9)	0.60	26	.784	*Sig.*	.260†	**.037**

Notes. Humidity values (%) are expressed as mean (SD). Between-group differences were compared using two-tailed independent samples *t*-tests. Within-group differences across study visits were compared using repeated-measures analysis of variance (RM-ANOVA). † = indicates that the assumption of sphericity was violated and a Greenhouse-Geisser correction was applied. For all tests, $\alpha = 0.05$ was considered statistically significant.

Light Levels in Testing Rooms

Data on ambient light levels during study visits were available for 100% of visits with HCs and BCS. Data is summarized in Table L-3. Comparison of light levels between groups at each visit with *t*-tests found that light levels were significantly higher for HCs than BCS at both Time 1 ($p < .007$). Light levels in the testing room were also higher for HCs at Time 2, but just missed the cut-off for statistical significance at $\alpha = 0.05$ ($p < .055$).

Comparison of light levels for HCs at Times 1, 2, and 3 using RM-ANOVA found significant effect of time on light levels ($F(2, 36) = 5.83$, $p = .006$; partial $\eta^2 = .245$; observed power = 84.2%). Post-hoc testing using a Bonferroni correction found that light levels in testing rooms were 300 lux brighter, on average, for HCs at Time 3 than at Time 1 ($p < .008$). By contrast, RM-ANOVA testing found no difference in the brightness of the testing rooms for BCS over the study ($F(2, 16) = .085$, $p = .919$; partial $\eta^2 = .011$; observed power = 6.1%).

Table L-3

Ambient Light Levels in Testing Rooms at Start of Visit for Female Breast Cancer Survivors (n = 9) and Healthy Female Controls (n = 20) at Time 1 (Week 0), Time 2 (Week 2 of Taxol®) and Time 3 (Week 6 of Taxol®)

	Between-Group Differences						*Within-Group Differences*	
	Breast Cancer (*n* = 9)	Healthy Female (*n* = 20)	*t*	*df*	Sig.		Breast Cancer (*n* = 9)	Healthy Female (*n* = 20)
Time 1	182.4 (109.8)	593.4 (593.4)	2.89	21.7	**.007**	*f*	.085	5.83
Time 2	177.9 (104.7)	436.0 (548.0)	2.03	21.9	**.055**	*df*	2, 16	2, 36

Time 3	180.0 (121.7)	143.3 (28.0)	-0.89	8.41	.397	*Sig.*	.919	**.006**

Notes. Light levels (in lux) are expressed as mean (SD). Between-group differences were compared using two-tailed independent samples *t*-tests. Within-group differences across study visits were compared using repeated-measures analysis of variance (RM-ANOVA). For all tests, $\alpha = 0.05$ was considered statistically significant.

Summary

Analysis of temperatures in the testing rooms found that ambient temperature was well-controlled during the study, varying less than one degree on average from the target of 25±1°C during the study. Humidity levels varied more across the study but were similar between groups and time points. Light levels varied the most, with evidence of significantly brighter light levels for HCs compared to BCS at Times 1 and Time 2. Analysis of light levels for HCs over time found that brightness in the testing room decreased by approximately 300 lux over the 6-week study. These differences can be explained by the decision to move some HCs to a room with better temperature-controls but dimmer lights as the study went on. Overall, results indicate that environmental conditions were well-controlled, and were not likely to contribute to any differences in axon reflex or axon flare size identified during the study.

APPENDIX M
DETAILED EXPLORATION OF ATTENUATED RESPONSE TO SKIN HEATING OBSERVED DURING EXPERIMENTS FOR AIM 1

Introduction

As described in Chapters Four and Five, during the study a large portion (~50%) of the data collected for Aim 1 on the axon reflex response had to be set aside during analysis. The reason for this is that during data collection, we observed not one, but two distinct responses to skin heating in the toe. Because of the negative impact that having to set aside this much data had on the study, an attempt was made to determine the factors that may have been responsible for the "attenuated response." As described below, although not originally planned, the data collected for Aim 2 on axon flares in the toe provided important insights into what may have caused the attenuated response to skin heating observed in Aim 1.

Background

As described in the Review of Literature (Chapter Two), the hyperemic flare response can be divided into two parts. The first portion of the flare response (known as the axon flare or LDI_{FLARE} (for the Laser Doppler Imagers (LDI) originally used to measure the response)) refers to the area of increased SkBF that develops in the skin around the heat probe during local skin heating. Research shows the size of this hyperemic flare (which can extend several inches from the site of the heat probe) is primarily caused by the activation of temperature-sensitive small-fiber nerves in the skin, which when heated release vasodilatory peptides that relax the muscle in the walls of the surrounding blood vessels (S. T. Krishnan & G. Rayman, 2004; P. R. Vas, A. Q. Green, & G. Rayman, 2012).

By contrast, the second portion of the axon flare is known as the *maximum hyperemic response* or LDI_{MAX}. Unlike the flare itself, which is primarily neurogenic, studies show that the vasodilation that occurs directly under the heat probe during skin heating is largely the result of non-neurogenic mechanisms (Green et al., 2010). The fact that the LDI_{MAX} is largely non-neurogenic in nature provided us with a convenient way to determine whether the low SkBF we observed in the surface of the toe during some participants during the study could have

been due to underlying changes in the blood vessels themselves (which could conceivably have been caused by the effects of the patient's initial cancer treatment with Adriamycin® and Cytoxan®).

Furthermore, because the LDI_{MAX} only provides information about the blood vessels below the heat probe after the heat probe had been removed (which had to be removed in order to make it possible to capture the size of the post-heating flare with the imager), the LDI_{MAX} data from the current study also provided a way to infer whether the way the heat probe had been attached to the skin could have unintentionally blocked SkBF, which also could potentially explain the diminished blood flow response observed in Aim 1.

To test this premise, we began by calculating the $LDI_{MAX's}$ for each participant at each visit. To calculate the LDI_{MAX}, we used the software included with the imager. Using the software, we drew a polygon the size of the heat probe on the image of the toe. This allowed us to estimate mean SkBF in the skin directly under the heat probe. Next, we entered the LDI_{MAX}'s for each participant at each visit into a database and averaged them across the three visits (Times 1, 2, and 3).

Results of the analyses found that the mean LDI_{MAX} for BCS in the study was 810±158 TPUs, and the mean LDI_{MAX}'s for HCs at all three visits was 755±193 TPUs. Comparison of LDI_{MAX}'s for participants in our study to those from other studies found that LDI_{MAX}'s in our study were similar (or larger) to LDI_{MAX}'s reported in individuals in other studies free from neuropathy (Baker et al., 2007; Green et al., 2010; Krishnan et al., 2004; S. T. M. Krishnan & G. Rayman, 2004; P. R. J. Vas et al., 2012; P. R. J. Vas & G. Rayman, 2013c). Furthermore, the fact that the attenuated response to skin heating only occurred when the heat probe was attached to the toe suggests that the attenuated response was due to the method used to use hold the heat probe on the skin (discussed below) and not to exposure to cancer therapy (which hypothetically would have impaired the vessel's ability to dilate regardless of whether the heat probe was on the skin or not).

As alluded to above, one explanation for the attenuated response observed during Aim 1 was that the "probe slip" used to hold the heat probe against the toe was too tight. If true, this could have constricted SkBF passing through the capillaries during laser Doppler

monitoring. Normally, local skin-heating protocols use laser Doppler to assess SkBF and an adhesive probe holder to hold the laser Doppler and heat probe against the skin. These holders, which fit around the base of the heat probe like a pedestal, use an adhesive strip to attach the heat probe to the skin. These probe holders can be very effective for holding the probe in place. However, testing by the researcher before the start of the study revealed that when the probe holders were removed during the switch-over between the end of the skin heating used to generate the axon reflex for Aim 1 and the imaging for the axon flare for Aim 2, the adhesive on the bottom of the probe holder would often stretch the skin, flooding the skin with excess blood flow, which would make it impossible to accurately determine the size of the post-heating flare.

To address this issue, the researcher developed an alternate method for holding the heat probe in place. Pieces of hypoallergenic tape were placed together, adhesive side-in, creating a non-adhesive strip that could be used to hold the heat probe against the toe surface like a belt around a person's waist. At the start of skin heating, the probe was inserted through the hold in the slip, and the probe slip was wrapped around the toe. Once in place, the probe slip was secured around the head of the toe using simple binder clips. This design allowed the laser Doppler/heat probe to be held securely in place during skin heating, while ensuring that apparatus could be released without disturbing the underlying tissue once skin heating was complete. However, the fact that LDI_{MAX}'s for the study showed that even in individuals displaying the attenuated response, blood flow beneath the heat probe was well within the expected range for 44 °C skin heating suggest that this design may have restricted blood flow to the toe during skin heating.

Studies have shown similar reductions in SkBF during laser Doppler monitoring during sustained pressure on the skin (Fromy, Abraham, & Saumet, 1998). However, in most individuals, when tissue is exposed to a constant low-level pressure (such as that used in our study), the blood vessels in the area being compressed respond by dilating. This *pressure-induced vasodilatory* (PIV) response is thought to be a defense-mechanism which protects the tissue from ischemia by increasing blood flow to the area (Fromy et al., 1998; Fromy, Merzeau, Abraham, & Saumet, 2000; Hsiu et al., 2008). The fact that none of the HCs or BCS in our

study who displayed an attenuated response displayed a PIV response is somewhat vexing. It is possible that the amount of pressure used to hold the probe in place in this study was enough to diminish the participant's response to skin heating but not enough to trigger a PIV response, which would have been detectable using the laser Doppler (Fromy et al., 1998; Fromy, Abraham, & Saumet, 2000; Hsiu et al., 2008; Patel, 1996).

To further investigate whether pressure from the probe slips used to hold the laser Doppler/heat probe against the skin in our study could have been responsible for the attenuated response observed in Aim 1, I enlisted help from engineers at the company that produces the imagers and skin heater used for the study, Moor Instruments. After explaining the issue, I requested that the engineers at the company perform an experiment. The goal of the experiment was to determine if engineers at Moor Instruments could replicate the attenuated response to skin heating we observed in glabrous skin. To test this, the experiment would compare SkBF using the same laser Doppler and skin heater and the same skin type (glabrous) under two conditions.

In the first condition, engineers would attach the laser Doppler/skin heater against the skin using an adhesive probe-holder, while placing no pressure on skin (a "no pressure" condition). In the second condition, the engineers would use tape to hold the laser Doppler/skin heater against the skin, mimicking the "pressure-added condition" from our experiment. The experiment was performed in a single individual using identical skin heating and laser Doppler equipment, heating length (40-minutes), temperature range (33 °C/42 °C/44 °C), and skin type (glabrous) but the thumb was used instead of the toe.

Results of the test showed a nearly identical pattern of diminished SkBF in the pressure-added condition as observed during the present study. The pattern was characterized by (1) muted baseline blood flow, which (2) showed little change over the 40-minute skin heating but which still displayed (3) a measurable increase in SkBF which corresponded to the rise in temperature in the heat probe at 42 °C and 44 °C. The engineer that served as the test subject reported being surprised by the amount of pressure needed to produce a drop in SkBF during their experiment. In particular, the engineers who performed the experiment noted that

in the pressure-added condition, an impression was clearly visible in the skin where the probe tip had been placed, which we also noted in our study.

In addition, the subject in the experiment reported that the pressure on their skin was "was fairly uncomfortable" (Personal Communication: Moor Instruments, 2017). While BCS in our study did not give any indication that pressure associated with the heat probe was uncomfortable, this may have been due to the fact that skin on the toe is thicker than on the finger, and that mechanoreceptors on the toe are used to having pressure on them during standing, which may have accounted for the difference in perceptions between participants in our study and the subject in the test performed by Moor Instruments.

Summary

The appearance of two distinct patterns of SkBF during local skin heating for Aim 1 presented an unexpected challenge for the study. Review of data on the axon flare response collected for Aim 2 suggested that the attenuated SkBF response we observed was not due to an inability of the blood vessels in the toe to dilate. This suggested that mechanical compression, caused by the method used to hold the heat probe in place, was likely to have been the cause of the attenuated response. Testing by the manufacturer of the heat probe suggests that this may have been the case, but because of the sample size of the additional test, further study will be needed to confirm if the attenuated SkBF response to local skin heating observed in this study was the result of mechanical compression or was due to other factors.

APPENDIX N
DISCUSSION OF CASE OF TEMPORARY THERMAL/MECHANICAL REPORTED BY PARTICIPANT DURING THE STUDY

Introduction

As described in the discussion of mean axon flare size in Chapter Five, data on the size of flares was missing from one BCS, who was removed from the skin-heating portion of the study at Time 3 because she developed temporary symptoms of thermal/mechanical hyperalgesia in the area of her right toe that had been heated with the heat probe. This occurrence, while limited to this one BCS in the sample, raises important questions about whether local skin heating is suitable for routine use as an early detection method for small-fiber TIPN.

Case Presentation

The case involved a 32-year-old BCS receiving weekly Taxol® for the study. Like other participants taking part in the study, the participant was being treated for first-time non-metastatic breast cancer (Table N-1). Results of the patient's initial mammogram identified a 1.8-centimeter mass in the upper portion of her left breast, which on biopsy was confirmed as a stage II *ductal carcinoma in situ* (DCIS). Subsequent immunohistochemical testing classified her cancer as HER2- and weakly ER+.

Based on these findings, the patient was prescribed 4 cycles of dose-sense (i.e., twice monthly) neoadjuvant therapy with doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2), followed by 12 cycles of weekly Taxol® (80 mg/m^2). The patient also started on monthly leuprolide acetate (7.5 mg) to minimize the impact of chemotherapy on her future fertility. The patient's past medical history was largely unremarkable but did show signs of pre-diabetes at her last primary care visit. Demographically, the patient was well-educated, employed, and married with one child.

Before starting Taxol®, the patient enrolled in our research study as a participant. At her first study visit (prior to her first Taxol® infusion), the patient reported no pain and showed no signs of neuropathy on physical examination (Table N-1). Two weeks later though, on the morning of her third Taxol® infusion (total exposure: 160 mg/m^2), the

participant had developed bilateral tingling in her feet and numbness in her hands. The participant also displayed signs of mild ankle hyperreflexia without clonus during physical examination.

Table N-1

Chemotherapy Exposure, Pain Levels, and Neuropathy Scores for Breast Cancer Survivor Receiving Weekly Taxol® that Developed Symptoms of Thermal / Mechanical Hyperalgesia following 40-Minute, 42-44 ℃ Local Skin Heating

Day of Taxol® therapy:	Study Visit 1 (Day 1)	Study Visit 2 (Day 14)	Study Visit 3 (Day 42)	Post-Study (Day 70)
Chemotherapy Exposure (in mg/m^2)				
Adriamycin®	195.0	-	-	-
Cytoxan®	1,950.0	-	-	-
Taxol®	0.0	159.5	478.5	717.75
Numeric Pain (rang: 0-10)				
Pain score	0	0	0	-
Item Score on TNSr-SF (range: 0-4[†])				
Tingling	0	1	4	-
Numbness	0	4	4	-
Neuropathic pain	0	0	1	-
Diminished vibration	0	0	0	-
Diminished reflexes	0	1	1	-
Total TNSr-SF Score (range: 0-20):	0	6	10	-

Notes. Values listed for doxorubicin, cyclophosphamide, and paclitaxel columns represent cumulative dose (in mg/m^2) the patient received by that point in time. [†]= Occurring after the end of the study

Although the skin heating portion of the visit proceeded without incident, 5 days later, the participant reported that she had developed a sensitive area on the palmar surface of the right toe where the heat probe had been placed. The participant described the area as "mildly painful." The pain came and went and was similar in quality to the sensation she felt during the skin heating. When asked to elaborate, the participant indicated that the pain was noticeable during walking and could be elicited by putting pressure on the toe from a standing position.

Management and Outcomes

Because of the potential to worsen the participant's TIPN or create a permanent sensitivity to heat in the participant's right toe, skin heating was not performed at her third visit, although data on the participant's overall pain levels as well as potential signs and

symptoms of TIPN were collected. Per protocol, the participant was monitored until the sensitivity resolved. The painful heat sensitivity persisted for approximately 4 weeks following the participant's last study visits (i.e., roughly 6 weeks after she first reported symptoms) (Figure N-1), at which time her symptoms resolved completely. During this time, the patient reported that her symptoms continued to be noticeable during activities of daily living, especially those involving heat such as taking a hot shower. When asked whether other temperature ranges such as cold elicited any pain or sensitivity, the participant indicated that they did not.

Discussion

Although inadvertent, the finding that for some BCS, Taxol® may actually *increase* sensitivity to local skin heating has important implications for the suitability of using skin heating to detect TIPN. Determining whether local skin heating is tolerable during weekly Taxol® therapy is critical to establishing the utility of the technique. The majority of BCS receiving Taxol® in our study found the 42-44 °C local skin heating tolerable (and in many cases, comfortable). However, as described above, at Time 3, one BCS had to be removed from the study because she developed a painful sensitivity to heat and touch (i.e., thermal and mechanical hyperalgesia) in her toe where the heat probe had been placed.

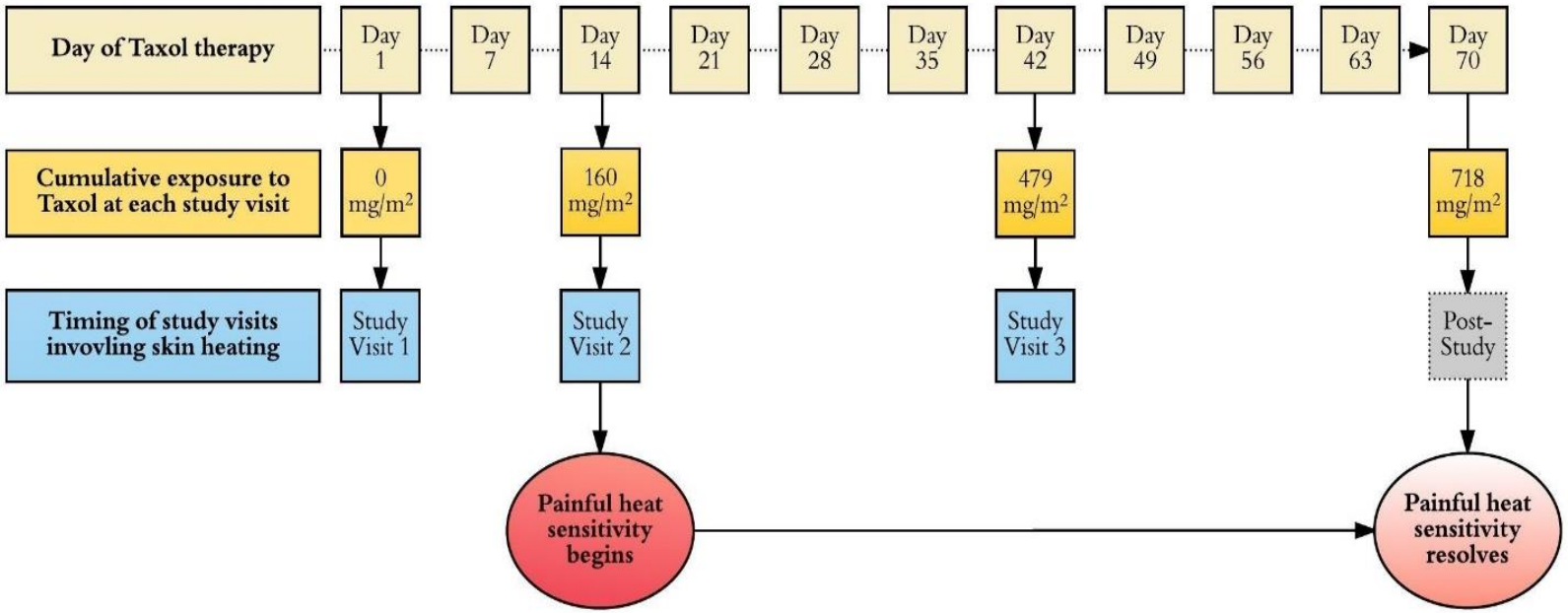
The BCS' aggressive TIPN (which became serious enough for her oncologist to temporarily stop her Taxol® at one point) suggests that this participant may have had a predisposition to TIPN. In addition, because at one point in the participant's medical history, she had been diagnosed as pre-diabetic, it is possible that she had pre-existing damage to the nerves in her feet that predisposed her to this outcome. However, the patient's young age, lack of continuing issues with blood glucose, and lack of clinically-detectable signs of neuropathy at her Time 1 visit argues against this. This suggests that the symptoms of hyperalgesia she developed were at least partially due to exposure to skin heating in the presence of weekly Taxol®. Regardless of the cause, the fact that this participant developed hyperalgesia in the portion of her toe that had been heated raises the question of whether local skin heating is a suitable method for detecting early signs of small-fiber TIPN in the larger population of BCS receiving Taxol®. This concern is especially pertinent because newer, more clinically-useful

skin-heating protocols such as the Modified LDI_{FLARE} protocol require temperatures as high as 47 °C to generate flares in a shorter time frame. In addition, since the effect of Taxol® on the tolerability of heat may depend in part on the stage of treatment, studies are needed to determine for *which* patients local skin heating is appropriate, as well as *when* during treatment the technique is most tolerable.

Summary

Local skin heating is a simple non-invasive technique that may be effective for monitoring small-fiber neuropathy in female breast cancer patients receiving Taxol®, but may not be appropriate for all patients. Research exploring which patients develop heat-sensitivity during Taxol therapy and whether this sensitivity to heat changes during Taxol® therapy is needed to understand for which patient's local skin heating is appropriate.

Figure N-1. Timeline Illustrating Onset and Resolution of Thermal/Mechanical Hyperalgesia in the Palmar Toe Surface in Relationship to Participant's Cumulative Taxol® Exposure and Timing of Study Visits Involving Local Skin Heating



REFERENCES

Abdullah, T. I., Iddon, J., Barr, L., Baildam, A. D., & Bundred, N. J. (1998). Prospective randomized controlled trial of preservation of the intercostobrachial nerve during axillary node clearance for breast cancer. *British Journal of Surgery, 85*(10), 1443-1445. doi:10.1046/j.1365-2168.1998.00843.x

Abraham, J. E., Guo, Q., Dorling, L., Tyrer, J., Ingle, S., Hardy, R., . . . Earl, H. M. (2014). Replication of genetic polymorphisms reported to be associated with Taxane-related sensory neuropathy in patients with early breast cancer treated with Paclitaxel. *Clinical Cancer Research, 20*(9), 2466-2475. doi:10.1158/1078-0432.ccr-13-3232

American Cancer Society. (2015). *Breast cancer facts & figures 2015-2016*. Retrieved from https://www.google.com/search?q=American+Cancer+Society+Facts+Figures+Breast+Cancer&ie=utf-8&oe=utf-8

American Cancer Society. (2017). *Cancer Facts & Figures: 2017*. Retrieved from: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-facts-and-figures-2017.pdf

American College of Surgeons. (2017). *National Cancer Database (NCDB) Public Benchmark Reports. Cases Diagnosed 2004-2014*. Retrieved from httpshttps://oliver.facs.org/BMPub/Docs/

Amichetti, M., & Caffo, O. (2003). Pain after quadrantectomy and radiotherapy for early-stage breast cancer: Incidence, characteristics and influence on quality of life. Results from a retrospective study. *Oncology, 65*(1), 23-28. https://doi.org/10.1159/000071201

Andersen, K. G., Aasvang, E. K., Kroman, N., & Kehlet, H. (2014). Intercostobrachial nerve handling and pain after axillary lymph node dissection for breast cancer. *Acta Anaesthesiologica Scandinavica, 58*(10), 1240-1248. doi:10.1111/aas.12393

Andersen, K. G., Jensen, M. B., Tvedskov, T. F., Kehlet, H., Gartner, R., & Kroman, N. (2013). Persistent pain, sensory disturbances and functional impairment after immediate or delayed axillary lymph node dissection. *European Journal Surgical Oncology, 39*(1), 31-35. doi:10.1016/j.ejso.2012.10.010

Anderson, B., & Sawyer, D. B. (2008). Predicting and preventing the cardiotoxicity of cancer therapy. *Expert Review of Cardiovascular Therapy, 6*(7), 1023-1033. doi:10.1586/14779072.6.7.1023

Aoki, K., Kondo, N., Shibasaki, M., Takano, S., & Katsuura, T. (1997). Circadian variation in skin blood flow responses to passive heat stress. *Physiol Behavior, 63*(1), 1-5. https://doi.org/10.1016/S0031-9384(97)00348-X

Argyriou, A. A., Koltzenburg, M., Polychronopoulos, P., Papapetropoulos, S., & Kalofonos, H. P. (2008). Peripheral nerve damage associated with administration of Taxanes in patients with cancer. *Critical Reviews in Oncology Hematology, 66*(3), 218-228. doi:10.1016/j.critrevonc.2008.01.008

Argyriou, A. A., Kyritsis, A. P., Makatsoris, T., & Kalofonos, H. P. (2014). Chemotherapy-induced peripheral neuropathy in adults: A comprehensive update of the literature. *Cancer Management Research, 6*, 135-147. doi:10.2147/cmar.s44261

Argyriou, A. A., Polychronopoulos, P., Iconomou, G., Koutras, A., Kalofonos, H. P., & Chroni, E. (2005). Paclitaxel plus Carboplatin-induced peripheral neuropathy. A prospective clinical and electrophysiological study in patients suffering from solid malignancies. *Journal of Neurology, 252*(12), 1459-1464. doi:10.1007/s00415-005-0887-8

Argyriou, A. A., Zolota, V., Kyriakopoulou, O., & Kalofonos, H. P. (2010). Toxic peripheral neuropathy associated with commonly used chemotherapeutic agents. *Journal of B.U.ON.: Official journal of the Balkan Union of Oncology, 15*(3), 435-446. PMID: 20941808

Arora, S., Smakowski, P., Frykberg, R. G., Simeone, L. R., Freeman, R., Logerfo, F. W., & Veves, A. (1998). Differences in foot and forearm skin microcirculation in diabetic patients with and without neuropathy. *Diabetes Care, 21*(8), 1339-1344. doi:10.2337/diacare.21.8.1339

Atherton, D. D., Facer, P., Roberts, K. M., Peter Misra, V., Chizh, B. A., Bountra, C., & Anand, P. (2007). Use of the novel contact heat evoked potential stimulator (CHEPS) for the assessment of small fibre neuropathy: Correlations with skin flare responses

and intra-epidermal nerve fibre counts. *BMC Neurology, 7*(1), 21. https://doi.org/10.1186/1471-2377-7-21

Augusto, C., Pietro, M., Cinzia, M., Sergio, C., Sara, C., Luca, G., & Scaioli, V. (2008). Peripheral neuropathy due to Paclitaxel: Study of the temporal relationships between the therapeutic schedule and the clinical quantitative score (QST) and comparison with neurophysiological findings. *Journal of Neuro-Oncology, 86*(1), 89-99. Retrieved from https://doi.org/10.1007/s11060-007-9438-8

Authier, N., Gillet, J. P., Fialip, J., Eschalier, A., & Coudore, F. (2000). Description of a short-term Taxol-induced nociceptive neuropathy in rats. *Brain Research, 887*(2), 239-249. https://doi.org/10.1016/S0006-8993(00)02910-3

Avila, E. K., Goenka, A., & Fontenla, S. (2011). Bilateral phrenic nerve dysfunction: A late complication of mantle radiation. *Journal of Neuro-Oncology, 103*(2), 393-395. https://doi.org/10.1007/s11060-010-0396-1

Backonja, M. M., & Galer, B. S. (1998). Pain assessment and evaluation of patients who have neuropathic pain. *Neurologic Clinics, 16*(4), 775-789. https://doi.org/10.1016/S0733-8619(05)70097-9

Baker, N., Green, A., Krishnan, S., & Rayman, G. (2007). Microvascular and C-fiber function in diabetic charcot neuropathy and diabetic peripheral neuropathy. *Diabetes Care, 30*(12), 3077-3079. https://doi.org/10.2337/dc07-1063

Bakitas, M. A. (2007). Background noise: The experience of chemotherapy-induced peripheral neuropathy. *Nursing Research, 56*(5), 323-331. doi:10.1097/01.NNR.0000289503.22414.79

Balcioglu, A. S., & Muderrisoglu, H. (2015). Diabetes and cardiac autonomic neuropathy: Clinical manifestations, cardiovascular consequences, diagnosis and treatment. *World Journal of Diabetes, 6*(1), 80-91. doi:10.4239/wjd.v6.i1.80

Bao, T., Basal, C., Seluzicki, C., Li, S. Q., Seidman, A. D., & Mao, J. J. (2016). Long-term chemotherapy-induced peripheral neuropathy among breast cancer survivors: Prevalence, risk factors, and fall risk. *Breast Cancer Research and Treatment*. doi:10.1007/s10549-016-3939-0

Baron, R., Haendler, G., & Schulte, H. (1997). Afferent large fiber polyneuropathy predicts the development of postherpetic neuralgia. *Pain, 73*(2), 231-238. doi:10.1016/S0304-3959(97)00105-X

Becker, D. J., Talwar, S., Levy, B. P., Thorn, M., Roitman, J., Blum, R. H., . . . Grossbard, M. L. (2013). Impact of oncology drug shortages on patient therapy: Unplanned treatment changes. *Journal of Oncology and Practice, 9*(4), e122-128. doi:10.1200/jop.2012.000799

Beijers, A., Mols, F., Dercksen, W., Driessen, C., & Vreugdenhil, G. (2014). Chemotherapy-induced peripheral neuropathy and impact on quality of life 6 months after treatment with chemotherapy. *Journal of Community Supportive Oncology, 12*(11), 401-406. doi:10.12788/jcso.0086

Beijers, A. J. M., Vreugdenhil, G., Oerlemans, S., Eurelings, M., Minnema, M. C., Eeltink, C. M., . . . Mols, F. (2016). Chemotherapy-induced neuropathy in multiple myeloma: Influence on quality of life and development of a questionnaire to compose common toxicity criteria grading for use in daily clinical practice. *Supportive Care in Cancer, 24*(6), 2411-2420. doi:10.1007/s00520-015-3032-y

Bennett, G. J., Liu, G. K., Xiao, W. H., Jin, H. W., & Siau, C. (2011). Terminal arbor degeneration - a novel lesion produced by the antineoplastic agent Paclitaxel. *European Journal of Neuroscience, 33*(9), 1667-1676. https://doi.org/10.1111/j.1460-9568.2011.07652.x

Berger, P. S., & Bataini, J. P. (1977). Radiation-induced cranial nerve palsy. *Cancer, 40*(1), 152-155. https://doi.org/10.1002/1097-0142(197707)40:1<152::AID-CNCR2820400125>3.0.CO;2-E

Berger, T., Malayer, R., Doppelbauer, A., Krajnik, G., Huber, H., Auff, E., & Pirker, R. (1997). Neurological monitoring of neurotoxicity induced by Paclitaxel/Cisplatin chemotherapy. *European Journal of Cancer, 33*(9), 1393-1399. http://dx.doi.org/10.1016/S0959-8049(97)00103-2

Berger, T., Malayeri, R., Doppelbauer, A., Krajnik, G., Huber, H., Auff, E., & Pirker, R. (1997). Neurological monitoring of neurotoxicity induced by Paclitaxel/Cisplatin

chemotherapy. *European Journal of Cancer, 33*(9), 1393-1399. https://doi.org/10.1016/S0959-8049(97)00103-2

Bhatnagar, B., Gilmore, S., Goloubeva, O., Pelser, C., Medeiros, M., Chumsri, S., . . . Bao, T. (2014). Chemotherapy dose reduction due to chemotherapy induced peripheral neuropathy in breast cancer patients receiving chemotherapy in the neoadjuvant or adjuvant settings: A single-center experience. *Springerplus, 3*, 366. doi:10.1186/2193-1801-3-366

Bickel, A., Heyer, G., Senger, C., Maihoefner, C., Heuss, D., Hilz, M. J., & Namer, B. (2009). C-fiber axon reflex flare size correlates with epidermal nerve fiber density in human skin biopsies. *Journal of the Peripheral Nervous System, 14*(4), 294-299. http://dx.doi.org/10.1111/j.1529-8027.2009.00241.x

Bickel, A., Heyer, G., Senger, C., Maihofner, C., Heuss, D., Hilz, M. J., & Namer, B. (2009). C-fiber axon reflex flare size correlates with epidermal nerve fiber density in human skin biopsies. *Journal of Peripheripheral Nervous System, 14*(4), 294-299. doi:10.1111/j.1529-8027.2009.00241.x

Bickel, A., Krämer, H. H., Hilz, M. J., Birklein, F., Neundörfer, B., & Schmelz, M. (2002). Assessment of the neurogenic flare reaction in small-fiber neuropathies. *Neurology, 59*(6), 917-919. https://doi.org/10.1212/WNL.59.6.917

Binda, D., Vanhoutte, E. K., Cavaletti, G., Cornblath, D. R., Postma, T. J., Frigeni, B., . . . Dorsey, S. G. (2013). Rasch-built Overall Disability Scale for patients with chemotherapy-induced peripheral neuropathy (CIPN-R-ODS). *European Journal of Cancer, 49*(13), 2910-2918. doi:10.1016/j.ejca.2013.04.004

Binner, M., Ross, D., & Browner, I. (2011). Chemotherapy-induced peripheral neuropathy: Assessment of oncology nurses' knowledge and practice. *Oncology Nursing Forum, 38*(4), 448-454. doi:10.1188/11.onf.448-454

Blaise, S., Roustit, M., Millet, C., & Cracowski, J. L. (2011). Effect of oral sildenafil on skin postocclusive reactive hyperemia in healthy volunteers. *Microcirculation, 18*(6), 448-451. doi:10.1111/j.1549-8719.2011.00105.x

Bocci, G., Di Paolo, A., & Danesi, R. (2013). The pharmacological bases of the antiangiogenic activity of Paclitaxel. *Angiogenesis, 16*(3), 481-492. doi:10.1007/s10456-013-9334-0

Boignard, A., Salvat-Melis, M., Carpentier, P. H., Minson, C. T., Grange, L., Duc, C., . . . Cracowski, J. L. (2005). Local hyperemia to heating is impaired in secondary Raynaud's phenomenon. *Arthritis Research and Therapy, 7*(5), R1103-1112. doi:10.1186/ar1785

Bouhassira, D., & Attal, N. (2011). Diagnosis and assessment of neuropathic pain: The saga of clinical tools. *Pain, 152*(3 Suppl), S74-83. doi:10.1016/j.pain.2010.11.027

Boyette-Davis, J., Xin, W., Zhang, H., & Dougherty, P. M. (2011). Intraepidermal nerve fiber loss corresponds to the development of Taxol-induced hyperalgesia and can be prevented by treatment with minocycline. *Pain, 152*(2), 308-313. doi:10.1016/j.pain.2010.10.030

Boyette-Davis, J. A., Eng, C., Wang, X. S., Cleeland, C. S., Wendelschafer-Crabb, G., Kennedy, W. R., . . . Dougherty, P. M. (2012a). Subclinical peripheral neuropathy is a common finding in colorectal cancer patients prior to chemotherapy. *Clinical Cancer Research, 18*(11), 3180-3187. doi:10.1158/1078-0432.ccr-12-0205

Boyette-Davis, J. A., Eng, C., Wang, X. S., Cleeland, C. S., Wendelschafer-Crabb, G., Kennedy, W. R., . . . Dougherty, P. M. (2012). Subclinical peripheral neuropathy is a common finding in colorectal cancer patients prior to chemotherapy. *Clinical Cancer Research, 18*(11), 3180-3187. doi:10.1158/1078-0432.ccr-12-0205

Brackstone, M., Fletcher, G. G., Dayes, I. S., Madarnas, Y., SenGupta, S. K., & Verma, S. (2015). Locoregional therapy of locally advanced breast cancer: A clinical practice guideline. *Current Oncology, 22*(Suppl 1), S54-66. doi:10.3747/co.22.2316

Breiner, A., Lovblom, L. E., Perkins, B. A., & Bril, V. (2014). Does the prevailing hypothesis that small-fiber dysfunction precede large-fiber dysfunction apply to type 1 diabetic patients? *Diabetes Care, 37*(5), 1418-1424. doi:10.2337/dc13-2005

Bridges, C. M., & Smith, E. M. (2014). What about Alice? Peripheral neuropathy from Taxane-containing treatment for advanced nonsmall cell lung cancer. *Support Care Cancer, 22*(9), 2581-2592. doi:10.1007/s00520-014-2317-x

Bromm, B., & Lorenz, J. (1998). Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology, 107*(4), 227-253. https://doi.org/10.1016/S0013-4694(98)00075-3

Kang, J. H., & Bruera, E. (2013). Neuropathic component of pain in cancer. In M. Hanna & Z. Zylicz (Eds.), *Cancer Pain* (pp. 165-190). London, UK: Springer.

Bruning, R. S. (2013). *The mechanisms and functional implications of altered microvascular vasoreactivity in healthy and essential hypertensive men and women* (Doctoral dissertation) Retrieved from http://ulib.iupui.edu/cgi-bin/proxy.pl?url=http://search.proquest.com/docview/1511655871?accountid=7398 http://ulib.iupui.edu/findit/openurl?ctx_ver=Z39.88-2004&ctx_enc=info:ofi/enc:UTF-8&rfr_id=info:sid/ProQuest+Dissertations+%26+Theses+A%26I&rft_val_fmt=info:ofi/fmt:kev:mtx:dissertation&rft.genre=dissertations+%26+theses&rft.jtitle=&rft.atitle=&rft.au=Bruning%2C+Rebecca+Sue&rft.aulast=Bruning&rft.aufirst=Rebecca&rft.date=2013-01-01&rft.volume=&rft.issue=&rft.spage=&rft.isbn=9781303781551&rft.btitle=&rft.title=The+mechanisms+and+functional+implications+of+altered+microvascular+vasoreactivity+in+healthy+and+essential+hypertensive+men+and+women&rft.issn=&rft_id=info:doi/ ProQuest Dissertations & Theses A&I database. (Accession number: 3585564)

Burns, R. J. (1978). Delayed radiation-induced damage to the brachial plexus. *Clinical and Experimental Neurology, 15*, 221-227. PMID: 756017

Caffo, O., Amichetti, M., Ferro, A., Lucenti, A., Valduga, F., & Galligioni, E. (2003). Pain and quality of life after surgery for breast cancer. *Breast Cancer Research and Treatment, 80*(1), 39-48. doi:10.1023/a:1024435101619

Calhoun, E. A., Chang, C. H., Welshman, E. E., Fishman, D. A., Lurain, J. R., & Bennett, C. L. (2001). Evaluating the total costs of chemotherapy-induced toxicity: Results from a pilot study with ovarian cancer patients. *Oncologist, 6*(5), 441-445. https://doi.org/10.1634/theoncologist.6-5-441

Calhoun, E. A., Welshman, E. E., Chang, C. H., Lurain, J. R., Fishman, D. A., Hunt, T. L., & Cella, D. (2003). Psychometric evaluation of the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity (Fact/GOG-Ntx) questionnaire for patients receiving systemic chemotherapy. *International Journal of Gynecological Cancer, 13*(6), 741-748. https://doi.org/10.1111/j.1525-1438.2003.13603.x

Cammisuli, S., Cavazzi, E., Baldissarro, E., & Leandri, M. (2016). Rehabilitation of balance disturbances due to chemotherapy induced peripheral neuropathy: A pilot study. *European Journal Physical and Rehabilitation Medicine, 52*(4), 479-488. PMID: 756017

Campbell, W. (2013). *Essentials of Electrodiagnostic Medicine*. New York, NY: Demos Medical Publishing.

Carbognin, L., Sperduti, I., Nortilli, R., Brunelli, M., Vicentini, C., Pellini, F., . . . Bria, E. (2015). Balancing activity and tolerability of neoadjuvant Paclitaxel- and docetaxel-based chemotherapy for HER2-positive early stage breast cancer: Sensitivity analysis of randomized trials. *Cancer Treatment Reviews, 41*(3), 262-270. doi:10.1016/j.ctrv.2015.02.003

Carmeliet, P., & Jain, R. K. (2011). Molecular mechanisms and clinical applications of angiogenesis. *Nature, 473*(7347), 298-307. doi:10.1038/nature10144

Carozzi, V., Chiorazzi, A., Canta, A., Oggioni, N., Gilardini, A., Rodriguez-Menendez, V., . . . Cavaletti, G. (2009). Effect of the chronic combined administration of Cisplatin and Paclitaxel in a rat model of peripheral neurotoxicity. *European Journal of Cancer, 45*(4), 656-665. doi:10.1016/j.ejca.2008.10.038

Carozzi, V. A., Canta, A., Oggioni, N., Sala, B., Chiorazzi, A., Meregalli, C., . . . Cavaletti, G. (2010). Neurophysiological and neuropathological characterization of new murine

models of chemotherapy-induced chronic peripheral neuropathies. *Experimental Neurology, 226*(2), 301-309. doi:10.1016/j.expneurol.2010.09.004

Carpenter, J. S., Andrykowski, M. A., Sloan, P., Cunningham, L., Cordova, M. J., Studts, J. L., . . . Kenady, D. E. (1998). Postmastectomy/postlumpectomy pain in breast cancer survivors. *Journal of Clinical Epidemiology, 51*(12), 1285-1292. https://doi.org/10.1046/j.1523-5394.1999.07208.x

Carpenter, J. S., Sloan, P., Andrykowski, M. A., McGrath, P., Sloan, D., Rexford, T., & Kenady, D.(1999). Risk factors for pain after mastectomy/lumpectomy. *Cancer Practice, 7*(2), 66-70. https://doi.org/101046/j.1523-5394.1999.07208.

Casanova-Molla, J., Grau-Junyent, J. M., Morales, M., & Valls-Sole, J. (2011). On the relationship between nociceptive evoked potentials and intraepidermal nerve fiber density in painful sensory polyneuropathies. *Pain, 152*(2), 410-418. doi:10.1016/j.pain.2010.11.012

Cata, J. P., Weng, H. R., Lee, B. N., Reuben, J. M., & Dougherty, P. M. (2006). Clinical and experimental findings in humans and animals with chemotherapy-induced peripheral neuropathy. *Minerva Anestesiologica, 72*(3), 151-169. Retrieved from https://www.researchgate.net/profile/James_Reuben/publication/7284654_Clinical_and_experimental_finding_in_humans_and_animals_with_chemotherapy-induced_peripheral_neuropathy/links/0c96051c9f4f8cdbbe000000/Clinical-and-experimental-finding-in-humans-and-animals-with-chemotherapy-induced-peripheral-neuropathy.pdf

Cavaletti, G., Alberti, P., Frigeni, B., Piatti, M., & Susani, E. (2011). Chemotherapy-induced neuropathy. *Current Treatment Options in Neurology, 13*(2), 180-190. doi:10.1007/s11940-010-0108-3

Cavaletti, G., Bogliun, G., Marzorati, L., Zincone, A., Marzola, M., Colombo, N., & Tredici, G. (1995). Peripheral neurotoxicity of Taxol in patients previously treated with cisplatin. *Cancer, 75*(5), 1141-1150. https://doi.org/10.1002/1097-0142(19950301)75:5<1141::AID-CNCR2820750514>3.0.CO;2-U

Cavaletti, G., Cornblath, D. R., Merkies, I. S. J., Postma, T. J., Rossi, E., Frigeni, B., . . . Grisold, W. (2013). The chemotherapy-induced peripheral neuropathy outcome measures standardization study: From consensus to the first validity and reliability findings. *Annals of Oncology, 24*(2), 454-462. https://doi.org/10.1002/1097-0142(19950301)75:5<1141::AID- CNCR2820750514>3.0.CO;2-U

Cavaletti, G., Frigeni, B., Lanzani, F., Mattavelli, L., Susani, E., Alberti, P., . . . Bidoli, P. (2010). Chemotherapy-induced peripheral neurotoxicity assessment: A critical revision of the currently available tools. *European Journal of Cancer, 46*(3), 479-494. doi:10.1016/j.ejca.2009.12.008

Cavaletti, G., Frigeni, B., Lanzani, F., Piatti, M., Rota, S., Briani, C., . . . Zanna, C. (2007b). The Total Neuropathy Score as an assessment tool for grading the course of chemotherapy-induced peripheral neurotoxicity: Comparison with the National Cancer Institute-Common Toxicity Scale. *Journal of Peripheral Nervous System, 12*(3), 210-215. doi:10.1111/j.1529-8027.2007.00141.x

Cavaletti, G., Jann, S., Pace, A., Plasmati, R., Siciliano, G., Briani, C., . . . Giussani, G. (2006). Multi-center assessment of the total neuropathy score for chemotherapy-induced peripheral neurotoxicity. *Journal of Peripheral Nervous System, 11*(2), 135-141. doi:10.1111/j.1085-9489.2006.00078.x

Cavaletti, G., & Marmiroli, P. (2012). Evaluation and monitoring of peripheral nerve function. *Handboook of Clinical Neurology, 104*, 163-171. doi:10.1016/b978-0-444-52138-5.00013-x

Center for Drug Evaluation and Research. (1998). *Approval letter for Taxol online: Food and Drug Administration (FDA)*. Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20262s024.cfm.

Centers for Disease Control and Prevention. (2017). *High blood pressure fact sheet: High blood pressure in the United States*. Retrieved from https://www.cdc.gov/dhdsp/data_statistics/fact_sheets/fs_bloodpressure.htm

Charkoudian, N., & Johnson, J. M. (1997). Modification of active cutaneous vasodilation by oral contraceptive hormones. *Journal of Applied Physiology (1985), 83*(6), 2012-2018. doi:10.1152/jappl.1997.83.6.2012

Charkoudian, N., Stephens, D. P., Pirkle, K. C., Kosiba, W. A., & Johnson, J. M. (1999). Influence of female reproductive hormones on local thermal control of skin blood flow. *Journal of Applied Physiology, 87*(5), 1719-1723. doi:10.1152/jappl.1999.87.5.1719

Chen, E. I., Crew, K. D., Trivedi, M., Awad, D., Maurer, M., Kalinsky, K., . . . Hershman, D. L. (2015). Identifying predictors of Taxane-induced peripheral neuropathy using mass spectrometry-based proteomics technology. *Plos One, 10*(12). doi:10.1371/journal.pone.0145816

Chico, I., Kang, M. H., Bergan, R., Abraham, J., Bakke, S., Meadows, B., . . . Bates, S. (2001). Phase I study of infusional Paclitaxel in combination with the p-glycoprotein antagonist PSC 833. *Journal of Clinical Oncology, 19*(3), 832-842. doi:10.1200/jco.2001.19.3.832

Chiu, N., Chiu, L., Chow, R., Lam, H., Verma, S., Pasetka, M., . . . DeAngelis, C. (2017). Taxane-induced arthralgia and myalgia: A literature review. *Journal of Oncology Pharmacy Practice, 23*(1), 56-67. doi:10.1177/1078155215627502

Choi, P. J., Brunt, V. E., Fujii, N., & Minson, C. T. (2014). New approach to measure cutaneous microvascular function: An improved test of NO-mediated vasodilation by thermal hyperemia. *Journal of Applied Physiology, 117*(3), 277-283. doi:10.1152/japplphysiol.01397.2013

Colleoni, M., Sun, Z., Price, K. N., Karlsson, P., Forbes, J. F., Thurlimann, B., . . . Goldhirsch, A. (2016). Annual hazard rates of recurrence for breast cancer during 24 years of follow-up: Results from the International Breast Cancer Study Group trials I to V. *Journal of Clinical Oncology, 34*(9), 927-935. doi:10.1200/jco.2015.62.3504

Cornblath, D. R., Chaudhry, V., Carter, K., Lee, D., Seysedadr, M., Miernicki, M., & Joh, T. (1999). Total neuropathy score: Validation and reliability study. *Neurology, 53*(8), 1660-1664. doi:10.1212/wnl.53.8.1660

Cracowski, J. L., Minson, C. T., Salvat-Melis, M., & Halliwill, J. R. (2006). Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends in Pharmacological Sciences, 27*(9), 503-508. doi:10.1016/j.tips.2006.07.008

Cruccu, G., Sommer, C., Anand, P., Attal, N., Baron, R., Garcia-Larrea, L., . . . Treede, R. D. (2010). EFNS guidelines on neuropathic pain assessment: Revised 2009. *European Journal of Neurology, 17*(8), 1010-1018. doi:10.1111/j.1468-1331.2010.02969.x

Darby, L. M., Meng, H., & Fehrenbacher, J. C. (2017). Paclitaxel inhibits the activity and membrane localization of PKCalpha and PKCbetaI/II to elicit a decrease in stimulated calcitonin gene-related peptide release from cultured sensory neurons. *Molecular and Cellular Neuroscience, 82*, 105-117. https://doi.org/10.1016/j.mcn.2017.04.001

Davis, L. L., Carpenter, J. S., & Otte, J. L. (2016). State of the science: Taxane-induced musculoskeletal pain. *Cancer Nursing, 39*(3), 187-196. doi:10.1097/ncc.0000000000000273

Del Bianco, P., Zavagno, G., Burelli, P., Scalco, G., Barutta, L., Carraro, P., . . . De Salvo, G. L. (2008). Morbidity comparison of sentinel lymph node biopsy versus conventional axillary lymph node dissection for breast cancer patients: Results of the sentinella-GIVOM Italian randomised clinical trial. *European Journal of Surgical Oncology, 34*(5), 508-513. doi:10.1016/j.ejso.2007.05.017

Demicheli, R., Retsky, M. W., Swartzendruber, D. E., & Bonadonna, G. (1997). Proposal for a new model of breast cancer metastatic development. *Annals of Oncology, 8*(11), 1075-1080. doi:10.1023/a:1008263116022

Deshpande, A. D., Harris-Hayes, M., & Schootman, M. (2008). Epidemiology of diabetes and diabetes-related complications. *Physical Therapy, 88*(11), 1254-1264. doi:10.2522/ptj.20080020

Devigili, G., Tugnoli, V., Penza, P., Camozzi, F., Lombardi, R., Melli, G., . . . Lauria, G. (2008). The diagnostic criteria for small fibre neuropathy: From symptoms to neuropathology. *Brain, 131*(7), 1912-1925. doi:10.1093/brain/awn093

Dina, O. A., Chen, X., Reichling, D., & Levine, J. D. (2001). Role of protein kinase Cepsilon and protein kinase A in a model of Paclitaxel-induced painful peripheral neuropathy in the rat. *Neuroscience, 108*(3), 507-515. doi:10.1016/s0306-4522(01)00425-0

Donovan, D. (2009). Management of peripheral neuropathy caused by microtubule inhibitors. *Clinical Journal of Oncology Nursing, 13*(6), 686-694. doi:10.1188/09.cjon.686-694

Dougherty, P. M., Cata, J. P., Cordella, J. V., Burton, A., & Weng, H. R. (2004). Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients. *Pain, 109*(1-2), 132-142. doi:10.1016/j.pain.2004.01.021

Dranitsaris, G., Yu, B., Wang, L., Sun, W., Zhou, Y., King, J., . . .Yuan, P. (2016). Abraxane(R) versus Taxol(R) for patients with advanced breast cancer: A prospective time and motion analysis from a Chinese health care perspective. *Journal of Oncology Pharmacy Practice, 22*(2), 205-211. doi:10.1177/1078155214556008

Duale, C., Ouchchane, L., Schoeffler, P., & Dubray, C. (2014). Neuropathic aspects of persistent postsurgical pain: A French multi-center survey with a 6-month prospective follow-up. *Journal of Pain, 15*(1), 24.e21-24.e20. doi:10.1016/j.jpain.2013.08.014

Dubin, A. E., & Patapoutian, A. (2010). Nociceptors: The sensors of the pain pathway. *Journal of Clinical Investigation, 120*(11), 3760-3772. doi:10.1172/jci42843

Dusch, M., Schley, M., Rukwied, R., & Schmelz, M. (2007). Rapid flare development evoked by current frequency-dependent stimulation analyzed by full-field laser perfusion imaging. *Neuroreport, 18*(11), 1101-1105. doi:10.1097/WNR.0b013e3281e72cff

Dworkin, R. H., Backonja, M., Rowbotham, M. C., Allen, R. R., Argoff, C. R., Bennett, G. J., . . . Weinstein, S. M. (2003). Advances in neuropathic pain: Diagnosis, mechanisms, and treatment recommendations. *Archives of Neurology, 60*(11), 1524-1534. doi:10.1001/archneur.60.11.1524

England, J. D., Gronseth, G. S., Franklin, G., Miller, R. G., Asbury, A. K., Carter, G. T., . . . Sumner, A. J. (2005). Distal symmetric polyneuropathy: A definition for clinical research. Report of the American Academy of Neurology, the American Association

of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Neurology, 64*(2), 199-207. doi:10.1212/01.wnl.0000149522.32823.ea

Farooqi, M. A., Lovblom, L. E., Lysy, Z., Ostrovski, I., Halpern, E. M., Ngo, M., . . . Perkins, B. A. (2016). Validation of cooling detection threshold as a marker of sensorimotor polyneuropathy in type 2 diabetes. *Journal of Diabetes Complications, 30*(4), 716-722. doi:10.1016/j.jdiacomp.2015.12.023

Fassoulaki, A., Sarantopoulos, C., Melemeni, A., & Hogan, Q. (2000). EMLA reduces acute and chronic pain after breast surgery for cancer. *Regional Anesthesia and Pain Medicine, 25*(4), 350-355. doi:10.1053/rapm.2000.7812

Feldman, E. L., Nave, K. A., Jensen, T. S., & Bennett, D. L. (2017). New horizons in diabetic neuropathy: Mechanisms, bioenergetics, and pain. *Neuron, 93*(6), 1296-1313. doi:10.1016/j.neuron.2017.02.005

Feldweg, A. M., Lee, C.-W., Matulonis, U. A., & Castells, M. (2005). Rapid desensitization for hypersensitivity reactions to Paclitaxel and Docetaxel: A new standard protocol used in 77 successful treatments. *Gynecologic Oncology, 96*(3), 824-829. http://dx.doi.org/10.1016/j.ygyno.2004.11.043

Ferdinand, K. C., & Armani, A. M. (2007). The management of hypertension in African Americans. *Critical Pathways in Cardiology, 6*(2), 67-71. doi:10.1097/HPC.0b013e318053da59

Fernandes, J., & Kumar, S. (2016). Effect of lower limb closed kinematic chain exercises on balance in patients with chemotherapy-induced peripheral neuropathy: A pilot study. *International Journal of Rehabilitation Research, 39*(4), 368-371. doi:10.1097/mrr.0000000000000196

Ferrari, G., Nalassamy, N., Downs, H., Dana, R., & Oaklander, A. L. (2013). Corneal innervation as a window to peripheral neuropathies. *Experimental Eye Research, 113*, 148-150. doi:10.1016/j.exer.2013.05.016

Flatters, S. J., & Bennett, G. J. (2006). Studies of peripheral sensory nerves in Paclitaxel-induced painful peripheral neuropathy: Evidence for mitochondrial dysfunction. *Pain, 122*(3), 245-257. doi:10.1016/j.pain.2006.01.037

Fontanella, C., Bolzonello, S., Lederer, B., & Aprile, G. (2014). Management of breast cancer patients with chemotherapy-induced neutropenia or febrile neutropenia. *Breast Care, 9*(4), 239-245. doi:10.1159/000366466

Fontes, F., Pereira, S., Castro-Lopes, J. M., & Lunet, N. (2016). A prospective study on the neurological complications of breast cancer and its treatment: Updated analysis three years after cancer diagnosis. *The Breast, 29*, 31-38. doi:10.1016/j.breast.2016.06.013

Food and Drug Administration. (2011). Package Insert for Taxol® (Paclitaxel). In *Food and Drug Administration* (pp. 53). Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020262s049lbl.pdf

Forsyth, P. A., Balmaceda, C., Peterson, K., Seidman, A. D., Brasher, P., & DeAngelis, L. M. (1997). Prospective study of Paclitaxel-induced peripheral neuropathy with quantitative sensory testing. *Journal of Neuro-Oncology, 35*(1), 47-53. doi:10.1023/a:1005805907311

Freeman, R. (2007). Autonomic peripheral neuropathy. *Neurologic Clinics, 25*(1), 277-301. doi:10.1016/j.ncl.2007.01.001

Freeman, S. R., Washington, S. J., Pritchard, T., Barr, L., Baildam, A. D., & Bundred, N. J. (2003). Long term results of a randomised prospective study of preservation of the intercostobrachial nerve. *European Journal of Surgical Oncology , 29*(3), 213-215. doi:10.1053/ejso.2002.1409Fromy, B., Abraham, P., & Saumet, J. L. (1998). Non-nociceptive capsaicin-sensitive nerve terminal stimulation allows for an original vasodilatory reflex in the human skin. *Brain Research, 811*(1-2), 166-168. https://doi.org/10.1016/S0006-8993(98)00973-1

Fromy, B., Abraham, P., & Saumet, J. L. (2000). Progressive calibrated pressure device to measure cutaneous blood flow changes to external pressure strain. *Brain Research Protocols, 5*(2), 198-203. doi:10.1016/s1385-299x(00)00013-1

Fromy, B., Merzeau, S., Abraham, P., & Saumet, J. L. (2000). Mechanisms of the cutaneous vasodilator response to local external pressure application in rats: Involvement of CGRP, neurokinins, prostaglandins and NO. *British Journal of Pharmacology, 131*(6), 1161-1171. doi:10.1038/sj.bjp.0703685

Fryar, C. D., Gu, Q., & Ogden, C. L. (2012). Anthropometric reference data for children and adults: United States, 2007-2010. *Vital and Health Statistics, 11*(252), 1-48. Retrieved from https://www.cdc.gov/nchs/data/series/sr_11/sr11_252.pdf

Galiè, E., Villani, V., Terrenato, I., & Pace, A. (2017). Tapentadol in neuropathic pain cancer patients: A prospective open label study. *Neurological Sciences*, *38*(10), 1747-1752. https://doi.org/10.1007/s10072-017-3035-1

Gandhi, S., Fletcher, G. G., Eisen, A., Mates, M., Freedman, O. C., Dent, S. F., & Trudeau, M. E. (2015). Adjuvant chemotherapy for early female breast cancer: A systematic review of the evidence for the 2014 Cancer Care Ontario systemic therapy guideline. *Current Oncology, 22*(Suppl 1), S82-94. doi:10.3747/co.22.2321

Gartner, R., Jensen, M. B., Nielsen, J., Ewertz, M., Kroman, N., & Kehlet, H. (2009). Prevalence of and factors associated with persistent pain following breast cancer surgery. *Journal of the American Medical Association, 302*(18), 1985-1992. doi:10.1001/jama.2009.1568

Ghersi, D., Wilcken, N., Simes, J., & Donoghue, E. (2005). Taxane containing regimens for metastatic breast cancer. *Cochrane Database of Systematic Reviews, 2*, 1-78. doi:10.1002/14651858.CD003366.pub2

Ghersi, D., Willson, M. L., Chan, M. M., Simes, J., Donoghue, E., & Wilcken, N. (2015). Taxane-containing regimens for metastatic breast cancer. *Cochrane Database Systematic Reviews, 6*,1-173. doi:10.1002/14651858.CD003366.pub3

Gianni, L., Munzone, E., Capri, G., Fulfaro, F., Tarenzi, E., Villani, F., . . . et al. (1995). Paclitaxel by 3-hour infusion in combination with bolus Doxorubicin in women with untreated metastatic breast cancer: High antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study. *Journal of Clinical Oncology, 13*(11), 2688-2699. http://doi.org/10.1200/JCO.1995.13.11.2688

Gianni, L., Munzone, E., Capri, G., Villani, F., Spreafico, C., Tarenzi, E., . . . et al. (1995). Paclitaxel in metastatic breast cancer: A trial of two doses by a 3-hour infusion in patients with disease recurrence after prior therapy with anthracyclines. *Journal of the National Cancer Institute, 87*(15), 1169-1175. https://doi.org/10.1093/jnci/87.15.1169

Gilardini, A., Avila, R. L., Oggioni, N., Rodriguez-Menendez, V., Bossi, M., Canta, A., . . . Kirschner, D. A. (2012). Myelin structure is unaltered in chemotherapy-induced peripheral neuropathy. *NeuroToxicology, 33*(1), 1-7. doi:10.1016/j.neuro.2011.10.010

Ginés, J., Sabater, E., Martorell, C., Grau, M., Monroy, M., & Casado, M. A. (2011). Efficacy of Taxanes as adjuvant treatment of breast cancer: A review and meta-analysis of randomised clinical trials. *Clinical and Translational Oncology, 13*(7), 485-498. doi:10.1007/s12094-011-0686-x

Ginsberg, J. (2003). Discovery of Camptothecin and Taxol: National historic chemical landmark [Press release]. Retrieved from https://www.acs.org/content/acs/en/education/whatischemistry/landmarks/camptothecintaxol.html

Gosk, J., Rutowski, R., Urban, M., Wiecek, R., & Rabczynski, J. (2007). Brachial plexus injuries after radiotherapy - analysis of 6 cases. *Folia Neuropathologica, 45*(1), 31-35. Retrieved from https://www.termedia.pl/Orginal-article-Brachial-plexus-injuries-after-radiotherapy-8211-analysis-of-6-cases,20,7663,0,1.html

Gottrup, H., Andersen, J., Arendt-Nielsen, L., & Jensen, T. S. (2000). Psychophysical examination in patients with post-mastectomy pain. *Pain, 87*(3), 275-284.

Gracias, N. (2011). *Paclitaxel alters the function of the small diameter sensory neurons* (Doctoral dissetation). Retrieved from http://ulib.iupui.edu/cgi-bin/proxy.pl?url=/docview/872087953?accountid=7398 Dissertations & Theses @ CIC Institutions; ProQuest Dissertations & Theses A&I database. (Accesssion number: 3456463)

Gracias, N., Cummins, T. R., Kelley, M. R., Basile, D. P., Iqbal, T., & Vasko, M. R. (2011). Vasodilatation in the rat dorsal hindpaw induced by activation of sensory neurons is reduced by Paclitaxel. *NeuroToxicology, 32*(1), 140-149. doi:10.1016/j.neuro.2010.09.006

Gracias, N. G., Cummins, T. R., Kelley, M. R., Basile, D. P., Iqbal, T., & Vasko, M. R. (2011). Vasodilatation in the rat dorsal hindpaw induced by activation of sensory

neurons is reduced by Paclitaxel. *NeuroToxicology, 32*(1), 140-149. http://dx.doi.org/10.1016/j.neuro.2010.09.006

Gralow, J., Rugo, H., Gradishar, W., O'Shaughnessy, J. A., Jahanzeb, M., Perez, E., & Tripathy, D. (2008). Novel Taxane formulations in the treatment of breast cancer: A thought leader discussion and consensus roundtable. *Clinical Breast Cancer, 8*(1), 33-37. doi:10.3816/CBC.2008.n.046

Green, A. Q., Krishnan, S., Finucane, F. M., & Rayman, G. (2010). Altered C-fiber function as an indicator of early peripheral neuropathy in individuals with impaired glucose tolerance. *Diabetes Care, 33*(1), 174-176. doi:10.2337/dc09-0101

Green, A. Q., Krishnan, S. T., & Rayman, G. (2009). C-fiber function assessed by the laser doppler imager flare technique and acetylcholine iontophoresis. *Muscle and Nerve, 40*(6), 985-991. doi:10.1002/mus.21333

Greenlee, H., Hershman, D. L., Shi, Z., Kwan, M. L., Ergas, I. J., Roh, J. M., & Kushi, L. H. (2017). BMI, lifestyle factors and Taxane-induced neuropathy in breast cancer patients: The pathways study. *Journal of the National Cancer Institute, 109*(2). doi:10.1093/jnci/djw206

Grem, J. L., Tutsch, K. D., Simon, K. J., Alberti, D. B., Willson, J. K., Tormey, D. C., . . . Trump, D. L. (1987). Phase I study of Taxol administered as a short i.v. infusion daily for 5 days. *Cancer Treatment Report, 71*(12), 1179-1184. PMID: 2891442

Griffith, K. A., Dorsey, S. G., Renn, C. L., Zhu, S., Johantgen, M. E., Cornblath, D. R., . . . Valsecchi, M. G. (2014). Correspondence between neurophysiological and clinical measurements of chemotherapy-induced peripheral neuropathy: Secondary analysis of data from the CI-PeriNomS study. *Journal of Peripheral Nervous System, 19*(2), 127-135. doi:10.1111/jns5.12064

Griffith, K. A., Dorsey, S. G., Renn, C. L., Zhu, S., Johantgen, M. E., Cornblath, D. R., . . . Valsecchi, M. G. (2014). Correspondence between neurophysiological and clinical measurements of chemotherapy-induced peripheral neuropathy: Secondary analysis of data from the CI-PeriNoms study. *Journal of Peripheral Nervous System*. doi:10.1111/jns5.12064

Griffith, K. A., Merkies, I. S., Hill, E. E., & Cornblath, D. R. (2010). Measures of chemotherapy-induced peripheral neuropathy: A systematic review of psychometric properties. *Journal of Peripheral Nervous System, 15*(4), 314-325. doi:10.1111/j.1529-8027.2010.00292.x

Grigoriev, I. S., Chernobelskaya, A. A., & Vorobjev, I. A. (1999). Nocodazole, Vinblastine and Taxol at low concentrations affect fibroblast locomotion and saltatory movements of organelles. *Membrane and Cell Biology, 13*(1), 23-48. Retrieved from http://cellmotility.genebee.msu.su/html/articles/grigoriev99.pdf

Gruener, G., & Dyck, P. J. (1994). Quantitative sensory testing: Methodology, applications, and future directions. *Journal of Clinical Neurophysiology, 11*(6), 568-583. doi:10.1097/00004691-199411000-00004

Han, Y., & Smith, M. T. (2013). Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). *Frontiers in Pharmacology, 4*, 156. doi:10.3389/fphar.2013.00156

Hassett, M. J., O'Malley, A. J., Pakes, J. R., Newhouse, J. P., & Earle, C. C. (2006). Frequency and cost of chemotherapy-related serious adverse effects in a population sample of women with breast cancer. *Journal of the National Cancer Institute, 98*(16), 1108-1117. doi:10.1093/jnci/djj305

Hausheer, F. H. (2008). Chemotherapy-induced peripheral neuropathy. In M. C. Perry (Ed.), *The chemotherapy source book* (4th ed.). Philadelphia, PA: Wolters Kluwer Health/Lippencott, Williams & Wilkins.

Hausheer, F. H., Schilsky, R. L., Bain, S., Berghorn, E. J., & Lieberman, F. (2006). Diagnosis, management, and evaluation of chemotherapy-induced peripheral neuropathy. *Seminars in Oncology, 33*(1), 15-49. doi:10.1053/j.seminoncol.2005.12.010

Henry, N. L., Azzouz, F., Desta, Z., Li, L., Nguyen, A. T., Lemler, S., . . . Storniolo, A. M. (2012). Predictors of aromatase inhibitor discontinuation as a result of treatment-emergent symptoms in early-stage breast cancer. *Journal of Clinical Oncology, 30*(9), 936-942. doi:10.1200/jco.2011.38.0261

Herman, R. M., Brower, J. B., Stoddard, D. G., Casano, A. R., Targovnik, J. H., Herman, J. H., & Tearse, P. (2007). Prevalence of somatic small fiber neuropathy in obesity. *International Journal of Obesity, 31*(2), 226-235. doi:10.1038/sj.ijo.0803418

Hershman, D. L., Lacchetti, C., Dworkin, R. H., Lavoie Smith, E. M., Bleeker, J., Cavaletti, G., . . . Loprinzi, C. L. (2014). Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline. *Journal of Clinical Oncology, 32*(18), 1941-1967. doi:10.1200/jco.2013.54.0914

Hershman, D. L., Weimer, L. H., Wang, A. T., Kranwinkel, G., Brafman, L., Fuentes, D., . . . Crew, K. D. (2011). Association between patient reported outcomes and quantitative sensory tests for measuring long-term neurotoxicity in breast cancer survivors treated with adjuvant Paclitaxel chemotherapy. *Breast Cancer Research and Treatment, 125*(3), 767-774. doi:10.1007/s10549-010-1278-0

Hertz, D. L., Motsinger-Reif, A. A., Drobish, A., Winham, S. J., McLeod, H. L., Carey, L. A., & Dees, E. C. (2012). CYP2C8*3 predicts benefit/risk profile in breast cancer patients receiving neoadjuvant Paclitaxel. *Breast Cancer Research and Treatment, 134*(1), 401-410. doi:10.1007/s10549-012-2054-0

Hess, L. M., Rajan, N., Winfree, K., Davey, P., Ball, M., Knox, H., & Graham, C. (2015). Cost analyses in the US and Japan: A cross-country comparative analysis applied to the PRONOUNCE Trial in non-squamous non-small cell lung cancer. *Advanced Therapy, 32*(12), 1248-1262. doi:10.1007/s12325-015-0270-9

Hile, E. S., Fitzgerald, G. K., & Studenski, S. A. (2010). Persistent mobility disability after neurotoxic chemotherapy. *Physical Therapy, 90*(11), 1649-1657. doi:10.2522/ptj.20090405

Hirayama, Y., Sasaki, J., Dosaka-Akita, H., & Ishitani, K. (2016). Survey of the management of chemotherapy-induced peripheral neuropathy in Japan: Japanese Society of Medical Oncology. *ESMO Open, 1*(3), e000053. doi:10.1136/esmoopen-2016-000053

Hodges, G. J., & Sparks, P. A. (2013). Contributions of endothelial nitric oxide synthase, noradrenaline, and neuropeptide Y to local warming-induced cutaneous

vasodilatation in men. *Microvascular Research, 90*, 128-134. doi:10.1016/j.mvr.2013.08.011

Holmes, F. A., Walters, R. S., Theriault, R. L., Forman, A. D., Newton, L. K., Raber, M. N., . . . Hortobagyi, G. N. (1991a). Phase-II trail of Taxol, an active-drug in the treatment of metastatic breast-cancer. *Journal of the National Cancer Institute, 83*(24), 1797-1805. doi:10.1093/jnci/83.24.1797-a

Hong, J. S., Tian, J., & Wu, L. H. (2014). The influence of chemotherapy-induced neurotoxicity on psychological distress and sleep disturbance in cancer patients. *Current Oncology, 21*(4), 174-180. doi:10.3747/co.21.1984

Houghton, B. L., Meendering, J. R., Wong, B. J., & Minson, C. T. (2006). Nitric oxide and noradrenaline contribute to the temperature threshold of the axon reflex response to gradual local heating in human skin. *Journal of Physiology, 572*(3), 811-820. doi:10.1113/jphysiol.2005.104067

Howard, J., & Hyman, A. A. (2003). Dynamics and mechanics of the microtubule plus end. *Nature, 422*(6933), 753-758. doi:10.1038/nature01600

Hsiu, H., Hsu, W. C., Chang, S. L., Hsu, C. L., Huang, S. M., & Lin, Y. Y. (2008). Microcirculatory effect of different skin contacting pressures around the blood pressure. *Physiological Measurement, 29*(12), 1421-1434. doi:10.1088/0967-3334/29/12/006

Huang, T. C., & Campbell, T. C. (2012). Comparison of weekly versus every 3 weeks Paclitaxel in the treatment of advanced solid tumors: A meta-analysis. *Cancer Treatment Review, 38*(6), 613-617. doi:10.1016/j.ctrv.2011.10.008

Huang, C. S., Wang, S. F., & Tsai, Y. F. (2012). Axon reflex-related hyperemia induced by short local heating is reproducible. *Microvascular Research, 84*(3), 351-355. doi:10.1016/j.mvr.2012.07.003

Huang, C. S., Wang, S. F., & Tsai, Y. F. (2013). Increasing acclimation period improves the reproducibility of short-heating local thermal hyperemia. *Microvascular Research, 85*, 93-98. doi:10.1016/j.mvr.2012.11.004

Ingemar Fredriksson, M. L., and Tomas Stromberg. (2012). Laser doppler flowmetry. In M. J. Leahy (Ed.), *Microcirculation Imaging* (pp. 67-84). Weinheim, Germany: Wiley-Blackwell.

Inoue, K., Ohkubo, F., Nagai, S., Tsuboi, M., Kubo, K., Kurozumi, K., . . . & Takei, H. (2013). Efficacy and tolerability of controlled-release oxycodone against nab-Paclitaxel-induced musculoskeletal pain in breast cancer patients. *Gan To Kagaku Ryoho. Cancer & Chemotherapy*, *40*(8), 1021-1025. PMID: 23986044

Iredahl, F., Lofberg, A., Sjoberg, F., Farnebo, S., & Tesselaar, E. (2015). Non-invasive measurement of skin microvascular response during pharmacological and physiological provocations. *Plos One, 10*(8), e0133760. doi:10.1371/journal.pone.0133760

Isah, T. (2016). Anticancer alkaloids from trees: Development into drugs. *Pharmacognsy Reviews, 10*(20), 90-99. doi:10.4103/0973-7847.194047

Ivens, D., Hoe, A. L., Podd, T. J., Hamilton, C. R., Taylor, I., & Royle, G. T. (1992). Assessment of morbidity from complete axillary dissection. *British Journal of Cancer, 66*(1), 136-138. doi:10.1038/bjc.1992.230

Jansen, C. E., Cooper, B. A., Dodd, M. J., & Miaskowski, C. A. (2011). A prospective longitudinal study of chemotherapy-induced cognitive changes in breast cancer patients. *Supportive Care in Cancer, 19*(10), 1647-1656. doi:10.1007/s00520-010-0997-4

Jimenez-Andrade, J. M., Peters, C. M., Mejia, N. A., Ghilardi, J. R., Kuskowski, M. A., & Mantyh, P. W. (2006). Sensory neurons and their supporting cells located in the trigeminal, thoracic, and lumbar ganglia differentially express markers of injury following intravenous administration of Paclitaxel in the rat. *Neuroscience Letters, 405*(1-2), 62-67. doi:10.1016/j.neulet.2006.06.043

Johansson, S., Svensson, H., & Denekamp, J. (2000). Timescale of evolution of late radiation injury after postoperative radiotherapy of breast cancer patients. *International Journal of Radiation Oncology Biology • Physics, 48*(3), 745-750. doi:10.1016/s0360-3016(00)00674-x

Johnson, C., Pankratz, V. S., Velazquez, A. I., Aakre, J. A., Loprinzi, C. L., Staff, N. P., . . . Yang, P. (2015). Candidate pathway-based genetic association study of platinum and platinum-Taxane related toxicity in a cohort of primary lung cancer patients. *Journal Neurological Science, 349*(1-2), 124-128. doi:10.1016/j.jns.2014.12.041

Jordan, M. A., & Wilson, L. (2004). Microtubules as a target for anticancer drugs. *Nature Reviews Cancer, 4*(4), 253-265. doi:10.1038/nrc1317

Kanbayashi, Y., Hosokawa, T., Okamoto, K., Konishi, H., Otsuji, E., Yoshikawa, T., . . . Taniwaki, M. (2010). Statistical identification of predictors for peripheral neuropathy associated with administration of Bortezomib, Taxanes, Oxaliplatin or Vincristine using ordered logistic regression analysis. *Anticancer Drugs, 21*(9), 877-881. doi:10.1097/CAD.0b013e32833db89d

Kawashiri, T., Egashira, N., Itoh, Y., Shimazoe, T., Ikegami, Y., Yano, T., . . . Oishi, R. (2009). Neurotropin reverses Paclitaxel-induced neuropathy without affecting anti-tumour efficacy. *European Journal of Cancer, 45*(1), 154-163. http://dx.doi.org/10.1016/j.ejca.2008.10.004

Kelly, P. J., Dinkin, M. J., Drappatz, J., O'Regan, K. N., & Weiss, S. E. (2011). Unexpected late radiation neurotoxicity following Bevacizumab use: A case series. *Journal of Neuro-Oncology, 102*(3), 485-490. doi:10.1007/s11060-010-0336-0

Kiernan, M. C. (2012). Emergence of a predictive clinical biomarker for diabetic neuropathy. *Diabetes, 61*, 1346-1347. doi:10.2337/db12-0193

Kim, H. Y., Kang, J. H., Youn, H. J., So, H. S., Song, C. E., Chae, S. Y., . . . Kim, J. Y. (2014). Reliability and validity of the Korean version of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire to assess chemotherapy-induced peripheral neuropathy. *Journal of the Korean Academy of Nursing, 44*(6), 735-742. doi:10.4040/jkan.2014.44.6.735

Kneis, S., Wehrle, A., Freyler, K., Lehmann, K., Rudolphi, B., Hildenbrand, B., . . . Ritzmann, R. (2016). Balance impairments and neuromuscular changes in breast cancer patients with chemotherapy-induced peripheral neuropathy. *Clinical Neurophysiology, 127*(2), 1481-1490. doi:10.1016/j.clinph.2015.07.022

Kosturakis, A. K., He, Z., Li, Y., Boyette-Davis, J. A., Shah, N., Thomas, S. K., . . . Dougherty, P. M. (2014). Subclinical peripheral neuropathy in patients with multiple myeloma before chemotherapy is correlated with decreased fingertip innervation density. *Journal of Clinical Oncology, 32*(28), 3156-3162. doi:10.1200/jco.2013.54.5418

Kramer, H. H., Rolke, R., Bickel, A., & Birklein, F. (2004). Thermal thresholds predict painfulness of diabetic neuropathies. *Diabetes Care, 27*(10), 2386-2391. doi:10.2337/diacare.27.10.2386

Krämer, H. H., Schmelz, M., Birklein, F., & Bickel, A. (2004). Electrically stimulated axon reflexes are diminished in diabetic small fiber neuropathies. *Diabetes, 53*(3), 769-774. doi:10.2337/diabetes.53.3.769

Kris, M. G., O'Connell, J. P., Gralla, R. J., Wertheim, M. S., Parente, R. M., Schiff, P. B., & Young, C. W. (1986). Phase I trial of Taxol given as a 3-hour infusion every 21 days. *Cancer Treatment Report, 70*(5), 605-607. https://doi.org/10.1016/0378-8741(86)90014-0

Krishnan, S. T., Baker, N. R., Carrington, A. L., & Rayman, G. (2004). Comparative roles of microvascular and nerve function in foot ulceration in type 2 diabetes. *Diabetes Care, 27*(6), 1343-1348. https://doi.org/10.2337/diacare.27.6.1343 Krishnan, S. T., Quattrini, C., Jeziorska, M., Malik, R. A., & Rayman, G. (2007). Neurovascular factors in wound healing in the foot skin of type 2 diabetic subjects. *Diabetes Care, 30*(12), 3058-3062. doi:10.2337/dc07-1421

Krishnan, S. T., Quattrini, C., Jeziorska, M., Malik, R. A., & Rayman, G. (2009). Abnormal LDI flare but normal quantitative sensory testing and dermal nerve fiber density in patients with painful diabetic neuropathy. *Diabetes Care, 32*(3), 451-455. doi:10.2337/dc08-1453

Krishnan, S. T., & Rayman, G. (2004). The LDI flare: A novel test of C-fiber function demonstrates early neuropathy in type 2 diabetes. *Diabetes Care, 27*(12), 2930-2935. https://doi.org/10.2337/diacare.27.12.2930

Krishnan, S. T. M., Quattrini, C., Jeziorska, M., Malik, R. A., & Rayman, G. (2009). Abnormal LDIflare but normal quantitative sensory testing and dermal nerve fiber

density in patients with painful diabetic neuropathy. *Diabetes Care, 32*(3), 451-455. doi:10.2337/dc08-1453

Krishnan, S. T. M., & Rayman, G. (2004). The LDIflare: A novel test of C-fiber function demonstrates early neuropathy in type 2 diabetes. *Diabetes Care, 27*(12), 2930-2935. doi:10.2337/diacare.27.12.2930

Kuehn, T., Klauss, W., Darsow, M., Regele, S., Flock, F., Maiterth, C., . . . Kreienberg, R. (2000). Long-term morbidity following axillary dissection in breast cancer patients--clinical assessment, significance for life quality and the impact of demographic, oncologic and therapeutic factors. *Breast Cancer Research and Treatment, 64*(3), 275-286. doi:10.1023/a:1026564723698

Kumar, N. (1981). Taxol-induced polymerization of purified tubulin. Mechanism of action. *Journal of Biological Chemistry, 256*(20), 10435-10441. Retrieved from http://www.jbc.org/

Kuroi, K., Shimozuma, K., Ohashi, Y., Hisamatsu, K., Masuda, N., Takeuchi, A., . . . Hausheer, F. H. (2009). Prospective assessment of chemotherapy-induced peripheral neuropathy due to weekly Paclitaxel in patients with advanced or metastatic breast cancer (CSP-HOR 02 study). *Supportive Care in Cancer, 17*(8), 1071-1080. doi:10.1016/S1470-2045(06)70910-X

Lam, S. W., Frederiks, C. N., van der Straaten, T., Honkoop, A. H., Guchelaar, H. J., & Boven, E. (2016). Genotypes of CYP2C8 and FGD4 and their association with peripheral neuropathy or early dose reduction in Paclitaxel-treated breast cancer patients. *British Journal of Cancer, 115*(11), 1335-1342. doi:10.1038/bjc.2016.326

Langford, D. J., Paul, S. M., West, C., Levine, J. D., Hamolsky, D., Elboim, C., . . . Miaskowski, C. (2014). Persistent breast pain following breast cancer surgery is associated with persistent sensory changes, pain interference, and functional impairments. *Journal of Pain, 15*(12), 1227-1237. doi:10.1016/j.jpain.2014.08.014

Latov, N. (2007). *Peripheral Neuropathy: When the Numbness, Weakness and Pain Won't Stop*. New York, NY: Demos Health.

Lavoie Smith, E. M. (2010). Chapter 4: Interventions for side-effects of treatment: Peripheral neuropathy. In L. Urquhart (Ed.), *A nurse's guide to caring for cancer survivors: Breast cancer*. Sudbery, MA: Jones & Bartlett Publishers.

Lavoie Smith, E. M., Bakitas, M. A., Homel, P., Fadul, C., Meyer, L., Skalla, K., & Bookbinder, M. (2009). Using quality improvement methodology to improve neuropathic pain screening and assessment in patients with cancer. *Journal of Cancer Education, 24*(2), 135-140. doi:10.1080/08858190902854715

Lavoie Smith, E. M., Barton, D. L., Qin, R., Steen, P. D., Aaronson, N. K., & Loprinzi, C. L. (2013). Assessing patient-reported peripheral neuropathy: The reliability and validity of the European Organization for Research and Treatment of Cancer QLQ-CIPN20 questionnaire. *Quality of Life Research, 22*(10), 2787-2799. doi:10.1007/s11136-013-0379-8

Lavoie Smith, E. M., Cohen, J. A., Pett, M. A., & Beck, S. L. (2011). The validity of neuropathy and neuropathic pain measures in patients with cancer. *Oncology Nursing Forum, 38*(2), 133-142. doi:10.1188/11.onf.133-142

Lee, J. J., & Swain, S. M. (2006). Peripheral neuropathy induced by microtubule-stabilizing agents. *Journal of Clinical Oncology, 24*(10), 1633-1642. doi:10.1200/JCO.2005.04.0543

Lei, J., & You, H. J. (2012). Variation of pain and vasomotor responses evoked by intramuscular infusion of hypertonic saline in human subjects: Influence of gender and its potential neural mechanisms. *Brain Research Bulletin, 87*(6), 564-570. doi:10.1016/j.brainresbull.2011.11.003

Lei, J., You, H. J., Andersen, O. K., Graven-Nielsen, T., & Arendt-Nielsen, L. (2008). Homotopic and heterotopic variation in skin blood flow and temperature following experimental muscle pain in humans. *Brain Research, 1232*, 85-93. doi:10.1016/j.brainres.2008.07.056

Leskela, S., Jara, C., Leandro-Garcia, L. J., Martinez, A., Garcia-Donas, J., Hernando, S., . . . Rodriguez-Antona, C. (2011). Polymorphisms in cytochromes P450 2C8 and 3A5 are associated with Paclitaxel neurotoxicity. *Pharmacogenomics Journal, 11*(2), 121-129. doi:10.1038/tpj.2010.13

Li Pi Shan, R., Nicolle, M., Chan, M., Ashworth, N., White, C., Winston, P., & Dukelow, S. (2016). Electrodiagnostic testing and treatment for carpal tunnel syndrome in Canada. *Canadian Journal of Neurological Sciences, 43*(1), 178-182. doi:10.1017/cjn.2015.323

Li, X., Lyu, P., Ren, Y., An, J., & Dong, Y. (2017). Arterial stiffness and cognitive impairment. *Journal of Neurological Sciences, 380*, 1-10. doi:10.1016/j.jns.2017.06.018

Lin, N. U., Parker, L. M., Come, S. E., Burstein, H. J., Haldoupis, M., Ryabin, N., . . . Shulman, L. N. (2007). Phase II study of CT-2103 as first- or second-line chemotherapy in patients with metastatic breast cancer: Unexpected incidence of hypersensitivity reactions. *Investigational New Drugs, 25*(4), 369-375. doi:10.1007/s10637-007-9034-y

Lin, Y.-S., Jen, Y.-M., & Lin, J.-C. (2002). Radiation-related cranial nerve palsy in patients with nasopharyngeal carcinoma. *Cancer, 95*(2), 404-409. doi:10.1002/cncr.10668

Lipton, R. B., Apfel, S. C., Dutcher, J. P., Rosenberg, R., Kaplan, J., Berger, A., . . . Schaumburg, H. H. (1989). Taxol produces a predominantly sensory neuropathy. *Neurology, 39*(3), 368-373. doi:10.1212/wnl.39.3.368

Liu, Y., Ye, G., Yan, D., Zhang, L., Fan, F., & Feng, J. (2017). Role of nab-Paclitaxel in metastatic breast cancer: A meta-analysis of randomized clinical trials. *Oncotarget, 8*(42), 72950. doi:10.18632/oncotarget.18900

Loprinzi, C. L., Reeves, B. N., Dakhil, S. R., Sloan, J. A., Wolf, S. L., Burger, K. N., . . . Lachance, D. H. (2011a). Natural history of Paclitaxel-associated acute pain syndrome: Prospective cohort study NCCTG N08C1. *Journal of Clinical Oncology, 29*(11), 1472-1478. doi:10.1200/jco.2010.33.0308

Loprinzi, C. L., Reeves, B. N., Dakhil, S. R., Sloan, J. A., Wolf, S. L., Burger, K. N., . . . Lachance, D. H. (2011b). Natural history of Paclitaxel-associated acute pain syndrome: Prospective cohort study NCCTG N08C1. *Journal of Clinical Oncology, 29*, 1472-1478. doi:10.1200/JCO.2010.33.0308

Lyman, G. H., Abella, E., & Pettengell, R. (2014). Risk factors for febrile neutropenia among patients with cancer receiving chemotherapy: A systematic review. *Critical Reviews of Oncology / Hematology, 90*(3), 190-199. doi:10.1016/j.critrevonc.2013.12.006

Lyman, G. H., Dale, D. C., Tomita, D., Whittaker, S., & Crawford, J. (2013). A retrospective evaluation of chemotherapy dose intensity and supportive care for early-stage breast cancer in a curative setting. *Breast Cancer Research and Treatment, 139*(3), 863-872. doi:10.1007/s10549-013-2582-2

Lysy, Z., Lovblom, L. E., Halpern, E. M., Ngo, M., Ng, E., Orszag, A., . . . Perkins, B. A. (2014). Measurement of cooling detection thresholds for identification of diabetic sensorimotor polyneuropathy in type 1 diabetes. *PLoS One, 9*(9), e106995. doi:10.1371/journal.pone.0106995

Mahe, G., Durand, S., Humeau-Heurtier, A., Leftheriotis, G., & Abraham, P. (2012). Impact of experimental conditions on noncontact laser recordings in microvascular studies. *Microcirculation, 19*(8), 669-675. doi:10.1111/j.1549-8719.2012.00205.x

Mahe, G., Durand, S., Humeau, A., Leftheriotis, G., Rousseau, P., & Abraham, P. (2012). Air movements interfere with laser speckle contrast imaging recordings. *Lasers in Medical Science, 27*(5), 1073-1076. doi:10.1007/s10103-011-1015-x

Mahe, G., Humeau-Heurtier, A., Durand, S., Leftheriotis, G., & Abraham, P. (2012). Assessment of skin microvascular function and dysfunction with laser speckle contrast imaging. *Circulation: Cardiovascular Imaging, 5*(1), 155-163. doi:10.1161/CIRCIMAGING.111.970418

Majithia, N., Loprinzi, C. L., & Smith, T. J. (2016). New Practical approaches to chemotherapy-induced neuropathic pain: Prevention, assessment, and treatment. *Oncology, 30*(11). Retrieved from http://www.cancernetwork.com/

Majithia, N., Temkin, S. M., Ruddy, K. J., Beutler, A. S., Hershman, D. L., & Loprinzi, C. L. (2016). National Cancer Institute-supported chemotherapy-induced peripheral neuropathy trials: Outcomes and lessons. *Support Care Cancer, 24*(3), 1439-1447. doi:10.1007/s00520-015-3063-4

Manfredi, J. J., Parness, J., & Horwitz, S. B. (1982). Taxol binds to cellular microtubules. *Journal of Cell Biology, 94*(3), 688-696. doi:10.1083/jcb.94.3.688

Markman, M. (2006b). Chemotherapy-induced peripheral neuropathy: Underreported and underappreciated. *Current Pain and Headache Reports, 10*(4), 275-278. doi:10.1007/s11916-006-0032-0

Marshall, T., Zipp, G. P., Battaglia, F., Moss, R., & Bryan, S. (2016). Effects of chemotherapy-induced-peripheral-neuropathy on spatiotemporal gait parameters and fall risk in cancer patients after the completion of chemotherapy drug treatment. *Archives of Physical Medicine and Rehabilitation, 97*(12), e13-e14. http://dx.doi.org/10.1016/j.apmr.2016.09.036

Matsuoka, A., Mitsuma, A., Maeda, O., Kajiyama, H., Kiyoi, H., Kodera, Y., . . . Ando, Y. (2016). Quantitative assessment of chemotherapy-induced peripheral neurotoxicity using a point-of-care nerve conduction device. *Cancer Science, 107*(10), 1453-1457. doi:10.1111/cas.13010

Maycock, L. A., Dillon, P., & Dixon, J. M. (1998). Morbidity related to intercostobrachial nerve damage following axillary surgery for breast cancer. *The Breast, 7*(4), 209-212. http://dx.doi.org/10.1016/S0960-9776(98)90110-2

McGarr, G. W., Hodges, G. J., & Cheung, S. S. (2017). Between-day reliability of local thermal hyperemia in the forearm and index finger using single-point laser Doppler flowmetry. *Microcirculation, 24*(8). doi:10.1111/micc.12395

McGuire, W. P., Rowinsky, E. K., Rosenshein, N. B., Grumbine, F. C., Ettinger, D. S., Armstrong, D. K., & Donehower, R. C. (1989). Taxol: A unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. *Annals of Internal Medicine, 111*(4), 273-279. doi:10.7326/0003-4819-111-4-273

Mejdahl, M. K., Andersen, K. G., Gartner, R., Kroman, N., & Kehlet, H. (2013). Persistent pain and sensory disturbances after treatment for breast cancer: Six-year nationwide follow-up study. *BMJ, 346*, f1865. doi:10.1136/bmj.f1865

Melli, G., Jack, C., Lambrinos, G. L., Ringkamp, M., & Hoke, A. (2006). Erythropoietin protects sensory axons against Paclitaxel-induced distal degeneration. *Neurobiology of Disease, 24*(3), 525-530. doi:10.1016/j.nbd.2006.08.014

Metzler-Wilson, K., Kellie, L. A., Tomc, C., Simpson, C., Sammons, D., & Wilson, T. E. (2012). Differential vasodilatory responses to local heating in facial, glabrous and hairy skin. *Clinical Physiology and Functional Imaging, 32*(5), 361-366. doi:10.1111/j.1475-097X.2012.01137.x

Miaskowski, C., Mastick, J., Paul, S. M., Topp, K., Smoot, B., Abrams, G., . . . Levine, J. D. (2017). Chemotherapy-Induced neuropathy in cancer Survivors. *Journal of Pain and Symptom Management, 54*(2), 204-218. doi:10.1016/j.jpainsymman.2016.12.342

Miaskowski, C., Paul, S. M., Cooper, B., West, C., Levine, J. D., Elboim, C., . . . Aouizerat, B. E. (2014). Identification of patient subgroups and risk factors for persistent arm/shoulder pain following breast cancer surgery. *European Journal of Oncology Nursing, 18*(3), 242-253. doi:10.1016/j.ejon.2013.12.002

Mielke, S., Mross, K., Gerds, T. A., Schmidt, A., Wasch, R., Berger, D. P., . . . Behringer, D. (2003). Comparative neurotoxicity of weekly non-break Paclitaxel infusions over 1 versus 3 h. *Anticancer Drugs, 14*(10), 785-792. doi:10.1097/01.cad.0000097709.69687.fb

Mielke, S., Sparreboom, A., Steinberg, S. M., Gelderblom, H., Unger, C., Behringer, D., & Mross, K. (2005). Association of Paclitaxel pharmacokinetics with the development of peripheral neuropathy in patients with advanced cancer. *Clinical Cancer Research, 11*(13), 4843-4850. doi:10.1158/1078-0432.ccr-05-0298

Miltenburg, N. C., & Boogerd, W. (2014). Chemotherapy-induced neuropathy: A comprehensive survey. *Cancer Treatment Review, 40*(7), 872-882. doi:10.1016/j.ctrv.2014.04.004

Minson, C. T. (2010). Thermal provocation to evaluate microvascular reactivity in human skin. *Journal of Applied Physiology, 109*(4), 1239-1246. doi:10.1152/japplphysiol.00407.2010

Minson, C. T., Berry, L. T., & Joyner, M. J. (2001). Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *Journal of Applied Physiology, 91*(4), 1619-1626. doi:10.1152/jappl.2001.91.4.1619

Minson, C. T., Holowatz, L. A., Wong, B. J., Kenney, W. L., & Wilkins, B. W. (2002). Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age

during local heating. *Journal of Applied Physiology, 93*(5), 1644-1649. doi:10.1152/japplphysiol.00229.2002

Mir, O., Alexandre, J., Tran, A., Durand, J. P., Pons, G., Treluyer, J. M., & Goldwasser, F. (2009). Relationship between GSTP1 Ile(105)Val polymorphism and docetaxel-induced peripheral neuropathy: Clinical evidence of a role of oxidative stress in Taxane toxicity. *Annals of Oncology, 20*(4), 736-740. doi:10.1093/annonc/mdn698

Mitchell, P. L., Goldstein, D., Michael, M., Beale, P., Friedlander, M., Zalcberg, J., . . . Clarke, S. (2006). Addition of gabapentin to a modified FOLFOX regimen does not reduce oxaliplatin-induced neurotoxicity. *Clinical Colorectal Cancer, 6*(2), 146-151. doi:10.3816/CCC.2006.n.032

Mo, M., Erdelyi, I., Szigeti-Buck, K., Benbow, J. H., & Ehrlich, B. E. (2012). Prevention of Paclitaxel-induced peripheral neuropathy by lithium pretreatment. *FASEB Journal, 26*(11), 4696-4709. doi:10.1096/fj.12-214643

Mols, F., van de Poll-Franse, L. V., Vreugdenhil, G., Beijers, A. J., Kieffer, J. M., Aaronson, N. K., & Husson, O. (2016). Reference data of the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-CIPN20 Questionnaire in the general Dutch population. *European Journal of Cancer, 69*, 28-38. doi:10.1016/j.ejca.2016.09.020

Moor Instruments. (2014). *Microvascular Responses to Skin Heating: An Introduction.* Retrieved from https://www.moor.co.uk/ckfinder/userfiles/files/Moor_Skin_Heating_Theory_Issue_1.pdf

Moor Instruments (2001). *LDI-Flare Assessment of C Fibre Function: An Early Assessment of Early Peripheral Neuropathy Application note #113*. Retrieved from https://us.moor.co.uk/ckfinder/userfiles/files/ENG%20Application%20note%20%23113%20LDI-Flare%20Assessment%20of%20C%20Fibre%20Function%20Issue%201.pdf

Motulsky, H. (1995). *Intuitive Biostatistics.* New York, NY: Oxford University Press, Inc.

Mross, K., Haring, B., Hollander, N., Mielke, S., Behringer, D., Massing, U., & Unger, C. (2002). Comparison of 1-hour and 3-hours Paclitaxel infusion pharmacokinetics: Results from a randomized trial. *Oncology Research and Treatment, 25*(6), 503-508. https://doi.org./10.1159/000068620

Nabavi Nouri, M., Ahmed, A., Bril, V., Orszag, A., Ng, E., Nwe, P., & Perkins, B. A. (2012). Diabetic neuropathy and axon reflex-mediated neurogenic vasodilatation in type 1 diabetes. *Plos One, 7*(4), e34807. doi:10.1371/journal.pone.0034807

Nabholtz, J. M., Vannetzel, J. M., Llory, J. F., & Bouffette, P. (2003). Advances in the use of Taxanes in the adjuvant therapy of breast cancer. *Clinical Breast Cancer, 4*(3), 187-192. https://doi.org/10.1016/S1526-8209(11)70623-3

Nagashima, M., Ooshiro, M., Moriyama, A., Sugishita, Y., Kadoya, K., Sato, A., . . . Katoh, R. (2014). Efficacy and tolerability of controlled-release oxycodone for oxaliplatin-induced peripheral neuropathy and the extension of FOLFOX therapy in advanced colorectal cancer patients. *Support Care Cancer, 22*(6), 1579-1584. doi:10.1007/s00520-014-2132-4

Nahman-Averbuch, H., Yarnitsky, D., Granovsky, Y., Sprecher, E., Steiner, M., Tzuk-Shina, T., & Pud, D. (2011). Pronociceptive pain modulation in patients with painful chemotherapy-induced polyneuropathy. *Journal of Pain and Symptom Management, 42*(2), 229-238. doi:10.1016/j.jpainsymman.2010.10.268

Nakata, T., & Yorifuji, H. (1999). Morphological evidence of the inhibitory effect of Taxol on the fast axonal transport. *Neuroscience Research, 35*(2), 113-122. http://dx.doi.org/10.1016/S0168-0102(99)00074-7

National Academies of Sciences and Medicine. (2016). *Appropriate use of advanced technologies for radiation therapy and surgery in oncology: Workshop summary* (p. 111). Washington, DC: National Academies Press.

National Comprehensive Cancer Network. (2014a). *Clinical practice guidelines in oncology* (NCCN Guidelines): *Colon cancer. Version 2. 2015*. Retrieved from http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

National Comprehensive Cancer Network. (2014b). *NCCN clinical practice guidelines in oncology* (NCCN Guidelines): Breast cancer. *Version 3. 2014*. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

National Comprehensive Cancer Network. (2015). *NCCN clinical practice guidelines in oncology:* (NCCN Guidelines): Breast cancer. *Version 2. 2015*. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

National Comprehensive Cancer Network. (2016). *NCCN clinical practice guidelines in oncology: Breast cancer. Version 2. 2016*. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

Nennesmo, I., & Reinholt, F. P. (1988). Effects of intraneural injection of Taxol on retrograde axonal transport and morphology of corresponding nerve cell bodies. *Virchows Archive B Cell Pathology Including Molecular Pathology, 55*(4), 241-246. doi:10.1007/BF02896582

Nieuwenhoff, M. D., Wu, Y., Huygen, F. J., Schouten, A. C., van der Helm, F. C., & Niehof, S. P. (2016). Reproducibility of axon reflex-related vasodilation assessed by dynamic thermal imaging in healthy subjects. *Microvascular Research, 106*, 1-7. doi:10.1016/j.mvr.2016.03.001

Noda, K., Akita, H., Ogata, M., & Saji, M. (2014). Paclitaxel-induced hyperalgesia modulates negative affective component of pain and NR1 receptor expression in the frontal cortex in rats. *Neuroscience Research, 80*, 32-37. doi:10.1016/j.neures.2014.01.002

Norton, L. (1997). Evolving concepts in the systemic drug therapy of breast cancer. *Seminars in Oncology, 24*(4 Suppl 10), S10-13-s10-10. PMID: 9275000. Retrieved from https://www.seminoncol.org/

Nouri, M. N., Ahmed, A., Bril, V., Orszag, A., Ng, E., Nwe, P., & Perkins, B. A. (2012). Diabetic neuropathy and axon reflex-mediated neurogenic vasodilatation in type 1 diabetes. *Plos One, 7*(4). doi:10.1371/journal.pone.0034807

Oberg, P. A. (1990). Laser-Doppler flowmetry. *Critical Reviews in Biomedical Engineering, 18*(2), 125-163. PMID: 9275000. Retrieved from

http://www.begellhouse.com/journals/critical-reviews-in-biomedical-engineering.html

Obermann, M., Katsarava, Z., Esser, S., Sommer, C., He, L., Selter, L., . . . Maschke, M. (2008). Correlation of epidermal nerve fiber density with pain-related evoked potentials in HIV neuropathy. *Pain, 138*(1), 79-86. doi:10.1016/j.pain.2007.11.009

Ocean, A. J., & Vahdat, L. T. (2004). Chemotherapy-induced peripheral neuropathy: Pathogenesis and emerging therapies. *Support Care Cancer, 12*(9), 619-625. doi:10.1007/s00520-004-0657-7

Openshaw, H., Beamon, K., Longmate, J., Synold, T., Slatkin, N. E., & Somlo, G. (2005). The effect of height on Paclitaxel nerve damage. *Journal of Neuro -Oncology, 74*(2), 207-210. doi:10.1007/s11060-004-6599-6

Osmani, K., Vignes, S., Aissi, M., Wade, F., Milani, P., Levy, B. I., & Kubis, N. (2012). Taxane-induced peripheral neuropathy has good long-term prognosis: A 1- to 13-year evaluation. *Journal of Neurology, 259*(9), 1936-1943. doi:10.1007/s00415-012-6442-5

Osmani, K., Vignes, S., Aissi, M., Wade, F., Milani, P., Lévy, B. I., & Kubis, N. (2012). Taxane-induced peripheral neuropathy has good long-term prognosis: A 1- to 13-year evaluation. *Journal of Neurology, 259*(9), 1936-1943. https://doi.org/10.1007/s00415-012-6442-5

Pachman, D. R., Qin, R., Seisler, D., Smith, E. M., Kaggal, S., Novotny, P., . . . Loprinzi, C. L. (2016). Comparison of Oxaliplatin and Paclitaxel-induced neuropathy (Alliance A151505). *Support Care Cancer, 24*(12), 5059-5068. doi:10.1007/s00520-016-3373-1

Paice, J. A. (2009). Clinical challenges: Chemotherapy-induced peripheral neuropathy. *Seminar in Oncology Nursing, 25*(2 Suppl 1), S8-19. doi:10.1016/j.soncn.2009.03.013

Pan, Y., & Kao, M. S. (2007). Discordance of clinical symptoms and electrophysiologic findings in Taxane plus platinum-induced neuropathy. *International Journal of Gynecological Cancer, 17*(2), 394-397. doi:10.1111/j.1525-1438.2006.00766.x

Park, S. B., Goldstein, D., Krishnan, A. V., Lin, C. S. Y., Friedlander, M. L., Cassidy, J., . . . Kiernan, M. C. (2013). Chemotherapy-induced peripheral neurotoxicity: A critical analysis. *CA: A Cancer Journal for Clinicians*. doi:10.1002/caac.21204

Park, S. B., Krishnan, A. V., Lin, C. S. Y., Goldstein, D., Friedlander, M., & Kiernan, M. C. (2008b). Mechanisms underlying chemotherapy-Induced neurotoxicity and the potential for neuroprotective strategies. *Current Medicinal Chemistry, 15*(29), 3081-3094. doi:10.2174/092986708786848569

Park, S. B., Lin, C. S., Krishnan, A. V., Friedlander, M. L., Lewis, C. R., & Kiernan, M. C. (2011a). Early, progressive, and sustained dysfunction of sensory axons underlies Paclitaxel-induced neuropathy. *Muscle and Nerve, 43*(3), 367-374. doi:10.1002/mus.21874

Park, S. B., Lin, C. S. Y., Krishnan, A. V., Friedlander, M. L., Lewis, C. R., & Kiernan, M. C. (2011b). Early, progressive, and sustained dysfunction of sensory axons underlies Paclitaxel-induced neuropathy. *Muscle and Nerve, 43*(3), 367-374.

Park, S. B., Lin, C. S., Krishnan, A. V., Goldstein, D., Friedlander, M. L., & Kiernan, M. C. (2011c). Long-term neuropathy after Oxaliplatin treatment: Challenging the dictum of reversibility. *Oncologist, 16*(5), 708-716. doi:10.1634/theoncologist.2010-0248

Parness, J., & Horwitz, S. B. (1981). Taxol binds to polymerized tubulin in vitro. *Journal of Cell Biology, 91*(2), 479-487. https://doi.org/10.1083/jcb.91.2.479

Pascual, D., Goicoechea, C., Burgos, E., & Martín, M. I. (2010). Antinociceptive effect of three common analgesic drugs on peripheral neuropathy induced by Paclitaxel in rats. *Pharmacology Biochemistry and Behavior, 95*(3), 331-337. http://dx.doi.org/10.1016/j.pbb.2010.02.009

Pasquier, E., Honore, S., Pourroy, B., Jordan, M. A., Lehmann, M., Briand, C., & Braguer, D. (2005). Antiangiogenic concentrations of Paclitaxel induce an increase in microtubule dynamics in endothelial cells but not in cancer cells. *Cancer Research, 65*(6), 2433-2440. doi:10.1158/0008-5472.can-04-2624

Passavanti, M. B., Pace, M. C., Barbarisi, A., D'Andrea, F., Grella, E., Nicoletti, G. F., & Aurilio, C. (2006). Pain and sensory dysfunction after breast cancer surgery: Neurometer CPT evaluation. *Anticancer Research, 26*(5b), 3839-3844. Retrieved from http://ar.iiarjournals.org/

Patel, S. S. (1996). *Effects of temperature on surface pressure induced changes in perfusion.* (Doctoral dissertation). Retrieved from https://search.proquest.com/pqdtglobal/docview/304310522/abstract/C665C2A690734B27PQ/1?accountid=7398

Pazdur, R., Kudelka, A. P., Kavanagh, J. J., Cohen, P. R., & Raber, M. N. (1993). The Taxoids: Paclitaxel (Taxol®) and Docetaxel (Taxotere®). *Cancer Treatment Reviews*, *19*(4), 351-386. doi:10.1016/0305-7372(93)90010-o

Pereira, S., Fontes, F., Sonin, T., Dias, T., Fragoso, M., Castro-Lopes, J. M., & Lunet, N. (2016). Chemotherapy-induced peripheral neuropathy after neoadjuvant or adjuvant treatment of breast cancer: A prospective cohort study. *Supportive Care in Cancer, 24*(4), 1571-1581. doi:10.1007/s00520-015-2935-y

Perez, E. A. (2009). Microtubule inhibitors: Differentiating tubulin-inhibiting agents based on mechanisms of action, clinical activity, and resistance. *Molecular Cancer Therapy, 8*(8), 2086-2095. doi:10.1158/1535-7163.mct-09-0366

Persohn, E., Canta, A., Schoepfer, S., Traebert, M., Mueller, L., Gilardini, A., . . .Cavaletti, G. (2005). Morphological and morphometric analysis of Paclitaxel and docetaxel-induced peripheral neuropathy in rats. *European Journal of Cancer, 41*(10), 1460-1466. doi:10.1016/j.ejca.2005.04.006

Peters, C. M., Jimenez-Andrade, J. M., Kuskowski, M. A., Ghilardi, J. R., & Mantyh, P. W. (2007). An evolving cellular pathology occurs in dorsal root ganglia, peripheral nerve and spinal cord following intravenous administration of Paclitaxel in the rat. *Brain Research, 1168*, 46-59. http://dx.doi.org/10.1016/j.brainres.2007.06.066

Pignata, S., De Placido, S., Biamonte, R., Scambia, G., Di Vagno, G., Colucci, G., . . . Perrone, F. (2006). Residual neurotoxicity in ovarian cancer patients in clinical remission after first-line chemotherapy with Carboplatin and Paclitaxel: The multi-center Italian trial in ovarian cancer (MITO-4) retrospective study. *BMC Cancer, 6*, 5. doi:10.1186/1471-2407-6-5

Pike, C. T., Birnbaum, H. G., Muehlenbein, C. E., Pohl, G. M., & Natale, R. B. (2012). Healthcare costs and work loss burden of patients with chemotherapy-associated

peripheral neuropathy in breast, ovarian, head and neck, and nonsmall cell lung cancer. *Chemotherapy Research and Practice, 2012*, 913848. doi:10.1155/2012/913848

Pittman, S. K., Gracias, N. G., Vasko, M. R., & Fehrenbacher, J. C. (2014). Paclitaxel alters the evoked release of calcitonin gene-related peptide from rat sensory neurons in culture. *Experimental Neurology, 253*, 146-153. doi:10.1016/j.expneurol.2013.12.011

Polomano, R. C., Mannes, A. J., Clark, U. S., & Bennett, G. J. (2001). A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, Paclitaxel. *Pain, 94*(3), 293-304. doi:10.1016/s0304-3959(01)00363-3

Polston, D. (2018). Skin biopsy for small fiber sensory neuropathy. *Diagnostics & Testing.* Retrieved from https://my.clevelandclinic.org/health/diagnostics/17479-skin-biopsy-for-small-fiber-sensory-neuropathy

Postma, T. J., Vermorken, J. B., Liefting, A. J., Pinedo, H. M., & Heimans, J. J. (1995). Paclitaxel-induced neuropathy. *Annals of Oncology, 6*(5), 489-494. doi:10.1093/oxfordjournals.annonc.a059220

Quasthoff, S., & Hartung, H. P. (2002). Chemotherapy-induced peripheral neuropathy. *Journal of Neurology, 249*(1), 9-17. doi:10.1007/pl00007853

Quock, J., Dea, G., Tanaka, M., Gandara, D., Lara, P., & Lau, D. (2002). Premedication strategy for weekly Paclitaxel. *Cancer Investigation, 20*(5-6), 666-672. https://doi.org/10.1081/CNV-120003535

Rao, R. D., Michalak, J. C., Sloan, J. A., Loprinzi, C. L., Soori, G. S., Nikcevich, D. A., . . . Wong, G. Y. (2007). Efficacy of gabapentin in the management of chemotherapy-induced peripheral neuropathy: A phase 3 randomized, double-blind, placebo-controlled, crossover trial (N00C3). *Cancer, 110*(9), 2110-2118. doi:10.1002/cncr.23008

Rao, S., Horwitz, S. B., & Ringel, I. (1992). Direct photoaffinity labeling of tubulin with Taxol. *Journal of the National Cancer Institute, 84*(10), 785-788. doi:10.1093/jnci/84.10.785

Reeves, B. N., Dakhil, S. R., Sloan, J. A., Wolf, S. L., Burger, K. N., Kamal, A., . . . Loprinzi, C. L. (2012). Further data supporting that Paclitaxel-associated acute pain syndrome is

associated with development of peripheral neuropathy. *Cancer, 118*(20), 5171-5178. https://doi:10.1002/cncr.27489

Rietman, J. S., Dijkstra, P. U., Geertzen, J. H., Baas, P., De Vries, J., Dolsma, W., . . . Hoekstra, H. J. (2003). Short-term morbidity of the upper limb after sentinel lymph node biopsy or axillary lymph node dissection for Stage I or II breast carcinoma. *Cancer, 98*(4), 690-696. doi:10.1002/cncr.11545

Rietman, J. S., Dijkstra, P. U., Geertzen, J. H., Baas, P., de Vries, J., Dolsma, W. V., . . . Hoekstra, H. J. (2004). Treatment-related upper limb morbidity 1 year after sentinel lymph node biopsy or axillary lymph node dissection for stage I or II breast cancer. *Annals of Surgical Oncology, 11*(11), 1018-1024. doi:10.1245/aso.2004.03.512

Rietman, J. S., Geertzen, J. H., Hoekstra, H. J., Baas, P., Dolsma, W. V., de Vries, J., . . . Dijkstra, P. U. (2006). Long term treatment related upper limb morbidity and quality of life after sentinel lymph node biopsy for stage I or II breast cancer. *European Journal of Surgical Oncology, 32*(2), 148-152. doi:10.1016/j.ejso.2005.11.008

Risinger, A. L., Riffle, S. M., Lopus, M., Jordan, M. A., Wilson, L., & Mooberry, S. L. (2014). The taccalonolides and Paclitaxel cause distinct effects on microtubule dynamics and aster formation. *Molecular Cancer, 13*, 41. doi:10.1186/1476-4598-13-41

Rizzo, R., Spaggiari, F., Indelli, M., Lelli, G., Baricordi, O. R., Rimessi, P., & Ferlini, A. (2010). Association of CYP1B1 with hypersensitivity induced by Taxane therapy in breast cancer patients. *Breast Cancer Research and Treatment, 124*(2), 593-598. doi:10.1007/s10549-010-1034-5

Robarge, J. (2015). *Aromatse inhibitors produce hypersensitivity in experimental models of pain: Studies in vivo and in isolated sensory neurons* (Doctoral dissertation). Retrieved from https://search.proquest.com/openview/bfae2943af4ea6efa2917117bd786718/1?pq-origsite=gscholar&cbl=18750&diss=y. (Accession number 3668050)

Robarge, J. D., Duarte, D. B., Shariati, B., Wang, R., Flockhart, D. A., & Vasko, M. R. (2016). Aromatase inhibitors augment nociceptive behaviors in rats and enhance the excitability of sensory neurons. *Experimental Neurology, 281*, 53-65. doi:10.1016/j.expneurol.2016.04.006

Rogers, G., & Oosthuyse, T. (2000). A comparison of the indirect estimate of mean arterial pressure calculated by the conventional equation and calculated to compensate for a change in heart rate. *International Journal of Sports Medicine, 21*(2), 90-95. doi:10.1055/s-2000-8865

Roustit, M., Blaise, S., Millet, C., & Cracowski, J. L. (2010). Reproducibility and methodological issues of skin post-occlusive and thermal hyperemia assessed by single-point laser Doppler flowmetry. *Microvascular Research, 79*(2), 102-108. doi:10.1016/j.mvr.2010.01.001

Roustit, M., & Cracowski, J. L. (2012). Non-invasive assessment of skin microvascular function in humans: An insight into methods. *Microcirculation, 19*(1), 47-64. doi:10.1111/j.1549-8719.2011.00129.x

Rowinsky, E. K., & Donehower, R. C. (1991). The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. *Pharmacology & Therapeutics, 52*(1), 35-84. doi:10.1016/0163-7258(91)90086-2

Roytta, M., & Raine, C. S. (1986). Taxol-induced neuropathy: Chronic effects of local injection. *Journal of Neurocytology, 15*(4), 483-496. doi:10.1007/BF01611731

Rugo, H. S., Barry, W. T., Moreno-Aspitia, A., Lyss, A. P., Cirrincione, C., Leung, E., . . . Winer, E. P. (2015). Randomized phase III trial of paclitaxel once per week compared with nanoparticle albumin-bound nab-paclitaxel once per week or ixabepilone with Bevacizumab as first-line chemotherapy for locally recurrent or metastatic breast cancer: CALGB 40502/NCCTG N063H (Alliance). *Journal of Clinical Oncology, 33*(21), 2361-2369. doi:10.1200/jco.2014.59.5298

Saad, M., Psimaras, D., Tafani, C., Sallansonnet-Froment, M., Calvet, J. H., Vilier, A., . . . Ricard, D. (2016a). Quick, non-invasive and quantitative assessment of small fiber neuropathy in patients receiving chemotherapy. *Journal of Neuro-Oncology, 127*(2), 373-380. doi:10.1007/s11060-015-2049-x

Saad, M., Psimaras, D., Tafani, C., Sallansonnet-Froment, M., Calvet, J. H., Vilier, A., . . . Ricard, D. (2016b). Quick, non-invasive and quantitative assessment of small fiber

neuropathy in patients receiving chemotherapy. *Journal of Neuro-Oncology, 127*(2), 373-380. doi:10.1007/s11060-015-2049-x

Sahenk, Z., Barohn, R., New, P., & Mendell, J. R. (1994). Taxol neuropathy. Electrodiagnostic and sural nerve biopsy findings. *Archives of Neurology, 51*(7), 726-729. doi:10.1001/archneur.1994.00540190110024

Schattschneider, J., Uphoff, J., Binder, A., Wasner, G., & Baron, R. (2006). No adrenergic sensitization of afferent neurons in painful sensory polyneuropathy. *Journal of Neurology, 253*(3), 280-286. doi:10.1007/s00415-005-0976-8

Schiff, P. B., Fant, J., & Horwitz, S. B. (1979). Promotion of microtubule assembly in vitro by Taxol. *Nature, 277*(5698), 665-667. doi:10.1038/277665a0

Schneider, B. P., Li, L., Radovich, M., Shen, F., Miller, K. D., Flockhart, D. A., . . . Sledge, G. W., Jr. (2015). Genome-wide association studies for Taxane-induced peripheral neuropathy in ECOG-5103 and ECOG-1199. *Clinical Cancer Research, 21*(22), 5082-5091. doi:10.1158/1078-0432.ccr-15-0586

Schneider, B. P., Wang, M., Radovich, M., Sledge, G. W., Badve, S., Thor, A., . . . Miller, K. D. (2008). Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of Paclitaxel compared with Paclitaxel plus Bevacizumab in advanced breast cancer: ECOG 2100. *Journal of Clinical Oncology, 26*(28), 4672-4678. doi:10.1200/jco.2008.16.1612

Schneider, B. P., Zhao, F., Wang, M., Stearns, V., Martino, S., Jones, V., . . . Sparano, J. A. (2012). Neuropathy is not associated with clinical outcomes in patients receiving adjuvant Taxane-containing therapy for operable breast cancer. *Journal of Clinical Oncology, 30*(25), 3051-3057. doi:10.1200/jco.2011.39.8446

Schneider, B. P., Zhao, F., Wang, M., Stearns, V., Martino, S., Jones, V., . . . Sparano, J. A. (2012). Neuropathy is not associated with clinical outcomes in patients receiving adjuvant Taxane-containing therapy for operable breast cancer. *Journal of Clinical Oncology, 30*(25), 3051-3057. doi:10.1200/JCO.2011.39.8446

Schulze, T., Mucke, J., Markwardt, J., Schlag, P. M., & Bembenek, A. (2006). Long-term morbidity of patients with early breast cancer after sentinel lymph node biopsy

compared to axillary lymph node dissection. *Journal of Surgical Oncology, 93*(2), 109-119. doi:10.1002/jso.20406

Seidman, A. D., Berry, D., Cirrincione, C., Harris, L., Muss, H., Marcom, P. K., . . . Hudis, C. (2008). Randomized phase III trial of weekly compared with every-3-weeks Paclitaxel for metastatic breast cancer, with trastuzumab for all HER-2 overexpressors and random assignment to trastuzumab or not in HER-2 nonoverexpressors: Final results of cancer and leukemia group B protocol 9840. *Journal of Clinical Oncology, 26*(10), 1642-1649. doi:10.1200/jco.2007.11.6699

Seretny, M., Currie, G. L., Sena, E. S., Ramnarine, S., Grant, R., MacLeod, M. R., . . . Fallon, M. (2014). Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. *Pain, 155*(12), 2461-2470. doi:10.1016/j.pain.2014.09.020

Sestak, I., Cuzick, J., Sapunar, F., Eastell, R., Forbes, J. F., Bianco, A. R., & Buzdar, A. U. (2008). Risk factors for joint symptoms in patients enrolled in the ATAC trial: A retrospective, exploratory analysis. *The Lancet Oncology, 9*(9), 866-872. http://dx.doi.org/10.1016/S1470-2045(08)70182-7

Sharma, S., Vas, P. R., & Rayman, G. (2015). Assessment of diabetic neuropathy using a point-of-care nerve conduction device shows significant associations with the LDIFLARE method and clinical neuropathy scoring. *Journal Diabetes Science Technology, 9*(1), 123-131. doi:10.1177/1932296814551044

Sharma, S., Venkitaraman, R., Vas, P. R., & Rayman, G. (2015). Assessment of chemotherapy-induced peripheral neuropathy using the LDIFLARE technique: A novel technique to detect neural small fiber dysfunction. *Brain and Behavior, 5*(7). Retrieved from http://doi.org./10.1002/brb3.354

Shimozuma, K., Ohashi, Y., Takeuchi, A., Aranishi, T., Morita, S., Kuroi, K., . . . Hausheer, F. H. (2012). Taxane-induced peripheral neuropathy and health-related quality of life in postoperative breast cancer patients undergoing adjuvant chemotherapy: N-SAS BC 02, a randomized clinical trial. *Support Care Cancer, 20*(12), 3355-3364. doi:10.1007/s00520-012-1492-x

Shipton, E. A. (2013). Skin matters: Identifying pain mechanisms and predicting treatment outcomes. *Neurology Research International, 2013*. Retrieved from http://doi.org./10.1155/2013/329364

Siao, P., & Cros, D. P. (2003). Quantitative sensory testing. *Physical Medicine and Rehabilitation Clinics, 14*(2), 261-286. doi:10.1016/S1047-9651(02)00122-5.

Siau, C., Xiao, W., & Bennett, G. J. (2006). Paclitaxel- and Vincristine-evoked painful peripheral neuropathies: Loss of epidermal innervation and activation of Langerhans cells. *Experimental Neurology , 201*(2), 507-514. doi:10.1016/j.expneurol.2006.05.007

Simon, N. B., Danso, M. A., Alberico, T. A., Basch, E., & Bennett, A. V. (2017). The prevalence and pattern of chemotherapy-induced peripheral neuropathy among women with breast cancer receiving care in a large community oncology practice. *Quality of Life Research, 26*(10), 2763-2772. doi:10.1007/s11136-017-1635-0

Sivaskandarajah, G. A., Halpern, E. M., Lovblom, L. E., Weisman, A., Orlov, S., Bril, V., & Perkins, B. A. (2013). Structure-function relationship between corneal nerves and conventional small-fiber tests in type 1 diabetes. *Diabetes Care, 36*(9), 2748-2755. doi:10.2337/dc12-2075

Smith, E. L., Whedon, M. B., & Bookbinder, M. (2002). Quality improvement of painful peripheral neuropathy. *Seminars in Oncology Nursing, 18*(1), 36-43. doi:10.1053/sonu.2002.30043

Smith, E. M. (2013a). Current methods for the assessment and management of Taxane-related neuropathy. *Clinical Journal of Oncology Nursing, 17* (1 Suppl), 22-34. doi:10.1188/13.cjon.s1.22-34

Smith, E. M., Beck, S. L., & Cohen, J. (2008). The total neuropathy score: A tool for measuring chemotherapy-induced peripheral neuropathy. *Oncology Nursing Forum, 35*(1), 96-102. doi:10.1188/08.onf.96-102

Smith, E. M., Campbell, G., Tofthagen, C., Kottschade, L., Collins, M. L., Warton, C., . . . Visovsky, C. (2014). Nursing knowledge, practice patterns, and learning preferences regarding chemotherapy-induced peripheral neuropathy. *Oncology Nursing Forum, 41*(6), 669-679. doi:10.1188/14.onf.669-679

Smith, E. M., Cohen, J. A., Pett, M. A., & Beck, S. L. (2010). The reliability and validity of a modified total neuropathy score-reduced and neuropathic pain severity items when used to measure chemotherapy-induced peripheral neuropathy in patients receiving Taxanes and Platinums. *Cancer Nursing, 33*(3), 173-183. doi:10.1097/NCC.0b013e3181c989a3

Smith, E. M., Pang, H., Cirrincione, C., Fleishman, S., Paskett, E. D., Ahles, T., . . . Shapiro, C. L. (2013). Effect of Duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: A randomized clinical trial. *Journal of the American Medical Association, 309*(13), 1359-136. doi:10.1001/jama.2013.2813

Smith, S. B., Crager, S. E., & Mogil, J. S. (2004). Paclitaxel-induced neuropathic hypersensitivity in mice: Responses in 10 inbred mouse strains. *Life Sciences, 74*(21), 2593-2604. doi:10.1016/j.lfs.2004.01.002

Song, S. J., Min, J., Suh, S. Y., Jung, S. H., Hahn, H. J., Im, S. A., & Lee, J. Y. (2017). Incidence of Taxane-induced peripheral neuropathy receiving treatment and prescription patterns in patients with breast cancer. *Support Care Cancer, 25*(7), 2241-2248. doi:10.1007/s00520-017-3631-x

Sparano, J. A., Wang, M. L., Martino, S., Jones, V., Perez, E. A., Saphner, T., . . . Davidson, N. E. (2008). Weekly Paclitaxel in the adjuvant treatment of breast cancer. *New England Journal of Medicine, 358*(16), 1663-1671. doi:10.1056/NEJMoa0707056

Speck, R. M., Sammel, M. D., Farrar, J. T., Hennessy, S., Mao, J. J., Stineman, M. G., & DeMichele, A. (2013). Impact of chemotherapy-induced peripheral neuropathy on treatment delivery in nonmetastatic breast cancer. *Journal of Oncology Practice, 9*(5), e234-240. doi:10.1200/jop.2012.000863

Spill, F., Guerrero, P., Alarcon, T., Maini, P. K., & Byrne, H. M. (2015). Mesoscopic and continuum modelling of angiogenesis. *Journal of Mathematical Biology, 70*(3), 485-532. doi:10.1007/s00285-014-0771-1

Srivastava, R. K., Srivastava, A. R., Korsmeyer, S. J., Nesterova, M., Cho-Chung, Y. S., & Longo, D. L. (1998). Involvement of microtubules in the regulation of Bcl2

phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. *Molecular and Cellular Biology, 18*(6), 3509-3517. doi:10.1128/MCB.18.6.3509

Gerson, S. L., Caimi, P. F., William, B. M., & Creger, R. J. (2018). Pharmacology and molecular mechanisms of antineoplastic agents for hematologic malignancies. In R. Hoffman, E. J. Benz., L. E. Silberstein, H. E. Heslop, J. I., Weitz, J. Anastasi, M. E. Salama, & S. A. Abutalib (Eds.), *Hematology: Basic principles and practice* (7th ed., pp. 849-912). Philadelphia, PA.: Elsevier, Inc.

Stephenson, F. (2004). A tale of Taxol. Florida State University Research in Review, *12*(3), 13-37. Retrieved from http://www.rinr.fsu.edu/fall2002/taxol.html

Stephenson, L. A., & Kolka, M. A. (1985). Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating. *American Journal of Physiology, 249*(2), R186-191. https://doi.org/10.1152/ajpregu.1985.249.2.R186

Stubblefield, M. D., Burstein, H. J., Burton, A. W., Custodio, C. M., Deng, G. E., Ho, M., . . . Von Roenn, J. H. (2009). NCCN task force report: Management of neuropathy in cancer. *Journal of the National Comprehensive Cancer Network, 7*(Suppl 5), S1-S26. doi:10.6004/jnccn.2009.0078

Stubblefield, M. D., McNeely, M. L., Alfano, C. M., & Mayer, D. K. (2012). A prospective surveillance model for physical rehabilitation of women with breast cancer: Chemotherapy-induced peripheral neuropathy. *Cancer, 118*(Suppl 8), 2250-2260. doi:10.1002/cncr.27463

Sweeney, C., Schmitz, K. H., Lazovich, D., Virnig, B. A., Wallace, R. B., & Folsom, A. R. (2006). Functional limitations in elderly female cancer survivors. *Journal of the National Cancer Institute, 98*(8), 521-529. doi:10.1093/jnci/djj130

Takemoto, S., Ushijima, K., Honda, K., Wada, H., Terada, A., Imaishi, H., & Kamura, T. (2012). Precise evaluation of chemotherapy-induced peripheral neuropathy using the visual analogue scale: A quantitative and comparative analysis of neuropathy occurring with Paclitaxel-Carboplatin and Docetaxel-Carboplatin therapy. *International Journal of Clinical Oncology, 17*(4), 367-372. doi:10.1007/s10147-011-0303-6

Tanabe, Y., Hashimoto, K., Shimizu, C., Hirakawa, A., Harano, K., Yunokawa, M., . . . Fujiwara, Y. (2013). Paclitaxel-induced peripheral neuropathy in patients receiving adjuvant chemotherapy for breast cancer. *International Journal of Clinical Oncology, 18*(1), 132-138. doi:10.1007/s10147-011-0352-x

Tanay, M. A., Armes, J., & Ream, E. (2016). The experience of chemotherapy-induced peripheral neuropathy in adult cancer patients: A qualitative thematic synthesis. *European Journal of Cancer Care*. doi:10.1111/ecc.12443

Tasmuth, T., von Smitten, K., Hietanen, P., Kataja, M., & Kalso, E. (1995). Pain and other symptoms after different treatment modalities of breast cancer. *Annals of Oncology, 6*(5), 453-459. https://doi.org/10.1093/oxfordjournals.annonc.a059215

Tasmuth, T., von Smitten, K., & Kalso, E. (1996). Pain and other symptoms during the first year after radical and conservative surgery for breast cancer. *British Journal of Cancer, 74*(12), 2024-2031. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2074824/pdf/brjcancer00028-0174.pdf

Tavee, J., & Zhou, L. (2009b). Small fiber neuropathy: A burning problem. *Cleveland Clinic Journal of Medicine, 76*(5), 297-305. doi:10.3949/ccjm.76a.08070

Tian, J., Chen, G. L., & Zhang, H. R. (2015). Sleep status of cervical cancer patients and predictors of poor sleep quality during adjuvant therapy. *Support Care Cancer, 23*(5), 1401-1408. doi:10.1007/s00520-014-2493-8

Tofthagen, C. (2010). Patient perceptions associated with chemotherapy-induced peripheral neuropathy. *Clinical Journal of Oncology Nursing, 14*(3), E22-28. doi:10.1188/10.cjon.e22-e28

Tofthagen, C., McAllister, R. D., & McMillan, S. C. (2011). Peripheral neuropathy in patients with colorectal cancer receiving oxaliplatin. *Clinical Journal of Oncology Nursing, 15*, 182-188. https://doi.org/10.1188/11.CJON.182-188

Tofthagen, C., McAllister, R. D., & Visovsky, C. (2013). Peripheral neuropathy caused by Paclitaxel and Docetaxel: An evaluation and comparison of symptoms. *Journal of the Advanced Practitioner in Oncology, 4*(4), 204-215. PMID: 25032002

Tofthagen, C., Overcash, J., & Kip, K. (2012). Falls in persons with chemotherapy-induced peripheral neuropathy. *Support Care Cancer, 20*(3), 583-589. doi:10.1007/s00520-011-1127-7

Tofthagen, C., Visovsky, C. M., & Hopgood, R. (2013a). Chemotherapy-induced peripheral neuropathy. *Clinical Journal of Oncology Nursing, 17*, 138-144.

Tofthagen, C., Visovsky, C. M., & Hopgood, R. (2013b). Chemotherapy-induced peripheral neuropathy: An algorithm to guide nursing management. *Clinical Journal of Oncology Nursing 17*(2), 138-144. doi:10.1188/13.cjon.138-144

Tonissi, F., Lattanzio, L., Merlano, M. C., Infante, L., Lo Nigro, C., & Garrone, O. (2015). The effect of Paclitaxel and nab-Paclitaxel in combination with anti-angiogenic therapy in breast cancer cell lines. *Investigational New Drugs, 33*(4), 801-809. doi:10.1007/s10637-015-0249-z

Truini, A., Panuccio, G., Galeotti, F., Maluccio, M. R., Sartucci, F., Avoli, M., & Cruccu, G. (2010). Laser-evoked potentials as a tool for assessing the efficacy of antinociceptive drugs. *European Journal of Pain, 14*(2), 222-225. https://doi.org/10.1016/j.ejpain.2009.05.001

Ueno, N. T., & Mamounas, E. P. (2016). Neoadjuvant nab-Paclitaxel in the treatment of breast cancer. *Breast Cancer Research and Treatment, 156*(3), 427-440. doi:10.1007/s10549-016-3778-z

Umapathi, T., Tan, W. L., Loke, S. C., Soon, P. C., Tavintharan, S., & Chan, Y. H. (2007). Intraepidermal nerve fiber density as a marker of early diabetic neuropathy. *Muscle and Nerve, 35*(5), 591-598. doi:10.1002/mus.20732

U.S. Department of Health and Human Services, National Cancer Institute (2014). *Success story: Taxol® (NSC 125973)*. Retrieved from http://dtp.nci.nih.gov/timeline/flash/success_stories/S2_taxol.htm

Vadalouca, A., Raptis, E., Moka, E., Zis, P., Sykioti, P., & Siafaka, I. (2012). Pharmacological treatment of neuropathic cancer pain: A comprehensive review of the current literature. *Pain Practice, 12*(3), 219-251. doi:10.1111/j.1533-2500.2011.00485.x

Valentini, M., & Parati, G. (2009). Variables influencing heart rate. *Progress in Cardiovascular Diseases, 52*(1), 11-19. doi:10.1016/j.pcad.2009.05.004

Valeriani, M., Le Pera, D., Niddam, D., Chen, A. C., & Arendt-Nielsen, L. (2002). Dipolar modelling of the scalp evoked potentials to painful contact heat stimulation of the human skin. *Neuroscience Letters, 318*(1), 44-48. doi:10.1016/s0304-3940(01)02466-1

Van der Schueren, B. J., de Hoon, J. N., Vanmolkot, F. H., Van Hecken, A., Depre, M., Kane, S. A., . . . Sinclair, S. R. (2007). Reproducibility of the capsaicin-induced dermal blood flow response as assessed by laser Doppler perfusion imaging. *British Journal of Clinical Pharmacology, 64*(5), 580-590. doi:10.1111/j.1365-2125.2007.02939.x

Van Duijnhoven, N. T., Janssen, T. W., Green, D. J., Minson, C. T., Hopman, M. T., & Thijssen, D. H. (2009). Effect of functional electrostimulation on impaired skin vasodilator responses to local heating in spinal cord injury. *Journal of Applied Physiology (1985), 106*(4), 1065-1071. doi:10.1152/japplphysiol.91611.2008

van Gerven, J. M., Moll, J. W., van den Bent, M. J., Bontenbal, M., van der Burg, M. E., Verweij, J., & Vecht, C. J. (1994). Paclitaxel (Taxol) induces cumulative mild neurotoxicity. *European Journal of Cancer, 30*(8), 1074-1077. doi:10.1016/0959-8049(94)90459-6

Vas, P. R., Green, A. Q., & Rayman, G. (2012). Small fibre dysfunction, microvascular complications and glycaemic control in type 1 diabetes: A case-control study. *Diabetologia, 55*(3), 795-800. doi:10.1007/s00125-011-2417-9

Vas, P. R., & Rayman, G. (2013a). The rate of decline in small fibre function assessed using axon reflex-mediated neurogenic vasodilatation and the importance of age related centile values to improve the detection of clinical neuropathy. *Plos One, 8*(7), e69920. doi:10.1371/journal.pone.0069920

Vas, P. R., & Rayman, G. (2013b). Validation of the modified LDIFlare technique: A simple and quick method to assess C-fiber function. *Muscle and Nerve, 47*(3), 351-356. doi:10.1002/mus.23532

Vas, P. R. J., Green, A. Q., & Rayman, G. (2012). Small fibre dysfunction, microvascular complications and glycaemic control in type 1 diabetes: A case-control study. *Diabetologia, 55*(3), 795-800. doi:10.1007/s00125-011-2417-9

Vas, P. R. J., & Rayman, G. (2013a). The rate of decline in small fibre function assessed using axon reflex-mediated neurogenic vasodilatation and the importance of age related centile values to improve the detection of clinical neuropathy. *Plos One, 8*(7). doi:10.1371/journal.pone.0069920

Vas, P. R. J., & Rayman, G. (2013c). Validation of the modified LDIFlare technique: A simple and quick method to assess C-fiber function. *Muscle and Nerve, 47*(3), 351-356. doi:10.1002/mus.23532

Vasquez, S., Guidon, M., McHugh, E., Lennon, O., Grogan, L., & Breathnach, O. S. (2014). Chemotherapy induced peripheral neuropathy: The modified total neuropathy score in clinical practice. *Irish Journal of Medical Science, 183*(1), 53-58. doi:10.1007/s11845-013-0971-5

Ventzel, L., Jensen, A. B., Jensen, A. R., Jensen, T. S., & Finnerup, N. B. (2016). Chemotherapy-induced pain and neuropathy: A prospective study in patients treated with adjuvant Oxaliplatin or Docetaxel. *Pain, 157*(3), 560-568. doi:10.1097/j.pain.0000000000000404

Veves, A., Akbari, C. M., Primavera, J., Donaghue, V. M., Zacharoulis, D., Chrzan, J. S., . . . Freeman, R. (1998). Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. *Diabetes, 47*(3), 457-463. doi:10.2337/diabetes.47.3.457

Vichaya, E. G., Wang, X. S., Boyette-Davis, J. A., Mendoza, T. R., He, Z., Thomas, S. K., . . . Dougherty, P. M. (2013a). Subclinical pretreatment sensory deficits appear to predict the development of pain and numbness in patients with multiple myeloma undergoing chemotherapy. *Cancer Chemotherapy and Pharmacology, 71*(6), 1531-1540. doi:10.1007/s00280-013-2152-7

Vilholm, O. J., Cold, S., Rasmussen, L., & Sindrup, S. H. (2009). Sensory function and pain in a population of patients treated for breast cancer. *Acta Anaesthesiologica Scandinavica, 53*(6), 800-806. doi:10.1111/j.1399-6576.2009.01938.x

Visovsky, C., Meyer, R. R., Roller, J., & Poppas, M. (2008a). Evaluation and management of peripheral neuropathy in diabetic patients with cancer. *Clinical Journal of Oncology Nursing, 12*(2), 243-247. doi:10.1188/08.cjon.243-247

Wald, N. J. (2001). The definition of screening. *Journal Medical Screening, 8*(1), 1. doi:10.1136/jms.8.1.1

Wall, M. E., & Wani, M. C. (1995). Camptothecin and Taxol: Discovery to clinic--thirteenth Bruce F. Cain Memorial Award Lecture. *Cancer Research, 55*(4), 753-760. Retrieved from http://cancerres.aacrjournals.org/content/canres/55/4/753.full.pdf

Wampler, M. A., Hamolsky, D., Hamel, K., Melisko, M., & Topp, K. S. (2005). Case report: Painful peripheral neuropathy following treatment with Docetaxel for breast cancer. *Clinical Journal of Oncology Nursing, 9*(2), 189-193. doi:10.1188/05.cjon.189-193

Wampler, M. A., Topp, K. S., Miaskowski, C., Byl, N. N., Rugo, H. S., & Hamel, K. (2007). Quantitative and clinical description of postural instability in women with breast cancer treated with Taxane chemotherapy. *Archives of Physical Medicine and Rehabilitation, 88*(8), 1002-1008. doi:10.1016/j.apmr.2007.05.007

Warren, S., Capra, N. F., Yezierski, R. P. (2018). The somatosensory system I: Tactile discrimination and position sense. In D. E. Haines & G. A. Mihailoff (Eds.), *Fundamental Neuroscience for Basic and Clinical Applications* (5th ed., pp. 243-257.e241). Philadelphia, PA: Elsevier, Inc.

Wiernik, P. H., Schwartz, E. L., Einzig, A., Strauman, J. J., Lipton, R. B., & Dutcher, J. P. (1987). Phase I trial of Taxol given as a 24-hour infusion every 21 days: Responses observed in metastatic melanoma. *Journal of Clinical Oncology, 5*(8), 1232-1239. doi:10.1200/jco.1987.5.8.1232

Wiernik, P. H., Schwartz, E. L., Strauman, J. J., Dutcher, J. P., Lipton, R. B., & Paietta, E. (1987). Phase I clinical and pharmacokinetic study of Taxol. *Cancer Research, 47*(9),

2486-2493. Retrieved from http://cancerres.aacrjournals.org/content/canres/47/9/2486.full.pdf

Wilkins, B. W., Holowatz, L. A., Wong, B. J., & Minson, C. T. (2003). Nitric oxide is not permissive for cutaneous active vasodilatation in humans. *Journal of Physiology, 548*(3), 963-969. doi:10.1113/jphysiol.2002.035931

Williams, C., & Bryant, A. (2011). Short versus long duration infusions of Paclitaxel for any advanced adenocarcinoma. *Cochrane Database Systematic Reviews, 5*, Cd003911. doi:10.1002/14651858.CD003911.pub2

Winer, E. P., Berry, D. A., Woolf, S., Duggan, D., Kornblith, A., Harris, L. N., . . . Norton, L. (2004). Failure of higher-dose Paclitaxel to improve outcome in patients with metastatic breast cancer: Cancer and leukemia group B trial 9342. *Journal of Clinical Oncology, 22*(11), 2061-2068. doi:10.1200/JCO.2004.08.048

Winters-Stone, K. M., Torgrimson, B., Horak, F., Eisner, A., Nail, L., Leo, M. C., . . . Luoh, S. W. (2011). Identifying factors associated with falls in postmenopausal breast cancer survivors: A multi-disciplinary approach. *Archives of Physical Medicine and Rehabilitation, 92*(4), 646-652. doi:10.1016/j.apmr.2010.10.039

Wolf, S., Barton, D., Kottschade, L., Grothey, A., & Loprinzi, C. (2008). Chemotherapy-induced peripheral neuropathy: Prevention and treatment strategies. *European Journal of Cancer, 44*(11), 1507-1515. http://dx.doi.org/10.1016/j.ejca.2008.04.018

Wolf, S. L., Barton, D. L., Qin, R., Wos, E. J., Sloan, J. A., Liu, H., . . . Loprinzi, C. L. (2012a). The relationship between numbness, tingling, and shooting/burning pain in patients with chemotherapy-induced peripheral neuropathy (CIPN) as measured by the EORTC QLQ-CIPN20 instrument, N06CA. *Support Care Cancer, 20*(3), 625-632. doi:10.1007/s00520-011-1141-9

Wolf, S. L., Barton, D. L., Qin, R., Wos, E. J., Sloan, J. A., Liu, H. S., . . . Loprinzi, C. L. (2012b). The relationship between numbness, tingling, and shooting/burning pain in patients with chemotherapy-induced peripheral neuropathy (CIPN) as measured by the EORTC QLQ-CIPN20 instrument, N06CA. *Supportive Care in Cancer, 20*(3), 625-632. doi:10.1007/s00520-011-1141-9

Wong, B. J., & Fieger, S. M. (2010). Transient receptor potential vanilloid type-1 (TRPV-1) channels contribute to cutaneous thermal hyperaemia in humans. *Journal of Physiology, 588*(21), 4317-4326. doi:10.1113/jphysiol.2010.195511

Wong, B. J., & Minson, C. T. (2011a). Altered thermal hyperaemia in human skin by prior desensitization of neurokinin-1 receptors. *Experimental Physiology, 96*(6), 599-609. doi:10.1113/expphysiol.2011.057356

Wong, B. J., Tublitz, N. J., & Minson, C. T. (2005). Neurokinin-1 receptor desensitization to consecutive microdialysis infusions of substance P in human skin. *Journal of Physiology, 568*(3), 1047-1056. doi:10.1113/jphysiol.2005.095372

Wong, B. J., Wilkins, B. W., Holowatz, L. A., & Minson, C. T. (2003). Nitric oxide synthase inhibition does not alter the reactive hyperemic response in the cutaneous circulation. *Journal of Applied Physiology (1985), 95*(2), 504-510. doi:10.1152/japplphysiol.00254.2003

Wong, B. J., Wilkins, B. W., & Minson, C. T. (2004). H1 but not H2 histamine receptor activation contributes to the rise in skin blood flow during whole body heating in humans. *Journal of Physiology, 560*(3), 941-948. doi:10.1113/jphysiol.2004.071779

Wong, B. J., Williams, S. J., & Minson, C. T. (2006). Minimal role for H1 and H2 histamine receptors in cutaneous thermal hyperemia to local heating in humans. *Journal of Applied Physiology (1985), 100*(2), 535-540. doi:10.1152/japplphysiol.00902.2005

Wong, M. C. (2010). *The feasibility of contact heat evoked potentials (CHEPs) in early detection of symptomatic diabetic distal symmetric polyneuropathy* (Doctoral dissertation). Retrieved from http://ulib.iupui.edu/cgi-bin/proxy.pl?url=http://search.proquest.com/docview/848641056?accountid=7398 ProQuest Dissertations & Theses A&I database. (Accession number 3441029)

Wong, R., & Sagar, S. (2006). Acupuncture treatment for chemotherapy-induced peripheral neuropathy--a case series. *Acupuncture in Medicine, 24*(2), 87-91. doi:10.1136/aim.24.2.87

Woodward, E. J., & Twelves, C. (2010). Scheduling of Taxanes: A review. *Current Clinical Pharmacology, 5*(3), 226-231. doi:10.2174/157488410791498725

World Health Organization. (2006). *Table 1: The International Classification of adult underweight, overweight and obesity according to BMI*. World Health Organization. Retrieved from http://apps.who.int/bmi/index.jsp?introPage=intro_3.html

Worthen-Chaudhari, L., Lamantia, M. T., Monfort, S., Chaudhari, A. M. W., & Lustberg, M. B. (2016). Novel balance interventions for chemotherapy-induced peripheral neuropathy: Argentine tango. *Archives of Physical Medicine and Rehabilitation, 97*(10), e119. http://dx.doi.org/10.1016/j.apmr.2016.08.372

Wozniak, K. M., Vornov, J. J., Wu, Y., Nomoto, K., Littlefield, B. A., DesJardins, C., . . . Slusher, B. S. (2016). Sustained accumulation of microtubule-binding chemotherapy drugs in the peripheral nervous system: Correlations with time course and neurotoxic severity. *Cancer Research, 76*(11), 3332-3339. doi:10.1158/0008-5472.CAN-15-2525

Wu, S. G., Huang, S. J., Zhou, J., Sun, J. Y., Guo, H., Li, F. Y., . . . He, Z. Y. (2014). Dosimetric analysis of the brachial plexus among patients with breast cancer treated with post-mastectomy radiotherapy to the ipsilateral supraclavicular area: Report of 3 cases of radiation-induced brachial plexus neuropathy. *Radiation Oncology, 9*(1), 292. doi:10.1186/s13014-014-0292-5

Xiao, W. H., & Bennett, G. J. (2008). Chemotherapy-evoked neuropathic pain: Abnormal spontaneous discharge in A-fiber and C-fiber primary afferent neurons and its suppression by acetyl-L-carnitine. *Pain, 135*(3), 262-270. doi:10.1016/j.pain.2007.06.001

Xu, J. J., Diaz, P., Bie, B., Astruc-Diaz, F., Wu, J., Yang, H., . . . Naguib, M. (2014). Spinal gene expression profiling and pathways analysis of a CB2 agonist (MDA7)-targeted prevention of Paclitaxel-induced neuropathy. *Neuroscience, 260*, 185-194. http://dx.doi.org/10.1016/j.neuroscience.2013.12.028

Yim, J., Petrofsky, J., Berk, L., Daher, N., & Lohman, E. (2012). Differences in endothelial function between Korean-Asians and Caucasians. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 18*(6), Cr337-343. doi:10.12659/MSM.882902.

Yim, J., Petrofsky, J., Berk, L., Daher, N., Lohman, E., Moss, A., & Cavalcanti, P. (2012). Protective effect of anti-oxidants on endothelial function in young Korean-Asians compared to Caucasians. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 18*(8), Cr467-479. doi:10.12659/MSM.883266

Zanville, N. R., Nudelman, K. N., Smith, D. J., Von Ah, D., McDonald, B. C., Champion, V. L., & Saykin, A. J. (2016). Evaluating the impact of chemotherapy-induced peripheral neuropathy symptoms (CIPN-sx) on perceived ability to work in breast cancer survivors during the first year post-treatment. *Support Care Cancer, 24*(11), 4779-4789. doi:10.1007/s00520-016-3329-5

Zasadil, L. M., Andersen, K. A., Yeum, D., Rocque, G. B., Wilke, L. G., Tevaarwerk, A. J., . . . Weaver, B. A. (2014). Cytotoxicity of Paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Science Translational Medicine, 6*(229), 229ra243. doi:10.1126/scitranslmed.3007965

Zeichner, S. B., Terawaki, H., & Gogineni, K. (2016). A review of systemic treatment in metastatic triple-negative breast cancer. *Breast Cancer, 10*, 25-36. doi:10.4137/bcbcr.s32783

Zhang, H., Boyette-Davis, J. A., Kosturakis, A. K., Li, Y., Yoon, S. Y., Walters, E. T., & Dougherty, P. M. (2013). Induction of monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR2 in primary sensory neurons contributes to Paclitaxel-induced peripheral neuropathy. *Journal of Pain, 14*(10), 1031-1044. doi:10.1016/j.jpain.2013.03.012

Zochodne, D. W. (2007). Diabetes mellitus and the peripheral nervous system: Manifestations and mechanisms. *Muscle and Nerve, 36*(2), 144-166. doi:10.1002/mus.20785

CURRICULUM VITAE

Noah Robert Zanville

Education

Institution	*Location*	*Degree/Field of Study*	*Year*
Indiana University	Indianapolis, IN	Ph.D., Clinical Nursing Science (GPA: 3.82)	2018
Indiana University School of Nursing	Indianapolis, IN	B.S.N., Nursing (Accelerated) (GPA: 3.38)	2009
Lane Community College	Eugene, OR	Pre-Nursing Coursework (GPA: N/A)	2007
University of Oregon	Eugene, OR	Bachelor of Arts, Philosophy (GPA: 3.33)	2000

Licensure

Registered Nurse, Indiana (2009 – Present)

Academic and Clinical Appointments

Pre-Doctoral Fellow (June 2015-June 2017) (Full-time)
Ruth L. Kirschstein National Research Service Award (National Institutes of Health)
Indiana University School of Nursing, Indianapolis, Indiana
Graduate Research Assistant (May 2014-October 2015) (10-20 hours/week)
Office of Assistant Dean for Operations & Community Partnerships
Indiana University School of Nursing, Indianapolis, Indiana
Pre-Doctoral Fellow (2013-2014) (Full-time)
Behavioral Oncology and Cancer Control Training Program (National Cancer Institute)
Indiana University School of Nursing, Indianapolis, Indiana
Graduate Research Assistant (August 2011-January 2013) (10-20 hours/week)
Center for Enhancing Quality of Life in Chronic Illness
Indiana University School of Nursing, Indianapolis, Indiana
Pre-Doctoral Fellow (2011-2010) (Full-time)
Training Program in Behavioral Nursing (National Institutes of Health)
Indiana University School of Nursing, Indianapolis, Indiana
Registered Nurse Case-Manager (November 2010-May 2011) (Full-time)
Enhanced Care Program, IU Health, Indianapolis, Indiana
Critical Care Nurse (October 2009-November 2011) (Full-time)
Transplant Intensive Care, Surgical Intensive Care Units
University Hospital/Simon Cancer Center, Indianapolis, Indiana
Research Assistant (September 2008-March 2009) (10-20 hours/week)
Fairbanks Center for Medical Ethics
Indiana University School of Nursing, Indianapolis, Indiana

Publications

1. Zanville, N.R., Nudelman, K.N.H., Smith, D.J., Von Ah, D. McDonald, B.C., Champion, V.L., Saykin, A.J. Evaluating the impact of chemotherapy-induced peripheral neuropathy symptoms (CIPN-sx) on perceived ability to work in breast cancer survivors after treatment. Accepted for Publication at *Supportive Care in Cancer*, May 29, 2016.
2. Nudelman, K.N.H., McDonald, B.C., Wang, Y., Smith, D.J. West, J.D., O'Neill, D. P., Zanville, N.R., Champion, V.L., Schneider, B.P., and Saykin, AJ. Cerebral Perfusion and Gray Matter Changes Associated with Chemotherapy-Induced Peripheral Neuropathy. *Journal of Clinical Oncology*, Mar 1; 34 (7):677-683.

Book Chapters

1. Vasko, M., Zanville, N.R., Shariati, B. Chapter 13: The Role of DNA Damage and Repair in Post-Mitotic Cells during Cancer Therapies. In: M.R. Kelly (Ed.): 'DNA Repair in Cancer Therapy.' Submitted for review on January 25, 2016.
2. Smith, E.L, Zanville, N.R. Chapter 21: Peripheral Neuropathy. In: C. Brown (Ed.): 'A Guide to Oncology Symptom Management.' Pittsburgh, PA: Oncology Nursing Society.

Invited Peer-Review Activities

Peer-Review for Scientific Manuscripts:

- *Journal of Medical Case Reports*. August 2016
- *Journal of Medical Case Reports*. October 2016
- *Journal of Medical Case Reports*. November 2016
- *Current Oncology.* August 2015

Peer-Review for Online Courses:

- *Oncology Nursing Society. Online course on Managing Cognitive Impairment.* 2013.

Posters

Zanville, N.R., Cohee, A. Storey, S. Champion, V.L. Comparing the Frequency of Upper-Extremity Neuropathy in Younger and Older Breast Cancer Survivors during Long-Term Survivorship (Poster). Presented at IU Simon Cancer Center Research Day, 2017.

- Winner, 3rd Place Award for "Best Graduate Student Poster" (Translational/Clinical Research Category)

Zanville, N.R., Nudelman K.N.H., Smith, D.J., McDonald, B.C., Champion, V.L., Saykin, A.J. The Impact of Chemotherapy-Induced Peripheral Neuropathy (CIPN) on Breast Cancer Survivor's Perceived Ability to Work during the First Year of Survivorship (Poster). Presented at IU Simon Cancer Center Research Day, 2016.

- Winner, 2nd Place Award for "Best Graduate Student Poster" (Translational/Clinical Research Category)

Nudelman, K.N.H., Wang, Y. McDonald, B.C., Conroy, S.K., Smith, D.J. West, J.D., O'Neill, D. P., Zanville, N.R., Champion, V.L., Schneider, B.P., and Saykin, AJ. "Nervous system sequelae of chemotherapy treatment: Associations and proposed mechanisms." Presented at American Society of Clinical Oncology Annual Meeting (May 30 - June 3, 2014).

Ansel B., Boyce, M., Fite, L., Simo, A., Zanville, N.R. "A Novel Nursing Intervention for Lower Extremity Radiculopathy (LER) Pain in Adults with Failed Back Surgery Syndrome (FBSS): Project Summary." Presented at the National Association of Clinical Nursing Specialists Conference, San Antonio, Texas (May 13, 2013), and American Society for Pain Management Nursing Annual Conference, Indianapolis, IN (October 13, 2013).

Public Presentations

Zanville, N.R. "Using Clinical/Informatics Partnerships to Improve Surveillance, Clinical Decision-Making and Predictive Modelling for CIPN and CIPN-Related Outcomes." Indiana University School of Computing and Informatics (Colloquia; 60-minutes). June 1, 2017. Indianapolis, Indiana.

Zanville, N.R. "Adapting Heat-Evoked Axon Flares for use in Women with Breast Cancer Receiving Neurotoxic Chemotherapy." Indiana Center for Vascular Biology & Medicine (Presentation; 60-minutes)). May 29, 2013. Indianapolis, Indiana.

Zanville, N.R. "Early Detection Methods for Taxane-Induced Peripheral Neuropathy." Behavioral Cooperative Oncology Group (Presentation; 30-minutes). September 13, 2014. East Lansing, Michigan.

Zanville, N.R. Early Detection Methods for Taxane-Induced Peripheral Neuropathy. 1-hour presentation to the Indiana Center for Vascular Biology & Medicine (ICVBM). Indianapolis, Indiana (August 28, 2013).

Zanville, N.R. Using Clinical-Informatics Partnerships to Improve Surveillance, Clinical Decision-Making and Predictive Modelling for CIPN and CIPN-Related Outcomes. 1-hour invited colloquia to Indiana University School of Computing and Informatics (SOIC). Indianapolis, Indiana (May 30, 2017).

Media

Featured Participant, an episode of American Association of the Colleges of Nursing's *AACN TV*® program, highlighting research at Indiana University School of Nursing. October 2017. Video available at https://www.aacntv.org/indiana-university

Moderator, Panelist, "Embracing Complexity: Managing Treatment-Related Symptoms." Plexus Institute (Podcast). January 21, 2015. Available online at http://plexusinstitute.org/healthcare-calls/

Panelist, "The Growing Crisis in Cancer Care." Plexus Institute (Podcast). June 27, 2014. Available at: https://itunes.apple.com/us/podcast/plexuscalls/id337545304?mt=2

Grants

National Institutes of Health (NIH). F-31 Ruth L. Kirschstein National Research Service Award (NRSA) Pre-Doctoral Fellowship (Award Number: 1F31NR015212-01A1). Award: $59,736.00. May 2015-May 2017

Midwest Nursing Research Society (MNRS)/Council for the Advancement of Nursing Science (CANS). Doctoral Dissertation Grant. Award: $5,000.00. May 2015-October 2016

Scholarships

Oncology Nursing Society (ONS): Connections Conference Scholarship, 2013

Indiana University School of Nursing: Nathaniel & Irene Aycock Nursing Scholarship, 2012

Awards

Indiana University School of Nursing: PhD Leadership Fellowship Award

Indiana University School of Nursing: Outstanding Graduate Student Award

Indiana University School of Nursing: Martel Plummer Leadership Award

Indiana University School of Nursing: IUPUI "Top 100" Award

Indiana University School of Nursing. Undergraduate BSN Class President, 2009