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A pilot clinical trial assessing the efficacy and safety of supplementation with a B complex vitamin to reduce the incidence of chemotherapy-induced peripheral neuropathy in patients diagnosed with a malignancy.

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Abstract

Introduction

Chemotherapy-induced peripheral neuropathy [CIPN] is a significant debilitating side effect resulting from the administration of neurotoxic chemotherapy agents. It is estimated that a third of all patients undergoing chemotherapy experience CIPN, with a third of those progressing to a permanent neuropathy. Patients experiencing moderate to severe CIPN report reduced quality of life, chronic discomfort and disruption of physical abilities for general life activities, which can be temporary or permanent. Moreover, CIPN can lead to a dose reduction or possible cessation of treatment that may adversely impact disease outcomes.

Methodology

In a single blind randomised placebo-controlled trial, newly diagnosed patients undergoing chemotherapy treatment with paclitaxel, oxaliplatin or vincristine were assessed for the safety and efficacy of an oral B group vitamin to reduce the incidence of CIPN. The primary outcome was the Total Neuropathy Score and secondary outcomes included B vitamin pathology, EORTC quality of life questionnaire, Brief Pain Inventory and Patient Neurotoxicity Questionnaire [PNQ].

Discussion

A total of 71 subjects were randomised from 121 evaluable patients (B vitamin n=38; placebo n=33). Participants between groups were matched for gender, chemotherapy agents, age and BMI. No statistical significance was found for the prevention of CIPN from vitamin B supplementation through total TNS score (p=0.73). On individual B vitamin analysis, Vitamin B12 was found to be statistically significant in reducing the onset and severity of CIPN (p=0.024) while vitamin B6 was not statistically significant (p=0.948). Statistical significance was recorded though for sensory peripheral neuropathy in the PNQ (12 weeks p=0.03; 24 weeks p=0.005; 36 weeks p=0.021). The risk estimate for the PNQ was also statistically significant with an OR=5.78, 95% CI = [1.63-20.5].

Conclusions

B vitamin supplementation throughout chemotherapy administration with neurotoxic agents was not superior to placebo (p>0.05) for the prevention of CIPN. Patient perception of reduced sensory peripheral neuropathy with B vitamin supplementation over placebo was statistically significant. Vitamin B12 was found to assist in reducing the onset and severity of CIPN. Furthermore, patients with moderate to severe CIPN may have a vitamin B12 deficiency that may lead to a worse symptomatic presentation.

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Declaration by author

This thesis is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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PUBLICATIONS DURING CANDIDATURE

1. **Journal Article**: An Orally administered B Group Vitamin supplement did not prevent the Development of Chemotherapy-Induced Peripheral Neuropathy [CIPN]. **Schloss J,** Colosimo M, Airey C, Masci P, Linnane A, Gromotenev H, Vitetta L. Submitted to Journal of Cancer and Clinical Oncology.

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5. **Journal Commentary:** Chemotherapy-Induced Peripheral Neuropathy and B Vitamins. **Schloss J,** Colosimo M, Vitetta L. ACNEM Journal. 2012: 31 (2): 24-28

6. **Abstract Publication**: Pilot trial assessing the efficacy and safety of a supplemental B vitamin complex to reduce the onset and severity of chemotherapy-induced peripheral neuropathy. 2015 ASCO (American Society of Oncology) Annual Meeting: 29th May – 2nd June. Chicago, Illinois, United States of America. Page: 125

7. **Abstract Publication**: Chemotherapy-Induced Peripheral Neuropathy (CIPN) and Vitamin B12 Deficiency. **Schloss J**, Colosimo M, Airey C, Linanne A, Vitetta L. 5th International Conference on the Science of Nutrition Medicine in Healthcare Proceedings. Melbourne, Queensland, Australia. May 2-3, 2015. Page: 74

8. **Abstract Publication:** Herbal Medicines and Chemotherapy-Induced Peripheral Neuropathy (CIPN): A critical literature review. **Schloss J,** Colosimo M, Vitetta L. National Herbal Association Australia: Inter-National Conference. Sydney, March 20-22, 2015 Page: 23

9. **Abstract Publication**: B Complex Vitamins Reduce the Onset and Severity of Chemotherapy-Induced Sensory Peripheral Neuropathy. **Schloss J,** Colosimo M, Airey C, Masci P, Linnane A, Vitetta L. 2014 Annual Conference & Exhibition of Functional Foods, Nutraceuticals, Natural Health Products and Dietary Supplements (ISNFF). Istanbul, Turkey. October 14-17, 2014. Page 111.

10. **Abstract Publication:** A pilot clinical trial assessing the efficacy and safety of supplementation with a B complex vitamin to reduce the incidence of chemotherapy-induced peripheral neuropathy [CIPN] in patients diagnosed with a malignancy – final results. **Schloss J,** Colosimo M, Airey C, Masci P, Linanne A, Vitetta L. 4th International Conference on the Science of Nutrition Medicine in Healthcare Proceedings. Gold Coast, Queensland, Australia. May 3-4, 2014. Page 150

11. **Abstract Publication:** B vitamin status in cancer patients undergoing administration of chemotherapy. **Schloss J**, Colosimo M, Masci P, Linanne A, Vitetta L. 3rd International Conference on the Science of Nutrition Medicine in Healthcare Proceedings. Sydney, NSW, Australia. May 3-5, 2013. Page 98.

12. **Abstract Publication:** Methodology for the Pilot Trial on The Efficacy and Safety of B Vitamins in Reducing Chemotherapy-Induced Peripheral Neuropathy. **Schloss J**, Colosimo M, Masci P, Linanne A, Vitetta L. Ist International Conference on the Science of Nutrition Medicine Proceedings. Sydney, NSW, Australia. May 13-15, 2011. Page 104.

13. **Abstract Publication:** Methodology for the Pilot Trial on The Efficacy and Safety of B Vitamins in Reducing Chemotherapy-Induced Peripheral Neuropathy. **Schloss J,** Colosimo M, Abrahams R, Masci P, Linanne A, Vitetta L. Australasian Integrative Medicine Association Conference Proceedings. Coolum, Queensland, Australia. Sept 3-5, 2010. Page 94.

14. **Clinical Roundup Article:** Cancer-Related Fatigue. **Schloss J.** 2014 J Altern and Com Ther. 2014:20(2): 102-103

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Contributions by Others to the Thesis

Conceptualisation of the Project:	A/Prof Luis Vitetta, Dr Maree Colosimo
Assistance with design and format:	A/Prof Luis Vitetta, Dr Sam Coulson, Shoshanna Beck, Dr Maree Colosimo, Prof Anthony Linanne, Dr Paul Masci
Statistical Plan and Assistance:	Helen Gramotov, A/Prof Luis Vitetta
Neurological testing:	Dr Caroline Airey
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Donation of investigation product and scholarship funds:	Bio Concepts Pty Ltd
Interpretation of results:	A/Prof Luis Vitetta

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Keywords

Chemotherapy-induced peripheral neuropathy

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Taxanes

Vincristine

Oxaliplatin

Peripheral neuropathy

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FoR code: 1109	Neurosciences	10%

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Abbreviations

(^{13}C) NMR spectra	Carbon 13 Nuclear magnetic resonance
µg	Micrograms
AC	Adriamycin and cyclophosphamide chemotherapy regime
AC-DT	Adriamycin, cyclophosphamide, Docetaxel, Herceptin chemotherapy regime
Acetyl CoA	Acetyl Coenzyme A
ACP	Acyl carrier protein
ADL	Activities of daily living
ADP-ribose	Adenosine diphosphate ribose
AIDs	Acquired immune deficiency syndrome
ALC	Acetyl-L-carnitine
ALL	Acute lymphoblastic leukaemia
ALT	Alanine aminotransferase
ANA	Antinuclear antibodies
Anti-CCP	Anti-cyclic citrulinated peptide antibody test
AST	Aspartate transaminase
ATP	Adenosine triphosphate
b.i.d	*bis in die* meaning to give medication twice a day
BD	Take medication twice a day
BMD	Bone mineral density
BMI	Body mass index
BPI	Brief Pain Inventory
CA	Cancer antigen
CAPEOx	Capecitabine and oxaliplatin
Cbl	Cobalamin (Vitamin B12)
CCM	Confocal microscope
CDP-choline	Cytidine-5-diphosphocholine
CEA	Carcinoembryonic antigen
CHCA	A-Cyano-4-hydroxycinnamic acid
CI	Confidence interval
CIPN	Chemotherapy-induced peripheral neuropathy
CML	N^5-Carboxymethyllysine

CNS	Central nervous system
CNT	Contact needle therapy
CoA	Coenzyme A
CoO	Carboxy group
CRP	C-reactive protein
CS	Case study
CTN	Clinical Trial Notification
DBRCT	Double-blind randomised clinical trial
DCIS	Ductal carcinoma in-situ
DHF	Dhydrofolate
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglia
DT	Docetaxel and Herceptin chemotherapy regime
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EMG	Electromyography
ENS	Electroneurological Conduction Studies
EORTC	European Organisation for Research and Treatment of Cancer
FAD	Favin adenine dinucleotide
$FADH_2$	Favin adenine dinucleotide (reduced form)
FBP	Folate binding protein
FMN	Favin mononucleotide
FOLFOX	5-fluorouracil, Leucovorin (folinic acid), Oxaliplatin
GGT	Gamma glutamyl transpeptidase
GJG	Goshajinkigan
GSH	Glutathione
GSSG	Oxidised glutathione
HC	Hapatocorrin
HCY	Homocysteine
HER2	Human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
HMM	Hexamethylmelamine chemotherapy
Holo TC	Holotranscobalamin
Holo-MCC	Holo-3-methylcrotonyl-CoA

Holo-PCC	Holo-propionyl-CoA carboxylase
HPLC	High performance liquid chromatography
HREC	Human Research Ethics Committee
HREC	Human Research Ethics Committee
HRLY	Hourly
Humulin NPH	Humulin neutral protamine Hagedorn (intermediate acting insulin)
IDC	Invasive ducal carcinoma
IF	Intrinsic factor
IHBI	Institute of Health and Biomedical Innovation
IL	Interleukin
IM	Intra-muscular
IPN	Induced peripheral neuropathy
IS	Internal standard
IV	Intravenous
IVF	*in vitro* fertilisation
LC/MS	Liquid chromatography / mass spectrometry
LD	Lactate dehydrogenase
LNF	Large nerve fibres
MALDI-MS	Mass-assisted laser desorption ionisation mass spectrometry
MEM	Mixed Effects Model
MMA	Methylmalonylic acid
MRP	Multi-drug resistance protein
MS	Methionine synthase
MS	Mass spectrometry
MTHFR	Methyl-tetrahydrofolate receptor
NAC	N-acetyl cysteine
NAD^+	Nicotinamide adenine dinucleotide
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
NB	Note bene meaning note well
NCI-CTC	National Cancer Institute – Common Toxicity Criteria
nCML	N-carboxymethyllysine
NCS	Nerve conduction studies
NFD	Nerve fibre density

NFL	Nerve fibre length
NF-κB	Nuclear factor kappa B
NGF	Nerve growth factor
$-NH_2$	Amino group
NHMRC	National Health and Medical Research Council
Nm	Newton meters
NMR	Nuclear Magnetic Resonance
NRCT	Non-randomised clinical trial
ns	Nano second
NSCLC	Non-small cell lung cancer
OCT	Ocular coherence tomography
OR	Odds ratio
P5P	Pyridoxal-5-Phosphate
PA	Princess Alexandra
PA HREC	Princess Alexandra Human Research Ethics Committee
PCFT	Proton-coupled folate transporter
PDH	Pyruvate dehydrogenase
PDXK	Pyridoxal kinase
PLA_2	Phospholipase A_2
PLP	Pyridoxal phosphate
PMP	Pyridoxamine 5-phosphate
PN	Peripheral neuropathy
PNP	Pyridoxine phosphate
PNQ	Patient Neurotoxicity Questionnaire
PNS	Peripheral nervous system
PP2A	Protein phosphatase 2A
PRN	Pro re nata: meaning prescribed medication taken as needed
proNGF	Pro-Nerve Growth Factor
QLQ	Quality of Life Questionnaire
QoL	Quality of Life
QPAH	Queensland Princess Alexandra Hospital
RAGE	Receptors for advanced glycation end products
RBWH	Royal Brisbane and Women's Hospital
RCF	Red cell folate

R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone
R-CHOP+M	Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone plus intrathecal methotrexate
RCT	Randomised clinical trial
RNA	Ribonucleic acid
RNFL	Right nerve fibre length
SAMe	S-adenosylmethionine
SD	Standard deviation
SMVT	Sodium-dependent multivitamin transporter
SNF	Small nerve fibres
t.i.d	*ter in die* means three times a day
T2DM	Type II Diabetes Mellitus
TC	Transcobalamin
TC	Taxatere (Docetaxel) and Carboplatin chemotherapy regime
TCA	Tricarboxylic acid cycle
TDP	Thiamine diphosphate
TDP	Thiamine diphosphate
tds	*ter die sumendum* means three times a day
TGA	Therapeutic goods act
THF	Tetrahydrofolate
THTR1 or THTR2	Thiamine transporter protein 1 or 2
TK	Transketolase
TMP	Thiamine monophosphate
TNF-α	Tumour necrosis factor alpha
TNS	Total neuropathy score
TPK	Thiamine pyrophosphokinase
UH	The University of Queensland HREC ethics approval
UQ	The University of Queensland
UQ HREC	University of Queensland Human Research Ethics Committee
USA	United States of America
V	Volt
WCC	White cell count
WHO	World Health Organization
αKGDH	Alpha ketoglutarate dehydrogenase

1 CHAPTER 1

1.1 PREFACE

This thesis presents the results of a randomised placebo-controlled clinical trial assessing the safety and efficacy of a B complex vitamin supplement in reducing the incidence of chemotherapy-induced peripheral neuropathy [CIPN]. The background chapter of this thesis explores the aetiology of CIPN, the chemotherapeutic agents that cause CIPN, the current assessment and diagnostic tools and B vitamin background.

1.2 INTRODUCTION

CIPN is a debilitating clinical condition that represents a side effect from neurotoxic anti-neoplastic agents. It is estimated that one third of all patients who undergo chemotherapy experience CIPN [2]. Patients experiencing moderate to severe CIPN report a reduced quality of life [3], chronic discomfort [4] and disruption of physical abilities for general life activities, which can be temporary or permanent [3].

Current medical treatment for CIPN, including amifostine, anticonvulsants and antidepressants, has limited efficacy and can induce multiple adverse effects. These side effects include hypotension, nausea and vomiting, flushing, dehydration, fatigue, dyspepsia, myalgia, somnolence and allergic reactions [2, 5-8]. The referred pain commonly experienced with CIPN is often resistant to standard analgesics [9].

Moreover, CIPN can lead to a dose reduction of the chemotherapy agent or possible cessation of treatment, which may have an adverse impact on cancer treatment and disease outcomes [2]. It is hypothesised that effective application of therapeutic doses of B group vitamins[1] will decrease the onset and severity of CIPN and may provide a significant benefit to cancer patients undergoing chemotherapy treatment.

Chemotherapy agents [neurotoxic class of agents] that target the peripheral nervous system inducing CIPN include the platinum compounds and the anti-tubulin drugs such as the taxane class, vinca alkaloids and epothilones [2-4, 10]. Other drugs used for cancer that can cause CIPN include thalidomide [3, 11] and proteasome inhibitors such as bortezomib [3, 11]. The neurotoxic side effects

- [1] B group vitamins are a group of water soluble vitamins that include vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxial), folic acid and vitamin B12 (cynacobalmin). This group can also include inositol, choline and biotin which have been included in this thesis.

of these drugs target the peripheral nervous system. The highest incidence of CIPN has been found to be induced from the platinum drugs: cisplatin (60%) and oxaliplatin (80%); and anti-tubulin drugs paclitaxel (60%) and vincristine (75%) [2-4, 12-14].

1.2.1 Aims and Scope of Thesis

1.2.1.1 Research Aim

The aim of this research is to demonstrate that a B group vitamin complex can significantly reduce the incidence of CIPN over placebo.

1.2.1.2 Research Hypotheses

To expand on the aim of this project, the following hypotheses were developed.

a) The delivery of an oral B vitamin complex will be efficacious in reducing the onset and severity of chemotherapy-induced peripheral neuropathy [CIPN].
b) Patients with low B vitamin status prior to chemotherapy administration will be at an increased risk of development and increased severity of CIPN.

1.2.1.3 Research Objectives

To assist in achieving the aims and hypotheses of this project, three trials have been completed. These include:

a) Absorption Trial: Examining if the chosen B vitamin supplement shows absorption of vitamin B12 in healthy volunteers.
b) Absorption and Interaction Trial: Examining the blood B vitamin status of patients undergoing chemotherapy over 72 hours compared to healthy controls.
c) Main Clinical Trial: A randomised placebo-controlled clinical trial assessing the safety and efficacy of a B complex vitamin in reducing the incidence of CIPN.

1.2.1.4 Significance and Scope of Thesis

Current evidence states that approximately a third of all patients who undergo chemotherapy experience CIPN and of those a third will have neuropathy permanently [2]. The incidence of CIPN varies depending on the neurotoxic agent and the mechanism of action of these agents are diverse. The mechanisms include damage to neuronal cell bodies in the dorsal root ganglion, axonal toxicity via transport deficits, energy failure and axonal membrane ion channel dysfunction [10]. Although some of the underlying mechanisms are understood, further understanding of the pathophysiology of CIPN will assist in the development of neuroprotective strategies. The current prevention and management of CIPN remains a clinical challenge for

medical practitioners. Research into agents that may prevent this debilitating side effect is clinically relevant to assist clinicians in cancer management and care.

1.2.1.5 Thesis Structure

The overall structure of the thesis is as follows:

Chapter 1: Covers background knowledge on CIPN, neurotoxic agents, current treatment and diagnostic methods; as well as B vitamins in relation to absorption, deficiency, toxicity and involvement with peripheral nerves.

Chapter 2: Reviews the current international literature relating to CIPN prevention and treatment.

Chapter 3: Examines two new diagnostic tools for CIPN.

Chapter 4: Describes the methodology, study design, sample selection and analysis employed for all three trials used for this project.

Chapter 5: Presents the results of Trial 1: Absorption Trial.

Chapter 6: Presents the results of Trial 2: Absorption and Interaction Trial.

Chapter 7: Presents the results of Trial 3: Main Clinical Trial.

Chapter 8: Presents case studies relevant to the results from the main clinical trial.

Chapter 9: Summarises the significant findings from the thesis, the limitations of the project, contributions to current knowledge, further recommendations and addresses future directions. All publications throughout this project, including publications still to be accepted, have been included in the appendices of this thesis.

1.3 BACKGROUND

1.3.1 Aetiology of CIPN

CIPN has been defined as grade 2 or higher according to the National Cancer Institute Common Toxicity Criteria manual (v2, 1999). Grade 2 includes symptoms such as numbness, tingling, pain or burning in the peripheries as well as motor function impairment that interferes with daily living activities. To examine the meaning of these symptoms the aetiology of the peripheral nervous system needs to be examined.

The peripheral nervous system (PNS) is the area where certain anti-neoplastic drugs accumulate. The PNS involves input and output neurons. The input neurons consist of sensory or afferent neurons that conduct nerve impulses from sensory receptors throughout the body to the central nervous system

(CNS). The output neurons consist of motor or efferent neurons. The motor neurons originate from the CNS and conduct nerve impulses from the CNS to the muscles and glands. The PNS can be further subdivided into the somatic nervous system, which is voluntary; and the autonomic nervous system, which is involuntary [15].

For the peripheral nervous system to be affected, a chemotherapy drug must first cross the blood-nerve barrier and, in addition, the nervous system must be sensitive to the drug. Patients with predisposing conditions such as type II diabetes mellitus (T2DM), HIV/AIDs, alcoholism or who are deficient in B vitamins may be more prone to the side effects of the drug on the peripheral nervous system; thereby increasing the risk of developing CIPN [12, 16]. Given that cancer is a multi-factorial family of diseases, a differential diagnosis of peripheral neuropathy [PN] experienced by cancer patients is necessary to ascertain whether the PN is from the chemotherapy agent. [Table 1-1]

Table 1-1: **The Differential Diagnosis of PN in Patients with Cancer [12]**

Cause	Neurotoxic Affect
Vitamin B12 deficiency	Large nerve fibre injury
Cachexia	Diffuse weakness
Chemotherapy	Small and/or large nerve fibre injury, fast to occur, cumulative
Charcot-Marie-Tooth disease	Large nerve fibre injury
T2DM	Small nerve fibre injury, slow to occur
Atherosclerotic ischemic disease	Sensory neuropathy in lower extremities
Paraneoplastic syndrome	Distal sensory or sensorimotor deficit
Thyroid dysfunction	Proximal and distal weakness, carpal tunnel syndrome
Alcoholic neuropathy	Numbness, paraesthesias

Peripheral nerve fibres are composed of small or large fibres. Small nerve fibres (SNF) are unmyelinated, comprised primarily of microtubules and have slow acting nerve impulses. SNF sense both pain and temperature. Large nerve fibres (LNF) are myelinated, composed mainly of neurofilaments that act as a framework for the axon and have fast acting nerve impulse. LNF sense

position, vibration and motor control [12]. Both of these fibres are targeted by chemotherapy drugs, which may explain why patients experience a variety of symptoms.

The symptoms of SNF CIPN include sensation of tingling, prickling, burning, decreased pin-prick, temperature and light-touch sensations. The symptoms of LNF CIPN include decreased vibratory sensation, proprioception, deep tendon reflexes and muscle strength [16].

The dorsal root ganglion [DRG] nerves, may also be affected by chemotherapy agents [3, 4, 12]. The DRG connect to either the dorsal column (large fibre) or spinothalamic tract (small fibre) in the spinal cord that acts to relay information to the sensory area of the brain [12].

Although each class of neurotoxic chemotherapy agents has a different effect on the nervous system, all induce a glove-stocking distribution of CIPN. This means that the point most distal from the trunk of the body is affected first e.g. fingers and toes, and then the sensations/pain progresses proximally towards the trunk [12]. Each agent has been found to specifically affect one type of fibre more than another. For example, cisplatin damages large fibres whereas paclitaxel and vincristine damages small fibres [12]. Figure 1-1 shows the locations on a nerve fibre where the neurotoxic agents accumulate and cause CIPN [10].

CIPN can be a temporary side effect from which a patient can take up to two years to experience full recovery. However, in approximately one third of cases, it can be a permanent consequence of chemotherapy drug administration. Symptoms may occur within hours, days or weeks after the introduction of the chemotherapy agent(s) that induce CIPN, with cumulative doses increasing the severity and length of time the patient experiences the side effect [12]. Cisplatin differs to other neurotoxic agents as it can induce a delayed CIPN several months after the drug has been administered, rather than a more immediate response [4].

The chemotherapy agents with the highest incidence rate of CIPN are oxaliplatin, paclitaxel and vincristine; consequently, these three agents have been selected for this pilot clinical trial [2-4, 12-14]. These agents were chosen due to the beneficial in-vitro studies conducted on vitamin B6 for oxaliplatin [17, 18] and the high incidence of CIPN over a short period of exposure from paclitaxel and vincristine [2].

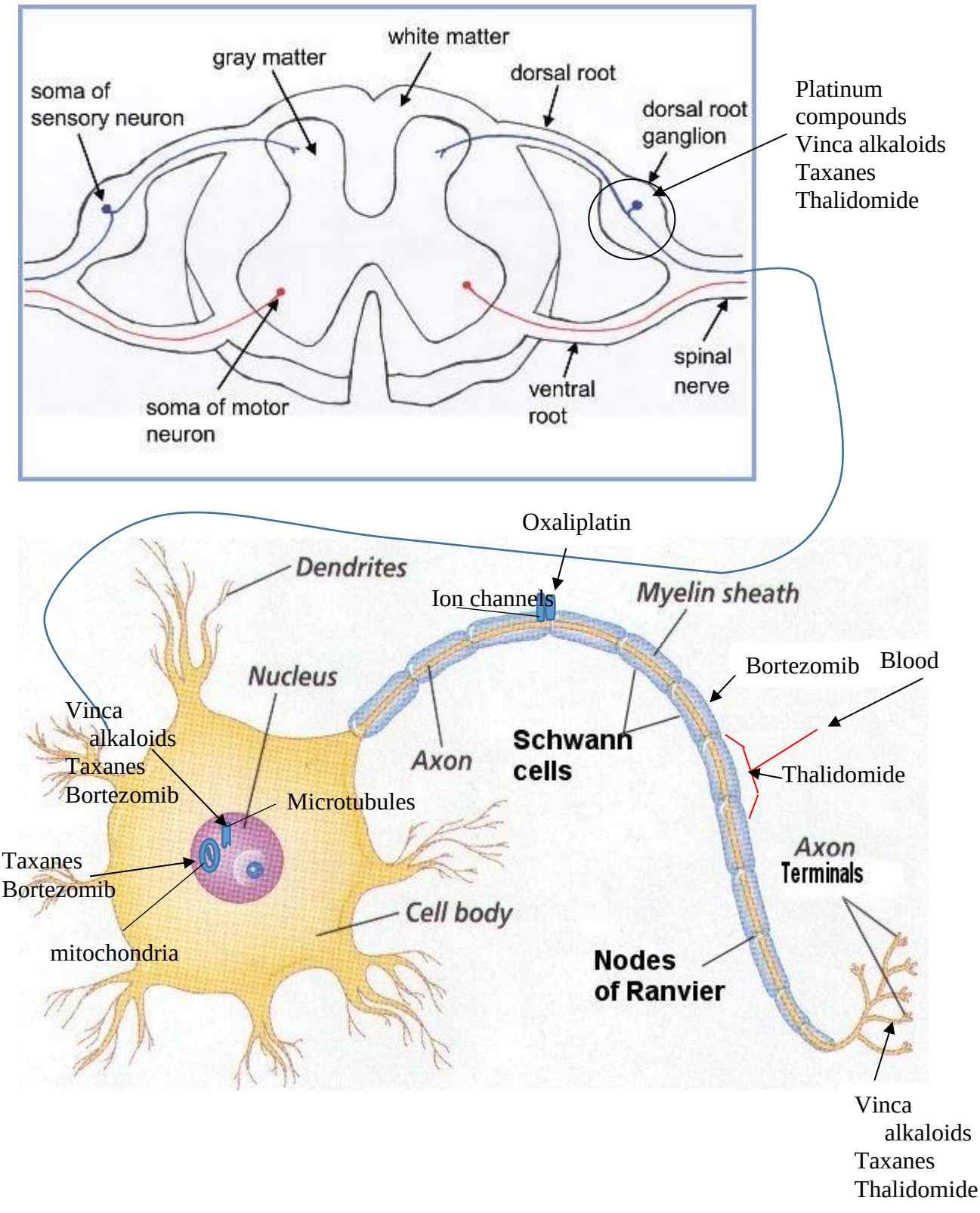


Figure 1-1: Diagrammatic Representation of a Nerve Cell with Sites of Chemotherapy-Induced Neurotoxicity Highlighting where the Chemotherapy Drugs exhibit their Affect. Information and Figures Extracted from references [10, 19, 20].

1.4 MECHANISM OF ACTION OF NEUROTOXIC CHEMOTHERAPY AGENTS

Neurotoxic chemotherapy agents can be divided into four main categories: alkylating and anti-tubulin agents, thalidomide and proteasome inhibitors as shown in Table 1-2. A common feature of these drugs is that they are unable to cross the blood-brain barrier thereby protecting the CNS; however, the PNS has no protection barrier making it susceptible to neurotoxicity [3].

Table 1-2: Neurotoxic Chemotherapy Agents and Incidence of CIPN

Chemotherapy Category	Chemotherapy Drug	Incidence of CIPN
Alkylating Agents		
Platinum compounds	Cisplatin (Platinol®)	59-92% [12-14]
	Carboplatin (Paraplatin®)	25% [4]
	Oxaliplatin (Eloxatin®)	Acute: 80-90 % Chronic: 15-25% [3, 4, 12]
Anti-tubulin Agents		
Taxane Class:	Paclitaxel (Taxol®)	60% [2, 12, 21]
	Docetaxel (Taxotere®)	50% [2, 12]
	Abraxane™	71% [22]
Vinca Alkaloid Class:	Vincristine (Onkovin®)	75% [2, 4, 23]
	Vinorelbine (Navelbine®)	25% [2, 4]
	Vindesine	
	Vinorelbine	
Epothilones:	Ixabepilone	60-71% [3, 24]
	Patupilone	
	MBS-310705	
	Epothilone D (KOS-862)	
	Sagopilone (ZK-EPO) [25]	
Other Categories		
Proteasome inhibitors	Bortezomib	35-50% [10, 11]
Thalidomide	Thalidomide	50-83% [11]

The following chemotherapy agents: the taxane class, oxaliplatin and vincristine were selected for the pilot trial conducted for this doctorate. This is due to the high incidence rate of CIPN experienced by patients who are exposed to these agents in addition to the fact that each agent has a different mechanism of action. Three different chemotherapy agents were chosen to ascertain if B vitamins were more efficacious taken in conjunction with a particular type of chemotherapy agent. If a positive result was recorded for a particular chemotherapy agent, further phase II trials would be considered for that chemotherapy agent. The molecular weight of the chemotherapy agents and the B vitamins can be seen in Appendix 1.

1.4.1 Platinum Compounds

Platinum compounds are alkylating agents used for breast, colon, lung, testicular, bladder and ovarian cancer due to their wide range of activity. These compounds include cisplatin, carboplatin and oxaliplatin [2]. The neurotoxicity from high doses of these drugs is reported to result from mechanisms that infiltrate the intra-cytoplasmic protein binding and ion channel interactions and neuronal apoptosis of the dorsal root ganglion [2, 3, 26]. Oxaliplatin has been chosen from these chemotherapy compounds due to its high incidence of CIPN [92%]. Furthermore, it was the chemotherapy agent used by Garg, et al [27] *in vitro* to test vitamin B6 and CIPN. A recent study by Coriat, et al [18] tested a derivative of vitamin B6 mangafodipir, on CIPN from oxaliplatin in both mice and twenty-three cancer patients with grade 2 CIPN. The results found that mangafodipir may prevent and/or relieve oxaliplatin-IPN. Results from both of these studies concluded that conducting clinical trials with vitamin B6 and oxaliplatin would be beneficial.

1.4.1.1 Oxaliplatin

The platinum compound, oxaliplatin has been found to have marked efficacy in the treatment of large bowel cancer when used in combination with 5-fluorouracil and leucovorin, also known as folinic acid (i.e. FOLFOX) [3]. The mechanism of action of oxaliplatin has been associated with several inter- and intra-strand cross links in DNA, especially two adjacent guanine or two adjacent guanine-adenine bases [4]. Oxaliplatin activates metabolites and reacts with small proteins with sulfhydryl groups such as glutathione, cysteine and methionine. In addition, it also binds to larger molecular weight proteins such as albumin and gamma globulins [3].

The neurotoxicity from oxaliplatin varies compared to the other platinum compounds. In addition to the cumulative dose neurotoxicity, oxaliplatin induces an acute neurotoxicity in approximately 80-90% of patients. This acute neurotoxicity can occur 30-60 minutes after administration [12] and includes dysaethesia and paraesthesia, predominately in the fingers, toes, pharyngolaryngeal tract, perioral and oral regions; furthermore, it is generally induced or aggravated by cold exposure [3, 4].

Most of these acute side effects resolve within a few hours or days. Some patients may also experience muscle cramps or spasms: with both the cramps and the acute neurotoxicity resulting from the drug-related inhibition of sodium channels [3].

The chronic neurotoxicity from oxaliplatin occurs in around 10-15% of patients after cumulative doses and normally resolves several months after oxaliplatin cessation. The symptoms of this CIPN include non-cold related dysesthesia; paraesthesias; superficial and deep sensory loss; and in some cases, sensory ataxia and functional impairment [3, 4]. Sural nerve biopsies in patients with this sensory neuropathy found evidence of axonal degeneration without any evidence of primary demyelination [3, 4]. [Figure 1-1]

1.4.2 Taxane Class

The taxane class consists of three drugs, paclitaxel, docetaxel and abraxane. All three chemotherapy agents are chemically similar [2]. The difference is that docetaxel is a semi-derived form of paclitaxel [4], whereas abraxane is paclitaxel biochemically attached to albumin as a base before administration [22]. The clinical features of the CIPN induced by paclitaxel, abraxane and docetaxel are qualitatively identical and the mechanism of action for all three agents is the same [2, 4]. Thus, the features of paclitaxel discussed below also refer to docetaxel and abraxane.

The mechanism of action for paclitaxel is via suppression of microtubules inducing mitotic arrest in dividing cells. A similar effect on axonal microtubules can interfere with axonal transport, which results in neuropathy mostly affecting small sensory fibres. With higher doses, motor function can also be affected [28].

Paclitaxel preferentially localises to the dorsal root ganglia and induces neuronal damage via necrosis as a result of inhibition of microtubule axonal transport. Demyelination has also been found as a result of paclitaxel administration [2]. Axonal damage has been hypothesised to be the main pathological change that occurs [3].

The neurotoxicity induced by paclitaxel is typically sensory, with deep tendon reflex loss. This causes a distal, symmetrical hypaesthesia in the upper and lower extremities with a length dependent distribution. Motor signs and symptoms may occur during treatment; however, these do not seem to be as clinically relevant as the sensory signs and symptoms experienced. Neuropathic pain very rarely occurs, although may still eventuate in certain individuals, with myalgia a frequent symptom in most patients [3]. The incidence of CIPN from paclitaxel is further increased with concurrent use of other neurotoxic agents such as carboplatin [2].

The CIPN induced by paclitaxel is mostly reversible, nevertheless in a minority of cases the neurotoxicity is persistent leading to permanent peripheral neuropathy [3]. [Figure 1-1]

1.4.3 Vinca Alkaloids

The vinca alkaloid group are anti-tubulin agents that bind to microtubules inducing cell cycle arrest. However, their action is opposite to that of the taxanes. Vinca alkaloids disassemble normal microtubular structures as they inhibit axonal microtubule-mediated transport, resulting in axonal degeneration and loss of epidermal innervations [2, 4].

The CIPN symptoms induced by vincristine are mainly sensory and can begin two weeks after the first dose with cumulative doses increasing the risk. CIPN is the main dose-limiting factor that affects vincristine administration with symptoms resolving slowly after cessation of the drug (approximately one third of cases have reported a temporary worsening of effects after cessation) and in some cases, leaving permanent neuropathy [2-4].

Vinca alkaloids are similar to the other neurotoxic agents in that sensory signs and symptoms are predominately found in the majority of patients. These symptoms are caused by inhibition of anterograde and retrograde axonal transport by microtubule damage leading to degeneration and atrophy of the peripheral nerve fibres. Both small and large fibres are affected, however a stronger effect on small fibres has been reported [12].

Mostly distal symmetrical hypaesthesia and dysesthesia involving all sensory modalities and both deep and superficial neuropathy have been reported with pain also noted [4]. Fifty-seven per cent of patients receiving vincristine reported paraesthesias in the hands or feet and 22-34% reported motor weakness or foot drop [23].

Myalgia or muscle cramps may occur in addition to reduced strength in the distal muscles, in those patients most severely affected by CIPN [3, 12]. Occasionally, severe impairment of motor function leading to tetraplegia has been reported in patients with pre-existing peripheral neuropathy [3]. There have also been rare reports of isolated peripheral nerve functional impairment associated with vincristine administration. [Figure 1-1]

A recent study assessed nutrient deficiencies in children, with or without CIPN from vincristine administration, during acute lymphoblastic leukaemia (ALL) treatment [29]. Jain, et al [29] focused on vitamin E, B12 and folate. Of the 80 children tested electrophysiologically within the study, neuropathy was seen in 27 (33.75%). None of the children were found to be deficient in vitamin E; Moreover, the prevalence of a folate ($p=0.48$) and vitamin B12 ($p=0.21$) deficiency was not statistically significant. Although not statistically significant, a number of children were found deficient in folate and/or vitamin B12. However, no mention was provided regarding vitamin replacement and their current CIPN status. This would have been a clinically relevant finding if the CIPN status decreased after B12 and folate supplementation.

Further case studies on children with leukaemia treatment and CIPN have been conducted looking at vitamin B6 [30, 31]. Both studies used pyridoxine and pyridostigmine for treatment of vincristine-IPN with success as all children had complete recovery between 1-2 weeks after commencing the intervention.

1.5 CIPN CURRENT TREATMENT OPTIONS

The prevention and management of CIPN is of major clinical importance to patients diagnosed with cancer and are undergoing therapy with neurotoxic chemotherapy agents. Several different substances for pharmacological neuro-protection have been tested e.g. Amifostine, but so far neither prophylactic strategies nor symptomatic treatments have proven useful [1, 4]. With the treatment of CIPN largely remaining ineffective, many patients undergo dose reduction of their chemotherapy treatment or cease treatment altogether putting them at risk for disease progression and treatment inefficacy [1].

Figure 1-2 shows a flow chart for clinical evaluation of suspected CIPN. Clinicians supervising patients undergoing chemotherapy treatment with neurotoxic agents are required to monitor CIPN development throughout treatment. Guidelines for evaluation of CIPN are necessary for standardisation.

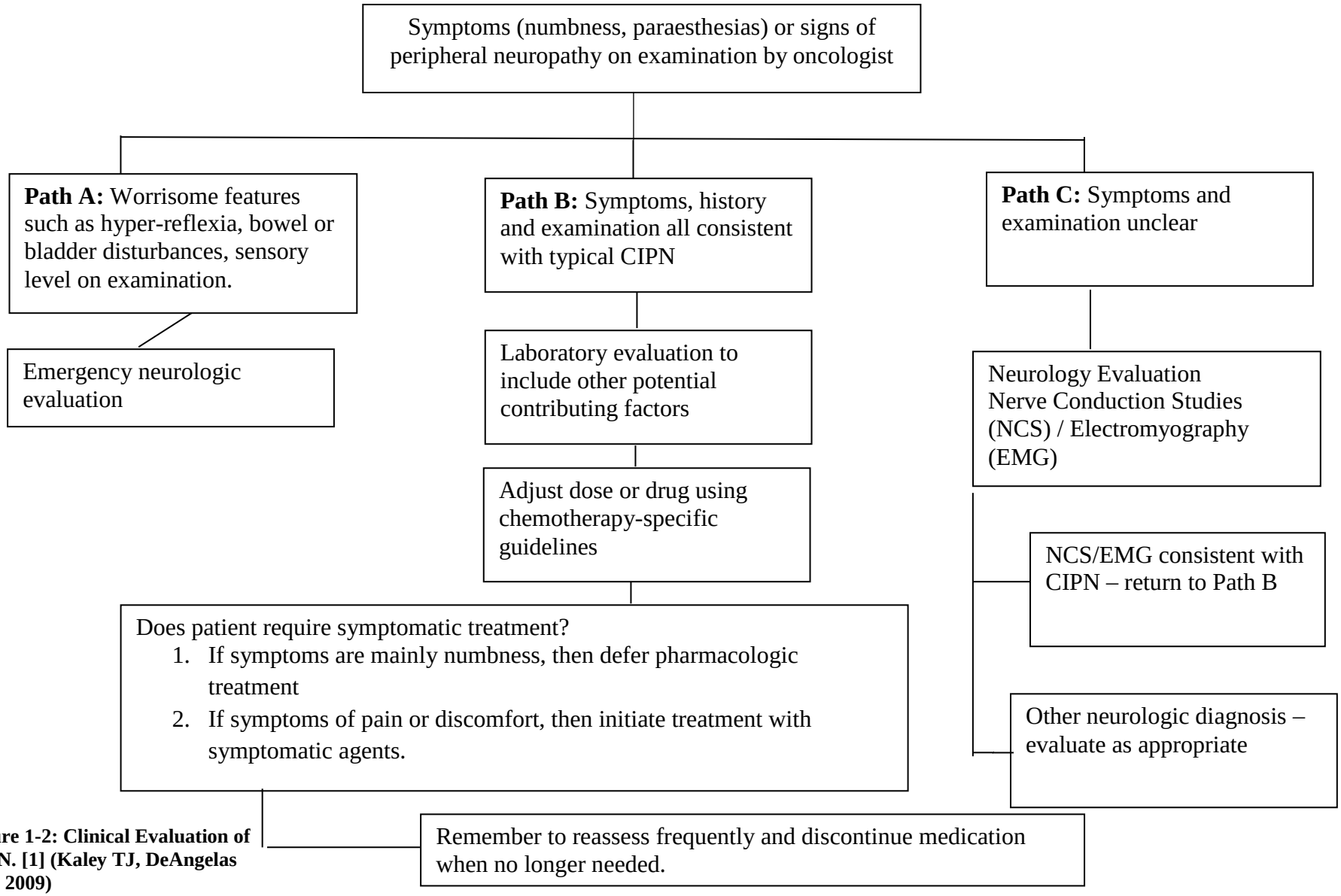


Figure 1-2: Clinical Evaluation of CIPN. [1] (Kaley TJ, DeAngelas LM. 2009)

1.6 CIPN DIAGNOSTIC TESTS AND ASSESSMENT OF CIPN

The grading or assessment of CIPN is critical for accurate clinical and instrumental monitoring. There is no 'gold standard' measurement tool that is used in either clinical trials or medical monitoring [32]. The lack of standardisation and reporting mechanisms has resulted in contention of CIPN data leading to CIPN being relatively under-diagnosed [33].

Clinicians using common toxicity scales such as the National Cancer Institute – Common Toxicity Criteria (NCI-CTC), most commonly grade CIPN. However, these scales rarely provide the detailed information on clinical and pathological aspects of peripheral neuropathy that clinicians require for clinical trials. Other measurement tools such as the Total Neuropathy Score [TNS] involve more detailed information pertaining to motor, sensory and autonomic signs and symptoms, determination of vibration perception thresholds and electrophysiological examination [34, 35].

Of the current measurement tools available, it is recommended that a variety of standardised tools be used; including a peripheral neuropathy scale, quality of life questionnaire, pain scale and patient's perspective questionnaire. The measurement tools recommended from the scientific literature include: TNS, EORTC quality of life questionnaire, a simple pain assessment and the Patients Neurotoxicity Questionnaire [34-36]. [Appendices 2, 3, 4 and 5.]

1.7 STRENGTHS AND WEAKNESSES CIPN DIAGNOSTIC AND ASSESSMENT TOOLS

As discussed, CIPN is generally assessed using the NCI-CTC scales however, it is known that significant inter-observer disagreements exist using these scales [34]. Table 1-3 outlines the available tools used to assess or measure CIPN, identifying their strengths, weaknesses, validation and psychometric properties. From the available literature, it is eveident the current existing scales are still limited when used individually for evaluating CIPN. The TNS and the FACT/GOG-Tnx have been found to be reliable, valid and relatively easy to use. However, there is limited information regarding physical limitations, symptom distress and effect on quality of life. Assessment for CIPN requires both objective and subjective data collected using both qualitative and quantitative methods. Moreover, it has been found that health-care professionals tend to underestimate and underreport the severity and frequency of CIPN. Hence, gaining information both from the patient and using a quantification tool conducted by a professional healthcare clinician is necessary to gather adequate information pertaining to CIPN detection and treatment. Therefore, further research into better instruments to assess and measure CIPN for clinical management needs to occur. The strengths and weaknesses of each assessment tool have been outlined in table 1-3.

Table 1-3 Assessment Tools for CIPN [34]

Assessment Tool	Strengths	Weaknesses
NCI-CTC	- Quick to use - Easy to administer - Good for screening process to assess those patients requiring further testing	- Subjective as it relies on observer's opinion, therefore inter-observer disagreement occurs - No quantitative assessment
World Health Organization (WHO) scale	- Records patients baseline data and treatment-related toxicity - Easy to administer	- Has never gained widespread use - No assignment of any clinical significance to each toxicity grade - Subjective rather than quantitative
ECOG scales	- Quick to use - Easy to administer	- Subjective as it relies on observer's opinion, therefore inter-observer disagreement occurs - No quantitative assessment
Ajani scale	- Included sensory and motor symptoms - Increased objective information - Introduced morbidity range	- Subjective as it relies on observer's opinion, therefore inter-observer disagreement occurs - No quantitative assessment
FACT/GOG-Ntx[2]	- Incorporates QoL questions - Gathers patient perception - Easy to administer	- Focussed mainly on sensory function - Patient or self-reported - No quantitative assessment - Limited mixed functional, impairment and symptom items
FACT-Taxane	- Demonstrates internal consistency reliability - Gathers patient perception	- Patient or self-reported - No quantitative assessment - Limited mixed functional, impairment and symptom items
Peripheral Neuropathy Scale (PNS)	- Gathers patient perception	- Patient or self-reported - No quantitative assessment
Oxaliplatin-associated neuropathy questionnaires	- Gathers patient perception	- Patient or self-reported - No quantitative assessment - Interpretation of results difficult due to descriptive discrimination between acute and chronic toxicities
SCIN[3]	- Quick to use - Easy to administer - Single items summed up between a score of 0-6	- Self-reported - Small scale divides into high (≥4) or low (≤3) - Limited to the occurrence of pain and tingling

[2] Functional Assessment of Cancer Therapy/Gynaecologic Oncology Group (GOG) – neurotoxicity (FACT/GOG-Ntx)

[3] SCIN - Scale for Chemotherapy-Induced Long Term Neurotoxicity

Assessment Tool	Strengths	Weaknesses
Patient Neurotoxicity Questionnaire (PNQ)	- Quick to use - Easy to administer - Gives patient perspective of CIPN	- Self-reported - Small scale - Limited diagnostic tool
EORTC QLQ-CIPN20[4]	- Reliable, reproducible and consistent results on QoL - Easy to administer - Gives patient perspective	- Self-reported
Total Neuropathy Score (TNS)	- Most comprehensive composite scale - Quantitative analysis by a neurologist - Indicates a higher sensitivity to CIPN - More accurate monitoring of detection and measurement of change - Incorporates nerve conduction studies - Incorporates both subjective and objective examinations	- Inadequately assesses pain - Inadequately assesses severity of CIPN - Limited QoL data - Requires a qualified neurologist to conduct the assessment

[37-39]

Griffith KA et al., (2010) conducted a systematic review on psychometric properties of measures for CIPN. [39] Table 1-4 shows an adapted table of the results listed in the review indicating quality, grading and psychometric tests. Griffith KA et al., (2010) analysed the available literature using a tailored quality tool and individual assessments of other important aspects of measure for integrity, such as responsiveness. The authors stated that both approaches were necessary to be able to differentiate measures scored in the higher range with the modified quality of diagnostic accuracy studies tool (QUADAS) [39].

The final outcomes from the evaluation of psychometric properties for measuring CIPN concluded that there were two well-tested, valid measures which scored moderately high that would be potentially useful in clinical trials and patient care. These were the FACT/GOG-Ntx and the TNS clinical versions. The FACT/GOG-Ntx was described as being a carefully developed subjective measure of CIPN-related quality of life measure and was considered important considering the high prevalence of this side effect. However, clinical measurement of CIPN is considered just as important.

[4] European Organisation of Research and Treatment of Cancer (EORTC) quality of life (QLQ)- chemotherapy-induced peripheral neuropathy (CIPN)20

The authors of the systematic review [39] found the TNS clinical versions incorporated both objective and subjective times and are likely to represent a more sensitive measure of CIPN. For best results, it concluded that incorporating both the FACT/GOG-Ntx and a TNS clinical version would offer the most promising measure of CIPN.

Table 1-4 Psychometric Measures for CIPN [39]

Study	Quality Score	Measure(s) evaluated	Reliability /Type	Validity / Type	Responsiveness	Results
Forsythe et al. (1997) [40]	4	QST	-	+/1	-	Found no correlation between cumulative paclitaxel dose and ES or QST changes in both upper and lower limbs. Median cumulative paclitaxel dose and SS correlated (P<0.01)
Postma et al. (1998) [41]	4	WHO ECOG NCI-CTC Ajani	+/2	-	+/1	Inter-observer agreement ranged from 81.1% to 94% for dichotomised grade 0-2 vs 3 on all scales Inter-observer agreement using ICC ranged from 0.37 (Ajani) to 0.75 (ECOG) Agreement on grade 3 ranged from 0% (WHO, Ajani) to 42% (ECOG, CTC)
Cavaletti et al. (2003) [42]	6	TNS TNSr	-	+/1	-	TNS score had a high correlation with NCI-CTC 2.0, Ajani and ECOG scores. TNS more sensitive in severe cases of CIPN.
Calhourn et al. (2003) [43]	5	FACT/GOG-Ntx	+/1	+/1	+/2	Significant correlation post-chemo and 3 months' post between FA/GOG-Ntx and reflexes and strength. P<0.05 for all. Cronbach's α = 0.84- 0.90
Cella et al. (2003) [44]	3	FACT/Taxane	+/1	+/1	+/1	Cronbach's α = 0.82-0.86 depending on treatment time point. Mean score changed from baseline to week 12 of treatment was 7.0 (P<0.001)

Study	Quality Score	Measure(s) evaluated	Reliability /Type	Validity / Type	Responsiveness	Results
Greimel et al. (2003) [45]	3	QLQ-OV28	+/1 and 3	+1/ and 4	+/2	Cronbach's α = 0.83. Peripheral neuropathy responsive from baseline to treatment (P<0.03). Test-retest reliability for chemotherapy group ICC = 0.88
Almadrones et al. (2004) [46]	5	PNS	+/1	+1 and 2	+/2	Cronbach's α = 0.91. Clinical sensitivity from T1 to T2 (P<0.05). The correlation between function and ICPN was P<0.05
Kopec et al. (2006) [47]	6	FACT/GOG-Ntx	+/1	+/1	+/1	Cronbach's α = 0.85. Factor structure indicated uni-dimensionality. Longitudinal score changes (P<0.0001). Correlated with NCI-Sanofi criteria
Oldenburg et al. (2006) [48]	7	SCIN	+/1 and 5	+/5	-	Cronbach's α = 0.72. Factor structure indicated three dimensions. Sensorsymptom items discriminated between patients with cisplatin and those receiving alternate treatments (P≤ 0.02)
Cavaletti et al. (2006) [49]	6	TNS TNSr	+/2	+/1	-	Moderate to high correlation between TNSr/TNSc and NCI-CTC 2.1, ECOG motor and sensory items (P <0.0001 for all)
Wampler et al. (2006) [50]	5	TNS mTNS	-	+1 and 3	-	TNS and mTNS correlated r = 0.99 (P<0.001). mTNS discriminated between groups, treatment vs control
Huang et al. (2007) [51]	5	FACT/GOG-Ntx	+/1	+/1	+/2	Cronbach's α ≥ 0.80 prior to cycles 1-7. Sensory items attributed to 80% of treatment differences and 63% of longitudinal changes in subscale score

Study	Quality Score	Measure(s) evaluated	Reliability /Type	Validity / Type	Responsiveness	Results
Cavaletti et al. (2007) [36]	5	TNS TNSc	+/1	+/1	+/2	TNS and TNSs correlated with NCI-CTC (P<0.001). Study 1 r =0.75, Study 2 r = 0.88
Shimozuma et al. (2009) [52]	4	PNQ	-	+/1	+/2	PNQ both sensory and motor scores correlated with FACT-GOG-ntx (r = 0.66 and 0.51 respectively). PNQ socres increased significantly with subsequent chemotherapy (P<0.0001)
Smith et al. (2010) [53]	5/5	TNSr/NPS	+1 and 2	+/1	-	Cronbach's α = 0.56 for TNSr and 0.96 for NPS. TNSr items eliminated the following factor analysis i.e. pin sensibility; strength and reflexes due to low inter-item correlations

NB: For reliability, validity and responsiveness: +, study tested this attribute; -, attribute not tested. For reliability type reported: 1 internal consistency; 2 inter-observer; 3 intra-observer; 4 test-retest; 5 split half. For validity type reported: 1 construct; 2 content; 3 discriminant; and for responsiveness: 1 effect size; 2 other.

1.8 DIAGNOSTIC TOOLS SELECTED FOR THIS THESIS

The measurement tools selected for this clinical trial were based on the literature suggesting a variety of measurement tools are required for accurate diagnosis, measurement and monitoring of CIPN development. It is recommended that the tools selected should include a peripheral neuropathy scale, quality of life questionnaire, pain scale and patient's perspective questionnaire. Hence, the TNS, EORTC Quality of Life Questionnaire, a simple pain assessment from MD Anderson used for cancer patients and the Patient Neurotoxicity Questionnaire were selected [34-36].

CIPN has been defined as grade 2 or higher according to the National Cancer Institute Common Toxicity Criteria manual (v2, 1999). Therefore, for this thesis as well as the clinical trial, a diagnosis of CIPN must be conducted by professional medical personnel (i.e. Neurologist) who uses a validated measurement tool(s) that can quantify peripheral nerve damage due to a chemotherapy agent administration that causes numbness, pain, or tingling, which interferes with the physical abilities of the patient. The major limiting issue of be able to diagnosis CIPN is that there is no defined standardisation approach to the diagnosis of CIPN. Hence, further quantification and standardisation of diagnosis needs to be confirmed. One group, CI-PERINOMS, are attempting to standardise the diagnosis of CIPN; however, further efforts are still required [54].

1.9 B VITAMINS

B vitamins are water-soluble vitamins that are required for a variety of functions within the human body, such as energy production from food and formation of red blood cells. Assessing the biochemistry and absorption of B vitamins is important when considering supplementation in compromised patients. The National Health and Medical Research Council's [NHMRC] [55] daily recommended doses and the suggested recommended upper dose are detailed in Appendix 6.

Currently, the B group vitamins encompass thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxal (vitamin B6), folic acid and cobalamin (vitamin B12) and biotin [56]. Other vitamins that are similar and were traditionally included in the B vitamin group include choline and inositol [39]. Both choline and inositol can be produced by the human body but have been included the in the selected B group supplement due to traditional classification and their functionality [58-60].

1.9.1 Mechanism of Action of B vitamins

The vitamin B complex [vitamins B1, 2, 3, 5, 6, 12, folate, choline and biotin] functions as coenzymes in several intermediary metabolic pathways for energy generation, blood cell formation and energy metabolism. Deficiencies in one or more of these B group vitamins may promote nerve dysfunction and nerve damage that can lead to peripheral neuropathy [61].

Vu, et al [62] found that certain chemotherapeutic agents induced a temporary deficiency in vitamin B12 through evaluations of holotranscobalamin II levels (Holo TC) [62]. It is possible that chemotherapy agents induce a B vitamin deficiency or temporarily lowers the B vitamin status. This may be a major component of the development of CIPN in patients undergoing treatment with neurotoxic chemotherapy agents.

Holo TC or active B12 test is an immunoassay that can be used for the detection of vitamin B12 deficiency instead of or in conjunction with total serum vitamin B12. Vitamin B12 in blood is bound to two proteins specifically in serum, transcobalamin (TC) and haptocorrin (HC). The majority of circulating vitamin B12 is bound to HC (70-90%); however, it is considered to be biologically unavailable to most cells. The remaining 10-30% of vitamin B12 is bound to holotranscobalamin (holo TC), which delivers the vitamin into metabolising cells from its site of absorption [71, 72]. The measurement of holo TC has been found to be more diagnostically accurate for detecting early vitamin B12 deficiency compared to total serum vitamin B12, which measures both HC and TC levels [63].

It is hypothesised that patients who are low or deficient in B vitamins would be more susceptible to developing CIPN and that treatment with B vitamins will aid in reducing the onset and severity of CIPN as well as promoting recovery.

1.9.2 Use of B vitamins in Cancer

Vitamin supplementation has been controversial for many years with differing views on its usefulness in both daily diet and for medical treatment. Currently, it is estimated that over 47% of males and 59% of females use dietary or vitamin supplementation for health benefits [64]. For decades now, numerous studies have reported that supplements do not have any health benefits or, alternatively, that they may trigger adverse health effects [64]. Nevertheless, many studies have also demonstrated the benefits of vitamin supplementation for certain conditions, for example using pre-natal folate supplementation to significantly decrease the incidence of foetal neural tube defects [65]. It is these equivocal and conflicting findings, regarding the administration of vitamin and dietary supplements

on health outcomes, that confuse consumers and clinicians alike. Vitamin supplementation in cancer is no different to any other health condition.

Conflicting evidence has been found for certain B vitamins in cancer research [66-68]. Moreover, the role of vitamin supplementation in those diagnosed with cancer is still unclear. The two main B vitamins researched for cancer are folic acid and vitamin B6 [69, 70]. The use of a B complex supplement in cancer has been limited in human trials, although epidemiologic studies suggest cancer patients are regularly administering these supplements [71]. Both vitamin B6 and folate have had numerous studies conducted *in vitro* and in animal models with less studies conducted on humans [69]. There have also been safety issues that have been brought to the forefront concerning supplementation or fortification [70].

Vitamin B6 investigations began around 1950 with researchers looking at the influence of vitamin B6 on oncogenesis and tumour progression [69]. The majority of these initial studies included *in vitro* and animal studies on immune-deficient rodents. The general consensus was that vitamin B6 exerted anti-neoplastic activity. In 2012, vitamin B6 was reported to exhibit a synergistic effect with certain chemotherapeutic drugs [72]. Small participant number clinical studies were then conducted and subsequently expanded.

The end-result found that cancer patients often manifest a decreased level of circulating P5P, or vitamin B6 vitamers, compared to age-matched healthy individuals. In addition, researchers also found that elevated circulating amounts of B6 vitamers in addition to high consumption of vitamin B6 correlated with a reduced incidence of several cancers. Furthermore, a high intra-tumoral expression level of pyridoxal kinase (PDXK – the enzyme that converts all forms of vitamin B6 to PLP) was reported to possibly improve disease outcome among lung cancer patients (NSCLC) [69].

The molecular and cellular mechanism of action underlying these observations still needs to be elucidated. There have been a number of well-established relationships identified between vitamin B6 and cancer; these include that a proficient metabolism of vitamin B6 is required to sustain the anabolic requirements of highly proliferating cells, such as tumour cells and the immune system [72, 74]. This assists in understanding why cancer patients can potentially suffer from a vitamin B6 deficiency and, furthermore, that cancer-associated immunosuppression may partially be derived from a vitamin B6 deficiency [69].

Vitamin B6 has been found to impinge on one-carbon metabolism and this may have an oncosuppressive activity by promoting DNA repair and genomic stability [75-77]. The metabolism of vitamin B6 has also been implicated in the adaptive response to a number of adverse conditions.

For example, multiple settings that malignant cells normally experience during tumour progression including nutrient deprivation and hypoxia [71].

Therefore, vitamin B6 use has been clinically related to cancer on a number of levels. From the studies discussed, it would be adventitious for people with cancer to take a low dose supplement of vitamin B6, approximately 40 mg daily. Interactions with chemotherapeutic agents still require further investigation with studies showing beneficial effects when administered in conjunction with cisplatin [72]. However, considering that B6 has affected dose response when given in high doses, further studies assessing dose still need to be completed [78].

Folic acid is the other B vitamin that has been extensively studied in relation to cancer [70]. The research on folate is more controversial compared to vitamin B6. A meta-analysis by Vollset, et al. [70] used individual participant data in a time-to-event analysis from large trials and found that there was no statistically significant excess risk from folate supplementation for any cancer. Vollset, et al. [70] state that any excess risk from folate supplementation found in prostate cancer patients was most likely a spurious exaggeration.

A main concern with folate supplementation and fortification is the increased risk of colorectal cancer [79]. In geographical areas such as the UK, food fortification with folate has not occurred due to the perceived increased risk of colorectal cancer. This increased risk of malignancy stems from clinical data indicating an increased risk of colorectal cancer incidence in the USA and Canada from 1996-98 when fortification of folate was introduced in those countries. It was estimated that there was between four to six additional cases of colorectal cancer, per 100,000 of the population, post fortification with folate; moreover, that this fortification could have resulted in an acceleration of progression from adenoma to carcinoma [79]. However, a more recent prospective cohort study found that the level of folate fortification in the USA was not detrimental and did not increase the risk of colorectal cancer [80].

Overall, the human evidence regarding folic acid and the risk of cancer has not been substantiated. The meta-analysis by Vollset, et al. [70] concluded that the aggregate of trials provided no statistically significant evidence of short-term effects of folic acid supplementation on overall cancer incidence or on the increased risk for any type of cancer. Hence, it is currently suggested that folic acid supplementation and fortification is safe and does not prevent or increase the risk of any particular type of cancer.

Recent research supports the use in low doses of B vitamin supplementation for patients diagnosed with cancer. Researchers suggest that dietary intake is a better form of consumption compared to supplementation.

1.9.3 B Vitamins Information and Research in Peripheral Neuropathy

1.9.3.1 Thiamine (Vitamin B1)

Thiamine, commonly designated as vitamin B1, was one of the first vitamins discovered and was identified as the dietary factor responsible for the development of beriberi. It is most commonly found in raw foods such as cereals, green vegetables, nuts, egg yolk and pork meat. Certain foods (e.g. breads, cereals and flours) are supplemented or fortified with thiamine, as heating destroys thiamine in foods [81]. Thiamine is decreased in the body by thyroid hormones, diabetes, ethanol, age and certain drugs such as 5-fluorouracil [81, 82]. The most common cause of a thiamine deficiency in affluent societies is chronic alcohol consumption leading to Wernicke-Korsakoff encephalopathy [83]. More recently, a link between thiamine deficiency and colon carcinogenesis [84] has also been reported.

Thiamine's chemical structure consists of a pyrimidine ring and a thiazole moiety linked by a methylene (CH_2) bridge as seen in Figure 1-3.

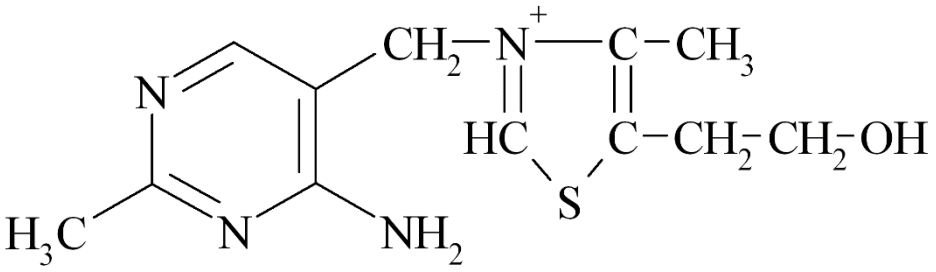


Figure 1-3: Chemical Structure of Thiamine [85]

<u>Digestion and Absorption of Thiamine</u>

Thiamine absorption primarily occurs in the jejunum or proximal small intestines. Thiamine predominately occurs in a nonphosphorylated form in plants and in a phosphorylated form such as thiamine diphosphate (TDP) in animal products [58]. In supplements, it is commonly found as thiamine hydrochloride and is hydrolysed by intestinal phosphatases to thiamine prior to absorption [58]. At low concentrations it requires transporters (i.e. THTR1 and THTR2 to cross the brush border) while at high concentration uptake occurs due to simple passive diffusion. The colon can also absorb thiamine through THTR1 transporters and thiamine/H+ exchange, which seems to be under the regulation of an intracellular calcium/cadmodulin mediated pathway. This could be the route of absorption from bacteria synthesised thiamine [81].

Absorption of thiamine is thought to be relatively high, however, there are certain anti-thiamine factors present in the diet that can interfere with thiamine's absorption. For example, fish preparation methods will influence thiamine absorption as raw fish contain thiaminases (deactivated by cooking). Polyhydroxyphenols, such as tannic and caffeic acids, are found in tea, coffee, betel nuts, certain fruits and vegetables (e.g. blueberries, black currants, Brussels sprouts and red cabbage). The polyhydroxyphenols in these foods can be deactivated by cooking or heating [58].

Within the mucosal cells, thiamine can be phosphorylated into a phosphate ester and transported across the basolateral border by active transport using both sodium and energy. Alcohol or ethanol ingestion interferes with the active transport of thiamine from the mucosal cells across the basolateral border but not the brush border thereby decreasing thiamine absorption [58]. Thiamine in the blood is mostly found in red blood cells (approximately 90%); and predominately exists as thiamine diphosphate (TDP) with small amounts of thiamine monophosphate (TMP). Whereas, in the plasma (approximately 10%) it is found in its free form, bound to albumin or as TMP [58].

Tissue Storage of Thiamine

A small amount of thiamine is stored in the body (approximately 30 mg per adult) with approximately 40% of the stored thiamine found in muscles. Additional thiamine storage sites include the brain, heart, liver and kidney [81].

Metabolism and Excretion of Thiamine

Excess thiamine, intact or metabolised, is excreted via the urine. Both TDP and TMP are excreted in their enzymic forms. Degradation of thiamine begins with the cleavage of the molecule into its pyrimidine and thiazole moieties. These two rings are further metabolised generating 20 or more metabolites, which are excreted in the urine in addition to TDP and TMP [58].

Function of Thiamine

Thiamine plays a number of roles in the human body, for example, coenzymes, energy transformation (particularly metabolism of glucose), synthesis of pentoses and NADPH as well as assisting in membrane and nerve conduction [58]. In particular, it is highly active in the mitochondria as it is involved in the dehydrogenase reactions as a cofactor for pyruvate, α-ketoglutarate and branched-chain ketoacid dehydrogenases. These enzymes catalyse the oxidative decarboxylation of α-ketoacids to release carbon dioxide; therefore, thiamine is central to mitochondrial energy production. Thiamine also acts as a cofactor for transketolase (TK), which is a reversible cytosolic enzyme that catalyzes the first and last step of the pentose phosphate pathway. This pathway plays a major role in cellular function, for example, the production of NADPH for maintaining cellular redox, glutathione (GSH)

levels, protein sulphydryl groups, fatty acid synthesis, and supplying ribose for nucleic acid synthesis [81].

Another function of thiamine is involvement in nerve membranes whereby TTP is thought to activate ion transport, in particular, chlorine but also participation in nerve impulse transmission via sodium channel regulation and release of acetylcholine [58].

Deficiency of Thiamine

A thiamine deficiency is commonly known as Beriberi. The first symptoms of a thiamine deficiency are loss of appetite and weight loss. As the deficiency continues or worsens, cardiovascular symptoms such as hypertrophy and altered heart rate are diagnosed; neurological symptoms such as apathy, confusion, decreased short-term memory and irritability [58].

There are three types of Beriberi that have been identified:

1. Dry Beriberi: this is predominately found in older adults particularly if they are consuming a high carbohydrate diet. It is characterised by muscle weakness and wasting especially in the lower limbs. This form of Beriberi is associated with PN and is characterised by sensorimotor, distal and axonal peripheral neuropathy. It is often associated with calf cramps, muscle tenderness and burning feet [96-98].
2. Wet Beriberi: involves the cardiovascular system such as right-side heart failure leading to respiratory involvement with oedema.
3. Acute Beriberi: is commonly observed in infants and has been primarily recorded in countries such as Japan [58].

As mentioned above, thiamine deficiencies have also been associated with high alcohol consumption and specific pharmaceutical drugs both of which can cause a peripheral neuropathy [81, 82]. In people with diabetes, a deficiency in thiamine has also been linked with multiple organ damage including diabetic peripheral neuropathy [86].

Toxicity of Thiamine

Currently, there are no reports of thiamine toxicity from oral ingestion of large doses of thiamine from supplementation i.e. 500mg daily for 1 month [87, 88]. However, headaches, convulsions, cardiac arrhythmias and anaphylactic shock have been associated with intravenous or intramuscular administration of thiamine [89]. The NHMRC has reported that there is no upper level or limit for oral thiamine and has not been documented as a health risk [90].

Assessment of Nutriture of Thiamine

Body fluid measurement of thiamine has not been routinely assessed by clinicians. However, Sullivan Nicholaides Pathology (www.snp.com.au) conducts assays on levels of vitamin B1, B2 and B6, which will be utilised for this trial. Other assays used to measure thiamine include a serum assessment involving the measurement of erythrocyte transketolase activity [transketolase is the thiamine-dependent enzyme of the hexose monophosphate shunt] or plasma thiamine pyrophosphokinase 1 [thiamine pyrophosphate synthesis in eukaryotes, requires thiamine pyrophosphokinase (TPK), which catalyses the transfer of a pyrophosphate group from ATP to thiamine] [91]. Urinary thiamine can also be measured, as excretion of thiamine decreases with reduced thiamine status [58]. The most common thiamine measure in the plasma is thiamine diphosphate, which is the form that that was chosen to measure for this trial.

Role of Thiamine in the Peripheral Nervous System

A thiamine deficiency can cause a peripheral neuropathy called beriberi neuropathy [92]. Beriberi affects the central nervous system, peripheral nervous system and cardiovascular system as previously outlined [93-95]. The peripheral neuropathy that develops from thiamine occurs after a prolonged mild to moderate period of thiamine deficiency [95].

Two studies conducted in Japan on beriberi neuropathy found that the neuropathy was due to axonal degeneration on large myelinated fibres rather than unmyelinated fibres. In addition, an accumulation of flattened sacs or tubuli in the axoplasm of large myelinated fibres was found [92, 96]. These PN changes were also displayed in thiamine deficient rats [97]. As a result of axonal degeneration from a thiamine deficiency, chromatolysis of DRG neurons and anterior horn cell neurons can occur [93].

The axonal degeneration from a beriberi neuropathy was comparable to that seen from vincristine sulphate, alcohol, triorthocresyl phosphate and acrylamide neuropathies [97]. Considering vincristine induces CIPN, a deficiency in thiamine displaying similar degeneration patterns may indicate the involvement of this nutrient. Active regeneration of peripheral nerves has been found in patients with a vitamin B1 deficiency receiving thiamine supplementation [97]. Consequently, it may assist in the regeneration of peripheral nerves from neurotoxic agents such as vincristine.

Thiamine administration has also been found to suppress thermal hyperalgesia [increased sensitivity to pain] by reducing hyper-excitability and lessening alterations of sodium currents in injured DRG neurons in rats [98]. This may be correlated to the injury incurred by oxaliplatin in the DRG as it affects voltage-gate sodium currents and causes axonal degeneration without evidence of primary demyelination [3, 4]. Therefore, thiamine administration could aid in the regeneration of axons, reduce pain and aid in balancing sodium currents.

1.9.3.2 Riboflavin (Vitamin B2)

Riboflavin, commonly known as vitamin B2 is the precursor to flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN); which are prosthetic groups essential for the activity of flavoenzymes, such as oxidases, reductases and dehyrodrogenases [81]. Riboflavin can be found in a number of foods especially those of animal origin. The major sources of riboflavin include eggs, lean meat, milk, broccoli, legumes and enriched bread and cereal products. Riboflavin can be destroyed by exposure to light [58, 81].

The structure of riboflavin consists of a flavin or isoalloxazine ring, which is attached to a ribitol or sugar alcohol side chain. The structure of riboflavin can be seen in Figure 1-4.

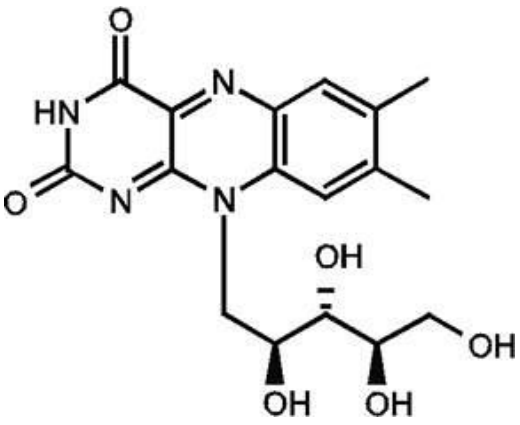


Figure 1-4: **Chemical Structure of Riboflavin [99]**

<u>Digestion and Absorption of Riboflavin</u>

Riboflavin is normally bound to proteins or found as FMN or FAD in foods. These protein bound riboflavins or flavoproteins, need to be released by stomach acid and gastric/intestinal proteases to free the riboflavin. The FMN and FAD foods forms or supplemental forms are hydrolyzed by FAD pyrophosphatase and FMN phosphatase on the brush border of the ileum enterocytes, which frees the riboflavin for transport into the enterocyte. Other enzymes may also assist in hydrolysing riboflavin from its attached phosphate such as nucleotide dephosphatase and alkaline phosphatase [81].

Small amounts of free riboflavin are absorbed into the enterocytes by saturable, energy-dependent carriers, primarily in the proximal small intestine; while large amounts found in supplemental administration are absorbed by diffusion. Once inside the enterocyte, it is phosphorylated to form FMN by flavokinases and requires ATP. Riboflavin then effluxes across the basolateral border and is transported to the liver by riboflavin transporters such as albumin. In the liver, riboflavin is converted to FMN and FAD. FAD is the predominant flavoenzyme transported to tissues. Before riboflavin can

transverse into most cells it must be converted to its free form, and then is phosphorylated inside the cell [58, 81].

Tissue Storage of Riboflavin

Approximately 50% of riboflavin is found in circulating plasma with 40% of it consisting of FAD and 10% FMN. Riboflavin is stored in small quantities in a variety of tissues with the greatest quantities found in the liver, kidney and the heart [58, 81].

Metabolism and Excretion of Riboflavin

Similar to thiamine, riboflavin and its metabolites are primarily excreted in the urine with only small amounts of unabsorbed riboflavin, or riboflavin metabolised by intestinal flora, excreted in faeces [58, 89]. Sixty to seventy percent of riboflavin is excreted into the urine intact, while the metabolites arise from tissue degradation of covalently bound flavins and the vitamin. This excretion can be seen in the urine a couple of hours after oral ingestion of a vitamin B2 supplement, as it is a fluorescent yellow compound. This is the noticeable change in colour of urine that individuals observe and report after taking a B vitamin supplement [58].

Function of Riboflavin

The flavoenzymes FMN and FAD function as coenzymes for a wide variety of enzyme reactions within the body. Both FMN and FAD act as an electron carrier, which plays an important role in mitochondrial energy production and cellular function.

The main biochemical functions of flavoproteins include [58]:

- in the *electron transport chain* as an electron carrier
- in the *oxidative decarboxylation of pyruvate* and *α-ketoglutarate* as an electron carrier
- as *succinate dehydrogenase* (a FAD flavoprotein) which removes electrons from succinate to form fumarate (forms $FADH_2$ from FAD). Coenzyme Q10 then pass the electrons into the electron transport chain
- as *Sphinganine oxidase* which requires FAD in sphingosine synthesis
- as *xanthine oxidase* as it requires FAD as a coenzyme, which aids the transfer of electrons directly to oxygen forming hydrogen peroxide
- as *aldehyde oxidase* as it requires FAD to convert aldehydes, such as pyridoxal (vitaminB6) to pyridoxic acid and retinal (vitamin A), to retinoic acid while passing electrons to oxygen and generating hydrogen peroxide

- for vitamin B6 metabolism as *pyridoxine phosphate oxidase* is dependent on FMN; this aids the conversion of pyridoxamine phosphate (PMP) and pyridoxine phosphate (PNP) to pyridoxal phosphate (PLP), which is the primary coenzyme form of vitamin B6.
- for the synthesis of *5-methyl tetrahydrofolate* (THF), the active form of folate requires $FADH_2$.
- *kynureninase monooxygenase* as it requires FAD, which is one of the steps in the synthesis of niacin from tryptophan.
- *choline metabolism* as it requires FAD for several enzymes: such as choline dehydrogenase, dimethylglycine dehydrogenase and sarcosine (also called monomethylglycine dehydrogenase).
- *thioredoxin reductase* as it is a flavoenzyme FAD
- *monoamine oxidase* as it is a FAD dependent enzyme required for the metabolism of several neurotransmitters, such as dopamine and other amines e.g. tyramine and histamine
  glutathione reductase as it is a FAD dependent enzyme used in the reduction of the oxidised form of glutathione (GSSG) to its reduced form GSH.

Deficiency of Riboflavin

The deficiency of riboflavin is called ariboflavinosis and is rarely found in isolation as a deficiency state. In most cases it is accompanied by other nutrient deficiencies [58]. The most common symptoms of a riboflavin deficiency are inflammation of the lip (cheilosis), corners of the mouth (angular stomatitis), tongue (glossitis), redness or bloody (hyperaemia) and swollen (oedema) mouth/oral cavity. Other symptoms or conditions associated with a riboflavin deficiency include seborrhoea dermatitis, anaemia, and peripheral neuropathy [58 81].

A severe deficiency of riboflavin may interfere with coenzyme and enzyme reactions that are dependent on riboflavin enzymes, such as the synthesis of vitamin B6 and niacin from tryptophan. Limited dietary intake without supplementation of riboflavin may cause a riboflavin deficiency in people with congenital heart disease, some cancers and those with an excessive alcohol intake [100]. Different conditions can also affect riboflavin status, such as thyroid disease, alters riboflavin metabolism and diabetes mellitus, trauma and stress increase excretion of riboflavin. A riboflavin deficiency may also be found in women taking the oral contraceptive pill compared to women who are not taking these drugs [58]. A deficiency in riboflavin can also affect iron metabolism. In a riboflavin deficient state heme formation in red blood cells is decreased, which results in normochromic and normocytic anaemia [101].

Toxicity of Riboflavin

Currently no toxicity of high oral supplementation of riboflavin has been identified [58].

Assessment of Nutriture of Riboflavin

The assessment of riboflavin status is usually measured by the *in vitro* stimulation of favin adenine dinucleotide (FAD) of the erythrocyte enzyme, glutathione reductase [102].

Role of Riboflavin in the Peripheral Nervous System

Riboflavin is not normally correlated to peripheral neuropathy except in chickens [103]. The only association that riboflavin has in the peripheral nervous system is its requirement in the conversion of inactive pyridoxine to its active form, pyridoxal-5-phosphate [104]. The role of pyridoxine, or vitamin B6, is discussed below.

1.9.3.3 Niacin/Nicotinamide/Nicotinic Acid (Vitamin B3)

Niacin was first discovered due to its deficiency state. In humans this is called pellagra and is a similar condition to what occurs in dogs called black tongue [58]. Vitamin B3 can be found in three forms; niacin (which is the generic term for the other two forms), nicotinic acid and nicotinamide. There is only a slight difference in structure as nicotinic acid is pyridine 3-carboxylic acid and nicotinamide is nicotinic acid amide [58]. The structure of vitamin B3 is presented in Figure 1-5.

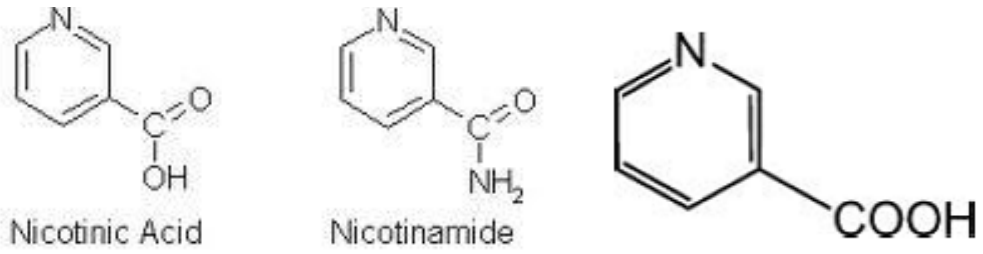


Figure 1-5: Chemical Structures of Nicotinic acid, Nicotinamide and Niacin [87, 88].

The best sources of niacin are meat, poultry and fish, especially tuna or halibut. Cereal products or enriched cereals and bread products, whole grains, seeds, legumes as well as coffee and tea contain reasonable amounts of niacin. There is a lesser amount found in green vegetables [58, 81].

In addition to being available in foods, niacin is synthesised by the liver and some other tissues from the amino acid tryptophan. Vitamin B2, B6 and iron are required for this reaction to occur in addition to adequate tryptophan consumption, as it is an essential amino acid. Approximately 1mg of niacin is produced from 60mg of dietary tryptophan [58].

Digestion and Absorption of Vitamin B3

Niacin exists as NAD^+ and $NADP^+$ in the majority of foods [81]. The digestion and absorption of niacin occurs due to NAD^+ and $NADP^+$ being hydrolysed by phyrophosphatases to release the phosphate bond and glycohydrolase to release free nicotinamide for absorption [58, 81].

Small amounts of nicotinamide and nicotinic acid can be absorbed in the stomach. However, the majority is absorbed in the small intestine by sodium dependant carrier-mediated (or facilitated) diffusion. At higher concentrations, which occur due to oral supplementation, niacin/nicotinamide/nicotinic acid are all absorbed by simple or passive diffusion [58, 81].

In the enterocytes, nicotinamide and nicotinic acid can be converted to NAD^+ where required and the rest of the nicotinamide and nicotinic acid diffuses out of the cells into the portal blood. Once in the plasma, approximately one third is bound to plasma proteins and is transported to the liver. The nicotinamide and nicotinic acid can transport across cell membranes by simple diffusion except for kidney tubules and red blood cells whereby it requires a carrier, and in the brain where it is energy dependant [58, 81]. Nicotinamide is the primary precursor for NAD^+ that can be synthesised in all tissues and cells. Nicotinic acid can also be synthesised to NAD^+ but this primarily occurs in the liver [58].

Tissue Storage of Vitamin B3

Very small amounts of niacin are stored within the body. NAD^+ or $NADP^+$ (NADPH which is its reduced form) are stored in small amounts in the liver but are not bound to enzymes. The NAD^+ and $NADP^+$ in cells are trapped and used for enzyme reactions within the cell. All excess is either stored in the liver, as previously mentioned, or excreted in urine [58].

Metabolism and Excretion of Vitamin B3

NAD^+ and $NADP^+$ within the cells undergo degradation by glycohydrolase producing ADP-ribose and nicotinamide. The released nicotinamide is transported to the liver where it is than methylated and oxidised into metabolites, such as N'methyl nicotinamide, N'methyl 2-pyridone5-carboxamide and small amounts of N'methyl 4-pyridone carboxamide. Nicotinic acid is metabolised to N'methyl nicotinic acid and all metabolites are excreted in the urine. Free nicotinamide and nicotinic acid are not excreted in the urine as both are actively reabsorbed from the glomerular filtration [58].

Function of Vitamin B3

Between 200 and 500 enzymes, primarily dehydrogenases, require the coenzyme forms of niacin NAD^+ and $NADP^+$. These compounds mainly act as a hydrogen donor or electron acceptor [58, 81]. Niacin coenzymes are essential for all of human energy production including [58, 81]:

- Glycolysis
- Oxidative decarboxylation of pyruvate to acetyl-CoA
- Oxidation of acetyl-CoA in the tricarboxylic acid cycle (TCA)
- B-oxidation of fatty acids.

In addition to mitochondrial energy production, NAD^+ and $NADP^+$ are involved in the following [58, 81]:

- Oxidation of ethanol
- Aldehyde dehydrogenase for catabolism of vitamin B6 from pyridoxal to pyridoxic acid
- Pentose phosphate pathway
- Mitochondrial membrane malate aspartate shuttle
- Fatty acid synthesis
- Cholesterol and steroid hormone synthesis
- Proline synthesis
- Deoxyribonucleotide synthesis
- Glutathione, vitamin C and thioredoxin regeneration
- Folate coenzyme synthesis (dihydrofolate [DHF], tetrahydrofolate [THF], 5-methyl THF, and 5,10-methylene [THF])
- Donor of adenosine diphosphate ribose (ADP-ribose) for posttranslational modification of proteins associated with chromosomes and the formation of cyclic ADP-ribose
- Other non-redox activity.

Deficiency of Vitamin B3

The term for the deficiency of niacin is pellagra. One of the signs and symptoms of pellagra is dermatitis. The dermatitis looks similar to sunburn at first and appears in areas exposed to the sun. The neurological signs and symptoms may include headaches, apathy, loss of memory, peripheral neuritis, paralysis of extremities, confusion, disorientation and dementia or delirium [58, 107].

The gastrointestinal signs and symptoms include glossitis, cheilosis, angular stomatitis, nausea, vomiting and diarrhoea. Treatment for pellagra is administration of 500 mg of nicotinamide daily for several weeks. If pellagra is untreated then death can occur [58, 107].

Several drugs, malabsorption disorders and excessive alcohol intake have been found to decrease niacin status possibly leading to a deficiency state. Some of the medications that have been found to decrease niacin status include azathioprine [108], isoniasid, 5-fluoro-uracil, pyrazinamide ehtionamide, hydantoins, phenobarbital, chloramphenicol and mercaptopurine [107].

The malabsorption disorders include chronic diarrhoea, inflammatory bowel disease, some intestinal cancers and Hartnup disease [58, 107].

Toxicity of Vitamin B3

Side effects from niacin toxicity have been documented in doses above 1,000 micrograms per day [109]. These side effects include a vasodilatory 'niacin flush', which is partly due to a histamine release, causing an uncomfortable flush with redness, burning, itching, tingling and headaches. It can also affect the gastrointestinal tract causing heartburn, nausea and possible vomiting. In severe cases liver injury (hepatic toxicity), high uric acid levels (possibly gout) and high blood glucose concentrations may occur [58, 109].

Due to the side effects from niacin toxicity, the NHMRC have set an upper level for niacin at 900 mg a day for men and women [90]. The total amount each participant will be receiving for this trial per day is 200 mg (100 mg per capsule).

Assessment of Nutriture of Vitamin B3

There are several methods that have been used to assess niacin status. In Australia, it is rarely assessed. Healthscope is the primary pathology company that conducts these tests/assays. Healthscope uses a 24-hour urine test to ascertain niacin levels, which seems to be the most common form of pathology assay undertaken for vitamin B3. Metabolites of the vitamin are tested in urine, such as N-methyl nicotinamide, as well as comparing N-methyl nicotinamide to creatinine ratio. This ratio has been criticised as being hard to interpret and normally just the metabolite is used. Red blood cell indicators may also be used to assess NAD concentrations in certain circumstances [58, 110].

Role of Vitamin B3 in the Peripheral Nervous System

Vitamin B3 has been related to a peripheral neuropathy associated with its deficiency state, namely pellagra and encephalopathy in alcoholics [111]. A retrospective study on 22 heavy alcoholics reported a post-mortem diffuse chromatolysis of neurons identical to that found in neurological pellagra [111]. Due to the fact that frequent co-existence of other alcoholic encephalopathies and neuropathies can be found in the same patient, pellagra encephalopathy that can cause a peripheral neuropathy can go unnoticed and treated with other nutrients such as thiamine (Vitamin B1) and pyridoxal (vitamin B6). In the case studies reported, fifteen patients were treated with thiamine and pyridoxine without niacin which appeared to aggravate their neurological state [111]. It is suggested that undetermined encephalopathy displaying peripheral neuropathy should be treated with niacin.

1.9.3.4 Pantothenic Acid (Vitamin B5)

Pantothenic acid, or vitamin B5, is essentially known for its role as a precursor of coenzyme A (CoA). Coenzyme A is a molecule used in enzymatic reactions, such as TCA cycle, heme synthesis and lipid metabolism [58, 81].

The structure consists of β-alanine and pantoic acid joined by a peptide bond or amide linkage as seen in Figure 1-6 [58].

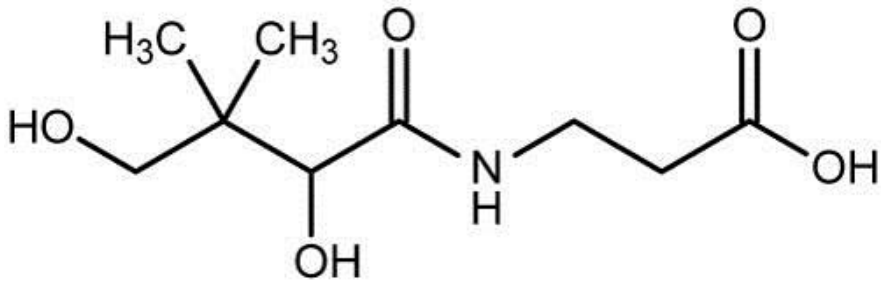


Figure 1-6: Chemical Structure of Pantothenic Acid [112]

Pantothenic acid is found widely within our foods and is present in virtually all plant and animal foods, therefore, making a deficiency unlikely. Some of the best sources of pantothenic acid include meats, particularly liver, egg yolk, and yoghurt, legumes including peanuts, whole-grain cereals, potatoes, mushrooms, broccoli, green leafy vegetables and avocadoes [58, 81].

Digestion and Absorption of Pantothenic Acid

Pantothenic acid found in foods is mainly found as CoA (approximately 85%) or in free form. To release the pantothenic acid from CoA, several steps are required during digestion to hydrolyse the CoA in the gastrointestinal tract. Phosphatases are firstly required to hydrolyse the CoA; pantetheinase, secreted by the intestinal mucosa, then splits the molecule to form pantothenic acid. It is then transported across the brush border by the sodium-dependent multivitamin transporter (SMVT). If ingested in high concentration, such as vitamin supplementation, it is absorbed by passive diffusion [58, 81].

After absorption it is transported to the liver where a certain percentage is converted to CoA. Pantothenic acid and CoA are both found in the blood. The same SMVT carrier is required to uptake the vitamin into cells throughout the body [58].

Tissue Storage of Pantothenic Acid

As most pantothenic acid in the body is used to synthesise CoA or resynthesise CoA, as well as be a component of 4'phosphopantetheine, it is mostly stored in these forms. These can be found in all cells due to mitochondrial activity, but is found primarily in high concentrations in the liver, adrenal gland, kidneys, brain and heart [58].

Metabolism and Excretion of Pantothenic Acid

Pantothenic acid is primarily excreted intact in the urine, as it does not appear to undergo metabolism prior to excretion. A small amount is also excreted in the faeces, but at this stage no metabolites have been identified in either the urine or the faeces [58].

Function of Pantothenic Acid

The primary function of pantothenic acid in the body is as a component of the acylation factors, CoA and 4'phosphopantetheine. As such, pantothenic acids have the following function within the body [58]:

As CoA [58]:

- Participates extensively in nutrient metabolism – carbohydrates, lipids and proteins
- Participates in the degradation reactions for energy production e.g. conversion of pyruvate to acetyl-CoA, the oxidative decarboxylation of α-ketoglutarate to succinyl-CoA
- Are important in the synthesis of cholesterol, bile salts, ketone bodies, fatty acids and steroid hormones
- Are involved in the synthetic reactions for the production of many vital compounds
- Are involved in the acetylation (donation of long-chain fatty acids) of some proteins, sugars and some drugs.

As Acyl Carrier Protein (ACP) [58]:

- 4'phosphopantetheine functions as the prosthetic group for acyl carrier protein which is a component of the fatty acid synthase complex
- The sulfhydryl group in the 4'phosphopantetheine binds and transfers acyl groups to other sulfhydryl groups located in the enzyme complex, therefore assisting the acyl chain being synthesised.

Deficiency of Pantothenic Acid

A pantothenic acid deficiency is not normally seen as it is found in a variety of foods; however, a deficiency state has been identified called 'Burning feet syndrome'. This is characterised by a

numbness of the toes and a sensation of burning in the feet. Warmth makes it worse and cold makes it feel better. Other deficiency symptoms may include vomiting, fatigue, weakness, restlessness and irritability [58].

This deficiency is normally found in conjunction with other nutrient deficiencies, for example, malnutrition or in conditions that may induce nutrient deficiencies such as alcoholism, diabetes mellitus or inflammatory bowel disease [58].

Toxicity of Pantothenic Acid

No toxicity or adverse effects of oral pantothenic acid have been reported to date. As there are no reported cases of toxicity in humans or animals the NHMRC have set no upper levels for oral pantothenic acid [90].

Assessment of Nutriture of Pantothenic Acid

Both blood and urine pantothenic acid concentrations can be used to assess vitamin B5 status. Urinary pantothenic acid has been found to be a superior indicator of status, as blood concentrations do not correlate well with changes in dietary pantothenic acid intake [58].

Role of Pantothenic acid in the Peripheral Nervous System

The main involvement of pantothenic acid and the peripheral nervous system involves 'burning feet syndrome' [113]. There is a long history of burning feet syndrome going back as far as the first British Burmese war in 1823. It was not until the 1900s, when Gopalan [114] discovered that complete relief was found with calcium pantothenate, that the relationship was made between burning feet syndrome and vitamin B5 therapy (possibly due to a deficiency). His discovery was then further substantiated by mice studies [115]. As a pantothenic acid deficiency is rare today, it may only been found in disorders such as alcoholism [116].

1.9.3.5 Pyridoxine (Vitamin B6)

The term pyridoxine or vitamin B6 incorporates the three forms pyridoxal, pyridoxine and pyridoxamine. These forms or vitamers are interchangeable as pyridoxine represents the alcohol form, pyridoxal the aldehyde form and pyridoxamine the amine form of vitamin B6. The phosphate bond form of all three vitamers is the major dietary source of the vitamin [58, 117].

The structure of pyridoxine is presented in Figure 1-7 [118].

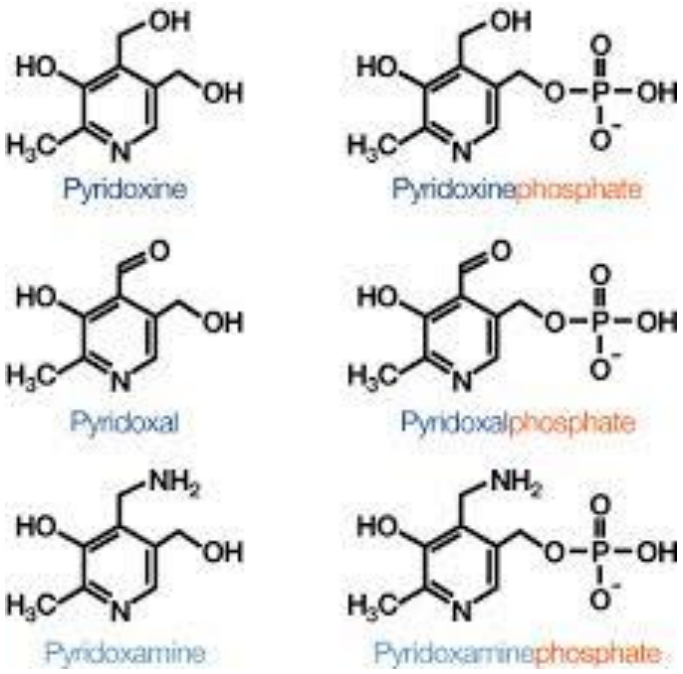


Figure 1-7: **Chemical Structures of Vitamin B6 or Pyridoxine and Associated Metabolites** [118].

Dietary sources of pyridoxine include meat, salmon, chicken, wholegrain products and vegetables as well as some fruits, for example, bananas, fortified cereals and nuts [58].

Digestion and Absorption of Pyridoxine

Due to the food source of pyridoxine being bound to phosphates, vitamin B6 needs to be dephosphorylated by alkaline phosphatase (which is zinc dependant) and occasionally by other phosphatases at the intestinal brush border to free the vitamers. All pyridoxine vitamers are absorbed primarily in the jejunum by passive diffusion. If supplemental phosphorylated forms, such as pyridoxal-5-phosphate, are ingested in high concentrations they can also be absorbed by passive diffusion without being dephosphorylated [58].

The pyridoxine may be rephosphorylated in the enterocyte cells, however, little metabolism occurs here and the vitamers need to be dephosphorylated before entering the portal vein. The main metabolism of pyridoxine occurs in the liver where all vitamers are converted to pydridoxal-5-phosphate (approximately 90% of total circulating vitamin B6). This conversion requires a FMN (vitamin B2) dependant enzyme, cytosolic pyridoxine phosphate oxidase [58, 117].

Tissue Storage of Pyridoxine

The primary site for pyridoxine storage is in skeletal muscles (75-80%), which are bound to glycogen phosphorylase and range in content from 40-185mg. The liver stores approximately 5-10%;

additionally, small amounts can be stored in the brain, kidney and spleen normally bound to enzymes [58].

Metabolism and Excretion of Pyridoxine

Pyridoxine is primarily excreted in the urine with very small amounts excreted in the faeces. The major metabolite of pyridoxine is 4-pyridoxic acid, which results from the oxidation of pyridoxal by a NAD-dependent, aldehyde dehydrogenase (vitamin B3) or FAD-dependent aldehyde oxidases (vitamin B2), in the liver and kidneys. This metabolite if measured in the urine is representative of recent ingestion rather than stored pyridoxine levels. Large supplemental ingestion of pyridoxine (100mg or larger) can result in urinary excretion of intact pyridoxine or 5-pyridoxic acid and lower amounts of urinary 5-pyridoxic acid excretion [58].

Function of Pyridoxine

The main function of the coenzyme form of pyridoxine is its role in enzyme activity, particularly with amino acids [58]. The main non-enzyme role of pyridoxine is its function in gene expression whereby it has been found to bind to DNA and, in some cases, modulate steroid hormone binding or transcription factor binding to regulatory regions on DNA [119].

The coenzyme function of pyridoxine is involved in the following reactions [58]:

- Transamination: Involves the transfer of an amino group ($-NH_2$) from one amino acid to a α-ketoacid. This is important for the synthesis of non-essential amino acids and the use of amino acid carbon skeletons for energy or glucose production.
- Deamination: Is the reaction whereby an amino group is removed from a compound and releases ammonia or ammonium ion.
- Decarboxylation: Involves the removal of a carboxy (COO-) group from an amino acid or other compound.
- Transulfhydration: Pyridoxine is required for two enzymes catalysing reactions in which cysteine is synthesised from methionine.
- Transelenation: Selenomethionine may be converted to selenocysteine, which requires a pyridoxine dependent enzyme, γ-lyase.
- Cleavage: There are a number of cleavage reactions, however, an example of one that requires pyridoxine is the removal of the hydroxymethyl group from serine to tetrahydrofolate (THF) to form glycine.
- Racemisation: Racemases that catalyse the interconversion of D- and L- amino acids require pyridoxine.

- Other Synthetic Reactions: Pyridoxine is also required as a coenzyme in the first step of heme synthesis, for animolevulinic acid synthetase, for sphingolipid synthesis, niacin synthesis and the synthesis of carnitine and taurine.
- Glycogen degradation: Pyridoxine is required for glycogen phosphorylase activity.

Deficiency of Pyridoxine

A pyridoxine deficiency is considered rare but may occur occasionally; mainly occurring in selected groups such as the elderly, alcoholics, infants exposed to severe heat treatment of milk formulas and people on a variety of drugs therapies (e.g. Isoniazid, penicillamine, corticosteroids, anticonvulsants and the oral contraceptive pill) [58].

The signs and symptoms of a pyridoxine deficiency include a seborrheic rash on the face, neck, shoulders and buttocks; weakness; fatigue; cheilosis; glossitis; angular stomatitis; peripheral neuropathy; seizures and convulsions; microcytic anaemia and hyperhomocysteinemia. A deficiency is usually treated with 100mg or more of vitamin 6 daily [58].

Toxicity of Pyridoxine

Pharmacological doses of pyridoxine have been found to induce a toxicity of vitamin B6. Excessive ingestion of greater than 200 mg/day for more than 5-6 months has been found to cause a sensory peripheral neuropathy, including an unsteady gait, tingling in the extremities and impaired tendon reflex. Doses of 2 grams/day have been found to cause peripheral neuropathy (tingling and numbness) in the feet and hands as well as impaired motor control or ataxia (loss of voluntary muscle control). High doses over a period of time may also cause degeneration of neurons (dorsal root ganglia) in the spinal cord, loss of myelination and degeneration of sensory fibres in peripheral nerves [120].

The upper level for adult men and women, as set by the NHMRC, is 50mg a day; which has been based on the results of studies involving long term oral administration of pyridoxine, as mentioned above. The dose prescribed in our clinical trial, being slightly higher than this upper level, was 60mg a day for between six to nine months. Participants were screened for both blood plasma levels of pyridoxine phosphate and peripheral neuropathy.

This dose was chosen considering that the B vitamin complex selected for this trial was a government approved nutrient supplement (Therapeutic Goods Act) and can be bought in Australia. The dose of two capsules a day was based on the vitamin B12 level of 1,000 mcg a day to assist nerve regeneration. In this way, the dose of one capsule was equivalent to 30mg of vitamin B6 and two capsules was equivalent to 60 mg. This dose, although slightly higher than the upper limit recognised by NHMRC, would only be administered for a set period of time: six to nine months. The research

states that this dose and duration are not associated with peripheral neuropathy development from a vitamin B6 toxicity, therefore, it was deemed safe to administer.

Assessment of Nutriture of Pyridoxine

Pyridoxine can be measured by determining plasma, erythrocyte or total blood pyridoxal-phosphate (PLP) levels or by urinary pyridoxal excretion. Other tests that can assist in determining pyridoxine status are erythrocyte tryptophan catabolites levels, aspartate aminotransferase and alanine aminotransferase activities, or plasma homocysteine. A loading test of 2 grams of oral tryptophan measuring urinary 4-xanthurenic acid can also be used. Urinary cystathionine and plasma homocysteine levels can also be measured after a dose of 3grams of methionine [117]. Homocysteine can also be an indicator for folate and vitamin B12 status, so is not a reliable indicator of vitamin B6 status [121]. The main pathology test utilised, clinically, is the plasma PLP test. A recent study on cancer utilised the sum of pyridoxial-5-phosphate, pyridoxal and 4-pyridoxic acid to define vitamin B6 status [122].

Role of Pyridoxine in the Peripheral Nervous System

Vitamin B6 directly affects the peripheral nervous system as both a deficiency and toxicity can cause peripheral neuropathy [123]. In relation to chemotherapy, two studies have been conducted with vitamin B6 and CIPN and positive results have been published [27, 78].

Wiernik, et al [78] conducted a clinical trial on low or moderate dose cisplatin and hexamethylmelamine in advanced ovarian carcinoma patients. Vitamin B6 was found to reduce CIPN [dose 100 mg of vitamin B6, pyridoxine hydrochloride, three times daily from days 1-21 of chemotherapy administration; equivalent to 300 mg/day]. However, it was also found to adversely affect response duration [78]. In 2010, Garg and Ackland found that vitamin B6 in cell culture examination was found to decrease CIPN associated with oxaliplatin administration, in the FOLFOX combination, without interfering with the anti-tumour activity [27]. It is recommended that further clinical trials be conducted on vitamin B6 and oxaliplatin administration for the prevention and treatment of CIPN.

Two journal articles, looking at various case studies, have also been published on pyridoxine and pyridostigmine in children with vincristine-induced neuropathy [124, 125]. Another case study has been published on a neonate with vincristine-induced peripheral neuropathy [126]. All case studies on these babies and children showed positive benefit in reducing the vincristine CIPN, with further

research required. In addition, a case report on an adult with vincristine induced cranial polyneuropathy treated with pyridoxine reported complete recovery after 2 weeks [127].

In developed countries a clinical deficiency of vitamin B6 is uncommon; however, several studies have shown that approximately 10-20% of the population show biochemical evidence of inadequate vitamin B6 nutrition [110]. PN from vitamin deficiencies, due to inadequate food intake, can be found in underdeveloped countries, however, in developed countries the most common causes of PN are alcohol, diabetes and medication use [129].

The medications known to cause PN, due to their antagonism to vitamin B6, are isoniasid (tuberculosis medication), hydralazine (anti-hypertensive), cycloserine (antibiotic for tuberculosis) and penicillamine (chelating agent used for rheumatoid arthritis). Other neurological side effects evident from these drugs include hyperkinesis, irritability, sleep disorders and seizures. Vitamin B6 administration has been found to reverse PN, and other neurological side effects from these drugs, without interfering with the efficacy of the medication [129].

The involvement of Vitamin B6 with peripheral nerves is through its role as a cofactor for neuronal protein synthesis [130]. The mechanism of action for pyridoxine on peripheral nerves is not confirmed, but it is known to be involved in numerous biochemical pathways of neural function. This includes neurotransmitter synthesis, amino acid metabolism and sphingolipid biosynthesis and degradation [131]. Similar to vitamin B1, the peripheral neuropathy related to a vitamin B6, deficiency occurs after an extended period of time [129].

A vitamin B6 toxicity has also been found to cause peripheral neuropathy [129, 131-135]. In 1983, Schaumburg et al [132] reported the development of PN in seven patients who were taking vitamin B6 in excess of 1g/day for several months. When the supplementation was ceased, the participants showed recovery of nerve function and reduced PN with a few patients experiencing persistent nerve damage. Further studies, with participants taking lower doses of vitamin B6 (90 mg-500 mg a day), also reported the development of sensory PN from vitamin B6 administration taken over one to five years. PN ceased once administration of B6 was discontinued, however, recovery took six months for most participants [133, 134].

The toxicity from vitamin B6 was found to be detrimental to the dorsal root ganglia, with subsequent degeneration of the sensory peripheral nerves [129, 132]. These are the same neurological areas that the neurotoxic chemotherapy agents accumulate [2, 4, 10, 34].

The dose that will be administered in this trial is 60 mg a day for nine months. According to the research, the administered dose for this trial is lower than the lowest reported dose to cause vitamin B6 induced PN (92 mg a day) and will be taken for less time (nine months versus one year).

The dose for this trial is also lower than the vitamin B6 administered in the Wiernik, et al [78] trial, which was 300 mg of vitamin B6 per day for 21 days after chemotherapy treatment. Although no toxicity from vitamin B6 was noted in Wiernik's trial, and CIPN was reduced, the dose of vitamin B6 was found to adversely affect response duration [78]. Therefore, a lower dose of vitamin B6 administration with chemotherapy treatment would be beneficial to trial for efficacy and safety.

1.9.3.6 Folic Acid (Vitamin B9)

The terms folic acid and folate are often used interchangeably, but are actually not interchangeable. Folic acid refers to the oxidised form found in supplements and fortified foods whereas folate refers to the reduced form found naturally in foods and biological tissue. Folate (pterolymonoglutamic acid or pterolglutamate) is composed of three parts, which are all required to be bound together for vitamin activity. These three parts include pteridine (2-NH_2-4-OH-6-CH_3 pterin), para-aminobenzoic acid (PABA) and glutamic acid. The primary pterolylmonoglutamates found in foods include 5-methyl tetrahydrofolate (THF) and 10-formyl THF, however, over 150 different forms of folate have been identified [58].

The chemical structure of folate is presented in Figure 1-8.

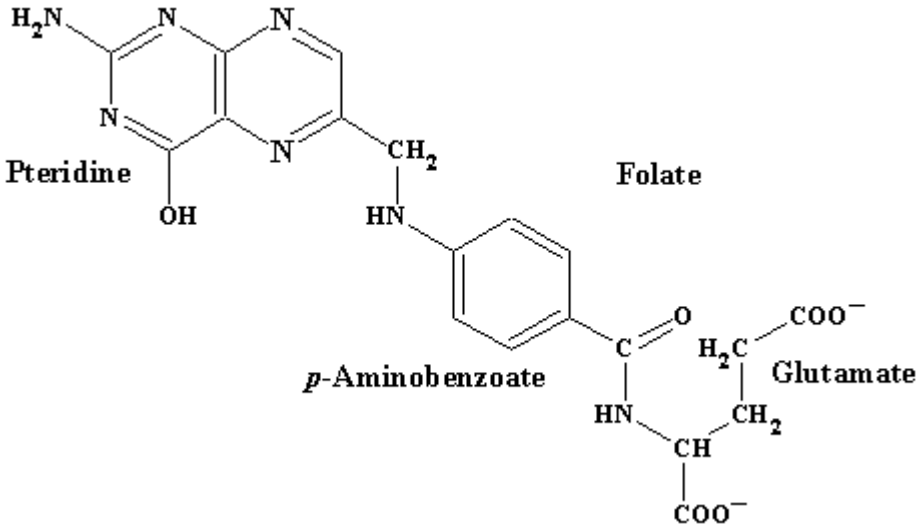


Figure 1-8: **The Chemical Structure of Folate [136]**

The main food sources of folate include unprocessed grains, oranges, dried beans, peas, eggs, mushrooms as well as green vegetables such as spinach, Brussels sprouts, broccoli, asparagus. Other good sources of folate include legumes, such as peanuts and lentils, fruits and liver [58, 117].

<u>Digestion and Absorption of Folate</u>

Folate found in foods is mostly bound to proteins and is required to be in its mono form or simple form for absorption. Consequently, polyglutamates cannot be absorbed without first being hydrolysed

by proteases to form monoglutamates. One of the main proteases found on the brush border that cleaves the polyglutamate into monoglutamine is called carboxypeptidase and is zinc dependent. A zinc deficiency has been found to impair folate absorption due to its requirement for this protease enzyme [58, 117].

The primary absorption into intestinal cells occurs in the proximal jejunum via the transporter proton-coupled folate transporter (PCFT) and 5-methyl THF. The PCFT is considered to be a high affinity folate carrier [137]. Inside the enterocyte, both folate and folic acid is converted to dihydrofolate (DHF), which is than reduced to generate THF. Transport across the basolateral border is active and carrier dependent, possibly involving multidrug resistance protein (MRP) 3. Folic acid can be found in the portal circulation as monoglutamates, not polyglutamates primarily as folate or 5-methyl THF with small amounts of dihydrofolate and formylated forms [137].

In systemic blood, folate is found as monoglutamates (e.g. THF, 5-methyl THF, or 10-formyl THF), either in free form (1/3) or bound to proteins (2/3) such as albumin, α-2 macroglobulin and high-affinity folate-binding protein. Red blood cells contain higher concentrations of folate than plasma; however, folate in red blood cells can only be obtained through erythropoiesis and is not taken up by mature red blood cells. Therefore, red cell folate pathology results represent long term folate status (approximately 2-3 months) compared to plasma [58].

Tissue Storage of Folate

Storage of folic acid in the body ranges between 11 to 28 mg with approximately half of the storage of folate found in the liver. Folic acid is normally found in the cytosol and mitochondria of cells in the polyglutamate forms of THF and 5-methyl THF [58].

Metabolism and Excretion of Folate

Folate excretion is through both urinary and faecal excretion. The body has the ability to retain needed folate via the kidneys by local folate binding proteins (FBPs) and tubular reabsorption. Excess folate is then excreted in the urine. Faecal excretion of folate is minimal [58].

Function of Folate

Folic acid functions as a coenzyme in both the mitochondria and cytosol as an acceptor of one carbon or single carbon groups. It is required for the synthesis of thymidylate, purines, methionine and other methylation related reactions. It is also required for the metabolism of amino acids, nucleotides (including pyrimidine synthesis) and metabolic pathways involving cell growth and survival, cell replication and DNA synthesis [58, 117].

There are certain genetic polymorphisms relating to folate-dependant enzymes that have mutations in the FAD-dependent methylene THF reductase enzyme (MTHFR – Methenyltetrahydrofolate receptor). These genetic polymorphisms can impair 5-methyl THF formation and, therefore, reduce the remethylation of homocysteine [58].

These folate mutations are now being assessed as possible risk factors for certain cancers such as breast cancer. A recent case-controlled study evaluated the MTHFR mutations as possible risk factors for breast cancer [138]. Weiwei, et al [138] found that there was a significant association between MTHFR C667T polymorphism, folate intake, vitamin B6 and breast cancer risk. There was also a significant interaction observed between MTHFR C667T polymorphism and folate intake on the risk of breast cancer. Although not assessed in this trial, further research into MTHFR, breast cancer and B vitamin supplementation is warranted.

Vitamin B12 and folate have a synergistic relationship whereby without vitamin B12 the methyl group of 5-methyl THF cannot be removed and by this means is trapped. Adequate amounts of vitamin B12 need to present for the enzyme methionine synthase, as the methyl group of vitamin B12 is required to convert homocysteine to methionine. In a low vitamin B12 status, it cannot accept the methyl group of 5-methyl THF and the folate is thus trapped. This means the cells have folate but not in a form they can use [58].

Deficiency of Folate

A deficiency of folate results in megaloblastic macrocytic anaemia. This means that fewer red blood cells are released into circulation and those released are large and immature. The signs and symptoms of this condition include fatigue, weakness, headaches, irritability, difficulty concentrating, shortness of breath and heart palpitations [58].

A diet devoid of folate can develop a folic acid deficiency within a month, with red blood cell folate concentrations diminishing after three to four months of low folate intake. Within approximately four to five months rapidly dividing cells become megaloblastic, the mean cell volume (MCV) increases, blood counts decrease and hyper segmentation of white blood cells occurs. The treatment for this deficiency is 1 to 5mg of folate daily [58].

A deficiency of folate impairs DNA synthesis, which can be seen initially in rapidly dividing cells such as the gastrointestinal tract. Hence, the first signs of a folate deficiency may be a bright red tongue, impaired absorption (due to shortening of villi) and diarrhoea. There are also certain conditions associated with an increased requirement of folate, for example excessive alcohol consumption and malabsorption disorders such as inflammatory bowel diseases. Pregnancy also requires more folate as a deficiency can cause neural tube defects [58].

Certain medications have also been found to affect folate status such as the diuretic furosemide, diphenylhydantoin or phenytoin (anticonvulsants), methotrexate (prevents THF synthesis), cholestyramine and sulfasalazine [58].

Toxicity of Folate

The upper level for adult intake in Australia is 1,000 mcg per day, according to the NHMRC [90]. Although not a toxicity, folate supplementation has been found to 'mask' a vitamin B12 deficiency; folate supplementation can alleviate the megablastic anemia but the neurological damage caused by the B12 deficiency continues and can be irreversible [90]. However, this concern has decreased with awareness and the normal testing of folate and vitamin B12 being conducted together.

Toxicity of folate supplementation with 15mg a day has been found to be associated with insomnia, malaise, irritability and gastrointestinal distress [90, 120, 139]. There have also been concerns that increased folic acid supplementation may accelerate the progression of pre-neoplastic lesions, which can increase the risk of colorectal cancer and possibly other forms of cancer in certain individuals [140-142].

There has also been a reported case of increased seizure frequency and activity with folic acid supplementation at a dosage of 800 mcg per day. The patient was on carbamazepine for symptomatic partial epilepsy with simple and complex seizures. The patient was planning to fall pregnant hence the folic acid supplementation; however, the paitent experienced generalised tonic-clonic seizures, for the first time, a few days after starting supplementation, which increased in frequency and severity [143].

Assessment of Nutriture for Folate

Folate status can be assessed by measuring plasma, serum or red blood cell folate concentrations. The plasma or serum folate concentrations reflect recent dietary intake, whereas the red blood cell folate concentrations reflect folate tissue status and are more representative of vitamin status [58].

Role of Folate in the Peripheral Nervous System

Vitamin B12 and folate are closely linked with specific functions in the body. This includes co-enzymatic one-carbon metabolism in the remethylation of homocysteine to methionine catalysed by the 5-methyltetrahydrofolate monoglutamate enzyme, activity in the nucleic acid pathways for the synthesis of thymidylate as well as purines and formation of methyl groups for biologic reactions [144, 145].

Deficiencies in either vitamin B12 or folate have been found to affect homocysteine levels due to their inter-relationship and the enzymes that are dependent on these vitamins [144-146]. Unlike

vitamin B12 deficiency, a folate deficiency does not cause primary peripheral neuropathy; however, it has been found to have a greater effect on cognitive abilities and the central nervous system rather than the peripheral nervous system [144-146]. Also, due to the inter-relationship between folate and vitamin B12, the neurologic manifestations displayed from a deficiency are indistinguishable between the two vitamins [93].

One case study has been published on the significance of a folate deficiency in alcoholic and nutritional neuropathies [147]. A 33 year-old woman, with chronic alcoholism, presented with acute progressive glove-stocking sensorimotor polyneuropathy and underwent a comprehensive clinical screening. Pathology results indicated on admission that her folate levels were reduced (0.4 in a range of 3.6-12.9) while her thiamine (2.2 in range of 2-7.2), riboflavin (15.2 in a range of 11.9-20.4) and cobalamin levels (955 in a range of 233-914) were within range. Laboratory findings also revealed macrocytic anaemia and liver dysfunction, while neurological testing indicated axonal neuropathy [147].

Two years previously, a patient presented with a previous peripheral neuropathy. No folate levels were assessed at that time, only levels for vitamins B1, B2 and B12 and all were found to be in range. The treatment, for her second admission of peripheral neuropathy, was administration of a multivitamin and intravenous folic acid 15mg daily. The patient gradually recovered and, although being bed ridden on admission, could walk after 3 months [147]. Therefore, patients presenting with alcoholism and peripheral neuropathy should undergo full screening and supplementation of a complete B vitamin might be a better treatment option. This case indicated that folate may play a role in peripheral neuropathy; however, as discussed previously, it may be indistinguishable between vitamin B12 and a folate deficiency unless laboratory tests can differentiate. Either way, supplementation with both or all of the B vitamins should assist in a B vitamin induced peripheral neuropathy.

1.9.3.7 Cobalamin (Vitamin B12)

Vitamin B12, or cobalamin, is the generic name for a group of compounds called corrinoids due to their corrin nucleus. The compounds that make up vitamin B12 include cyanocobalamin, hydroxocobalamin, aquo- or hydrocobalamin, nitrocobalamin, 5'-deoxyadenosylcobalamin and methylcobalamin [58, 117].

The best dietary sources of vitamin B12 include: organ meats such as liver; red meat such as beef, lamb, kangaroo; white meat such as pork, poultry, fish, shellfish (especially clams and oysters); as well as eggs. Dairy products such as cheese, cottage cheese and yoghurt have smaller amounts of

vitamin B12. Some foods are also fortified with vitamin B12 [58, 117]. Only bacteria or microorganisms can synthesise cobalamin while animals and plants cannot and so require vitamin B12 from an external source [58].

Bioavailability of vitamin B12 from food sources varies depending on the food and the age of the person; medication (such as antacids), surgery and digestive function. The range is approximately 40-80%, however, this maybe reduced tremendously if there is an intrinsic factor antibody issue; lower bowel resection or damage of the ileum as seen in Crohn's disease; atrophic gastritis; achlorhydria; metformin administration or chronic use of antacids. Other conditions that may interfere with vitamin B12 absorption include pancreatic insufficiency and Zollinger-Ellison syndrome, which results in a more acidic intestinal pH thereby impeding the release of vitamin B12 from the R proteins [58, 148-152]. Parasitic infestation such as tapeworms may also cause a deficiency as the parasite uses vitamin B12 therefore limiting the absorption of B12 to the human incubator [58].

The structure of vitamin B12, cyanocobalamin, which is the form found in most supplements is presented in Figure 1-9. The active forms, or coenzyme forms, of vitamin B12 in the body include methylcobalamin and adenosylcobalamin (5'-deoxyadensoyl-cobalamin) [58].

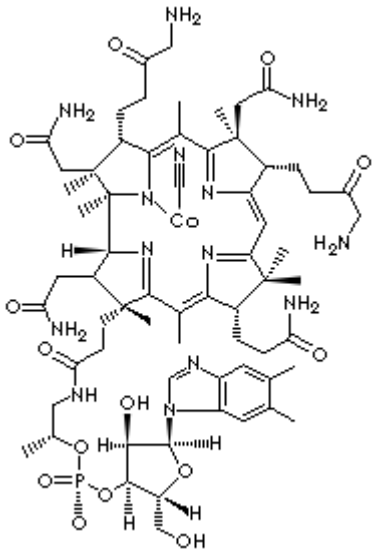


Figure 1-9: **The Chemical Structure of Cyanocobalamin [153]**

Digestion and Absorption of Cobalamin

The digestion and absorption of vitamin B12 is quite complex involving a number of steps in a particular sequence. Initially, the digestion requires the actions of gastric proteolytic enzymes, pepsin and hydrochloric acid, in the stomach to break the vitamin B12 away from amino acids. Next vitamin B12 binds to R proteins found in the saliva and gastric juices. The R proteins are said to protect and carry the vitamin away from bacterial use. Within the small intestine, the R protein-B12 complex is

hydrolysed by pancreatic protease which frees the vitamin B12. After B12 is released it then binds to intrinsic factor (IF) within the small intestine. The B12-IF complex travels from the duodenum to the ileum where it binds to a protein receptor called cubilin or IF receptor. If there are defects or damage to the receptors, this will result in vitamin B12 malabsorption as vitamin B12 is absorbed through the ileum [58, 154].

Inside the enterocyte, B12 is released from the B12-IF complex and is transported across the ileum's basolateral membrane; it then binds to transcobalamin II, the transport protein for B12 [58].

When vitamin B12 is given in pharmacological doses (1,000-2,000 µg) approximately 1-3% may be absorbed by passive diffusion [58]. The enterohepatic circulation of vitamin B12 is important as it accounts for part of the long half-life of the vitamin. Vitamin B12 is excreted in bile, however, once in the small intestine it can reattach to intrinsic factor and be re-absorbed. Therefore, malabsorption issues not only interfere with ingested vitamin B12 but also the enterohepatic reabsorption cycle. This may increase the amount of vitamin B12 needed for body requirements [58].

After absorption, vitamin B12 has been found to circulate for about 3 to 4 hours with peak levels of the vitamin occurring around 4 to 8 hours after ingestion. Vitamin B12 is circulated around the body by transport proteins called transcobalamin (TC) and haptocorrin (HC), like proteins [58]. Refer to the Holo TC section on page 37 for further information.

A mutation in the TCII has been found whereby a substitution of cytosine for guanine at the base pair 776 occurs. This substitution results in an insertion of arginine instead of proline, which decreases the ability of TCII to bind and transport vitamin B12 to tissues. It is estimated that approximately 20% of the population is homozygous for the GG variant; this can result in low serum vitamin B12 and high homocysteine levels, which is linked with an increased cardiovascular risk factor [155].

Tissue Storage of Cobalamin

Unlike most B vitamins, vitamin B12 can be stored and retained in the body for long periods of time, consequently a deficiency may not appear for 3 to 5 years. Approximately 2 to 4 mg are stored in the body, mainly the liver (50%), with small amounts stored in the muscles, bone, kidneys, heart, brain and spleen [58].

Metabolism and Excretion of Cobalamin

Little or no degradation of vitamin B12 occurs prior to excretion. The majority of vitamin B12 is excreted in bile, with only very small amounts lost in daily urine and trace amounts found in dermal losses [58].

Function of Cobalamin

Vitamin B12 has two enzymatic reactions that have been recognised in humans. One requires methylcobalamin and the other, adenosylcobalamin.

1. Methylcobalamin is required for the conversion of homocysteine into methionine. Polymorphisms in this reaction have been linked with an increased risk of neural tube defects in people with suboptimal vitamin B12 status in conjunction with individuals who have the 5,10-methylene tetrahydrofolate reductase (MTHFR) mutation. This results in reduced methionine synthase activity and therefore decreased methionine production [58].
2. Adensosylcobalamin is required for the methylmalonyl-CoA mutase conversation, which converts L-methylmalonyl-CoA to succinyl-CoA. A deficiency in vitamin B12 has been found to impair mutase activity, resulting in methylmalonyl-CoA and methyl malonic acid accumulating in the body. Methyl malonic acid status has been used to assist in determining vitamin B12 status, either deficiency or response to treatment [58].

Deficiency of Cobalamin

The deficiency for vitamin B12 is similar to folate in that it results in megaloblastic macrocytic anaemia as described on page 61. In regards to a vitamin B12 deficiency, the manifestations occur in stages:

1. Serum B12 concentrations decrease (serum B12 concentrations may remain normal until storage has been depleted).
2. Cell concentrations of B12 diminish which affects the activity of both the B12 dependent enzymes.
3. DNA synthesis decreases while plasma homocysteine and methyl malonic acid concentrations increase.
4. Morphological and functional changes occur in blood cells resulting in megaloblastic macrocytic anaemia.

The signs and symptoms of vitamin B12 deficiency affect the body's hematologic and neurologic systems. The hematologic signs and symptoms include anaemia, low blood leukocyte and thrombocyte counts, skin pallor, fatigue, shortness of breath and palpitations. The neurological signs and symptoms, which may be irreversible, include peripheral neuropathy: numbness of extremities; abnormal gait; increased loss of coordination; loss of proprioception; loss of vibration senses or touch in the ankles and toes; swelling of myelinated fibres and demyelination; irritability; memory loss; disorientation; psychosis and dementia [58].

Treatment of a B12 deficiency is administration of 1,000 mcg of vitamin B12 via either oral ingestion and/or intramuscular injections.

Toxicity of Cobalamin

Currently there is no upper limit or toxicity noted for vitamin B12. There is no evidence to date that high amounts of vitamin B12 represents a health risk; with no adverse effects having been reported with excess vitamin B12 intake or supplementation. There is also no evidence to indicate that high levels of vitamin B12 are beneficial [90].

Assessment of Nutriture of Cobalamin

There are a number of different pathological assessments that can be conducted to ascertain vitamin B12 status. These include: serum vitamin B12, holo Transcobalamin, homocysteine levels, methyl malonic acid levels and the Shillings test (rarely used now). At present, the laboratory tests used have limitations; due to technical issues or due to biological basis resulting in the inability to attain perfect sensitivity, specificity or predictive value [156, 157]. Further testing of intrinsic factor antibodies or parenteral cell antibodies is more indicative of a vitamin B12 deficiency but not functional considering other factors can also cause a B12 deficiency, as discussed before.

1. Serum vitamin B12: A snapshot of total concentration in the blood (range 200-900 pg/ml or pmol/ml) [58, 157].
2. Holo TC: Measurement of holotranscobalamin, which is the metabolically active component of vitamin B12. It indicates the transport of vitamin B12 from the liver to the tissues and may detect an early B12 deficiency [157, 158].
3. Homocysteine (HCY): Measurement of homocysteine in blood as a biomarker for vitamin B12. However, it is not specific as other vitamins, such as folate, vitamin B6 and folate polymorphisms e.g. MTHFR, also affect it. High homocysteine levels do not always correlate with a B12 deficiency [157].
4. Methylmalonylic acid (MMA): Measurement of MMA in the blood as a biomarker for vitamin B12. This is similar to HCY, however, is slightly more specific than HCY for a B12 deficiency [157].
5. Shillings test: A multistep assay involving the administration of radioactive vitamin B12, which is capable of assessing the aetiology of cobalamin malabsorption. As pernicious anaemia is the most common cause of cobalamin deficiency in Western populations, IF-blocking antibodies and antiparenteral cell antibody assays [157] have replaced this test.

Role of Cobalamin in the Peripheral Nervous System

A vitamin B12 deficiency is known to cause primary demyelinating peripheral neuropathy without any other causal factors [159, 160]. A deficiency of vitamin B12 causes neuronal demyelination, axonal degeneration and reduced nerve conduction velocity in the peripheral nervous system; and, if not treated, will result in neuronal death and permanent damage [160-162].

The mechanism of action of vitamin B12 on peripheral nerves is based on its catalytic role. The biochemistry of vitamin B12 is associated with two enzymes that are cobalamin dependent.

These include mitochondrial L-methymalonyl-coenzyme A (CoA) mutase and cytoplasmic homocysteine methyl transferase (also known as methionine synthase) [163, 164]. Reduced activities of these two enzymes alone have been linked to the neurological manifestations of a vitamin B12 deficiency [163].

Cobalamin deficiency has neurological manifestations in the brain and peripheral nerves. Scalabrino, et al [163] reported that the severity of the neuropathological features of white matter in the spinal cord of totally gastrectomised rats does not correlate with the progressive accumulation of methylmalonylic acid (MMA) and homocysteine (HCY) in the serum or the spinal cord [162]. The authors hypothesised that the accumulation of these neurotoxic metabolites from chronic cobalamin deficiency was unlikely to be the main mechanism of action to cause the subacute combined degeneration like lesions. In addition, children who have an inherited mutation of the mitochondrial L-methylmalonyl CoA mutase enzyme, and as a result suffer from methyl malonic aciduria not due to cobalamin deficiency, do not express typical subacute combined degeneration lesions [165].

This indicates that there could be other underlying mechanisms that could affect both central and peripheral nerves, other than high MMA and HCY levels. Research into the mechanisms of action of these nerves has revealed that nerve damage from a vitamin B12 deficiency may be via its effect on glial cells (Schwann cells are the peripheral nerve glia cells) as well as the impact of certain cytokines and growth factors [163]. Nerve cells such as the glial cells synthesise and release certain cytokines and growth factors due to injury or following certain triggers. This neuroglial pattern of cytokine and growth factor production can lead to demyelination and phlogosis from cytokines such as tumour necrosis factor alpha (TNF-α) [163].

A cobalamin deficiency therefore causes an imbalance of cytokines and growth factors that can affect glial cells and nerve myelination. Abnormally high levels of TNF-α and abnormally low levels of epidermal growth factor (EGF) were found in serum and cerebrospinal fluid of humans who were cobalamin deficient.

It was concluded that this imbalance of TNF-α and EGF can cause demyelination leading to nerve damage from a vitamin B12 deficiency [163]. Figure 1-10 represents a vitamin B12 deficiency in adults due to an imbalance of TNF-α and EGF [163].

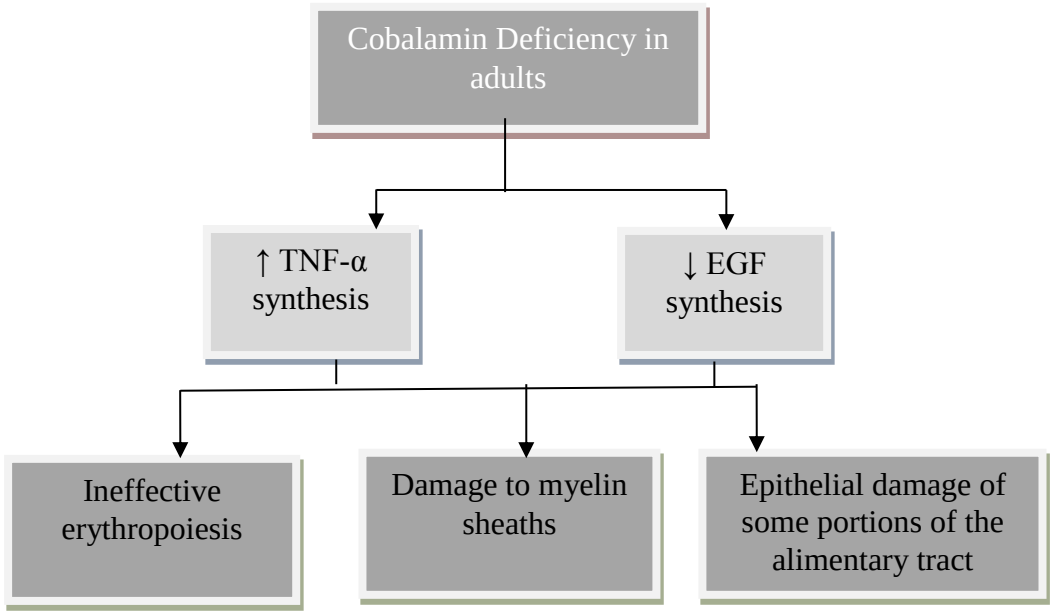


Figure 1-10: **Flow Chart of the Adverse Effect of a Vitamin B12 Deficiency in Adults [163]**

Other pathways involved in cobalamin deficiency that affect peripheral nerves includes the accumulation of $N^{\acute{\epsilon}}$-carboxymethyllysine (nCML) in sural nerves, the receptors for advanced glycation end products (RAGE) pathway and its activation of the pro-inflammatory transcription factor NF-κB [162]. NF-κB proteins have also been found to regulate gene expression in various cell types in both acute and chronic inflammatory response [162].

NF-κB activation can be related to the previous research of TNF-α production by glial cells. NF-κB is an important component when activated in the pathogenesis of inflammatory neuropathies. Its activation results in production of other pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 [162]. nCML-induced RAGE-activation is also a non-self-limiting way to activate NF-κB and the inflammatory response [162].

A study addressing various types of peripheral neuropathies found that the perineurial cells in the peripheral nervous system contain significant amounts of nCML and moderate amounts of RAGE and NF-κB in a vitamin B12 deficiency. In addition, the mononuclear cells were found to have significantly high amounts of RAGE, nCML and NF-κB [162].

Although these findings were from a study with a small sample size (n=5) the results warrant further investigation, since they indicate that inflammation may play a major role in the development of peripheral neuropathy from a vitamin B12 deficiency.

Furthermore, an in-vitro study found that neuroblastoma cells that were deficient in B12 showed slower proliferation, but faster differentiation. This was through interacting signalling pathways related to an increased expression of catalytic protein phosphatase 2A (PP2A), pro-nerve growth factor (proNGF) and two tumour necrosis factor alpha converting enzymes [166].

Hence, cytokines and growth factors may play an important role in the development of peripheral neuropathy induced from a vitamin B12 deficiency. [Table 1-5]

Table 1-5: The End Result of Enzymes from a B12 Deficiency on the Peripheral Nervous System:

Cobalamin (Cbl) deficiency in Humans		
Biochemical abnormalities	**Cytokine and growth factor abnormalities**	**Neurophysiological abnormalities**
↓ Cbl levels in serum ↑Methylmalonylic acid (MMA) levels in serum ↑ Homocysteine (HCY) levels in serum ↑ N^5-Carboxymethyllysine (nCML) ↑Advanced glycation end product (RAGE)	↑ Tumour necrosis factor alpha (TNF-α) levels in serum ↓ Epidermal growth factor (EGF) levels in serum ↑ Nuclear-factor kappa B (NFκB) ↑Interleukin -1β (IL-1β)	↓ Nerve conduction velocity in PNS

Information adapted from [162-164, 166]

Peripheral neuropathy that is caused by a deficit of vitamin B12 has been reported reversed or markedly improved by administration of vitamin B12 [160, 168-173]. Even in patients with other conditions that can cause peripheral neuropathy such as type II diabetes mellitus, administration of vitamin B12 has been beneficial for nerve regeneration and myelinogenesis [167].

In 1992 Yaqub, et al [167] conducted a double-blind randomised study on 50 diabetic patients (39 were non-insulin dependent and 11 were insulin dependent) that displayed diabetic neuropathy [167]. The patients were randomised to receive either placebo or methylcobalamin 2 capsules 3 times a day

equivalent to 1500 µg a day (250 µg per capsule) for 4 months. Evaluation was achieved via nerve conduction studies and a special scoring system adapted from the Peripheral Neurology Score (PNS). The results showed a significant improvement in somatic and autonomic symptoms in the supplemental group compared to placebo therefore showing efficacy in the treatment of diabetic neuropathy [167].

Another study compared oral versus intramuscular cobalamin treatment for megoblastic anaemia in a 90-day single-centre, prospective, randomised, open-label study [174]. Seventy patients (10 dropped out) were randomised to receive either oral or intramuscular (IM) cyanocobalamin at 1mg each. For the first 10 days, they received 1 mg every day either orally or via IM. After the first 10 days, both treatments were administered once a week for 4 weeks and then once a month for life. Serum B12 haematology tests were conducted at days 0 and 90, and complete blood counts were completed on days 0, 10 and 30. Results indicated that both oral and IM cobalamin treatment were equally effective for megablastic anaemia and cobalamin deficiency related conditions [174].

Therefore to achieve nerve regeneration and myelinogenesis, at least 1,000 µg of cyanocobalamin or methylcobalamin is required [167, 173, 174].

1.9.3.8 Choline

Choline is one of the B vitamins that the body can manufacture in the liver without dietary intake although dietary intake is required to maintain choline status. Choline is manufactured from methylation of the amino acid serine using S-adenosyl methionine (SAMe). Choline can also be found in food but is mostly found as part of the phospholipid lecithin (phophatidyl choline) [58]. The structure of choline is presented in Figure 1-11.

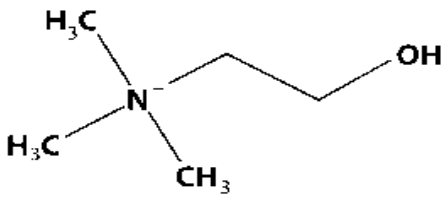


Figure 1-11: Chemical Structure of Choline [175].

Foods rich in choline include eggs, liver and other organ meats, muscle meats, shrimp, cod, salmon, wheat germ and legumes such as soybeans and peanuts. Lecithin is also available in shops which can be added to foods and is also used as an emulsifier in other foods [58].

Digestion and Absorption of Choline

Digestion of choline from the diet as lecithin occurs in the small intestines where it is hydrolysed by intestinal mucosa to glycerophophoryl choline. The choline component is than absorbed in the small intestines via transporter proteins into the enterocyte with no other components found to be competitive for absorption. The liver takes up the majority of the absorbed choline with small amounts being transported to the peripheral tissues via intestinal lymphatics [176].

Tissue Storage of Choline

The majority of choline is stored within the liver as phosphatidylcholine and sphingomyelin. The kidney and the brain also store some choline with a specific transporter required to transport the choline across the blood brain barrier. Excess choline intake is then excreted in urine [176].

Function of Choline

There are several functions of choline including [58]:

- Is required for the synthesises of sphingomyelin and phosphatidyl choline
- Is part of the neurotransmitter, acetylcholine
- Assists as a methyl donor
- Aids in the formation of platelet aggregating factor
- Aids in the secretion of very-low-density lipoproteins from the liver

Deficiency of Choline

Diets that have found to be devoid of choline indicate a decrease in plasma choline and phosphatidylcholine concentrations in addition to alterations in some liver enzymes. Animal studies have shown that a diet devoid in choline increase the development of fatty liver accompanied by some hepatic necrosis. In addition, low intakes of choline and betaine have been linked with increased inflammation [58].

Toxicity of Choline

Although no adverse events have been found, the NHMRC have set the upper level for adult male and females at 3,500 mg/day [90].

Assessment of Nutriture of Choline

Choline assessment is not routinely conducted. However, for specific research and analysis, pathology tests have been used. As choline is a polar, non-volatile molecule, which lacks a chromophore, it cannot be assessed or measured by immunoassay due to its small size. Hence, other methods have been used such as homogeneous chemiluminescent enzyme assays and more importantly, liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been quantified [177,178].

Role of Choline in the Peripheral Nervous System

Choline's role in the nervous system mainly revolves around its incorporation in the peripheral neurotransmitter, acetylcholine [58]. To date, choline has not been found to be involved with the development or treatment of peripheral neuropathy. Two studies on rats have assessed the topical application of cytidine-5'-diphophocholine (CDP-choline) to improve nerve regeneration and functional recovery of sciatic nerve injury [179, 180]. Both studies found that it was of benefit for nerve recovery and regeneration, however intra-application is not applicable to human nerves in regards to peripheral neuropathy. Nevertheless, CDP-choline maybe a potential treatment internally for nerve regeneration and recovery but further research is required.

1.9.3.9 Biotin

Biotin was first discovered after a link was established between raw eggs consumption and hair loss, dermatitis and various neuromuscular problems. A substance in liver, which was later found to be biotin was able to cure and prevent the problems caused by eating raw eggs [58].

The chemical structure of biotin or biotinyllysine is presented in Figure 1-12.

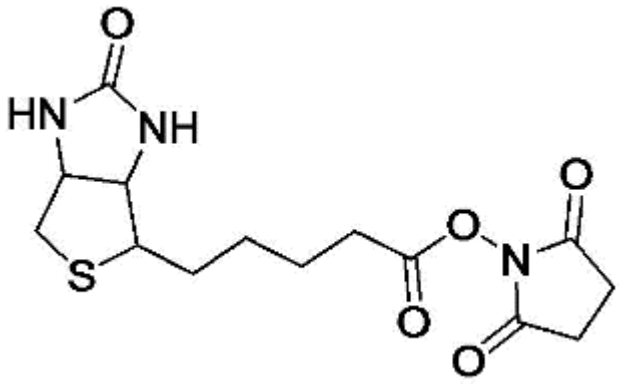


Figure 1-12: **Chemical Structure of Biotin [181]**

Biotin sources can be found from both food and the manufacture from intestinal bacteria in the colon. The best sources of biotin include liver, soy beans, egg yolk, legumes, nuts, salmon and some cereals. The whites of raw eggs contain a component called avidin, which is a glycoprotein.

Avidin can irreversibly bind to biotin in a tight noncovalent bond, which in turn prevents the absorption of biotin. Cooking of an egg or the white of an egg destabilises the avidin and it cannot bind to the biotin. The cooking process does not interfere with biotin so therefore, it can still be absorbed [58].

Digestion and Absorption of Biotin

Biotin within the diet is usually found bound to protein therefore requires proteases in the small intestine to break the bond to assist with absorption. Biotinidase is an enzyme found on the surface of the brush border as well in pancreatic secretion. It assists with the hydrolysation of the biocytin to free biotin for absorption [58].

The absorption of biotin varies. The absorption of biotin from the diet across the brush border in the small intestines and colon as well into the liver is carrier mediated and sodium dependent while pharmacological doses is absorbed by passive diffusion. The biotin manufacture and absorption from the colon bacteria is not adequate to maintain the requirements of the body, so dietary intake is essential. Alcohol interferes with biotin absorption in the intestines [58].

Tissue Storage of Biotin

Small amounts of biotin is stored in the muscle, liver and brain [58].

Metabolism and Excretion of Biotin

The metabolism or catabolism of biotin occurs due to proteases with only small amounts of metabolites formed. These metabolites are excreted in urine while unabsorbed intestinal bacteria synthesised biotin and unabsorbed dietary biotin is excreted in faeces [58]. Smoking has been found to increase the catabolism of biotin in women [182].

Function of Biotin

Biotin functions as both a coenzyme and in non-coenzyme capacities which include cell proliferation and gene expression. The functions of biotin include [58]:

Four Coenzyme Roles of Biotin [58, 183]:

- *1 and 2 Pyruvate carboxylase*: a mitochondrial enzyme that catalyses the carboxylation of pyruvate to oxaloacetate

- *Acetyl-CoA Carboxylase*: Is the rate limiting enzyme involved in energy production from the fatty acid synthesis converting malonyl-CoA to acetyl-CoA
- *Propionyl-CoA Carboxylase:* is another mitochondrial enzyme important for the catabolism of the amino acids isoleucine, threonine and methionine as well as odd-number-chain fatty acids (found in fish) to produce propionyl-CoA.
- *β-methylcrotonyl-CoA carboxylase:* is an important enzyme in the catabolism of leucine

Non-coenzyme Roles of Biotin [58, 183]:

- *Biotinylations of proteins and gene expression:* Biotin has been found to influence multiple cellular functions through biotinylation of proteins, in particular nonhistone and histone proteins.
- *Cell cycle:* Biotin is required for cells to progress normally through the cell cycle with a biotin deficiency appearing to cause cell arrest at the G1 phase.

Deficiency of Biotin

Biotin can be manufactured by gastrointestinal bacteria and subsequently absorbed [58], however human biotin requirements are still unknown, therefore a deficiency or sufficiency may be difficult to assess [183]. Nonetheless, deficiencies can occur due to genetic mutations (e.g. biotinidase and holocarboxylase synthetase), high raw egg intake, excessive chronic alcoholism and gastrointestinal disorders such as inflammatory bowel disease. Other states that may cause biotin status to decline include pregnancy and anticonvulsant medication (e.g. phenobarbital, phenytoin or carbamazepine) [58].

Deficiency signs and symptoms can include neurologic symptoms such as lethargy, paraesthesia in extremities (such as pins and needles), hypnotonia (reduced muscle tone), depression and hallucinations. Other signs and symptoms include a red, scaly dermatitis found around the eyes, nose and mouth, anorexia, nausea, alopecia (hair loss), and muscle pain. Death can occur if the biotin deficiency is not treated. Treatment is normally a therapeutic dose of biotin of 10 mg or more daily [58].

Toxicity of Biotin

Currently the NHMRC has found that there is insufficient evidence of adverse effects or toxicity in both human and animal studies to set an upper level or limit [90].

Assessment of Nutriture of Biotin

Both blood and urine can be used for the assessment of biotin. In blood analysis, biotinylated holo-3-methylcrotonyl-CoA (holo-MCC) and holo-PCC from lymphocyte extracts are easily detectable in streptavidin blots and gel densitometry analysis and are reliable markers for biotin status.

Urine excretion of biotin has been found to be beneficial in detecting biotin supplemented individuals however, it is not a good marker to distinguish a deficiency [183].

Role of Biotin in the Peripheral Nervous System

Currently, no research has been conducted on biotin and CIPN, but there has been studies conducted for uremic [184, 185] and diabetic neuropathy [186]. For uremic neuropathy, a small study conducted on nine patients undergoing dialysis that were suffering from PN were supplemented 10 mg of biotin in three divided doses for 1-4 years [184]. There was a marked improvement reported for paraesthesia, restless legs and difficulty walking in all patients within three months of supplementation. One patient who was unable to walk for three months improved significantly within six months of supplementation of biotin [184].

The same researchers who trialled the biotin treatment for dialysis patients, also began trying biotin for diabetic patients. A case report on three diabetic patients experiencing severe diabetic PN was treated with 10 mg of biotin via intramuscular injections daily for six weeks. This was followed up by injections three times a week for another six weeks and oral administration of 5 mg daily. All three patients noted improvements with 4-8 weeks although nerve conduction studies found that it had only slightly improved or not at all [186].

1.9.3.10 Inositol

Inositol was originally thought of as a B vitamin although now it is considered to be an essential cell constituent. The human body has the ability to synthesis inositol so the need for external intake may not be necessary. Moreover, it can be found in nearly all plant and animal cells, both in free form and combined with other compounds [59, 60].

The chemical structure of inositol is presented in Figure 1-13.

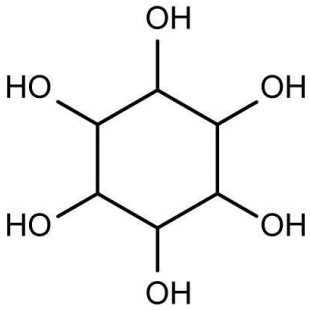


Figure 1-13: **The Chemical Structure of Inositol [187]**

The main food sources of inositol include grains, beef liver, legumes, milk, fruits, nuts, organ meats, vegetables and brewer's yeast.

Absorption of Inositol

As mentioned previously the body can produce inositol but we can also absorb it from our diet. The actual absorption pathway has not been completely documented however it is known that as a phytate it will block absorption of certain nutrients but as inositol it can aid the absorption of minerals such as zinc. A vitamin B complex also aids the absorption of inositol. Certain drinks such as alcohol, coffee, any caffeine drinks can all inhibit inositol absorption. Tobacco has also been found to inhibit inositol absorption [60].

Tissue Storage of Inositol

Inositol can be found in every cell in the body. Higher concentrations can be found in the brain, cerebrospinal fluid, skeletal and heart muscles, liver, kidneys and in the male reproductive organs, particularly the semen [60].

Metabolism and Excretion of Inositol

Inositol excretion is mainly through urine [188].

Function of Inositol

There are a number of functions that inositol plays within the body. These include [188]:

1. As phosphatidylinositol in all cell membranes
2. As a second messenger in cells
3. Mediation of cell responses to stimuli
4. Aiding nerve transmissions
5. Aiding regulation of enzyme activity
6. May also affect the function of lipid-transporting molecules

Deficiency of Inositol

An inositol deficiency is considered rare due to the fact that humans can produce it within their bodies and its widespread availability in foods [60].

Toxicity of Inositol

No toxicities have been reported related to inositol supplementation. Currently it is under review by the TGA with no negative studies being found on supplementation. One case report in 2010 [189] has documented a high dose of 3grams of inositol for 4 years to a 62 year female suffering from bipolar disorder for 30 years. It was found to be effective and safe [90].

Assessment of Nutriture of Inositol

The assessment method used will depend on which function of inositol is required. Both plasma and urine samples can be tested such as myo-inositol in plasma [190] and urine for inositol phosphoglycan P-type [191]. Inositol is not normally tested except for trials.

Role of Inositol in the Peripheral Nervous System

Inositol, in particular myo-inositol has been assessed for diabetic peripheral neuropathy. As mentioned in chapter one, myo-inositol is an important constituent of the phospholipids that make up cell membranes including nerve cells [192]. Nerve myo-inositol levels have been tested with numerous in vitro studies indicating reduced myo-inositol levels in diabetic peripheral neuropathy [193-197]. A rat study experimented with nerve myo-inositol levels and high glucose states [180]. They found that induced cellular myo-inositol depletion did occur in peripheral nerves without hyperglycaemia or insulin deficiency and this reproduced a biochemical, electrophysiological and structural defect that characterised early onset diabetic neuropathy. They still emphasised that this depletion was also in conjunction with other metabolic, vascular and neurotrophic insults that all contribute to the pathogenesis of diabetic neuropathy [198].

A clinical trial on sural nerve biopsies was conducted on 30 males, n=10 with type 1 diabetes (5 with clinically diagnosed peripheral neuropathy), n=10 with impaired glucose tolerance and n=10 with normal glucose tolerance [199]. This study found that nerve myo-inositol levels were significantly lower in participants with diabetes who had neuropathy. In addition, participants with normal or impaired glucose tolerance who had high nerve myo-inositol levels were found to be associated with nerve regeneration due to increased nerve fibre density [199].

Two small clinical trials have been conducted on oral supplemental myo-inositol and diabetic neuropathy [200, 201]. A double-blind placebo-controlled RCT with 28 participants with diabetes

with early subclinical diabetic neuropathy were supplemented with 2 grams of oral myo-inositol or placebo three times daily for two months. The results found no significant changes in nerve conduction velocities, vibratory perception or amplitude of action potentials in either group. High blood and muscle tissue levels were found in the myo-inositol supplemented group but nerve myo-inositol levels were not evaluated [200].

The other trial was also a double-blind placebo-controlled RCT but on only 7 participants with clinical signs of diabetic neuropathy (n=3) or subclinical neurophysiological signs (n=4). These participants were supplemented with 500 mg of myo-inositol or placebo twice daily for 14 days, and then crossed-over. Each group had 2 phases of the placebo (14 days = 1 phase) and 1 phase of myo-inositol. Results found no significant differences in the nerve conduction velocities during either placebo or myo-inositol phases however, action potentials in the median, sural and popliteal nerves increased in amplitude by 76%, 160% and 40% respectively during the myo-inositol supplementation phase [201].

Although the initial potential to prevent or reverse diabetic peripheral neuropathy was seen, the clinical evidence is limited. Therefore it is hard to correlate if inositol would be beneficial or not for CIPN. No research has been conducted on CIPN and inositol to date and with the clinical research on diabetic PN not showing promise it may not be a selected nutrient for further research.

1.10 Rationale: The Focus of the Study

The relevance of CIPN is that it can affect chemotherapy dose and continuation of treatment, which is of clinical importance. Investigating agents that can assist with CIPN treatment and prevention has significant scientific merit. Several different substances hypothesised for pharmacological neuro-protection (*e.g.* Amifostine) have been trialled, however, neither prophylactic strategies nor symptomatic treatments have proven useful [4, 28].

The neurotoxic chemotherapy agents included in this clinical trial are oxaliplatin, paclitaxel and vincristine due to their high incidence of inducing CIPN. The consequence of developing CIPN from these drugs includes dose reduction, cessation of treatment and for approximately a third of patient's permanent peripheral neuropathy, which can affect quality of life and physical functionality [2-4, 12].

To date, no research on B group vitamins and CIPN has been undertaken. B group vitamins have the potential to decrease the onset and severity of CIPN. A deficiency or low status of B group vitamins may biochemically affect the health of nerve fibres and ganglia that neurotoxic chemotherapy agents can adversely modulate. It is expected that the administration of a B group vitamin supplementation

will decrease the severity and onset of CIPN and therefore increase patient compliance and quality of life.

A literature search to ascertain previous research on agents to prevent or treat CIPN has been conducted in Chapter 2. These include pharmaceutical, neutraceutical and other agents such as herbal medicine and acupuncture. A basis of current literature assists in giving a grounding knowledge in what has been trialled and the outcomes have been achieved.

2 CHAPTER 2 – LITERATURE REVIEW

Modern cancer treatments have been reported to prolong life, however, treatment-related complications such as CIPN are detrimental for patients and increasing in frequency. Extensive clinical trials, epidemiological studies and standardised diagnostic criteria still have not produced established methods for prevention and/or treatment [202]. Listed below are the studies conducted on pharmaceutical agents, nutriceuticals and other complementary medicines trialled to assist in the prevention and/or treatment of CIPN.

2.1 RESEARCH ON MEDICAL DRUGS FOR THE TREATMENT AND/OR PREVENTION OF CIPN

Amifostine is a pharmaceutical agent that has had numerous studies conducted on it for the prevention of CIPN [6, 7, 203-215]. The results are contentious with two studies cancelled due to harmful side effects, for example. hypotension [209, 211]; while other studies reported an increased incidence of worsened nausea and vomiting and no improvement on quality of life [6, 207]. All other pharmaceutical agents showed negative or minimal prevention or treatment for CIPN.

Results the current literature on pharmaceutical agents can be found in **Table 2-1 Clinical Studies Investigating the Efficacy of Pharmaceuticals for the Prevention of CIPN.**

Methods

A systematic search of the literature was conducted using PubMed, the Cochrane Library, Science Direct, Scopus, EMBASE, MEDLINE and CINAHL.

Search Terms

Articles were identified using the search terms, "chemotherapy" OR "Cisplatin" OR "Taxanes" OR "Paclitaxel" OR "Docetaxel" OR "Oxaliplatin" OR "Carboplatin" OR "Platinum compounds" OR "Proteasome inhibitors" AND "induced peripheral neuropathy" OR "CIPN" AND "Treatment" OR "Prevention".

The Inclusion criteria for this review were: 1) An RCT and/or cross-over clinical trial that used either a placebo comparator or current anti–CIPN treatment as a control; 2) Human participants diagnosed with a cancer and administered chemotherapy; 3) The use of a pharmaceutical agent as the main intervention and specifically investigating its effects on reducing the primary outcome i.e., CIPN; and 4) The clinical study was published in English.

The overall body of evidence (based on a summary of the individual studies) (Table 2-3) evaluated within this review was assessed using a separate tool, the Australian National health and Medical Research Council's (NHMRC) body of clinical evidence assessment matrix. This is an assessment tool that assigns a level/grade (Level I: strongest evidence to level IV: weakest evidence) based on the strength of the published study [216]. The consort diagram can be seen in figure 2-1.

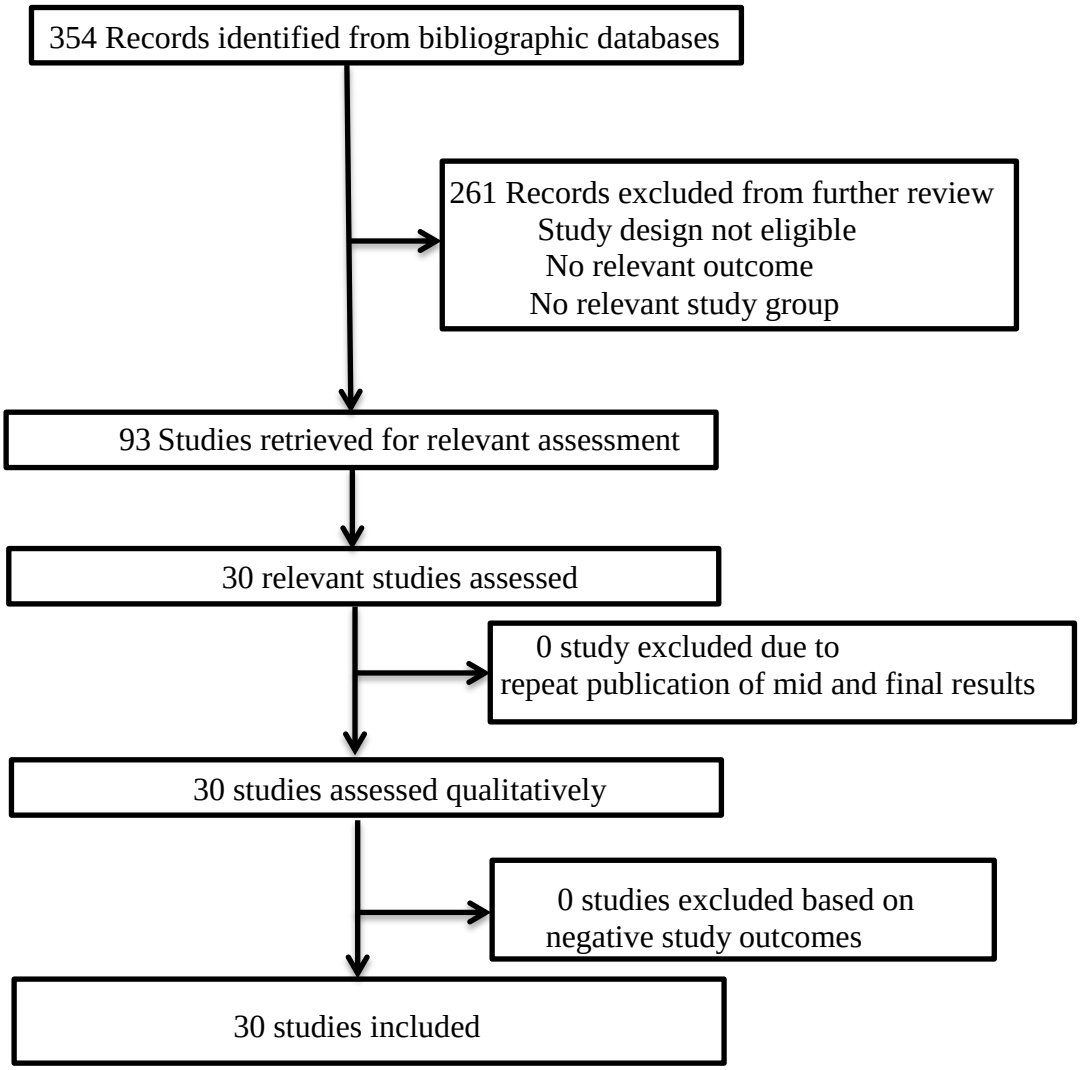


Figure 2-1: Flow of information for systematic review on pharmaceutical agents.

2.1.1 Pharmaceuticals and CIPN research

2.1.1.1 Amifostine (WR2721)

Fifteen trials have currently been conducted on Amifostine and CIPN [6, 7, 203-215] with mixed results. Of the trials conducted there was one pilot trial [206], three non-randomised clinical trials [209, 210, 212], six randomised controlled trials [7, 203-205, 211, 215], one phase II open label trial

[214], three phase II double-blind placebo-controlled clinical trials [6, 208, 213] and one multi-centre phase III trial [207].

The types of chemotherapy varied amongst the trials with cisplatin resulting in minor protection for ototoxicity [7, 212] and general CIPN [204, 205, 214]. Oxaliplatin also showed minor protection for CIPN in one trial [206], which used subcutaneous administration of amifostine. Paclitaxel resulted in two negative responses [210, 213] indicating that it is not a clinical option for paclitaxel alone. Moreover, a trial conducted on paclitaxel and cisplatin was ceased due to the number of patients experiencing CIPN exceeded the pre-determined threshold [209]. However, the combination of paclitaxel and carboplatin indicated minor protection for severe CIPN [207, 211, 215] with one trial resulting in no benefit [6]. Only one trial on cisplatin administered for five consecutive days resulted in a significantly lower incidence of CIPN [203].

Most trials noted side effects including hypotension, nausea, vomiting, dizziness, flushing, anxiety, palpitations, and sneezing. Overall, the possible protective effect from amifostine is counteracted by its own side effects.

2.1.1.2 Recombinant Human Leukaemia Inhibitory Factor (rhuLIF)

One trial has been conducted on this compound with no benefit found for CIPN [217]. In addition, adverse reactions were noted including injection site reactions, light-headedness, rigor/chills, myocardial ischemia and hypotension [217].

2.1.1.3 Anticonvulsants

Two anticonvulsants have been trialled for CIPN, carbamazepine and oxcarbazepine [218-221] for oxaliplatin-IPN. Two trials, one with 10 participants [218] and another with 32 participants [220] showed mild or possible prevention compared to two trials, which found no benefit [219, 221].

2.1.1.4 Gabapentin (Neurontin)

Two trials have been conducted with gabapentin for the treatment of cancer and/or CIPN pain [5, 8]. Ross, et al. [8] found it may benefit cancer pain but was not specific for CIPN. Rao, et al. [5] who trialled gabapentin specifically for CIPN in 115 participants found that it failed to show any benefit for CIPN symptoms.

2.1.1.5 Lamotrigine

A phase III RCT has been conducted on lamotrigine [222] for the treatment of CIPN in 131 participants. No difference was seen between the treatment and placebo arms and therefore was found to not be an effective agent for relieving neuropathic symptoms from CIPN.

2.1.1.6 Pregabalin

An open label trial was conducted on pregabelin (Lyrica) [223] as a treatment for oxaliplatin induced peripheral neuropathy for 23 cancer patients. The target dose of 150 mg three times a day (TDS) provided the most benefits (5/23 patients reached target dose) with improvements observed between two-six weeks of treatment.

Forty-eight percent of patients CIPN improved by one to two grades bringing the authors to the conclusion that pregabalin can significantly reduce the severity of sensory peripheral neuropathy from oxaliplatin administration. Pregabalin has fewer side effects than gabapentin so may be better options for patients.

2.1.1.7 Antidepressants

A number of antidepressants have been trialled for the treatment of CIPN including tricyclic antidepressants such as amitriptyline [223] and nortriptyline [225], venlafaxine [226-228] and duloxetine [229, 230]. Three of the published papers on venlafaxine and one of the duloxetine papers were case studies [226-229] with most indicating some benefit. The tricyclic antidepressants [224, 225] did not demonstrate improvement in CIPN particularly sensory neuropathic symptoms and participants also noted a number of side effects from these drugs. A recent phase III trial on duloxetine [230] assessing 231 participants for treatment of CIPN has been the first trial that has shown statistical significance for improving pain from CIPN compared to placebo. Further research to support this trial is warranted but it does show promise for patients experiencing CIPN pain from taxanes or oxaliplatin.

2.1.1.8 Calcium Channel Blockers

A retrospective study was conducted on 116 participants who underwent administration of FOLFOX6 regime [231]. The data on these patients was assessed for acute CIPN development and calcium channel blocker concurrent administration. From the information obtained, it was hypothesised that calcium channel blockers may inhibit the development of CIPN but further research is required. To conduct a RCT would be difficult considering that these drugs lower blood pressure and not all patients undergoing chemotherapy have high blood pressure. If they do, they have normally been prescribed a specific drug or more which may not be a calcium channel blocker. Further research will most likely be more retrospective analysis on a larger population base.

2.1.2 Strengths and Weaknesses and Gaps in the Research

Clinical trials conducted on medical drugs involve a scientific study that involves patients and/or non-patient volunteers. They confirm if a medicine is safe and effective to introduce as a new treatment

for a particular disease or condition [232]. One of the major problems with protective or treatment trials for CIPN is that each neurotoxic drug has a different mechanism of action and the actual cause of the neuropathy is unknown other than that fact that the chemotherapy agent accumulates in the peripheral nerves. In addition, the patients have or may have had cancer which in itself can present a myriad of other physiological and biochemical issues.

The major weaknesses of the trials conducted on CIPN from medicinal drugs include the fact that the drugs themselves can induce their own side effects in addition to the side effects experienced by the chemotherapy agents. Moreover, most trials used the NCI-CTC as their measurement tool which is subjective rather than quantitative. The NCI-CTC is an assessment tool used by medical doctors or staff which is based on an examiner's interpretation rather than a formal neurological evaluation. A study by Frigeni et al., (2011) examined the difference between the NCI-CTC and the Total Neuropathy Score (TNS) to identify any discrepancies in diagnosis of CIPN. The authors found that the NCI-CTC evaluation when performed by experienced examiners may overestimate the occurrence of motor neuropathy due to confounding factors such as fatigue, depression and cachexia. They concluded that a more formal neurological assessment of patients with CIPN is advised rather than using an examiner interpretation of the NCI-CTC [338].

Considering that there are more cancer survivors and that these neurotoxic drugs can create a permanent consequence, it is important to see that further research is being conducted on both protection and treatment trials. One of the gaps in the research is that the drugs being trialled have been mostly used for other peripheral neuropathies which are different to CIPN. Other peripheral neuropathies such as diabetic, HIV or alcoholic induced peripheral neuropathy [12] are normally slow developing neuropathies whereas CIPN although accumulative can be a fast developing side effect [12]. Hence, the research gap is looking at different possible mechanisms of action that could be involved in the development or predisposition of CIPN. One of these possibilities is the fact that a deficiency in certain B vitamins such as vitamin B1, B6 and B12 can cause a peripheral neuropathy. Therefore, if chemotherapy drugs and/or poor diet during chemotherapy lowers these vitamins into a deficiency or near deficiency state, it may predispose the patient to the development of CIPN.

Another opportunity may involve prophylactic anti-inflammatory drugs. Although normal pain relief does not reduce the pain associated with CIPN, no specific anti-inflammatory drug has been trialled as a protective agent. This is one of the main mechanisms of action that has been identified in the nutrients and the herbs trialled for CIPN that have shown a possible beneficial protective effect [233-246]. The anti-inflammatory component may or may not protect patients against CIPN but it would be an interesting aspect to investigate. Another aspect that may be interesting to research is the relationship between lower limb oedema and the incidence of CIPN.

2.1.3 Outcomes for Pharmaceutical Agents and CIPN

From the trials conducted, no pharmaceutical agent has been found to prevent CIPN. Several agents have been found to have a limited benefit, for the treatment of CIPN however, further trials are required. Possible beneficial treatment options include duloxetine (Cymbalta) and other anti-depressants such as venlafaxine (Effexor). Currently clinicians have also been using pregabalin (Lyrica) with some benefit although only one open label trial has been conducted on CIPN.

Table 2-1: **Clinical Studies Investigating the Efficacy of Selected Pharmaceuticals for the Prevention of CIPN.**

Pharmaceuticals	Study Type / Level of Evidence	Level of Evidence	Number of Pts[5]	Chemotherapy and Duration	Results
Amifostine (WR2721)	RCT [1203]	Level III-1	69	5 consecutive days of cisplatin	WR 2721 had a significantly lower incidence of CIPN (incidence was 25% versus 49%)
	NRCT Phase 1 trial [212]	Level III-2	11	Cisplatin: duration not noted	Possible protection for ototoxicity from cisplatin
	RCT [204]	Level III-1	242	6 cycles every 3 weeks cisplatin/ CPP[6]	Pre-treatment with amifostine showed a reduction in accumulative toxicities (p=0.029).
	RCT [205]	Level III-1	74	6 cycles weekly (3 mth follow up) of cisplatin	Diminished incidence of subclinical CIPN in the amifostine arm (p=0.03) with no protection shown for renal or ototoxicity.
	RCT Phase II [213]	Level III-1	40	3 cycles every 3 weeks of taxol	No differences observed in neurotoxicity
	Pilot trial [206]	Level III-3	15	Raltitrexed/OxP[7] every 3 wks Irinotecan/OxP given day 1,14 every 4 wks.	Counteracted oxaliplatin CIPN as 10/15 patients had reduced the severity of CIPN.
	Phase II open label trial [214]	Level III-2	37	Cisplatin every 3 weeks until disease progression	No tumour protection or reduced toxicity
	Multi-centre Phase III RCT[207]	Level III-1	187	6 cycles every 3 weeks of carbo/taxol[8]	May be protective for grade 3 and 4 neurotoxicity (3.7% vs 7.2%; P-0.02)

[5] Pts - Participants
[6] CPP - cyclophosphamide
[7] OxP – oxaliplatin
[8] Carbo/Taxol is a combination of carboplatin and paclitaxel

	DBRCT [208]	Level II	60	2 cycles every 3 wks of carbo/taxol, then radiation, then 6 weekly taxol	No significant protective effects
	RCT [215]	Level III-1	38	6 cycles of carbo/taxol every 3 weeks	May reduce the incidence of CIPN
	Multi-centre NRCT [209]	Level III-2	27	6 cycles of paclitaxel and cisplatin every 3 weeks	4/21 had grade 2-4 neurotoxicity, 3/21 had grade 3 CIPN – Study terminated
	NRCT [210]	Level III-2	31	Doxorubicin, paclitaxel and CPP over 9 days	No significant effect was seen.
	RCT [211]	Level III-1	90	6 cycles every 3 weeks of carbo/taxol	Only minor protective effects
	DBRCT Phase II [6]	Level II	71	Carbo/taxol with or without epirubicin	Found to improve sensory neuropathy according to the NCI-CTC (P=0.0046)
	RCT [7]	Level III-1	97	4 cycles of cisplatin/ vincristine /CPP every 3 weeks	May significantly reduce the risk of severe ototoxicity
Recombinant human leukaemia inhibitory factor (rhuLIF)	DBRCT [217]	Level II	117	Carbo/taxol 4-6 cycles every 3 weeks	No differences noted
Anticonvulsants (carbamazepine/ oxcarbazepine)	NRCT [218]	Level III-3	10	FOLFOX (OxP) 12 cycles every 2 weeks	Carbamazepine may provide a possible prevention
	NRCT [219]	Level III-3	25	FOLFOX (OxP) 12 cycles every 2 weeks	Carbamazepine did not provide symptomatic relief or prevention
	Open label RCT [220]	Level III-2	32	FOLFOX (OxP) 12 cycles every 2 weeks	Oxcarbazepine was lower than the control with 5/16 (31.2%) versus 12/16 (75%) getting CIPN
	RCT [221]	Level III-1	36	FOLFOX (OxP) 6 weekly	No major differences were found between the two groups.

Gabapentin (Neurontin)	Open label NRCT [8]	Level III-2	62	15 days	Only 2/25 had CIPN pain
	DBRCT multi-centre [5]	Level II	115	CIPN >1 month Tx= 6 weeks plus a 2 weeks washout	Gabapentin failed to show any benefit to treat CIPN symptoms
Lamotrigine	RCT Phase III [222]	Level II	131	CIPN >1 month Tx = 10 weeks than 4 week tapering off drug	No differences seen in the two groups
Pregabelin (lyrica)	Open label [223]	Level III-2	23	150mg TDS for 6 weeks	Significantly reduced the severity of oxaliplatin-induced sensory neuropathy in patients that reached target dose (22%)
<u>Antidepressants</u>: **1. Tricyclic antidepressants e.g. amitriptyline and nortriptyline.**	DBRCT cross over trial [225]	Level II	51	Cisplatin CIPN pain >1 mth: 4 weeks, 1 week washout	Very modest effect of nortriptyline on the relief of painful CIPN.
	DBRCT [224]	Level II	44	CIPN 8 weeks	Amitriptyline did not demonstrate any improvement for sensory neuropathic symptoms
2.Venlafaxine	CS [227]	Level VI	1	CIPN from Carbo/Taxol	60yo women developed CIPN after 3rd cycle. Venlafaxine reduced CIPN.
	CS [226]	Level VI	1	CIPN from OxP	33yo patient developed CIPN after 3 cycles, exacerbated by cold. Venlafaxine reduced CIPN
	CS [228]	Level VI	2	CIPN from FOLFOX (OxP)	82yo women: assisted quick recovery. 53yo man: Venlafaxine had no beneficial effect.
3. Duloxetine	CS [229]	Level VI	1	CIPN from paclitaxel	68yo man: decreased CIPN in 5 months.
	DBRCT cross over trial Phase III [230]	Level II	231	CIPN – treatment for 12 weeks	Statistically significant in improving pain from CIPN compared to placebo
Calcium channel blockers	Retrospective study [231]	Level III-2	116	Data analysed retrospectively from FOLFOX6 regime	Had significantly lower incidence curve for acute CIPN but not for cumulative chronic CIPN.

DBRCT: Double Blind Randomised Controlled Trial; SBRCT: Single Blinded Randomised Controlled Trial; RCT: Randomised Controlled Trial; NRCT: Non Randomised Clinical Trial; CS: Case Study; TNS: Total Neuropathy Score

2.2 RESEARCH ON NUTRACEUTICALS FOR THE TREATMENT AND/OR PREVENTION OF CIPN

Twenty-four clinical trials examining a variety of agents on the prevention and symptomatic treatment of CIPN have been conducted. To date, few nutraceuticals have proven successful in preventing CIPN development. One nutraceutical, acetyl-L-carnitine [247, 248] has shown promise in a preliminary trial as a neuroprotective agent for the treatment of CIPN after chemotherapy administration, but further studies are required. A new study looking at omega 3-fatty acids showed a statistically significant difference for paclitaxel induced neuropathy although further studies are required to confirm this finding [237].

Results of all the studies can be found in **Table 2-3: Clinical Studies Investigating the Efficacy of Selected Nutraceuticals for the Prevention of CIPN.** Information pertaining to this section has been published: **Review:** Nutraceuticals and Chemotherapy-Induced Peripheral Neuropathy (CIPN): a Systematic Literature Review. **Schloss J**, Colosimo M, Airey C, Vitetta L. Clin Nutr 2013: 32(6):888-93 [249]

2.2.1 Study Characteristics

The studies selected for this review include randomised controlled trials, open label trials and one retrospective study. The randomised controlled trials identified are divided into eleven double blind placebo controlled trials, five randomised controlled trials including a randomised open label with a blind assessment plus two non-randomised controlled trials. Four open label trials were identified which did not contain a placebo component.

The results of the included studies were mixed. Several nutraceuticals showed initial positive results in reducing CIPN however further studies found that they were either not significant or indicated possible interference with the chemotherapy agent's response rate. No nutraceutical currently has been found to significantly show protection or treatment for CIPN.

Methodology

A systematic search of the literature was conducted using PubMed, the Cochrane Library, Science Direct, Scopus, EMBASE, MEDLINE and CINAHL.

Search Terms

Articles were identified using the search terms, "chemotherapy" OR "Cisplatin" OR "Taxanes" OR "Paclitaxel" OR "Docetaxel" OR "Oxaliplatin" OR "Carboplatin" OR "Platinum compounds" OR "Proteasome inhibitors" AND "induced peripheral neuropathy" OR "CIPN" AND "nutrient" OR

"vitamin" OR "mineral" OR "Acetyl-L-Carnitine" OR "Glutamine" OR "Vitamin E" OR "Alpha Lipoic acid" OR "Magnesium" OR "Calcium" OR "Vitamin B6" OR "B vitamins" OR "Omega-3 fatty acids".

The Inclusion criteria for this review were: 1) An RCT and/or cross-over clinical trial that used either a placebo comparator or current anti–CIPN treatment as a control; 2) Human participants diagnosed with a cancer and administered chemotherapy; 3) The use of a nutraceutical supplement as the main intervention and specifically investigating its effects on reducing the primary outcome i.e., CIPN; and 4) The clinical study was published in English.

The overall body of evidence (based on a summary of the individual studies) (Table 2-3) evaluated within this review was assessed using a separate tool, the Australian National health and Medical Research Council's (NHMRC) body of clinical evidence assessment matrix. This is an assessment tool that assigns a level/grade (Level I: strongest evidence to level IV: weakest evidence) based on the strength of the published study [216]. The consort diagram can be seen in figure 2-2.

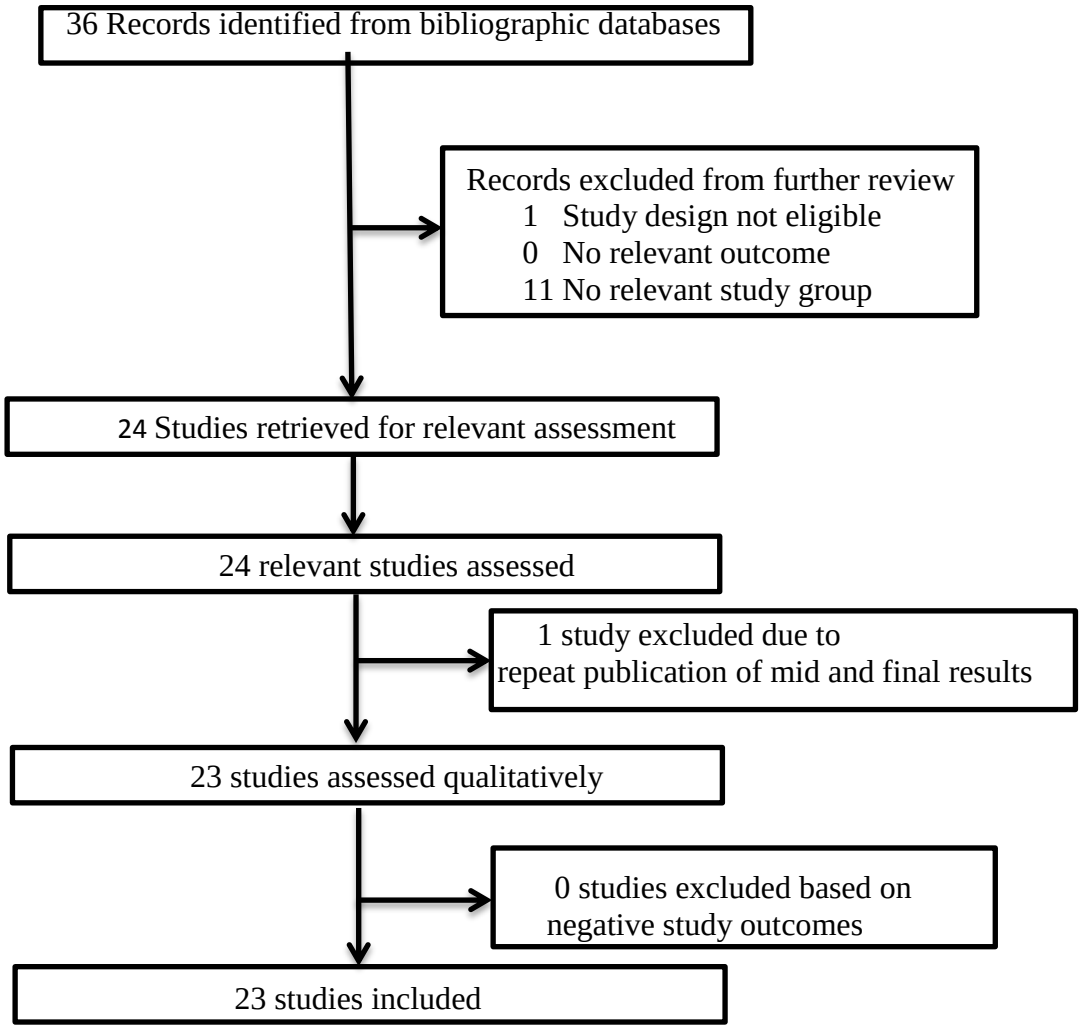


Figure 2-2: Flow of information for systematic review on nutriceuticals.

2.2.2 Nutriceuticals and CIPN Research

2.2.2.1 Magnesium and Calcium

Magnesium and calcium infusions with oxaliplatin also showed initial positive results in a retrospective study [250]. Three clinical trials [251-253] reported that efficacy was not enhanced with one trial (n=174) being terminated due to the treatment group reporting a significant lower response rate compared to placebo [251].

2.2.2.2 Vitamin E

Vitamin E was demonstrated to have positive results in three RCT's showing a significant reduction in the relative risk of cisplatin induced PN, especially ototoxicity [233-236, 254]. However, a phase III multi-centre trial combining vitamin E with taxanes, cisplatin, carboplatin, oxaliplatin or combination concluded that vitamin E did not appear to reduce the incidence of sensory neuropathy [254]. Vitamin E efficacy was limited to those patients administered cisplatin (only 8 from the 207 cohort) during the phase III study. Previous studies have indicated that vitamin E seems to be more protective for cisplatin ototoxicity compared to other neurotoxic agents [255].

A recent pilot trial with oxaliplatin induced peripheral neuropathy also failed to show protective effect of vitamin E [256]. In contrast, this study showed an increased number of participants developing grade 1 and 2 acute CIPN from oxaliplatin. There was also a major confounding factor with this study as each patient (either on vitamin E or placebo) also received infusions of calcium and magnesium with each administration of chemotherapy. This may have played a key factor with possible interactions occurring with the vitamin E and could explain why the placebo (68% vs 83%) [256], experienced less CIPN considering previous trials on magnesium/calcium infusion and oxaliplatin [250].

2.2.2.3 Lipoic Acid

One open label study was conducted with alpha lipoic acid (n=15) co-administered with oxaliplatin [257]. This treatment combination showed a trend toward a reduction in the severity of oxaliplatin induced CIPN in 8 out of 15 patients but the trend was not statistically significant [257]. The second trial was a randomised, double-blind placebo-controlled trial (n=243) who were randomised to either 600mg of alpha lipoic acid (n= 122) or placebo three times a day (n= 121). It was measured by the FACT/GOG-Ntx and the NCI-CTC. Only 70 (29%) of the 243 participants completed the trial (24 weeks) with both arms having comparable dropout rates. It was found that lipoic acid was not statistically significant in reducing neurotoxicity from cisplatin or oxaliplatin [258].

2.2.2.4 N-Acetyl Cysteine

N-acetyl cysteine (NAC) administration to cancer patients has been based on the assumption that NAC can increase glutathione production, an effect that may decrease cyto-toxicity [259].

In a further study conducted with NAC no significant changes on electrophysiological testing on both sensory and motor nerves were found between the two groups [259].

2.2.2.5 Glutathione

Glutathione has been reported to show positive results in reducing CIPN [260]. Intravenous glutathione administered before cisplatin administration reported promising outcomes for the prevention of cisplatin induced PN without reducing the anti-tumour activity of the chemotherapeutic agent [261-263]. A positive result was also found in two studies with oxaliplatin [264, 265]. Both studies reported a significant reduction in oxaliplatin induced PN, mainly relevant to sural sensory nerve reduction. These results present clinical data that indicates that glutathione administration may aid in the prevention of CIPN. However, additional higher quality studies are required, as these trials were limited due to a high participant dropout rate and without long-term follow–up.

2.2.2.6 Glutamine

Two published clinical studies [266, 267] on the administration of glutamine with paclitaxel are the results from the same clinical trial. The first study is from an early publication of results [266] with final results being published in the second study [267]. These studies reported that the patients on glutamine tended to have fewer symptoms than those on placebo however the trend in the nerve conduction studies was not statistically significant. There was a drift in the results that indicated that glutamine decreased the severity of dysesthesia in the fingers and toes. An additional study [268] with glutamine and oxaliplatin co-administration reported that glutamine may reduce the incidence and severity of oxaliplatin induced CIPN. No significant differences were found between the two groups [268].

2.2.2.7 Acetyl–L–Carnitine

Two open label studies reported that acetyl–L–carnitine (ALC) may be a treatment option for paclitaxel and cisplatin induced CIPN [247, 269]. A phase III trial demonstrated that ALC did not provide a positive benefit for the prevention of CIPN [270]. The study concluded that patients should be discouraged from using ALC during treatment with taxane therapy. Notwithstanding this recommendation it may be an option for the treatment of CIPN rather than prevention in patients that already may be experiencing CIPN.

2.2.2.8 Vitamin B6

One DBRCT with vitamin B6 found that it significantly reduced CIPN from cisplatin and hexamtheylmelamin administration [78]. Furthermore the results indicated that the high dose vitamin B6 (100 mg) administered may affect response duration and that it requires further investigation [78]. A recent study on oxaliplatin used a derivative of vitamin B6, mangafodipir [18] which found it beneficial in preventing or alleviating oxaliplatin-IPN.

2.2.2.9 Omega–3 Fatty Acids

A recent DBRCT investigating the effect of omega–3 fatty acids on paclitaxel induced peripheral neuropathy found that it significantly reduced the incidence of CIPN from paclitaxel by 70% [237]. The results of the reduced TNS found that 21 out of 30 patients (70%) did not develop CIPN while 9 patients (30%) did develop CIPN in the omega 3 fatty acid supplemented group. In the placebo group out of 11 out of 26 patients (40.7%) did not develop CIPN. The only statistical significance found in the NCS parameters was the sural nerve conduction test. A significant difference was observed for the sural a-SAP score between the omega-3 supplemented group compared to placebo ($p=0.015$). A sharp decrease of the sural a-SAP as seen in the placebo group compared to the intervention group. Further testing in larger randomised clinical trials is required however omega–3 fatty acids do show promise for protection against paclitaxel induced peripheral neuropathy [237].

2.2.3 Adverse Events and Adherence

There were limited adverse events noted from the nutraceuticals trialled for CIPN. The reported adverse events were from the use of acetyl-l-carnitine. Two patients reported acetyl-l-carnitine induced mild nausea [247] and one patient reported insomnia related to acetyl-l-carnitine treatment [271].

The nutraceuticals trialled were generally well tolerated according to the journal articles with only one study stating that two participants stopped their vitamin E supplementation after one month [272]. No reason was given as to why they had stopped taking the vitamin E.

2.2.4 Confounding Factors for Research on Nutraceuticals and CIPN

All studies indicated but did not state confounding factors associated with the clinical trials. These may have been listed under why patients didn't complete the trial or why they withdrew or other toxicities associated with the chemotherapy administration. All of these factors may have implications on the nutraceutical administration by affecting absorption, metabolism or compliance. One study reported the difficulty in reproducing or quantifying peripheral neuropathy as a confounding factor [263].

The main confounding factors reported in the studies from Table 2-3 are presented in Table 2-2

Table 2-2: **Reported Confounding Factors in Studies on Nutraceuticals and CIPN.**

Confounding Factors	n
Grade 3 or 4 nausea and vomiting	4
Grade 3 or 4 diarrhoea	3
Haematological toxicity (including transient hepatic failure)	2
Stomatitis	2
If the treatment was first, second or third line treatment	2
Cardiac and/or neurocerebellar adverse events	2
Use of Alternative treatments	1

2.2.5 Possible Drug-Interactions with High Doses of Nutraceuticals

Three studies found that there could be possible drug interactions with high dose nutraceuticals. The intravenous magnesium and calcium trial with FOLFOX regime was terminated due to the fact that the treatment group reported a significant lower response rate compared to placebo [251]. The authors concluded that a possible drug interaction occurred between the chemotherapy regime and the magnesium and calcium infusions however no further investigations were continued to ascertain the mechanism of action.

One study on glutamine stated that there could have been a protective effect from the glutamine on the tumour from the cytotoxic effects from the paclitaxel although no decrease in response rate was noted in the trial [266]. Again, no further testing was undertaken to ascertain this variable.

The study on vitamin B6 did show a significant decrease in the response rate with cisplatin and HMM [78]. Authors stated that this was directly due to the vitamin B6 supplementation as it occurred in all participants administered the high dose of vitamin B6. However, it also showed that vitamin B6 reduced response rate. No investigations were undertaken to ascertain how this occurred or the possible mechanism of action although the authors stated that further investigations were warranted.

2.2.6 Review Limitations

Several limitations have been identified within the selected studies. Limitations identified in the retrospective study [250] include no randomisation of patients, no blinding and patients, medical and nursing staff may have been biased in their reporting or assessment of signs and symptoms. All studies that did not include a placebo noted the limitation of not having a placebo-effect.

Assessment of CIPN is varied amongst all studies. This makes it difficult when comparing studies and results. There is no "gold standard assessment" of CIPN therefore clinical trials use a variety of assessment tools to quantify CIPN development and severity. These include the National Cancer Institute Common Toxicity Criteria (NCI-CTC), total neuropathy score (TNS), nerve conduction studies and/or electrophysiological investigations and quality of life questionnaires. Refer to chapter 1 section 1.7, p 1-37 for further evaluation of assessment tools.

The NCI-CTC is a clinician-based grading scale whereby the assessor may mix impairment, disability and quality of life measures which can lead to different interpretations of results and demonstrates observer objectivity and possible bias [34]. The NCI-CTC is the most commonly used assessment tool in the selected studies. The TNS is stated to be the most comprehensive assessment tool available at this time [273] with only one study using this assessment tool as their main measurement of results [256]. The nerve conduction studies and electrophysiological investigations are the other main assessment tool used within these studies. These provide information on compound action potential amplitude and conduction velocity but do not provide information regarding ion channel function or resting membrane potential and are still dependent on the skill of the neurologist conducting the assessment [274].

Another limitation involves the activities of daily living (ADL) or assessment of ADL. This is because the level of symptoms or interference of daily activity is generally subjective and signs on a physical examination may not always be predictive of whether ADL is affected or if it is important for that patient's life.

2.2.7 Discussion on Outcomes on Nutraceuticals and CIPN

Currently there are no established neuroprotective nutraceuticals or treatment options for CIPN. Results are inconsistent requiring further investigation to confirm efficacy and safety. There are several nutraceuticals which have shown promise for selective neurotoxic chemotherapy agents such as vitamin E with cisplatin, intravenous glutathione for oxaliplatin administration, vitamin B6 with HMM and cisplatin although it did interfere with response rate and omega-3 fatty acids for paclitaxel. Acetyl-L-carnitine has also shown promise as a treatment option for CIPN with further research required in large randomised controlled trials.

Table 2-3: **Clinical Studies Investigating the Efficacy of Selected Nutraceuticals for the Prevention of CIPN.**

Nutraceuticals	Study Type	Level of Evidence	Number of Pts	Duration	Results
Magnesium / Calcium	Retrospective study [250]	Level III-2	161	3 cycles of FOLFOX every 2 weeks	Grade 3 or 4 CIPN in Ca/Mg group recovered significantly quicker
	DBRCT [251]	Level II	174	8 cycles of FOLFOX plus bevacizumab every two weeks	Trial was terminated
	Open label [252]	Level III-3	14	FOLFOX 12 cycles every two weeks	CIPN occurred in 8 participants (57.1%)
	DBRCT [253]	Level II	35	FOLFOX6 12 cycles given every two weeks	Not significant
Vitamin E	DBRCT [233]	Level II	47	Cisplatin 6 cycles and 3 months after	Significantly lower incidence of CIPN [vitamin E group 30.7% vs control group 85.7]
	RCT [234] Open label blind assessment	Level III-1	40	6 cycles of cisplatin, paclitaxel or combination plus 3 months after	CIPN developed in 4/16 (25%) of vitamin E group compared to 11/15 (73.3%) of control group.
	RCT [235] Open label blind assessment	Level III-1	35	6 cycles of cumulative cisplatin based regimes	This study includes the final results of the published 2005 paper
	DBRCT [236]	Level II	108	Cisplatin – given before, during and 3 months after chemotherapy	Significantly lower incidence of CIPN [vitamin E 5.9% vs placebo 41.9%]
	DBRCT [254] Multi-centre	Level II	207	4 days after chemotherapy, during and 1 month after	No significant differences found
	RCT [256]	Level III-1	34	OxP 12 cycles every 2 weeks	No significant decrease in the incidence of acute CIPN
Alpha Lipoic Acid	Open label [257]	Level III-3	15	≥grade 2 CIPN after 6 cycles of OxP till recovery	Reduced severity of CIPN in 8/15(53%)
	DBRCT [258]	Level II	243	Oxaliplatin or cisplatin: stratified to no prior exposure to platinum compounds, prior exposure to <399mg/m^2 cisplatin or <750mg/m^2 oxaliplatin lastly exposure to	Was found ineffective in preventing neurotoxicity from oxaliplatin and cisplatin

				>400mg/m^2 of cisplatin or >750mg/m^2 of oxaliplatin	
N-acetyl cysteine (NAC)	RCT [259]	Level III-1	14	FOLFOX 12 cycles every 2 weeks	No significant change.
Glutathione	DBRCT [261]	Level II	50	Weekly cisplatin for 15 weeks	A trend towards prevention of CIPN
	RCT [262]	Level III-1	33	Weekly cisplatin for 9 weeks	No major differences observed, trend towards neuroprotection
	DBRCT [263]	Level II	151	Cisplatin every 3 weeks for 6 cycles	Not significant
	DBRCT [264]	Level II	52	Oxaliplatin every 2 weeks for 12 cycles	Not statistically significant but was for placebo sural nerve conduction
	RCT [265]	Level III-1	27	Oxaliplatin every 2 weeks for 12 cycles	Statistically significant reduction of neurotoxicity (P=0.0037).
Glutamine	NRCT [266, 267]	Level III-2	47	Given orally for 4 days starting 24 hours after paclitaxel completion	Not statistically significant
	RCT [268]	Level III-1	88	6 cycles FOLFOX regime over 6 months	May reduced severity of CIPN
Acetyl-L-Carntine (ALC)	Open label [247]	Level III-2	25	8 weeks	May provide an option for treatment of CIPN
	Open label [269]	Level III-2	26	10 days	Slight improvement in CIPN
	DBRCT [270] multi-centre	Level II	409	24 weeks	No evidence of a positive impact
Vitamin B6	DBRCT [78]	Level II	248	Between 1-70 months	Significantly reduced CIPN however adversely affect response duration.
	Open label [18]	Level III-1	23	Treatment for 8 cycles of oxaliplatin (16 weeks)	May prevent and/or relieve oxaliplatin-IPN
Omega 3 Fatty Acids (Omega 3FA)	DBRCT [237]	Level II	69	Paclitaxel every 3 week for 4 cycles and one month after	Statistically significant protection of CIPN

DBRCT: Double Blind Randomised Controlled Trial; SBRCT: Single Blinded Randomised Controlled Trial; RCT: Randomised Controlled Trial; NRCT: Non Randomised Clinical Trial; CS: Case Study; TNS: Total Neuropathy Score

2.3 OTHER COMPLEMENTARY THERAPIES FOR CIPN

A number of complementary therapies other than nutraceuticals have been tested for CIPN. These include acupuncture [275], herbal medicine [276] and contact needle therapy [277]. Acupuncture has the most clinical trials whilst herbal medicine has limited research mainly based on in vitro and animal studies. The contact needle therapy is one pilot trial with more research needed.

2.3.1 Acupuncture and CIPN

A 2013 systemic review on experimental and clinical acupuncture in CIPN [275] identified 3989 articles on acupuncture, however only 98 were relevant to CIPN. Of those, there were only 7 clinical trials [278-284] and 1 experimental study [285] which was a rat study. The overall conclusion from the systemic review [275] was that acupuncture generally had positive effects on CIPN by reducing the pain score in most studies. The limitations identified in the trials reviewed include small sample size, poor controls or no controls, poor randomisation and lack of blinding. Further studies are required to confirm benefit from acupuncture but they require more rigorous randomised controlled clinical trials.

One paper has been published on contact needle therapy and CIPN which involves case studies on 6 patients [277]. Contact needle therapy (CNT) is a form of traditional Japanese acupuncture. It uses disposable needles that are not inserted but only settle on the acupuncture point to perform the least stimulus compared to other forms of acupuncture which insert the fine needles through the skin. The latter is used to unblock the meridian and improve the patients' condition no matter what the disease by regulating the flow of Qi [277].

The results of the CNT case studies found that all patients displayed some improvement with 4/6 patients showing apparent improvement in pain [277]. To confirm these preliminary results, well-designed clinical trials with adequate power and sample size are required.

A recent RCT on electroacupuncture found that the electroacupunture point's trialled were not superior to placebo and no significant differences were seen in sensory nerve conduction studies or quality of life [286]. The authors concluded that as other acupuncture studies have found benefit contrary to their findings, the effect of acupuncture remains unclear with further trials required.

2.3.2 Herbal Medicine and CIPN

A total of 5614 journal articles were identified (1543 in English and 1997 in Chinese or Japanese). These were retrieved through electronic search and examination of references in reviews. Dissemination of the articles and abstracts decreased the total of articles from 3540 to 34 relevant journal articles. These results found 6 single herbs [238, 245, 287-296], one extract [297], one

receptor agonist [298, 299] and 8 combinations of herbs [300-318]. All studies were analysed for common scientific characteristics however lower levels of evidence was used as rigorous randomised clinical trials were limited. The majority of the journal articles identified were animal studies [n=17]. Human trials consisted of one multi-centre, randomised double-blind placebo-controlled trial, six randomised trials, six retrospective studies, one uncontrolled study and three case reports found, (see Table 2-4). Information pertaining to the herbs reported for CIPN has been accepted in Critical Reviews in Food Science and Nutrition. Herbal Medicines and Chemotherapy-Induced Peripheral Neuropathy (CIPN): a Critical Literature Review. **Schloss J,** Colosimo M, Vitetta L. [319]

The main problem with research involving herbal extracts or medicinal herbs is the understanding of the mechanism of actions and the fact that the herbs contain a number of active compounds. Moreover, Asian herbal therapies contain a combination of multiple herbs, which adds to the complexity of study analysis, data interpretation and an assessment of benefit. Isolation of one extract which is common in scientific research is unable to be conducted with herbal medicine research in most situations as the combination of compounds or combination of herbs such as Asian herbal therapies are seen to work in synergy.

Therefore, the studies conducted on herbal medicines and CIPN to date have not yielded clinical evidence to support the use of herbal medicines in the prevention or treatment of CIPN. However, some herbal medicines and combination of herbs such as Goshajinkigan (Japanese Kampo medicine) show promise with further research required.

Methodology

Selection Criteria

The Inclusion criteria for this review were:

1) Any type of human trial e.g. RCT, retrospective, case study;
2) Animal studies;
3) The use of a herb or combination of herbs as the main intervention and specifically investigating its effects on reducing the primary outcome i.e. CIPN; and
4) The journal article or abstract must be written in English (a number of Asian journal articles had the abstract in English but the journal article in their language i.e. Japanese or Chinese.)

Databases

The following databases were used to retrieve journal articles: PubMed, the Cochrane Library, Science Direct, Scopus, EMBASE, MEDLINE, CNKI, CINAHL and Google Scholar.

Search Terms

Electronic databases were searched using the following search terms, "chemotherapy-induced peripheral neuropathy" OR "Cisplatin" OR "Taxanes" OR "Paclitaxel" OR "Docetaxel" OR "Oxaliplatin" OR "Carboplatin" OR "Platinum compounds" OR "Proteasome inhibitors" AND "peripheral neuropathy" OR "CIPN" AND "herb" OR "cannabis" OR "chamomile" OR "ginkgo" OR "sweet bee venom" OR "turmeric" OR "sage" OR "hypericum" OR "herbal medicines" OR "Chinese herbal medicines" OR "ayurvedic herbal medicines".

The overall body of evidence evaluated within this review was primarily assessed using a separate tool, the Australian National Health and Medical Research Council's (NHMRC) body of clinical evidence assessment matrix. This is an assessment tool that assigns a level/grade (Level I: strongest evidence to level IV: weakest evidence) based on the strength of the published study [216]. Supportive evidence was obtained from animal studies.

Risk bias assessment

The risk bias of both animal and human studies was assessed using the Cochrane Risk of Bias Assessment tool (http://handbook.cochrane.org/, part 2, Chapter 8). All studies were reviewed by two researchers.

Data Synthesis

All human clinical trial data (excluding case studies) was analysed using RevMan version 5.2.7 to quantify and compare the efficacy outcomes of the intervention versus control.

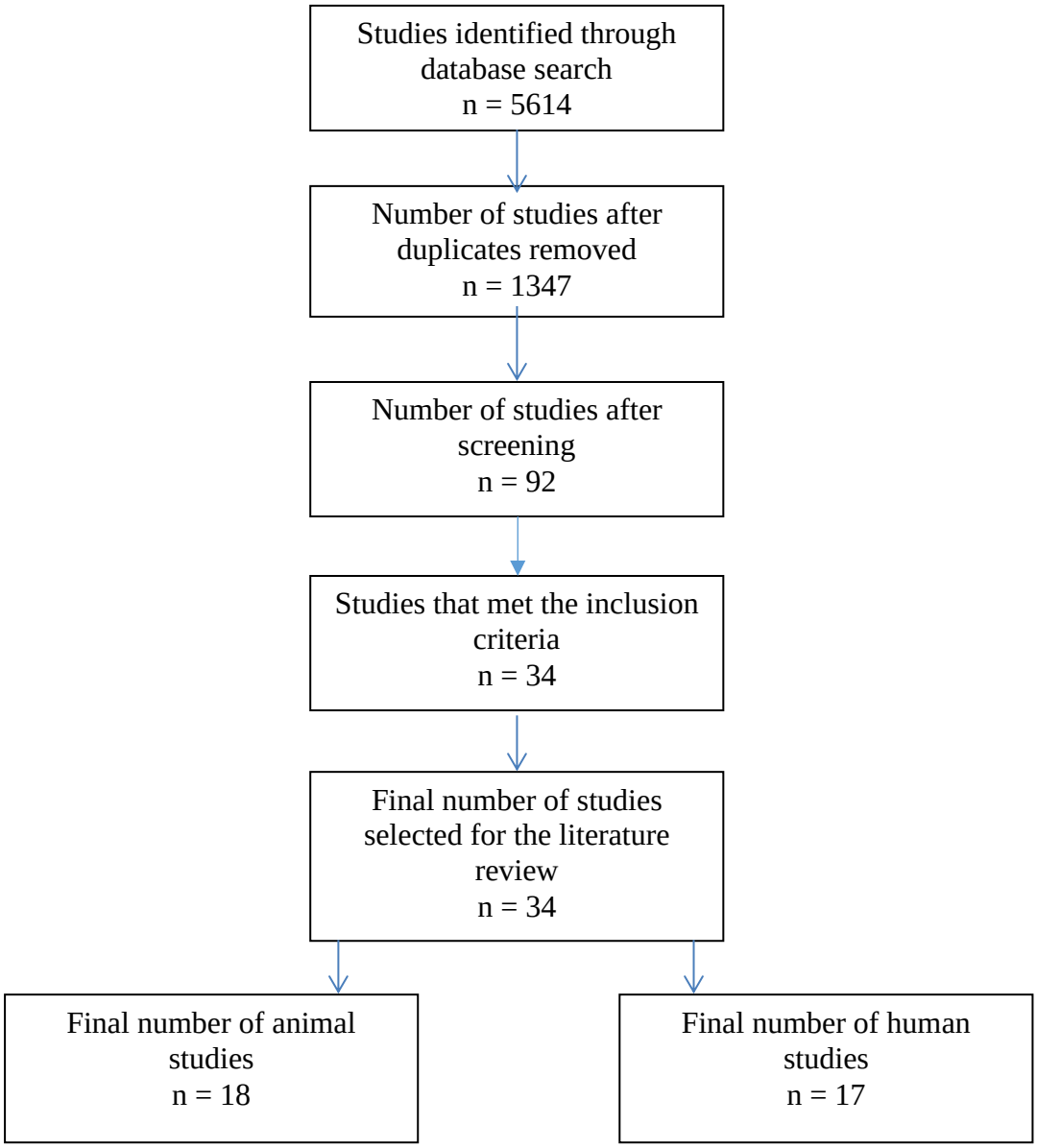


Figure 2-3: Flow chart of study selection for herbal medicines.

2.3.2.1 Single Herbal Medicines on Human Studies and CIPN

2.3.2.1.1 Ginkgo biloba (EGb 761)

One retrospective study has been conducted on human beings administered oxaliplatin [292]. The retrospective analysis was conducted on 17 colorectal patients who were being treated with either FOLFOX or CAPEOX chemotherapy regimens for either adjuvant or metastatic treatment. Gingko biloba 120mg b.i.d was given orally either after cycle 1 or 2 of treatment. They concluded that Gingko biloba appeared to decrease the intensity and duration of the acute dysesthesias caused by oxaliplatin

and have initiated a phase II RCT to confirm results. To date, no results have been published from this phase II trial which apparently started in 2004 [292].

2.3.2.1.2 Sweet Bee Venom (Pharmacopuncture)

Two journal articles outlining case studies using sweet bee venom for CIPN have been found with the agent used being identified as melittin, an extracted active ingredient from the bee venom that has the allergens removed such as phospholipase A_2 (PLA_2), hyaluronidase and histamine. Melittin is a low molecular weight peptide and is reported to have analgesic, anti-inflammatory and anti-cancer effects [240, 242, 246].

The first article outlined five case reports receiving a one week course of treatment with sweet bee venom pharmacopuncture. No side effects were experienced and results indicated clinical improvement of CIPN [294]. Another case series on 11 patients were treated for three weeks with six sweet bee venom pharmacopuncture treatments. Results showed a reduction in the WHO CIPN grade and PNQ score indicating a reduction in CIPN [295]. Further studies are required to confirm their findings.

2.3.2.2 Combination Herbal Medicine on Human Studies and CIPN

Many of the combination herbal formulas used in this literature review have been obtained from Chinese journals which only have an abstract printed in English. Obtaining the complete information pertaining to the clinical trial or observation has been limited due to inability to read Chinese. Therefore, conclusions on the results of some of the trials have been obtained from the authors without being able to quantify their conclusions. This has also limited access to statistical analysis, measurement tools, analysis and how they measured their primary outcomes. This is a major limitation and makes all results recorded from abstracts biased due to the author's perspective with no ability to clarify their conclusions.

2.3.2.2.1 Bu Yang Huan Wu (Chinese)

This formula consists of *Astragulus membranaceus radix, Angelica sinesis radix, Prunus persicae semen, Paeoniae rubra radix, Ligustici chuanxiong rhizome, Lumbricus terrestris, Spatholobi caulis, Curcuma radix, Chaenomeles lagenaria fructus* and *Achyranthes bidentatae radix.* In traditional Chinese medicine (TCM) it is said to tonify the yang and restore the five-tenths decoction [320]. This decoction was used in an RCT of 84 participants (intervention n=44, control n=40) for the treatment of CIPN after oxaliplatin administration. Results indicated that Bu Yang Huan Wu reduced the development of CIPN in the treatment group. Due to the main journal article being written in Chinese

rather than English, statistical analysis and measurement tools to ascertain this positive result are unable to be quantified. The information obtained was from an abstract written in English [314].

2.3.2.2.2 Modified Bu Yang Huan Wu (Chinese)

The modified formula used was *Bu Yang Huan Wu* plus *Liuwei Di Huang* which contains the herbs: *Astragulus membranaceus radix, Ligustrum lucidum fructus, Paeoniae rubra radix, Lumbricus terrestris, Prunus persicae semen, Rehmanniae viride radix, Corni officinalis fructus, Dioscorea opposite radix, Alismatis rhizome, Poria alba, Spatholobi caulis, Scolopendra, Mori fructus, Glycyrrhizae radix, Dipsaci fructus, Lycii fructus, Coicis semen, Atractylodis Rhizome, Phellodendri cortex, Scorpio, Moi ramulus* and *Cyathula officinalis.*

A RCT was conducted using this decoction on 32 patients with existing CIPN from various chemotherapy agents. The treatment was compared to 32 patients who were treated daily with 2500 µg of vitamin B1 orally in addition to intramuscular injections of 100 mg of vitamin B1. The Chinese herbal formula was found to be significantly more effective compared to the vitamin B1 treatment ($P<0.05$) [301]. Information pertaining to this observation was from an abstract as the main journal article was published in Chinese. Therefore measurement techniques and the magnitude of the differences seen in the results are unable to be expanded on.

2.3.2.2.3 Modified Chai Hu Long Gu Mu Li Wan (Chinese)

This modified Chinese oral combination of herbs consists of *Psuedostellaria heterophylla, Pinelliae rhizome, Glycyrrhizae radix, Scutellaria baicalensis radix, Bupleuri radix, Fossiliaossis mastoid, Ostreae concha, Rubia cordifolia radix, Scutellariae barbatae herba* and *Fritillariae thunbergia bulbi.* This combination was used in a RCT of 48 patients with ovarian cancer undergoing paclitaxel administration. They were divided into a control group of paclitaxel only or a treatment group of paclitaxel plus a combination of the oral Chinese herbal decoction and an external washing of the feet with Chinese herbs (*Astragulus membranaceus radix, Angelica sinensis radix, Paeoniae radix, lumbricus terrestris, Ligustici chuanxiong Rhizome, Prunus persicae semen* and *Carthami flos*.) Results indicated that the incidence rate of CIPN in the treatment group was nearly half compared to the paclitaxel only group. Therefore according to the authors this Chinese formula may help in preventing paclitaxel CIPN [311]. Further information pertaining to the clinical trial is unavailable due to the main journal article being written in Chinese.

2.3.2.2.4 Geramii Herba plus Aconiti Radix

A RCT was conducted on 58 patients experiencing CIPN from oxalipatin, taxol or capecitabine. 30 patients were assigned to the treatment group and 28 to the control group. After one week of

treatment, the external application of the two herbs was found to reduce pain, paraesthesia and swelling. The authors concluded that *Geramii herba* plus *Aconiti radix* may relieve neuropathy and improve quality of life. No species of these two herbs were mentioned in the abstract nor any statistical significance [313]. The main journal article was written in Chinese hence, further information is difficult to ascertain.

2.3.2.2.5 Goshajinkigan (Japan), Niu Che Sen Qi Wan (Chinese), Pilula renales plantaginis et achyranthis (Lat.)

Six human trials have been conducted on Goshajinkigan [GJG]. Firstly, GJG was invested in a non-controlled trail on 14 patients receiving oxaliplatin. GJG was administered every day after the first oxaliplatin infusion and results indicated that it seemed to prevent acute oxaliplatin-IPN [312]. Two retrospective studies were conducted on FOLFOX (oxaliplatin) and GJG [308, 310]. The first trial was conducted on 45 patients with 22 patients receiving GJG with their FOLFOX regime compared to 23 who did not receive GJG.

They found that the incidence of grade 3 CIPN in the GJG group was significantly lower than in the control group ($P<0.01$, log-rank test [310].

The second study investigated 90 patients undergoing FOLFOX for metastatic colorectal cancer. There were four groups: 1) FOLFOX plus GJG; 2) FOLFOX plus GJG plus Ca^{2+}/Mg^{2+}; 3) FOLFOX plus Ca^{2+}/Mg^{2+}; 4) FOLFOX only. Results included the incidence rate for each group which were 91% for FOLFOX only, 100% in FOLFOX plus Ca^{2+}/Mg^{2+}, 79% in FOLFOX plus GJG plus Ca^{2+}/Mg^{2+} and 50% in FOLFOX plus GJG. The authors concluded that GJG reduced the neurotoxicity of oxaliplatin without affecting the response rate [308].

In another retrospective trial, GJG was investigated for paclitaxel-IPN in 82 breast and gynaecological cancer patients. The investigators concluded that GJG was possibly effective for the treatment and the prevention of paclitaxel-IPN was seemed more effective if administered at the beginning of chemotherapy treatment [318]. Another human trial on GJG involved a prospective RCT on paclitaxel/carboplatin treatment for 29 ovarian or endometrial cancer patients. They were divided into two groups; 1) 14 patients received vitamin B12; 2) 15 patients received vitamin B12 and GJG and were given treatment for six weeks. Grade 3 CIPN was observed in 2/14 patients receiving vitamin B12 compared to 0/15 receiving vitamin B12/GJG. It was concluded that GJG inhibits the progression of CIPN but further trials are warranted [307].

A recent phase II multi-centre, randomised, double-blind, placebo-controlled trial was conducted and published on GJG and oxaliplatin-induced PN [321]. In this trial, patients undergoing FOLFOX for colorectal cancer was randomised to either receive oral GJG (7.5 g) or matching placebo daily. The

severity of CIPN was assessed, using the common toxicity criteria for adverse events, every two weeks until the 8th cycle and then every 4 weeks thereafter. The primary endpoint was the incidence of grade 2 CIPN or greater before the 8th cycle. The incidence of grade 2 or greater CIPN was 39% in the GJG arm and 51% in the placebo arm. The authors concluded that GJG shows promise in delaying the onset of grade 2 or greater CIPN without impairing FOLFOX efficacy [321].

2.3.2.2.6 Keishikajutsubuto (Japanese), Gui Zhi Jia Shu Fu Tang (Chinese), Decoctum ramulorum cassia cum atractylodis macrocephae et aconite (Lat)

This oriental formula contains *Cinamomi cortex, Aconiti lateralis praeparata tuber, Zingiberis rhizome, Jujubae fructus, Glycyrrhizae radix* and *Atracylodis macrocephalae rhizome*. A non-controlled trial was conducted investigating this herbal formula on 11 patients with metastatic colorectal cancer undergoing FOLFOX administration. A reduction of CIPN was observed in 5 cases (45.5%) after cessation of chemotherapy [317].

2.3.2.2.7 Ogikeishigomotsuto (Japanese), Huang Qi Wu Wu Tang (Chinese), Decotum quinque medicamentorum cum astragalo (Lat.), Astragulus and Cinnamon Five herb combination (English)

This oriental herbal combination contains *Astragalus membranaceus radix, cinnamomi cortex, Paeonia alba radix, Jujubae fructus* and *Zingiberis Rhizome*. A single case study was published using this formula for neuropathic pain induced by oxaliplatin. It showed a positive effect in reducing the pain and the patient was allowed to continue chemotherapy treatment with oxaliplatin [315].

2.3.2.2.8 Shakuyakukanzoto (Japanese), Shao Yao Gan Cao Tang (Chinese), Formula glycyrrhizae et paeonia (Lat), Peony and Licorice Decoction (English)

Two studies human studies have been conducted on this formula, a retrospective case analysis [302] and a retrospective clinical trial comparison [306]. The retrospective case analysis investigated 23 patients with paclitaxel-IPN and observed that this combination had a positive effect on the neuropathic pain experienced by these ovarian cancer patients [302]. Lastly the retrospective clinical trial compared Shakuyakukanzoto (peony and licorice) to Goshajinkigan (GJG). This was a preventive study and investigated 20 metastatic colorectal cancer patients administered Shakuyakukanzoto in conjunction with FOLFOX compared to 24 patients administered GJG. The Shakuyakukanzoto group was found to prevent 65% CIPN compared to 50% in the GJG group. It was concluded that both formulas may prevent oxaliplatin-IPN [306].

Table 2-4: Human Clinical Studies with Herbal Medicines for the Treatment and or Prevention of CIPN

Medicinal Herb	Study Type	No Pts[9]	T[10]	C[11]	NHMRC Rating	Chemotherapy Agent	Results
Single Medicinal Herbs							
Ginkgo biloba	RS [292]	17	N/A[12]	N/A	Level IIIb	Oxaliplatin	Possible neuroprotection
Sweet bee venom (pharmacopuncture)	Case series [294]	5	N/A	N/A	Level IV	Taxol, carbo/taxol[13]	Injecting into the acupoint decreased pain and neuropathy (treatment)
	Case series [295]	11	N/A	N/A	Level IV	Taxol, carbo/taxol	Injecting into the acupoint decreased pain and neuropathy (treatment)
Combination Herbal Studies							
Bu Yang Huan Wu (Chinese)	RCT [314]	84	44	40	Level II	Oxaliplatin	Reduced development of CIPN
Modified Bu yang *Huan Wu* (Chinese)	RCT [301]	64	32	32	Level IIIa	Different chemotherapies	Reduced development of CIPN
Modified *Chai Hu Long Gu Mu Li Wan* (Chinese)	RCT [311]	48	N/A	N/A	Level II	Paclitaxel	Possible neuroprotection
Geramii herba plus *Aconiti radix*	RCT prospective [313]	58	30	28	Level II	Oxaliplatin	Reduced neuropathic pain

[9] Pts: Participants
[10] T: Treatment group
[11] C: Control group or placebo
[12] N/A: Not applicable
[13] Carbo/Taxol: Carboplatin and Paclitaxol chemotherapy combination

Medicinal Herb	Study Type	No Pts	T	C	NHMRC Rating	Chemotherapy Agent	Results
Goshajinkigan GJG (Japan)	NRCT [312]	14	N/A	N/A	Level IIIb	Oxaliplatin	Reduced acute neurotoxicity
	RS [310]	45	22	23	Level IIIa	Folfox	Possible neuroprotection
	RS [308]	90	11	44	Level IIIa	Folfox	Possible neuroprotection, no change of anticancer activity
			CaMg[14] = 21	CaMg = 14			
	RS [318]	82	N/A	N/A	Level IIIb	Paclitaxel	Possible neuroprotection, better when administered early
	RCT prospective study [307]	29	Vit B12 + I = 15	Vit B12 = 14	Level IIIa	Carbo/Taxol	Less severe neurotoxicity, better in combined group
	DBRCT [321]	89	44	45	Level II	Folfox	Acceptable safety margin and indicates a delay in onset of grade 2 or higher CIPN without impairing FOLFOX efficacy.
Keishikajutsubuto (Japanese)	Uncontrolled study [317]	11	N/A	N/A	Level IIIb	Folfox	76.6% mean improvement
Ogikeishigomotsuto (Japanese)	Case report [315]	1	N/A	N/A	Level IV	Oxaliplatin	Reduced neuropathic pain
Shakuyakukanzoto (Japanese) Peony and Licorice Decoction (English)	Retrospective case analysis [302]	23	N/A	N/A	Level IV	Paclitaxel	Reduced neuropathic pain
	RS comparison [306]	44	20 (SKK)	24 (GJG)	Level IIIa	Folfox	50% response in Shakuyu-kanzoto and 65% in Goshajinkigan on prevention of neurotoxicity.

DBRCT: Double Blind Randomised Controlled Trial; SBRCT: Single Blinded Randomised Controlled Trial; RCT: Randomised Controlled Trial; NRCT: Non Randomised Clinical Trial; CS: Case Study; TNS: Total Neuropathy Score; RS: Retrospective Study

[14] CaMg: Calcium and magnesium infusion

2.3.2.3 Discussion on Outcomes on Herbal Medicine and CIPN

Current improvements in the detection and treatment of cancer strongly correlates with increases survival rates of those patients diagnosed with a malignant disease [322]. With an increased survival rate, long-term side effects from chemotherapy and other medical treatments has raised significant awareness as it can affect quality of life and clinical outcomes [2, 3, 229]. CIPN is an important side effect that can affect quality of life and can be a permanent consequence of treatment [2-4]. Currently, there are no standard recommended treatments or prophylactic options for CIPN that employ pharmacologicals, nutraceuticals or herbal medicines. Agents that have shown promise such as duloxetine [229, 230], vitamin E [233, 234, 236, 323], omega 3 fatty acids [237] and Asian herbal medicines in particular Goshajinkigan [300, 303, 304, 307-310, 312, 316, 318] require further research, however, in order to assess the strength of efficacy.

Traditional scientific research is based on a single agent or active compound; herbal medicines though can be comprised of numerous active compounds with synergistic efficacy. For example in the use of Asian herbal medicines, a combination of herbs can be employed [309]. This combination may give a multi-targeted approach that complicates the identification and elucidation of the active compound and the mechanism of action. Nevertheless, warranted research on single herbal extracts and compounds through validated clinical studies can still provide useful data.

Investigations carried out with herbal medicines for CIPN found no neuroprotection or treatment when a single compound or herb was studied. Animal models provide basic research for further studies but do not guarantee that the herb or compound will be efficacious in human clinical trials. Herbal medicines such as *Ginkgo biloba* [292] and *curcumin* [238] warrant further research as they have reported a positive clinical likelihood from animal studies.

An important and common mechanism of action within the identified medicinal herbs trialled for CIPN is their anti-inflammatory activity [237-246]. The anti-inflammatory activity of the herbs may be a plausible mechanism of action that assists with the protection and treatment of CIPN. Medical treatment of peripheral neuropathy has involved non-steroidal anti-inflammatory drugs for the inflammation and pain associated with this condition [1]. This potential mechanism of action warrants further research, which may attribute to other herbal remedies, nutrients or pharmaceuticals being trialled for CIPN.

The Asian herbal combination remedies investigated for CIPN were difficult to analysis as a number of the journal articles were written in their Asian language rather than English. Therefore, the information obtained for twelve out of the nineteen herbal combinations journal articles was extracted from abstracts. From these Asian herbal combinations, Goshajinkigan (GJG) is the herbal medicine

that has been trialled extensively through animal and human clinical trials [300, 303, 304, 307-310, 312, 316, 318]. Of the clinical trials, three were retrospective studies using controls for Folfox and paclitaxel administration [308, 310, 318], one RCT [307] comparing vitamin B12 administration with the herbal combination and a recent phase II multi-centre, randomised, double-blind, placebo-controlled trial conducted as an adjuvant treatment with FOLFOX [309]. All studies concluded that this herbal combination may provide neuroprotection however, as this is a Japanese combination and has been trialled in Japan, it may be difficult to transfer into other countries depending on their National regulatory body. Another limitation with GJG is that not all details of its mechanism of action have been clearly identified and for certain herbs, their effects are unknown [276].

All other trials with the Asian combination herbs were animal models, case studies, retrospective studies and limited RCT's so the quality of evidence is very low. Without further randomised clinical trials that are written in English and well established, they are not recommended for use.

Two herbal treatments have been investigated for peripheral neuropathy that may be considered for use in CIPN. These include St John's wort and capsaicin cream. St John's Wort (*Hypericum perforaturn)* was selected to trial for PN as tricyclic antidepressant medication is used medically for its treatment. In a crossover DBRCT, 54 patients with or without diabetes were investigated for treatment with St John's Wort (SJW) for diabetic PN. Participants were randomly assigned to either SJW (900mcg hypericin/tablet) or placebo for five weeks. They were then crossed over to the other treatment for an additional five weeks. No statistical significant was found in pain indexes however a trend was noted for an improved overall pain score [324].

Capsaicin has been investigated as a topical application for diabetic [325-329] and HIV peripheral neuropathy [330-332]. The majority of studies have been conducted on diabetes PN with most resulting in statistical significance [325-329]. The results indicate that capsaicin cream may provide relief for chronic, intractable pain and reduce dependence on opioids however the main side effect of burning at the site of application may be of concern [329]. It is recommended that it is not used as a monotherapy but in combination with oral medication to assist this condition.

Herbal treatments may offer a different aspect to assisting CIPN however the main problems with research involving herbal extracts or medicinal herbs is the understanding of the mechanism of actions and the fact that the herbs contain a number of active compounds. Moreover, Asian herbal therapies contain a combination of multiple herbs, which adds to the complexity of trying to analysis the results and what was beneficial. Isolation of one extract which is common in scientific research is unable to be conducted with herbal medicine research as the combination of compounds or combination of herbs such as Asian herbal therapies are seen to work in synergy.

2.4 CONCLUSION

Currently, no pharmaceutical, neutraceutical or complementary agent has been found beneficial in preventing or treating CIPN. Certain pharmaceutical agents have shown potential for the treatment of CIPN pain as with duloxetine (Cymbalta) and other anti-depressants such as venlafaxine (Effexor) although Effexor was case study based. Pregabalin is the pharmaceutical drug of choice for clinicians at present based on an open label trial. This has been chosen due to lower dose and less side effects compared to gabapentin.

For nutraceuticals, vitamin E shows potential for prevention of cisplatin-induced ototoxicity, intravenous glutathione for oxaliplatin administration, vitamin B6 for both oxaliplatin and cisplatin and omega three fatty acids for paclitaxel administration. Acetyl-L-carnitine may provide some relief as a treatment option for CIPN but should not be used as a preventative agent. Acupuncture may be of benefit for some patients and Goshajinkigan may be of benefit for protection of oxaliplatin-IPN for patients in Japan.

Another option for clinicians includes topical application. A combination of baclofen 10 mg, amitriptyline HCL 40 mg (3%) and ketamine 20 mg (1.5%) in a base of pluronic lecithin organogel and was found to be beneficial for sensory neuropathy over placebo ($p=0.053$) and decreased motor neuropathy symptoms ($p=0.021$) [333]. However, a phase III RCT trial on 462 patients with CIPN found no difference in six weeks of treatment with 2% ketamine and 4% amitriptyline cream ($p=0.363$) [334]. Recent case studies on menthol topically (1%) have found it beneficial for bortezomib [335] and carboplatin CIPN [336]. Moreover, as mentioned, high dose topical capsaicin cream may show benefit although no studies for CIPN have been conducted.

Clinicians and researchers also have no gold standard diagnosis technique for CIPN. The true incidence and severity of CIPN in clinical trials has been questioned and could be masked by non-standardised reporting [337]. New techniques in diagnosing and reporting CIPN require further investigation. Chapter three examines two new techniques that maybe beneficial for diagnosing and reporting CIPN development.

3 CHAPTER 3 – OTHER POSSIBLE TECHNIQUES TO MEASURE CIPN

The oncologists, haematologist oncologists or medical practitioners, using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) scale [338], commonly diagnose CIPN. As discussed in chapter 1, page 36, this assessment tool is subjective depending on the medical practitioner's perception. This chapter explores two possible techniques that could be incorporated into a CIPN diagnosis or used as an assessment tool. These include a corneal confocal microscopy (CCM) and two analytical techniques, nuclear magnetic resonance (NMR) and mass spectrometry (MS). A collaboration was formed between the researchers of the B vitamin and CIPN trial and ophthalmologist researchers from QUT. It was decided that a group of participants from the B vitamin trial be tested to find any correlation between the neurologists CIPN assessment and the ophthalmologists results.

3.1 CORNEAL CONFOCAL MICROSCOPY

The corneal confocal microscopy (CCM) is a technique used by ophthalmologist's that can be used to estimate the overall density of the corneal innervation [339]. This is achieved by assessing the nerve fibre length (NFL) and nerve fibre density (NFD) in the Bowman's layer. The tortuosity coefficient is a dimensionless measurement that reflects the nerve irregularity or curving and aims to assess the corneal nerve structure [339]. Basically the CCM is a high magnification microscope that can be used to look at the nerves in the front of the eye. This technique has been validated as a neuropathic diagnostic tool particularly for diabetic patients [340] and has been selected to trial for CIPN assessment.

3.1.1 Ophthalmic Markers for the Possible Diagnosis of CIPN

A collaboration was formed with the Queensland University of Technology involving Dr Nicola Pritchard, Dr Katie Edwards and Professor Nathan Efron who have been working on the LANDMark study for diabetes [341]. It was proposed that the CCM and the ocular coherence tomography (OCT) which is used to assess the nerves and tissues at the back of the eye [342] might be able to detect small nerve damage from chemotherapy similar to what they are finding for diabetic patients. As the nerve conduction studies that the neurologist uses assess large nerves, the only assessment of small nerve damage is the pin sensibility test which is not quantitative as it's based on what the patient can feel, therefore subjective. Validating a quantitative method of assessing small nerve damage such as the CCM or OCT would be of assistance for medical practitioners treating CIPN.

3.1.2 Rationale

CIPN [3] may reveal corresponding ocular changes in the corneal [340, 341] and nerve layers of the retina [342] similar to the nerve damage seen in diabetes. Conducing CCM and OCT assessments may allow researchers to investigate the relationship between the nerves of the eye and the peripheral nerves of the body in patients who undergo chemotherapy with neurotoxic agents. The measures of nerves made by these techniques may be related to the damage of nerves in the peripheral limbs therefore quantifying small nerve damage associated with CIPN.

In collaboration with the LANDMark study and the B vitamin and CIPN trial, it was aimed to investigate the following:

- Changes in corneal (front of eye) nerve counts before and after chemotherapy
- Changes in retinal (back of eye) nerve layer thickness before and after chemotherapy

The results of this pilot study were aimed at developing a better understanding of small fibre peripheral nerves in the arms and legs in patients suffering from nerve damage, and hoped to determine the extent to which these changes are associated with the clinical signs and symptoms of the condition. The significance of this pilot study was that it should reveal the potential for these eye tests to serve as sensitive, rapid, repeatable, 'patient-friendly' eye tests for the detection, diagnosis and monitoring of the progression of nerve damage. Understanding these aspects of the nerves may provide healthcare professionals with a quick, simple, cost-effective and repeatable means to identify patients at risk, anticipate and monitor deterioration, and assess new treatments for nerve damage.

3.1.3 Hypotheses

1. Corneal and retinal nerve parameters are reduced in individual who have undergone chemotherapy.
2. The amount of nerve tissue reduces as severity of impairment increases.
3. Corneal and retinal nerve parameters are associated with established measures of neuropathy.

3.1.4 Study Population

The participants in this pilot study were recruited from patients attending a chemotherapy-induced neuropathy study at the Princess Alexandra Hospital in Brisbane, Australia. It was estimated that 20 individuals who were undergoing chemotherapy would participate in this extended research, however, a sample of only three presented to participate for the study. Ophthalmologist researchers evaluating the number of participants required to meet statistical relevance for the B vitamin study (90 plus 50% attrition rate) statically determined the study sample. As this is a pilot trial to see if there is relevance in using this technique to analysis small nerve cell damage, a study size of 20% of the B

vitamin group was determined which is equivalent to 18-27 people including the attrition calculation. Therefore, an estimation of 20 patients were determined to be recruited from the B vitamin trial.

3.1.5 Study Design

This was a cross-sectional, observational, unmasked study. The ophthalmic researcher was aware of the status of the participant. Relevant medical and ocular history and demographic measures were ascertained and participants were asked to undergo corneal confocal microscopy (CCM) and, in some instances, ocular coherence tomography (OCT). Patients with ocular disease, for example, glaucoma or a peripheral neuropathy due to another disease were not eligible. The participant was assessed prior to commencing chemotherapy (or as close to this date as possible), and 6 and 12 weeks post-treatment.

The primary objective was to establish pilot data exploring the hypothesis that morphology of corneal and retinal nerves are altered in patients with CIPN. An estimate of association between the structural measures and standard tests of neuropathy was explored. The two primary structural outcome markers are morphometric measures obtained from:

1. Corneal nerve fibre length (CNFL) – length of nerves/mm^2 of corneal tissue from CCM images of the corneal sub-basal nerve plexus.
2. Retinal nerve fibre layer, ganglion cell complex and overall retinal thickness (μ) using OCT of the retinal layers.

The measures of CIPN include nerve conduction studies (NCS), neuropathy disability score and neuropathy symptom score. The secondary objectives of the study were to determine the effect size to establish if progression of CIPN could be monitored, the sensitivity, specificity of CCM and OCT in CIPN and identify risk factors of CIPN associated with corneal and retinal nerve damage, observed using CCM and OCT.

3.1.6 Methods

All tests were carried out according to the LANDMark standard operating procedures manual.

3.1.6.1 Corneal Confocal Microscopy (CCM)

Participants were first examined with a slit lamp biomicroscope to ensure the anterior eye was healthy. After anesthetising the cornea, images of the sub-basal nerve plexus was captured from one eye of each participant using a commercially available laser scanning CCM, the Heidelberg HRT III with Rostock Cornea Module. Images were captured using the section capture mode of the CCM, while fixates a stationary target with the contralateral eye. Corneal nerve morphology was determined

using automated software: the amount of nerve tissue (corneal nerve fibre length mm/mm2) and corneal nerve branch density (#/mm2) were assessed in the 400 x 400 um image.

3.1.6.2 Optical Coherence Tomography (OCT)

Optical coherence tomography (OCT) is a non-invasive and reliable technique allowing quantitative analysis of retinal morphology and is generally used to qualitatively assess retinal pathology or to measure retinal layer thicknesses. High axial and transverse resolution and rapid image capture capacity enable accurate identification and thickness measurement of individual retinal layers. The RTVue 100 (Optovue Inc, USA) is a Fourier domain OCT, which was used to collect data for all participants in this study. It has an axial resolution of 5 micron and a transverse resolution of 15 micron. Separate macula, optic nerve head and peri papillary scans were obtained, providing quantitative data for RNFL, ganglion cell layer and full retinal thickness at multiple sites, as well as (optic nerve head) neuro-retinal rim, and cup area and volume data. .

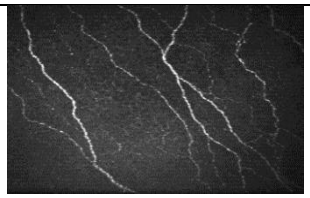
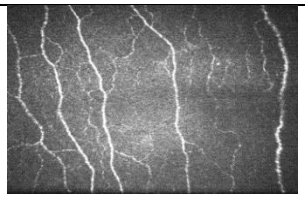
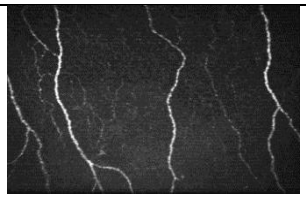
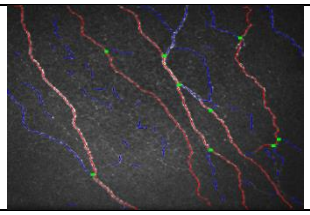
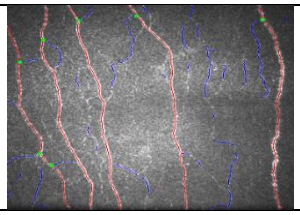
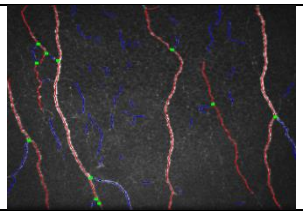
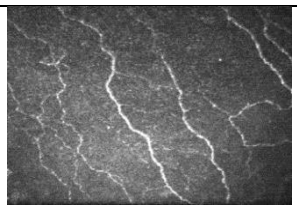
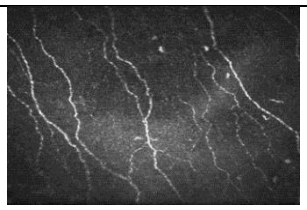
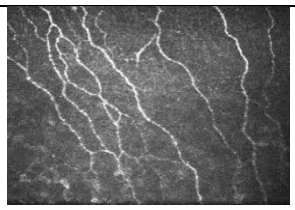
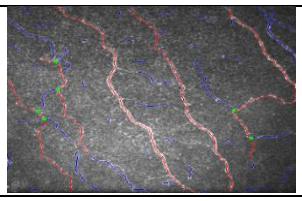
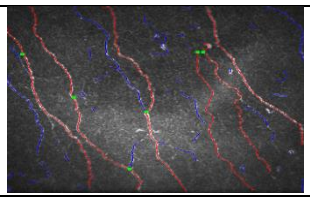
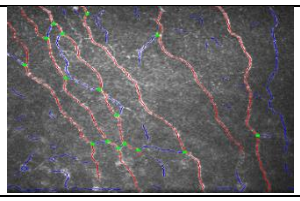
3.1.6.3 Neuropathy Measures

A qualified electrophysiology technician at IHBI measured the peroneal and sural nerve conduction velocity, amplitude and latencies. Neuropathy disability score involves sensation to heat, cold, vibration and reflex testing. These tests took approximately 15 minutes to perform.

3.1.7 Results

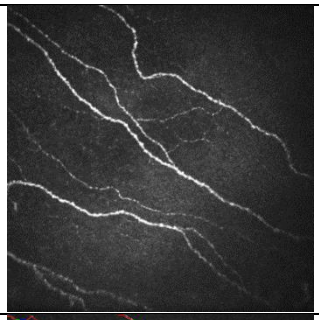
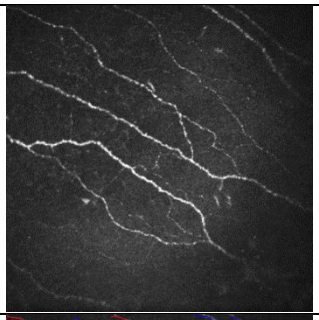
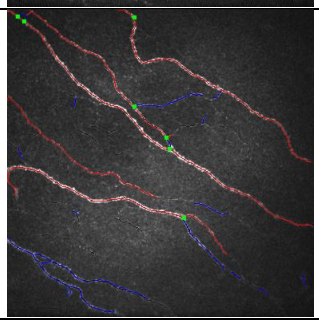
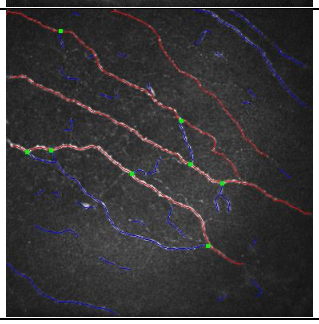
Participant sample results are presented in the form of case summaries in Table 3-1. Neuropathy symptoms were reported after chemotherapy had started in two of the three participants; neuropathy measures from the CCM did not support the symptoms the patients were experiencing. Corneal nerve fibre length did not vary between the three visits (paired t-test p=0.61 and 0.69 for pre-chemo vs. 6-week post and pre-chemo vs. 12 week post, respectively). The only clinically relevant change noted was in patient PA070LxW where a change of 5 mm.mm^2 and 3.3 mm/mm^2 was seen from baseline to the 6 weeks post-chemotherapy examination. The QUT researcher's unpublished research from the LANDMARK study assessing diabetes neuropathy established that less than 14 mm/mm^2 is indicative of neuropathy in diabetic patients [343]. The CNFL is calculated by the sum of the blue and red lines from baseline to the next measurement point.

Table 3-1: Case Summaries using CCM on Three Participants Pre- and Post-Chemotherapy

ID: PA034 PTE	**Pre-chemo**	**6 weeks post-chemo**	**12 weeks post-chemo**
69 year old male	Consent dated 27/6/12		
Sample raw CCM image[a]			
Sample processed CCM image			
Corneal nerve fibre length mm/mm^{2} [b]	20.0 ± 0.4	16.3 ± 4.8	18.1 ± 2.9
Neuropathy measures	Nil symptoms reported Sural 53.4 m/s (normal) NDS[d] 1 (normal)	Nil symptoms reported NDS[d] 1 (normal)	Nil symptoms reported Sural nerve CV[f] 56.9 m/s (normal)
ID: PA067 DxH	**Pre-chemo**	**6 weeks post-chemo**	**12 weeks post-chemo**
54 year old female	Consent dated 18/3/13		
Sample raw CCM image[a]			
Sample processed CCM image			
Corneal nerve fibre length mm/mm^{2} [b]	18.0 ± 1.4	21.4 ± 1.2	19.0 ± 3.3
Neuropathy	Nil symptoms reported	Symptoms reported	Symptoms reported DNSS[e] 4 (abnormal) Sural nerve CV[f] 45.3m/s (normal)

Legend	
	White lines represent nerves
	Blue line represents nerve branches
	Red line represents nerve trunks
	Green dots represent nerve bifurcations

Table 3-1 continued

ID PA070 LxW	Pre-chemo	6 weeks post-chemo	12 weeks post-chemo
58 year old female	Consent dated 23/4/13		
Sample raw CCM image[a]			No visit
Sample processed CCM image			No visit
Corneal nerve fibre length mm/mm^2 [b]	17.7 ± 2.3	14.4 ± 3.2	No visit
Neuropathy	Nil symptoms reported	Symptoms reported Sural nerve CV[f] 40.9 m/s (abnormal)	No visit

b average of 8 central images
d NDS = neuropathy disability score, 0 (nil) to 10 (severe)
e DNSS = diabetic neuropathy symptom score, 0 (nil) – 4 (severe)
f CV = conduction velocity

ID PA070 LxW pre-chemotherapy also had her OCT conducted showing her ocular fundus which can be seen in Figure 3-1. The tomography report is reported in Figure 3-2.

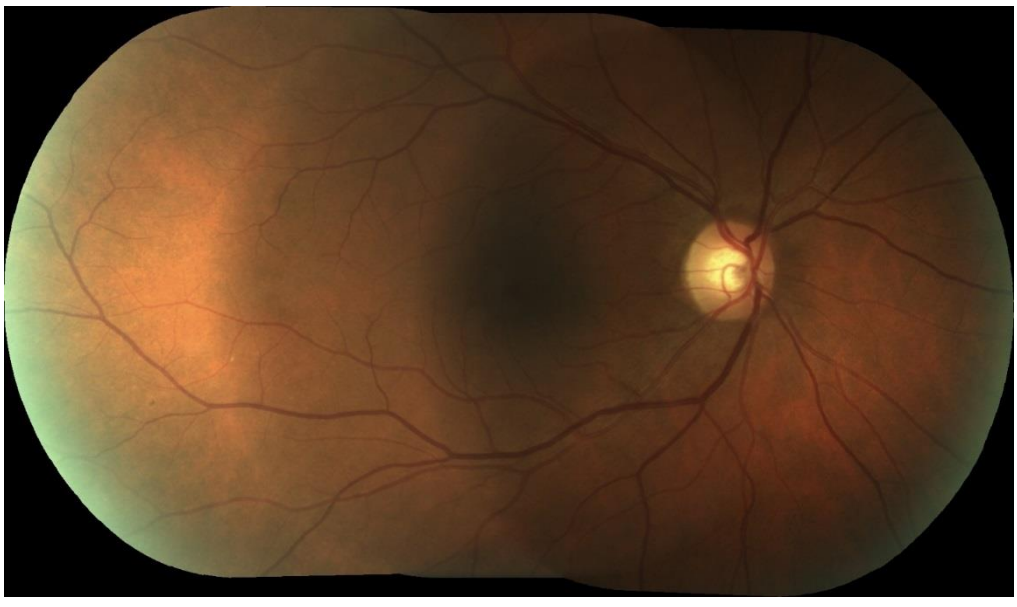


Figure 3-1: PA079 LxW Pre-Chemotherapy Ocular Fundus Picture using OCT.

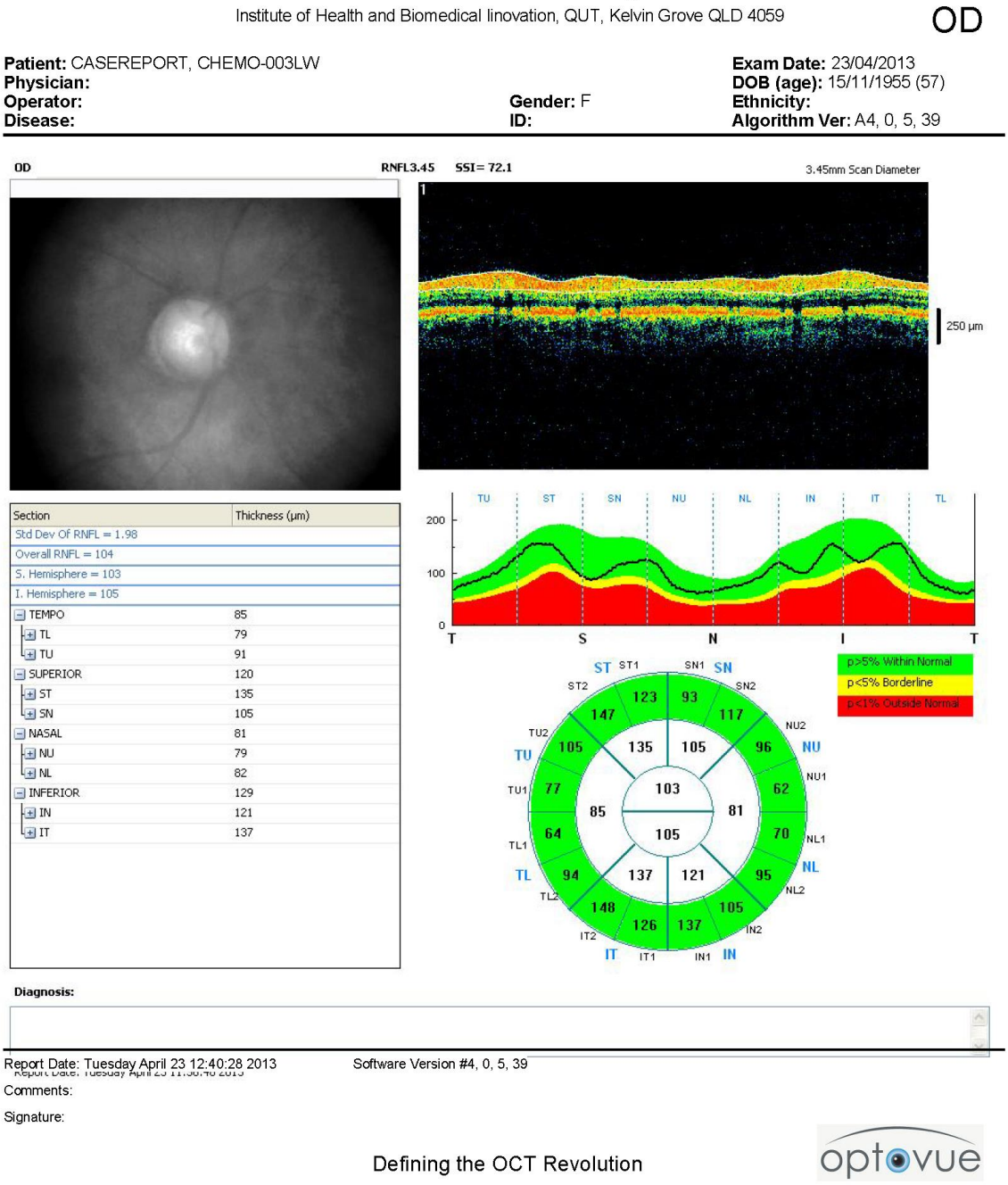


Figure 3-2: Ocular Coherence Tomography Report for PA070 LxW

3.1.8 Conclusion on CCM and OCT for CIPN Diagnosis

Because only three participants from the B vitamin study participated in this collaboration, few conclusions can be drawn from this pilot work. The main obstacle found for patients not participating in this collaboration was the fact that they had to physically go to Kelvin Grove, a suburb on the other side of Brisbane from The Princess Alexandra Hospital before their chemotherapy regime commenced. Although this may not be a major obstacle to some, certain factors need to be considered. Participants in the B vitamin trial were recruited from a public hospital. This meant that a number of patient's have difficulty with transport, as either they didn't drive or were unable to drive. Moreover, they may not be able to drive due to surgery. In addition before commencing chemotherapy the patients have a number of medical tests, and appointments that they need to attend and it is common that patients may experience anxiety before commencing chemotherapy. Lastly, most of the participants lived on the south side of Brisbane rather than the north side, which was prohibitive. These factors need to be considered if further research is to be conducted.

For the conclusions of this pilot work, the QUT researchers were unable to determine if corneal nerve mass was reduced in participants who underwent chemotherapy. Anecdotally, it did not appear that the amount of nerve tissue reduced was as severe an impairment, nor were corneal nerve parameters associated with established measures of neuropathy. Further investigation in a larger number of participants is required to establish the goals for this pilot study.

3.2 MASS SPECTROMETRY AND NUCLEAR MAGNETIC RESONANCE (NMR) ANALYSIS OF B VITAMINS

Nuclear magnetic resonance (NMR) spectrometry is an analytical technique for determining the structure of organic compounds [344]. There are a number of spectroscopic methods but only NMR has the ability to do a complete analysis and interpretation of the entire spectrum. NMR observes the specific quantum mechanical magnetic properties of nuclei of atoms. All nuclei have a magnetic field which absorbs and re-emits electromagnetic radiation and this energy resonates at a particular frequency which depends on the strength of the magnetic field and its properties [344]

Mass spectrometry (MS) is another analytical technique that produces a spectra of a mass of molecules from a sample i.e. solid, liquid or gas such as urine, plasma or food [345]. To measure the characteristics of individual molecules, a MS converts them into ions that can move around and be manipulated by an external electric and magnetic field.

Three essential functions of a MS involves [345]:

1. Ion source: A small sample is ionised (usually to cations due to a loss of an electron).
2. Mass Analyser: Ions are sorted and separated according to their mass and charge
3. The Detector: Separated ions are then measured and results displayed on a chart usually represented by bars.

The technique of NMR and MS were planned for this thesis but time prevented their completion. The samples collected will be used in future analysis of correlation between B vitamins and CIPN. To assess the current literature on these techniques, studies that have been conducted on B vitamins and patients with CIPN have been collated.

3.2.1 B Vitamins and Mass Spectrometry

3.2.1.1 B vitamin Complex

Five studies have been conducted on B vitamins or a selection of B vitamins using mass spectrometry [346-350]. The substances they were tested on include nutritional yeast [346], nutritional supplements [347], human milk [348] and infant formula [349]. The last article was a review comparing various mass spectrometry methods [350]. All studies found that mass spectrometry could achieve quantification of B vitamins. The greatest challenge found was the interference by other compounds in the samples and that the LC-MS/MS means more expensive equipment and requires experienced personnel.

3.2.1.2 Vitamin B6

Five studies have been conducted on vitamin B6 using different mass spectrometry machines and samples. The first study was also used in the B vitamin mix from human breast milk [348] which could quantify each vitamin from the sample. The blood samples that were assessed by NMR and Tandem MS could only detect certain metabolites in people with a vitamin B6 deficiency [351]. Plasma, urine and CSF was found to be a good sample to ascertain certain B6 metabolite's such as α-aminodipic semialdehyde in urine and pepecolic acid in plasma and cerebral spinal fluid [352, 353]. Whole blood was found to be the best sample to detect pyridoxal phosphate (PLP) [354].

3.2.1.3 Folate

Only two studies using mass spectrometry were found. It was determined that both were fast, reliable and sensitive for folate vitamers, homocysteine and methyl malonic acid [355, 356].

3.2.1.4 Vitamin B12

Five studies have been conducted using different forms of mass spectrometry machines to detect different forms and metabolites of vitamin B12. All studies concluded that mass spectrometry was an effective method to detect vitamin B12 in all forms in addition to homocysteine and methyl malonic acid [357-361]. Depending on what determinates were being examined determined what type of sample was used but all were found to be appropriate samples to detect the vitamin B12 they were assessing.

3.2.1.5 Betaine (Choline)

Only one study has been conducted on choline, which used urine and blood as samples and the LC-MS/MS machine. This method was found to be reliable and accurate in quantifying choline [362].

3.2.2 Discussion on Outcomes on NMR and MS and B vitamins

The literature search found a number of studies that have been conducted using MS to assess B vitamins; however, only four studies were found on NMR and B vitamin status [351, 363-365]. Two of the four studies were useful for assisting us for techniques linked with B vitamin identification using NMR [351, 365]. One study which was conducted in 1974, focused on the spectra for the vitamin B6 group looking at pyridoxal, pyridoxal 5'-phosphate, pyridoxamine, pyridoxamine 5'-phosphate and pyridoxine [365]. The other study also assessed vitamin B6 and found that NMR was effective in classifying samples according to vitamin B6 status and identifying discriminating features [351]. This indicates that NMR analysis should observe all forms of vitamin B6 in the analysis however no other information has been found for other B vitamins using NMR.

A number of studies using MS for B vitamin analysis were identified as mentioned above. Four studies addressing various samples was conducted on vitamin B complexes [346-349], a review on folate, vitamin B6 and B12 was conducted on methods for analysis in humans [350], four studies were conducted on vitamin B6 [351-354], two on folate [355, 356], five on vitamin B12 [357-361] and one on betaine (from choline) [362]. These studies have been outlined below in Table 3-2.

According to the research on mass spectrometry and NMR, these methods should be able to detect peaks indicated by B vitamin supplementation and their vitamirs as well as bi-products, metabolite's and other related compounds.

Table 3-2: **Mass Spectrometry Analysis on B Vitamins**

Vitamin	Samples Used for Analysis	Type of Mass Spectrometry	Efficacy
B complex vitamins [346]	Nutritional Yeast	LC/MS – TOF[15]	Fine separation of peaks of all studied vitamins was achieved. NAD+ and CoA could not be achieved with any of the studied enzyme preparations.
B vitamins [347]	Nutritional supplements	Flow-injection ESI-Ms/MS[16]	B vitamins down to the nanogram per gram level could be achieved.
B Vitamins [348]	Human milk	UPLC-MS/MS[17]	Quantitation of all vitamins was achieved even in human milk from severely depleted women.
B vitamins [349]	Infant formula	LC-MS/MS[18]	The accuracy was demonstrated and achieved a good spike recovery of the vitamins (100+/- 6%)
Vitamin B12, B6 and folate [350]	Review	MA[19], CI or ELI[20], LC with UV, fluorescence or electrochemical detection, LC-MS/MS, (GC)-MS[21]	Long term, LC-MS/MS have the potential to replace the 'automated methods' of chemiluminescent and immunoassays which often under report and require the establishment of method specific reference ranges for correct data interpretation.
Vitamin B6 [351]	Blood samples	NMR, Tandem MS	Certain metabolites may be detected connected with a vitamin B6 deficiency.
Vitamin B6 [352]	Plasma	LC-MS/MS	LC-MS/MS was used to analysis compounds relevant to one-carbon metabolism after a vitamin B6 restriction (diet)
Vitamin B6 [353]	Urine and plasma, cerebrospinal fluid	LC-MS/MS and (HPLC)/MS/MS[22]	LC-MS/MS can measure the presence of α-aminoadipic semialdehyde in urine. (HPLC)/MS/MS can measure pepecolic acid in plasma and/or cerebrospinal fluid
Vitamin B6 [354]	Whole blood	LC-ESI-MS/MS	An appropriate method to determine PLP in whole blood
Folate [355]	Plasma	LC-MS/MS	LC-MS/MS was used to measure concentrations of homocysteine and methyl malonic acid
Folate [356]	Whole blood	UPLC-MS/MS	A fast, reliable and sensitive assay using UPLC-MS/MS for the folate vitamers

[15] LC/MS – TOF - Liquid chromatography with mass spectrometry – time of flight and stable isotope assay
[16] Flow-injection ESI-Ms/MS - Flow-injection electrospray ionization tandem mass spectrometry
[17] UPLC-MS/MS - Ultra-performance liquid chromatography tandem mass-spectrometry
[18] LC-MS/MS - Liquid chromatography/tandem mass spectrometry
[19] MA- Microbiological assay
[20] CI or ELI - Chemiluminescent or enzyme-linked immunoassay
[21] (GC)- MS - Gas chromatography mass spectrometry
[22] (HPLC)/MS/MS – High Performance Liquid chromatography tandem mass spectrometry

Vitamin B12 [357]	Plasma and serum	HPLC-MS/MS, (GC)-MS	IM B12 causes swift and significant changes in plasma aminothiols
Vitamin B12 [358]	Plasma	LC/MS/MS	Found to be an accurate and precise method to measure hydroxycobalamin and cyanocobalamin.
Vitamin B12 [359]	Urine, Blood	(GS)-MS, LC-MS/MS	Both methods provided accurate and precise information
Vitamin B12 [360]	Blood, serum	LC-MS/MS	MMA has been considered a gold standard in the diagnosis of metabolic vitamin B12 diagnosis so was used to validate the Holo TC accuracy
Vitamin B12 [361]	Serum	UPLC-MS/MS	This method can be used to detect MMA in neonates and infants using lower amounts of serum
Betaine (choline) [362]	Urine, blood	LC-MS/MS	This method provided reliable, accurate and precise information.

3.2.3 Mass spectrometry and NMR analysis of CIPN

To date, no studies have been conducted on patients who are experiencing CIPN using mass spectrometry or NMR analysis.

3.2.4 Conclusion on NMR and MS for CIPN and B Vitamins

NMR and MS analysis are valid and have been found to be accurate in measuring samples for B vitamins and chemotherapy agents. These techniques are suggested for further research in this area.

The following trials have been conducted without examining samples using NMR and MS due to time restraints. The methodology for the three trials has been explained in Chapter 4.

4 CHAPTER 4 – RESEARCH PLAN AND METHODOLOGY OVERVIEW

4.1 PURPOSE OF STUDY AND OBJECTIVES

4.1.1 Purpose

The main purpose of this thesis was to evaluate the efficacy and safety of supplemental B group vitamins to reduce the incidence and severity of chemotherapy-induced peripheral neuropathy.

4.1.2 Hypothesis

To investigate the therapeutic efficacy and safety of an oral B vitamin complex to assist in reducing the onset and severity of chemotherapy-induced peripheral neuropathy [CIPN]. CIPN will be defined as grade 2 or higher according to the National Cancer Institute Common Toxicity Criteria manual (v2, 1999) or a score higher than 22 on the TNS. This means numbness, tingling, pain or burning in the peripheries in addition motor function impairment that interferes with daily living activities.

4.1.3 Aims

1. To demonstrate that a B group vitamin complex can significantly reduce the incidence of CIPN over placebo.
2. To document the status of B group vitamins in patients diagnosed with cancer that undergoes chemotherapy.

4.2 OUTCOMES

4.2.1 Primary Outcomes for main trial

- Total neuropathy score (TNS) is a validated test to assess CIPN that is conducted by an independent Neurologist. The comparison of this measure between the B vitamin group and the placebo group is the primary outcome.

4.2.2 Secondary Outcomes for main trial

- MD Anderson Brief Pain Inventory
- EORTC Quality of Life Questionnaire
- Patient Neurotoxicity Questionnaire and patient diaries
- Blood pathology of B vitamin status (vitamin B1, B2, B6, B12 and folate)

The comparison between the B vitamin and the placebo arms using these measurement outcomes comprises the secondary outcomes.

4.3 INVESTIGATIONAL PLAN

4.3.1 Overall Study Design

This study was a single-blinded, randomised, placebo-controlled trial. Outcomes were assessed before, after and 3 months' post-chemotherapy administration.

4.3.2 Study Sites

The trial was conducted at the Princess Alexandra Hospital, Brisbane, Australia.

4.3.3 Trial Conduct

Conduct of all researchers, investigators and personnel involved in the study were in line with the National Statement on Ethical Conduct in Research Involving Humans and the Declaration of Helsinki.

4.3.4 Population

The population for this trial were newly diagnosed cancer patients undergoing chemotherapy treatment and who were between the ages of 18 and 80. No restriction were given to ethnicity, social, and financial background. People who were excluded from the trial131 included pregnant and breast feeding ladies, people who had established cognitive impairment, alcoholism, intellectual disability or severe mental illness. All subjects were required to sign a written consent form before participation in the clinical trial(s).

4.3.5 Eligibility Criteria

4.3.5.1 Inclusion Criteria

1. Newly diagnosed with a neoplastic disease;
2. Prescribed chemotherapy treatment with oxaliplatin, taxanes or vincristine.
3. Aged 18 years old or older.

4.3.5.2 Exclusion Criteria

1. Participants prescribed any other concurrent investigational product
2. Participants who were currently experiencing peripheral neuropathy
3. Had undergone chemotherapy treatment before with a neurotoxic agent
4. Had undergone chemotherapy treatment within the last 5 years
5. Pregnant or breast feeding women

6. Any patient with established cognitive impairment, diagnosed alcoholism, intellectual disability or severe mental illness
7. Any patient found to be deficient in vitamin B12 or folic acid
8. Any patients taking concurrent multivitamins, nutritional and herbal supplements or fish oils

4.3.6 Study Treatment

4.3.6.1 Vitamin B Complex

The investigational product was a registered product with the Therapeutic Goods Act [TGA] of Australia. The dose regime was one capsule after/with breakfast and dinner. The dose per day was equivalent to 2 capsules administered daily. The combined daily dose can be seen in Table 4-1.

Table 4-1: B Vitamin Supplement which is Equivalent to Two Capsules Taken Daily.

B Vitamin	Amount
Biotin	1000 μg
Calcium folinate (folinic acid)	1000 μg
Calcium pantothenate (vitamin B5)	327.4 mg
Choline bitartrate	200 mg
Cyanocobalamin (vitamin B12)	1000 μg
Inositol	1000 μg
Nicotinamide (vitamin B3)	160 mg
Nicotinic acid (vitamin B3)	40 mg
Pyridoxal-5-phosphate (vitamin B6)	20 mg
Pyridoxal hydrochloride (vitamin B6)	40 mg
Riboflavin 5-phosphate (vitamin B2)	40 mg
Thiamine hydrochloride (vitamin B1)	100 mg

The dose for this trial was based on research conducted on deficiency states of vitamin B1, B6 and B12 and administered dosages required to correct these deficiencies [161, 162, 164-168]. Due to the fact that a toxicity of vitamin B6 may cause a peripheral neuropathy (dose above 95 mg a day for over a year) and that a high dose of 300 mg a day was found to interfere with tumour duration [159], the dose of vitamin B6 was suggested to be below 70 mg a day (dose in this trial is 60 mg a day).

Oral administration was selected over intra muscular injections as it included the entire B group vitamins rather than just vitamin B12. As these vitamins are water soluble, separating the dose to one capsule after breakfast and one capsule after dinner was required to maintain saturation levels. Patients were advised to take the capsules after or with food to decrease the risk of flushing and irritation on the digestive system which could have been sensitive due to chemotherapy administration.

The dosage period of three months after chemotherapy cessation was selected as delayed peripheral neuropathy or 'coasting' has been found to develop in some patients, particularly those receiving platinum drugs [169, 170].

4.3.6.2 Placebo

The placebo capsules were identical to the B vitamin capsules in appearance. They contained microcrystalline cellulose with a small amount of beta-carotene for colour.

4.3.7 Concomitant Treatment

All medically prescribed medications was allowed prior and throughout the trial period and recorded in the case report form and in the participant diaries. Any rescue medications or changes in medication was permitted and recorded in both the case report form and participant diaries.

Other complementary medications or trial medications were not permitted one week before and during the trial period. The only complementary therapy permitted supplement wise was a vitamin or mineral the patient had been found deficient in such as vitamin D. If a participant wanted to engage in other therapies, researchers had to have consented. Therapies such as acupuncture were not permitted, however massage, physiotherapy, occupational therapy, psychology or counseling were permitted throughout the trial period.

4.3.8 Medication

All participants received chemotherapy agents and adjunct medication. Each participant varied in regards to what chemotherapy agents they were administered and what adjunct medication they were given. All chemotherapy and adjunct medication was recorded on the participant's case file and diaries. Any changes in medication were recorded in the participant diaries and case file.

Current medication that participants had been prescribed was continued throughout the trial unless changed by their medical practitioners.

4.3.9 Withdrawal Criteria

Participants in this trial were considered ‘high risk patients’ as they have been diagnosed with a potentially life threatening disease and had undergone treatment with chemotherapy agents known to cause toxicity. Therefore, participants could have withdrawn from the trial due to disabling or life-threatening events, death, the clinical opinion of the treating doctor that discontinuation from the trial would be in the best interests of the patient, and by voluntary participant withdrawal.

A potential 50% withdrawal rate has been accounted for however the attrition rate at the end of trial was 14%. People did withdraw due to reactions to chemotherapy, death and disease progression. These have been listed in chapter 7, tables 7-1, 7-2.

If participants decided to withdraw from the study, they were asked to notify a member of the research team before they withdrew. This notice allowed the person or the research supervisor to inform them if there were any special requirements linked to withdrawing. Abrupt termination of the study supplement was unlikely to cause any adverse health effects.

All data collected was utilised as ‘intention to treat’. If a participant withdrew or was lost to follow up, all data collected at the point of withdrawal including personal, health information and testing was kept by the researchers. Survival data on all participants withdrawn or lost to follow-up was obtained. If a change in chemotherapy treatment or adverse reaction occurred, participants were asked to finish their participation with the last blood pathology test and a neurology test after their chemotherapy regime had been completed. Participants were contacted by phone, or next of kin were contacted by phone up to four times before being truly lost to follow up. Participants who passed away were not contacted.

4.3.10 Study Duration

Participants were required to have signed a consent form to participant in this study prior to their chemotherapy administration, during their chemotherapy regime and three months’ post-chemotherapy. This entailed either six or nine months in total per participant.

4.3.11 Treatment Assignment and Randomisation

An independent statistician, using a computer generated randomisation sequence, conducted randomisation. Due to the trial analysing three different chemotherapy drugs, a three-block randomisation on two variables was conducted with the overall number of intervention and placebo allocations found to be as close as possible. The statistician with study investigators kept blinded by the sequence allocated the randomisation sequence. See Figure 4-1 for allocation of participants.

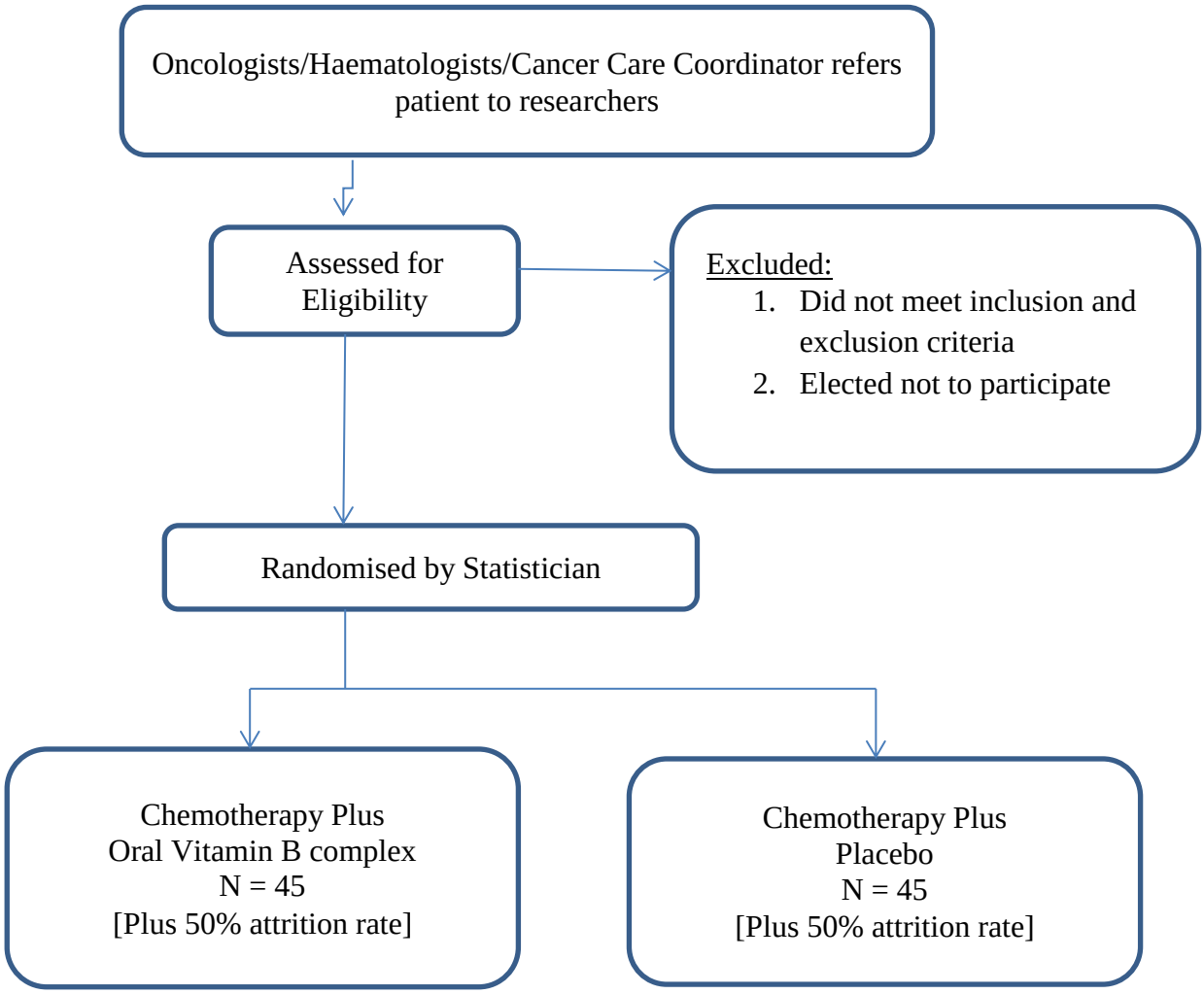


Figure 4-1: Flow Chart of Allocation of Participants

4.3.12 Discontinuation

Discontinuation of the trial occurred due to ethical and university time requirements and length of time of recruitment. The trial from start of recruitment to the finish of the last participant was two and half years.

Due to the small numbers recruited for this trial, no outside monitoring of interim analysis was organised. This trial was monitored from governance from the Princess Alexandra Hospital Human Research Ethics Committee twice throughout the recruitment of this trial. Results were also assessed after twenty-five participants had been completed.

Ethical and Statistical implications included:

1. Number of participants experiencing severe CIPN in either group. If over 50% were experiencing severe CIPN ((a score of over 22 from the TNS results quantified as severe CIPN the trial would have been terminated.

The statistical analysis utilised to confirm these results were:

a) Proportion percentage of severe CIPN reported comparing B vitamin arm versus placebo arm. If there were over 50% of the number participants experiencing CIPN and there was a similar percent proportions between the two arms than the trial would have been terminated.
b) An interim analysis of the data was conducted. The mean primary outcome score [TNS] was used to measure if there was a beneficial, harmful or null trend at participant recruitment points of n=25 for the B group vitamin intervention over placebo. The baseline TNS score of a ± 25% change on the primary outcome was assessed. The researcher group agreed that the null hypothesis for this clinical trial would refer to a default position and would indicate that there is no difference between the two treatments (namely, B vitamins versus placebo). This then indicated that the potential medical treatment with B group vitamins would have had no effect. The net effect would have resulted in the clinical trial cessation for a null or harmful outcome of the interim analysis.

2. Disease progression, adverse events and number of deaths were recorded and analysed to confirm if they were related to the intervention being trialed.

Safety concerns included:

1. If data or results indicated patient safety was at risk or that there was interference with the chemotherapy agent, the trial would have been terminated.
2. If patient medical treatment was deemed to be compromised, the trial would have been terminated.

Recruitment:

1. Due to the complexity of the inclusion and exclusion criteria, the potential participants emotional state of being newly diagnosed with cancer and having to undergo treatment, other research trials requiring similar patients and the referral of patients from oncologists/hospital staff, the trial was discontinued after 70 participants had been recruited due to time restraints.

4.3.13 Data Identification

All data was de-identified. All participants were given a trial number e.g. PA045 paperwork included the participant's initials and trial number. Not all publications and educational information/material pertaining to the trial identified any subject participating in this trial. Every effort was made to conceal the patient's identity and maintain anonymity and confidentiality of their personal details.

Any information obtained in connection with this research project that can identify a participant remained confidential and was only used for the purpose of this research project. It will only be disclosed with the participant's permission, except as required by law. Notification of involvement in this trial was given to the participant's oncologist and the signed consent form, blood pathology results and neurology test results were included in the participants hospital health file.

4.4 STUDY METHODOLOGY

4.4.1 Clinical Trials

Three aspects of the trial were required to support the pilot trial. These include:

1. Absorption study: to ascertain absorption of vitamin B12 from selected B vitamin complex. (Chapter 5)
2. Absorption/interaction study: B group vitamin status directly after administration of chemotherapy with or without B group vitamin administration. (Chapter 6)
3. Main clinical trial: Randomised clinical trial (Chapter 7)

4.4.2 Study Schedule for Main Trial

The patient flow chart can be seen on Figure 4-2, which outlines the flow of patients from recruitment.

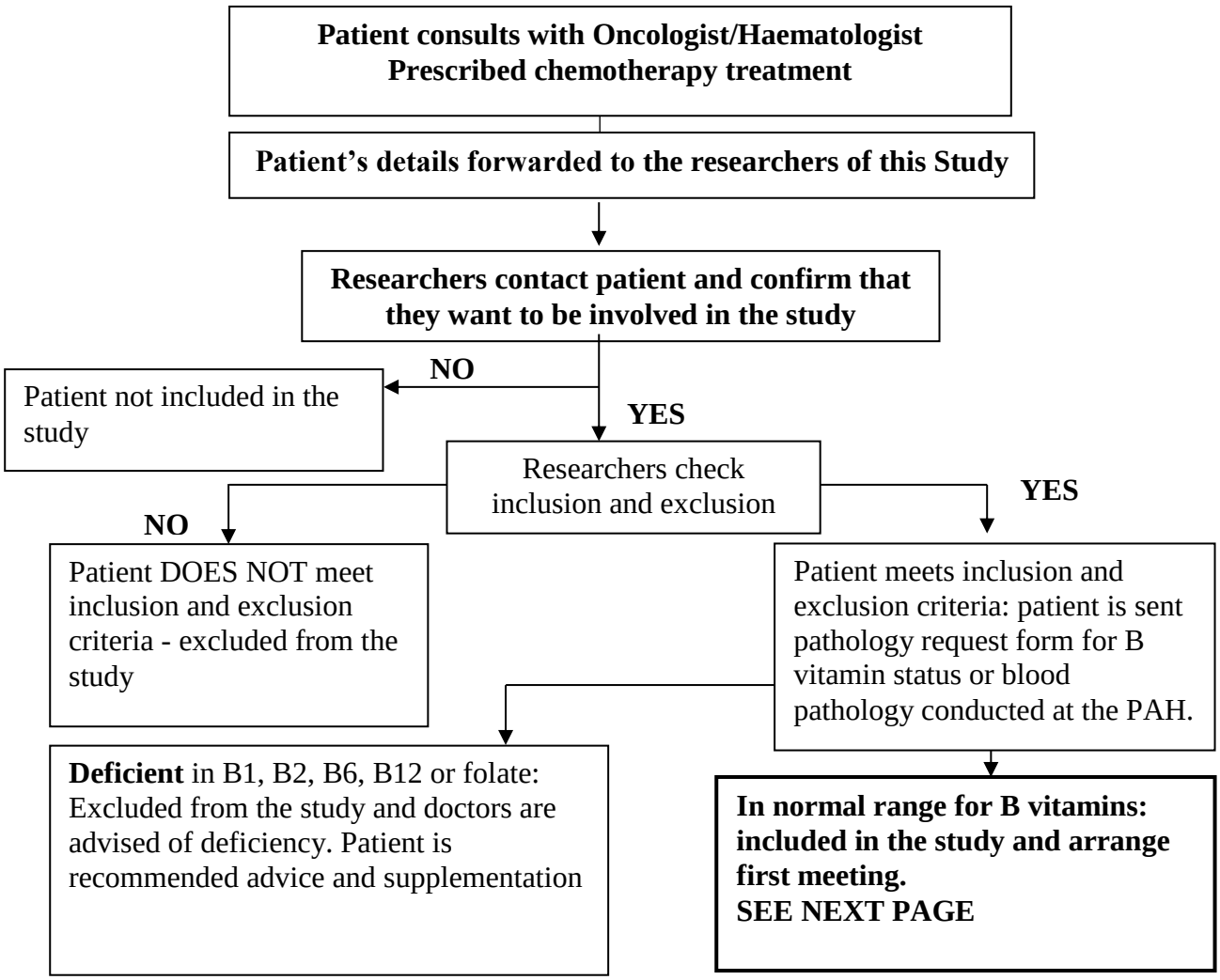
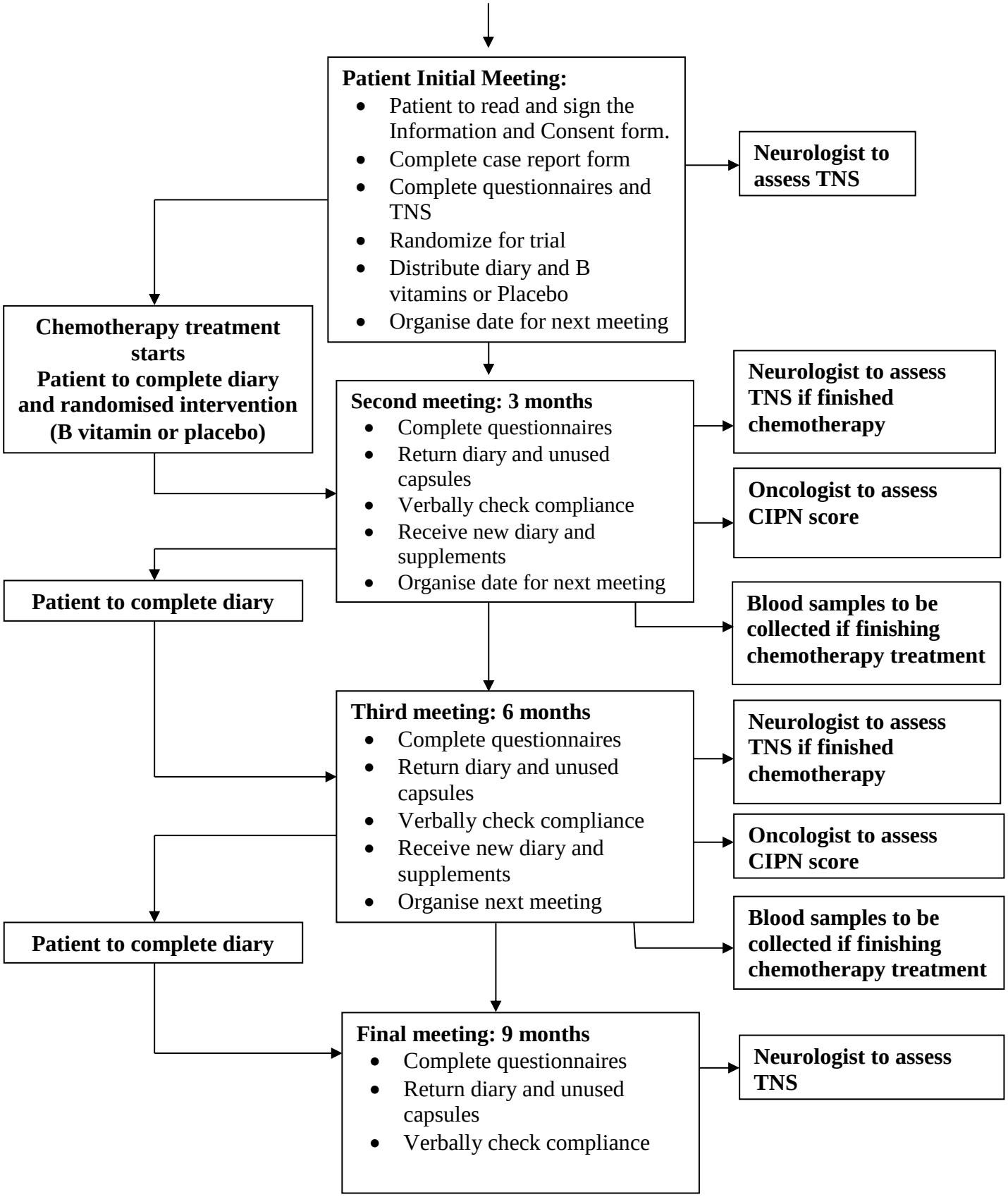


Figure 4-2: Patient Flow Chart for Main Trial (con't next page)


Patient Initial Meeting:
Patient to read and sign the Information and Consent form.
Complete case report form
Complete questionnaires and TNS
Randomize for trial
Distribute diary and B vitamins or Placebo
Organise date for next meeting
Neurologist to assess TNS
Chemotherapy treatment starts
Patient to complete diary and randomised intervention (B vitamin or placebo)
Second meeting: 3 months
Complete questionnaires
Return diary and unused capsules
Verbally check compliance
Receive new diary and supplements
Organise date for next meeting
Neurologist to assess TNS if finished chemotherapy
Oncologist to assess CIPN score
Blood samples to be collected if finishing chemotherapy treatment
Patient to complete diary
Third meeting: 6 months
Complete questionnaires
Return diary and unused capsules
Verbally check compliance
Receive new diary and supplements
Organise next meeting
Neurologist to assess TNS if finished chemotherapy
Oncologist to assess CIPN score
Blood samples to be collected if finishing chemotherapy treatment
Patient to complete diary
Final meeting: 9 months
Complete questionnaires
Return diary and unused capsules
Verbally check compliance
Neurologist to assess TNS

4.4.3 Screening

Participants were recruited through selected oncologists, haematologists and cancer care coordinators at The Princess Alexandra Hospital and various other hospitals located in Brisbane. Possible participants contact details were forwarded to the researchers from the doctors and cancer care coordinators via email, phone or in person or hospital's record system. The researchers contacted the possible participant to screen the patient (inclusion and exclusion criteria) and confirm participation. If participants meet inclusion and exclusion criteria and were interested in participating, a blood test for B vitamin status was conducted and they were asked to read through the participant information sheet thoroughly. They were given the opportunity to ask any questions in regards to the trial and were asked if they understood the requirements and information given.

From the blood test results, if a participant was found to be deficient in either vitamin B12 or folate, the participant was excluded from the study. If a prospective participant's vitamin B12 or folate were found to be within normal range, then inclusion in the study was satisfied and consent provided. Screening and neurology testing was subsequently organised. One participant was excluded to excessive high vitamin B6 levels although not on the exclusion list, this was a risk of peripheral neuropathy due to high vitamin B6. Two consent forms were signed upon full comprehension of the study. A copy of the consent form was provided to the participant, and the original document was retained and filed by the researchers. A copy of the signed consent form was put in the patients hospital file to indicate their involvement in this trial.

Participation in this research project was voluntary. Participants had the right to not participate or withdraw at any stage. Participation or withdrawal did not affect their treatment or their relationship with their medical practitioners.

4.4.4 Neurology Testing

Participants underwent a neurological test from an independent neurologist. The tests included a validated neuropathy score called the TNS see Appendix 2 in addition to nerve conduction studies. This incorporated qualitative and quantitative information regarding sensory and motor neuropathy, pin prick test, vibration testing, reflexes, motor and sensory nerves including the sural nerve and peroneal nerve and autonomic nerve system functioning.

4.4.5 Questionnaires Conducted

4.4.5.1 Patient Neurotoxicity Questionnaire (PNQ)

The Patient Neurotoxicity Questionnaire (PNQ) is a validated questionnaire developed to assess the incidence and severity of CIPN as reported directly by patients rather than physicians.

The PNQ consists of two items involving sensory and motor function and was initially developed for use in registration trials of potential neuroprotective agents as a measure of CIPN as a clinically significant end point. Physicians usually under report and under estimate the severity of CIPN symptoms compared with patients so it is important to support the view of patients undergoing chemotherapy with neurotoxic agents. See Appendix 3.

4.4.5.2 EORTC Quality of Life Questionnaire (QLQ) - C30 (version 3)

The EORTC QLQ-C30 is a quality of life questionnaire specifically designed to assess cancer patients. The EORTC QLQ-C30 is a copy write instrument and the version 3 is the most recent, version, which is to be used for all new studies. As CIPN can affect a patient's quality of life, this is an important aspect that requires monitoring. See Appendix 4.

4.4.5.3 MD Anderson Brief Pain Inventory (BPI)

The brief pain inventory was selected as pain has been reported from CIPN patients and it is not recorded in other assessment tools used. The BPI short form is a validated tool used in clinical trials to assess the severity of pain and impact of pain on daily functions. It also assesses the severity of pain, impact of pain on daily function and location of pain in addition to pain medications and amount of pain relief in the past 24 hours. See Appendix 5.

4.4.6 Blood Analysis

Blood samples from participants in the main trial were used to investigate their vitamin B1 (thiamin diphosphate), B2 (FAD), B6 (P5P), B12 (Holotranscobalamin- Holo TC) and folate (red cell folate) status on two occasions: before and after chemotherapy. Participants had blood taken before they commenced chemotherapy and supplementation and two to three weeks after chemotherapy has ceased. Participants were asked to abstain from alcohol, cigarettes, tea and coffee for 24 hours before sample collection.

Licensed phlebotomists collected blood from the Princess Alexandra Hospital or Sullivan Nicolaides pathology in Brisbane, Australia. For each participant at both venipunctures session, a lithium heparin tube (no gel) of blood was collected in addition to standard blood collection tubes for B vitamin analysis (heparin, ETDA and serum). The blood samples were centrifuged, 2 tubes were kept at room temperature and the plasma from the others were aliquoted and frozen at -20ºC by Princess Alexandra Hospital and/or Sullivan Nicolaides pathology staff. A research assistant collected the samples and brought to the Princess Alexandra Hospital for metabolomics experiments (the analysis will now occur in Sydney after thesis submission therefore samples will be packed accordingly and posted).

Staff from Sullivan Nicolaides Pathology Group performed additional pathology assays. The assays used by Sullivan Nicolaides are presented in Appendix 6.

4.4.7 Monitoring of Participant Compliance

Participant compliance was self-recorded in a participant diary (completed daily). The researchers counted the remaining capsules at each interview appointment to calculate the number of doses missed. B vitamin status was determined after chemotherapy has ceased through blood tests as a measure of adherence to the investigational product.

4.4.8 Timeline

Details of the study procedure are detailed below in chronological order. Each visit consisted of the following:

Visit 1 – Baseline and Beginning of Treatment

The first visit was conducted a week or more before the first cycle of chemotherapy treatment commenced. During this visit, written informed consent to participate in the study was collected. A blood test was performed to determine B vitamin status, a case report was filled out and 3 questionnaires regarding quality of life, pain and perception of neurotoxicity was completed. A registered neurologist conducted a neurology test that included the TNS. Study medication was provided to the participant and all information was recorded in a study diary given to the participant. A further appointment was made twelve weeks later depending on treatment cycles

Visit 2 – Mid Treatment (week 12)

The second visit involved the collection of any unused study medication and the participant receiving new study medication, retrieving the first diary and distributing a new diary, completing the same three questionnaires on quality of life, pain and perception of neurotoxicity and recording any health conditions or changes in medication from their treatment over the last three months. If the participant had completed their chemotherapy regime, blood was drawn to determine B vitamin status and the neurologist conducted the neurology tests including the TNS. A further appointment was made twelve weeks later depending on treatment cycles.

Visit 3 – Mid Treatment (week 24)

The third visit involved the collection of any unused study medication and the participant receiving new study medication, retrieving the second diary and distributing a new diary, completing the same three questionnaires on quality of life, pain and perception of neurotoxicity and recording any health conditions or changes in medication from their treatment over the last three months. If the participant had completed their chemotherapy regime, blood was drawn to determine B vitamin status and the

neurologist conducted the neurology tests including the TNS. A further appointment was made twelve weeks later if appropriate.

Visit 4 – End of Treatment (week 36)

The last visit involved the collection of any unused study medication, retrieving the last diary, completing the same three questionnaires on quality of life, pain and perception of neurotoxicity and recording any health conditions or changes in medication over the last three months. A neurology test was conducted by the neurologist and included the TNS. Follow up six months later occurred if possible in regards to survival, cancer re-occurrence and CIPN.

4.4.9 Invitation to Participate in Related Research

Twenty people were invited to assist with a new diagnostic test for CIPN. It is commercially available technology used to examine the nerves in the eyes, namely a corneal confocal microscope (CCM) and an ocular coherence tomography (OCT) that are being investigated for assessing and diagnosing peripheral neuropathy in diabetes. See chapter 3 for rationale and explanation.

The requirements for the twenty participants involved them attending three testing appointments at the Queensland University of Technology [QUT] Institute of Health & Biomedical Innovation [IHBI] at Kelvin Grove. The appointments were approximately 30 minutes in duration and were conducted at:

1. Baseline – before chemotherapy
2. After chemotherapy cessation
3. Three months after chemotherapy cessation

4.5 STATISTICAL ANALYSIS

All available data was treated as an intention-to-treat analysis. Due to the nature of the dependent variables the statistical analysis was based on t-tests, analysis of variance and multiple logistic regression. All analysis was conducted with software from STATA for Windows version 11.0 [College Station, Texas] and IBM SPSS Statistics 20 (SPSS Inc, Chicago).

The statistical analysis incorporated the following:

1. **Comparison of Participants who experienced CIPN**: B vitamins versus Placebo. This was analysed using the test of proportions of people experiencing CIPN between the treatment groups. A univariate analysis using Fisher's exact test was used due to the small sample size.

2. **Comparison of Participants with Severe CIPN**: B vitamins versus Placebo. Independent Samples t-test (or Mann-Whitney U test if data is non-normal) was used to compare severity of CIPN between treatment groups.

3. **Scores of the TNS/Electro Neurology Tests** before chemotherapy, after chemotherapy and three months' post-chemotherapy was compared between B vitamin and placebo groups. Mixed Effects Model was used for sub scores and the total score of the TNS.

4. **Scores of the Pain Questionnaire** before chemotherapy, three, six and nine months was compared between B vitamin and placebo groups. A score of pain and a score of pain interference will be calculated at each interval. Independent Samples t-test and mixed effects model was utilised for the analysis. Pain medication was recorded and a graphical comparison between treatment groups was generated. Differentiation was made between cancer pain and CIPN pain.

5. **Scores of the Quality of Life Questionnaire** before chemotherapy, three, six and nine months was compared between B vitamin and placebo groups in general. A sub group analysis of those participants who developed CIPN and those who didn't. An Independent Samples t-test and Mixed Effects Model was utilised for analysis on the general QLQ scores.

6. **Scores of the Patient Neurotoxicity Questionnaire** before chemotherapy, three, six and nine months was compared between B vitamin and placebo groups. Independent Samples t-test and Mixed Effects Model was used to compare between treatment groups at each time point.

7. **Comparison of Treatment Groups for Each Chemotherapy Drug** was generated for those participants who developed CIPN compared to those who did not. A Test of proportions between the treatment groups was used for analysis in addition to Fisher's exact test.

8. **Chemotherapy Dose Reduction** was assessed. A test of proportions between the treatment groups was used for analysis if required. Allergy response to the chemotherapy agents or other reasons will be identified if the cause of dose reduction or cessation is not due to CIPN.

9. **B Vitamin Status** before and after chemotherapy was assessed. Each B vitamin (vitamin B1, B2, B6, folate and B12) was compared to baseline between treatment groups. Either an Independent Samples t-test was utilised for analysis. Separate analysis occurred for interaction/absorption trial comparing hourly time points of B vitamin status between chemotherapy patients and healthy controls.

10. **B Vitamin Prophylaxis Effect on Tumour Response Rate.** Analysis of tumour response rate was conducted comparing B vitamin versus Placebo arms. The landmark approach will be utilised for 6 months after the end of intervention (end of the trial). The log rank test was used.

NB: Additional statistical analysis was conducted on data at the end of the trial as seen appropriate.

4.5.1 Subject Population(s) for Analysis

The subject population(s) whose data was used for analysis included:

- All-randomised population: Any participant who was randomised into the study, regardless of whether they received the investigation product or the placebo.
- All-treated population: Any participant randomised into the study that received at least 3 months of the investigational product or placebo.

4.5.2 Significance

The criterion of significance for this trial is set at 0.05.

4.5.3 Power Calculation for Sample Size

For the sample size of this research project, there are no published data on B group vitamins and CIPN. The calculation for our sample size was obtained from published studies on other nutraceutical and pharmaceutical agents such as magnesium/calcium infusions, acetyl-L-carnitine, glutathione, vitamin E, glutamine, amifositine and other drugs that have been conducted for CIPN.

Out of 52 human studies published, 7 were eligible to be used for the calculation of sample size. Calculations were based on comparison of two independent proportions of patients expected to have CIPN. Only 1 study used the TNS score as their measurement tool [215], the others used the National Cancer Institute – Common Toxicity Criteria. Research states that the TNS is the best measurement tool for CIPN.

Results: The power of our study was set at 80% and the significance level at 0.05 and the alpha is two sided.

The statistical calculator used is: http://www.stat.ubc.ca/~rollin/stats/ssize/

Table 4-2: **Power Analysis and Sample Size for B vitamin and CIPN Trial**

Author and Year	Intervention group	Placebo group	Chemotherapy Assessed
Argyriou., et al. (2005) [215]	N = 16 25%	N=15 73.3%	Cisplatin, Paclitaxel or combination
Wang., et al. (2007) [247]	N=42 Grade 1: 16.7% Grade 3-4: 11.9%	N=44 Grade 1: 38.6% Grade 3-4: 31.8%	Oxaliplatin
Pace., et al. (2010) [217]	N=17 5.9%	N=24 41.7%	Cisplatin
Argyriou., et al. (2006) [216]	N=14 21.4%	N=16 68.5%	Cisplatin
Gamelin., et al. (2004) [230]	N=96 Grade 3: 7% Total: 20%	N=65 Grade 3: 26% Total: 45%	Oxaliplatin
Gedlicka., et al. (2002) [237]	N=15 53%		Oxaliplatin
Milla., et al. (2009) [244]	N=14 Grade 1: 50% Grade 2: 50%	N=13 Grade 2: 69.2% Grade 3: 30.7%	Oxaliplatin

The average participant numbers from the studies cited above for:

Grade 1 N=30

Grade 2-4 N=35

We are predicting that the proportion of patients expected to develop no CIPN, Grade 1 CIPN and Grade 2-4 CIPN to be approximately 38% [n=13.3≈13], 42% [n=14.7≈15], and 20% [n=7] respectively. These calculations were based on average proportion percentages from the 7 studies above.

Hence, the requisite number for this pilot trial was 90 participants plus 50% attrition rate equating to 135 participants. The study endeavored to recruit an equal number of participants per group.

4.6 DISCUSSION

This clinical trial was designed to evaluate the efficacy and safety of B group vitamins in the prevention and treatment of CIPN development. By decreasing the incidence of this chemotherapy-induced side effect, it was anticipated that the patient's chemotherapy treatment regime, expected outcome, quality of life and physical ability for general life would be improved.

It was hypothesised that participants low in B vitamins before the commencement of chemotherapy treatment were at an increased risk of developing CIPN and that administration of B group vitamins could decrease CIPN incidence and/or severity. In addition, it was thought that the chemotherapy agents may lower the patients B group vitamin status therefore making them susceptible to the development of CIPN. That only occurred in one patient (refer to chapter 8) and final blood pathology results indicate only a slight decrease in certain B vitamins.

Limitations for this clinical trial included adverse reactions to chemotherapy agents that were not CIPN related, emotional/mental/physical exertion and stress involved with cancer diagnosis and treatment, mucositis associated with chemotherapy treatment that may alter B vitamin absorption and the mortality rate of high-risk cancer patients. An example of a non-related CIPN reaction includes allergic or hypersensitive reactions [366, 367] or significant myelosuppression [368] that may affect chemotherapy dose and treatment continuation. Two patients had quite adverse reactions to the chemotherapy. One developed Sweet's syndrome and the other had pulmonary artery spasms. Approximately eight participants had mild allergic reactions to the chemotherapy agents.

B group vitamins were assumed to have the potential to decrease the onset and severity of CIPN as a deficiency of B vitamins biochemically affects the health of nerve fibres and ganglia that neurotoxic chemotherapy agents can further adversely modulate. Reducing this chemotherapy-induced side effect is clinically relevant for disease outcome and quality of life.

The results of each trial can be seen in chapter 5, 6 and 7.

5 CHAPTER 5 – STUDY 1: ABSORPTION STUDY

5.1 INTRODUCTION

A majority of vitamin supplements in Australia, although approved by the Therapeutics Goods Act (TGA - Australia's governing body), are not externally tested for absorption. To ascertain if the B vitamin supplement chosen for this clinical trial was absorbed, an absorption study was devised. Initially the study focused on vitamin B12 as being the major B vitamin that could potentially decrease the incidence of CIPN. Hence, this pilot study tested serum vitamin B12 levels. Additionally, vitamin B12 has the potential to be poorly absorbed due to the complexity required for its absorption and utilisation (see chapter 1, page 64-65).

Initially a small absorption study was conducted on two healthy volunteers to ascertain the vitamin B12 status after administration. The first 24 hours with the two volunteers also included red cell folate, however only serum vitamin B12 was assessed for this pilot study for the rest of the volunteers due to availability of the assay.

Two participants were not enough to guarantee the selected supplement showed absorption or indicate significance. A further eight healthy volunteers underwent testing to quantify the absorption of the supplement giving a total of ten participants.

5.2 METHODS

5.2.1 Purpose

To ascertain the absorption of vitamin B12 from the selected B group vitamin supplement.

5.2.2 Design

The masking of this trial was open with the study duration being 3 weeks for each person. The end-point classification was the serum vitamin B12 status comparing baseline to 3 weeks after supplementation of B vitamins equivalent to 1,000 µg of vitamin B12 daily.

5.2.3 Participants

Ten generally healthy volunteers consisting of 5 male and 5 females were selected that meet the following inclusion and exclusion criteria:

5.2.3.1 Key Inclusion Criteria

1. Over the age of 18 years old
2. Healthy with no known acute illness (chronic illnesses such as diabetes, cardiovascular disease and arthritis are allowed if managed or in a stable situation.)

5.2.3.2 Key Exclusion Criteria

1. Participants who have been prescribed any other concurrent investigational product such as a vitamin, mineral or pharmaceutical agent for another trial.
2. Currently experiencing peripheral neuropathy
3. Have undergone previous chemotherapy treatment
4. Pregnant or breast feeding women
5. Any participant with established cognitive impairment, diagnosed alcoholism, intellectual disability or severe mental illness.
6. Any participant taking concurrent multivitamins, nutritional and/or herbal supplements or fish oils

5.2.3.3 Recruitment

Recruitment of health volunteers was established through the University of Queensland, School of Medicine students and staff located at the Princess Alexandra Hospital, Translational Research Institute. Recruitment was through word of mouth and researchers contacting staff and students.

5.2.4 Dose and Duration of Supplementation

Each participant had fasting blood drawn from a phlebotomist from the University of Queensland or the Princess Alexandra Hospital before supplementation for baseline vitamin B12 status. One capsule was then administered after breakfast, which was equivalent to 500 µg of both vitamin B12 and folate (as folinic acid). Bloods were then taken 3 hours, 6 hours and 24 hours after the initial administration of the B vitamin capsule. The last blood request was taken three weeks after the initial 24 hours. Each participant was administered one capsule twice a day after food equivalent to 1,000 µg/day of vitamin B12. Bloods are then drawn again to ascertain vitamin B12 status after administration.

During the first 24 hours a diet that restricted vitamin B12 was implemented. See Appendix 8.

5.2.5 Study Site and Ethics Approval

The study site was the University of Queensland and the Princess Alexandra Hospital, Brisbane, Australia. The University of Queensland Ethics Committee (UH 2010000749) approved the ethics for this project.

5.2.6 Study Outcomes

The primary end-point was the comparison of the baseline serum vitamin B12 status compared to the 3-week serum B12 status.

5.3 RESULTS

The demographics of the healthy volunteers seen in Table 5-1 showed an equal number of males to females. Their average age was 38 years old and they were in a generally healthy weight range and BMI (healthy BMI = 23-27). They had minimal caffeine and alcohol intake and none of the volunteers smoked.

Table 5-1: **Demographics of Healthy Volunteers for the B12 Absorption Study**

Healthy Volunteers	**Demographics**
Gender	Females x 5 Males x 5
Age (years)	Mean ± SD 38 ± 12.45
Weight (kg)	Mean ± SD 76.5 ± 13.52
BMI	Mean ± SD 24.38 ± 2.84
Caffeine intake (per day)	Median ± SD 1.5 cups ± 1.16
Alcohol intake (per week)	Median ± SD 3 standard drinks ± 1.77
Smoking (per day)	Median ± SD 0 ± 0

Table 5-2 contains the full results of the samples collected from the healthy volunteers. Levels varied over the twenty-four period however, all participants increased from baseline in the three week period of supplementation except participant PO5. This is the only participant that was vegetarian. Considering there is a higher prevalence of a vitamin B12 deficiency found in vegetarians [369] the initial baseline result of 750 pmol/L may represent a false result or reading considering the vitamin B12 status decreased after supplementation was commenced (500 µg). One capsule was taken after breakfast the same day as noted by researchers and the results three, six and twenty-four hours after were all lower than the baseline result. The results of PO5 after three weeks of supplementation can be surmised that the participant was not being compliant with taking the recommended dose although they stated that they were approximately 73% compliant.

Table 5-2: **Results from the B12 Absorption Study including Vitamin B12 and Folate**

Patient Tests	Baseline	3 hours	6 hours	24 hours	3 weeks taking B vitamins
P01 Serum B12	212	216	216	208	657
P02 Serum B12	500	603	620	693	819
PO3 Serum B12	318	368	358	314	790
PO4 Serum B12	401	426	466	456	900
PO5 Serum B12	750	570	580	610	570
PO6 Serum B12	256	321	303	366	640
PO7 Serum B12	285	282	303	312	520
PO08 Serum B12	191	194	208	177	272
PO09 Serum B12	263	269	280	357	425
PO10 Serum B12	311	279	335	347	414
Patient Tests	**Baseline**	**3 hours**	**6 hours**	**24 hours**	**3 weeks taking B vitamins**
P01 RCF	14.85	13.55	13.65	13.82	N/A
P02 RCF	25.22	22.94	23.18	23.53	N/A

The blood results of the red cell folate collected for the two initial healthy volunteers as seen in Figure 5-1 showed no difference after 500 µg of folinic acid was ingested. No further testing was conducted on folate for the remainder of the pilot study.

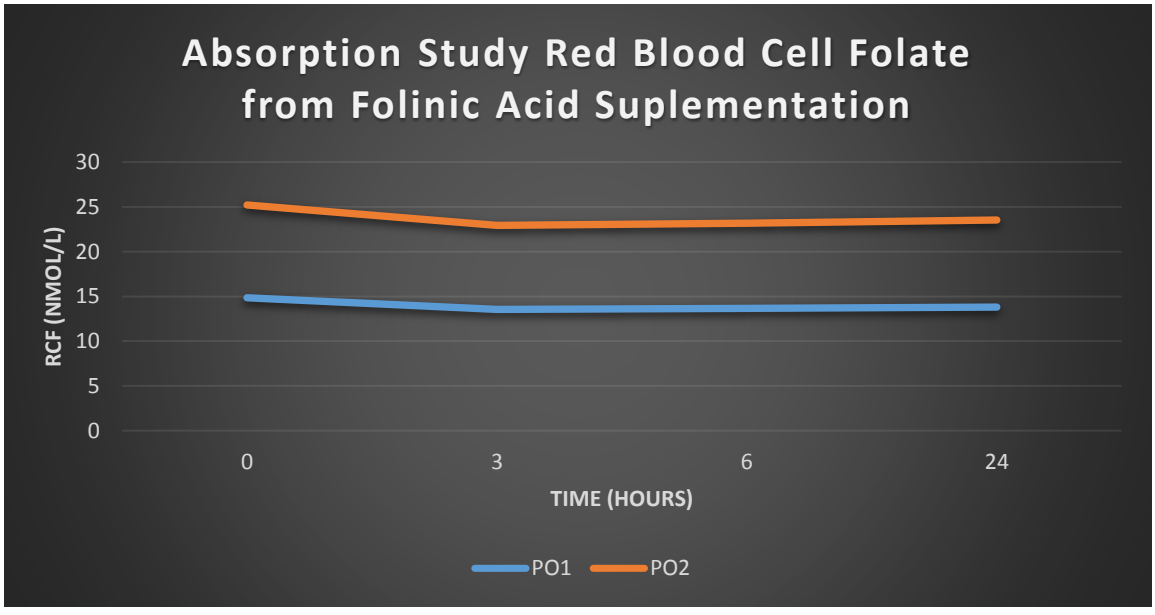


Figure 5-1: **Red Cell Folate (RCF) Results from 2 Participants over 24 hours.**

The final-results of the absorption study seen in Figure 5-2 shows that the selected oral B vitamin supplement does increase serum vitamin B12 levels in healthy volunteers after three weeks of supplementation of two capsules a day (equivalent to 1,000 µg/day). Minimal absorption of vitamin B12 was seen in blood results over twenty-four hours after 500 µg was ingested.

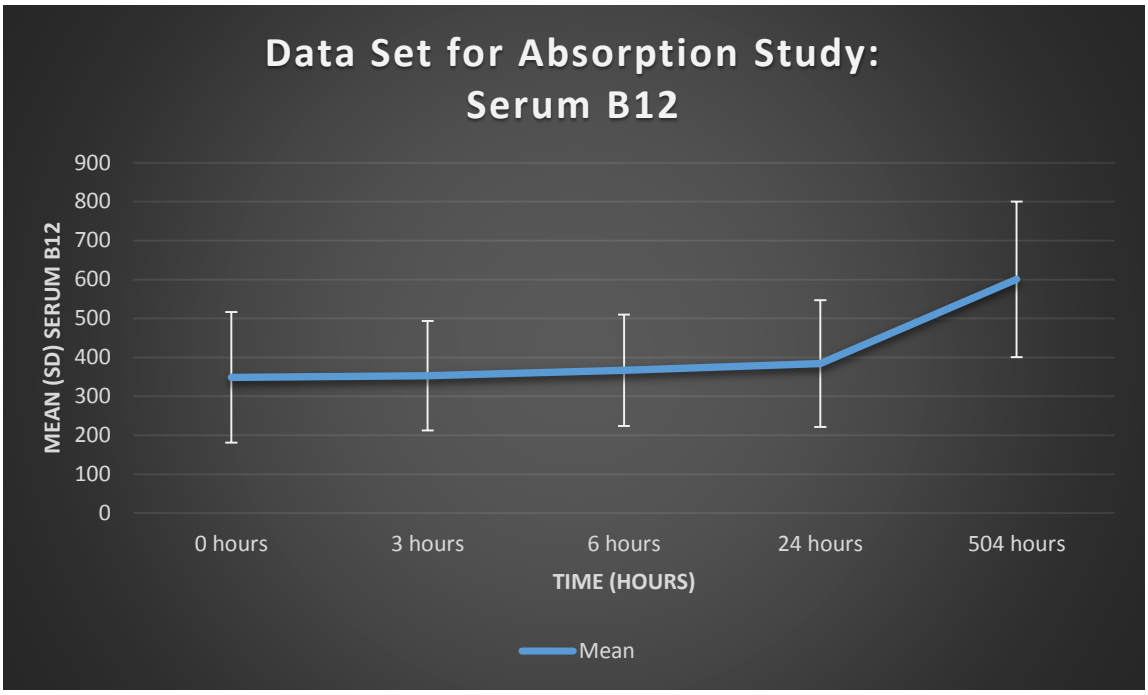


Figure 5-2: **Vitamin B12 Results over Three Week Supplementation**

5.4 DISCUSSION

Ten healthy volunteers commenced and completed the absorption study. The demographics of these volunteers can be seen in Table 5-1. There were equal male and female participants with an average age of 38 years old. All were within an adequate BMI of around 24 so therefore were not overweight and consumed on average one to two cups of coffee a day and around 2 to 4 standard alcoholic drinks a week. No participants used tobacco products and only two participants had been prescribed medications. One participant was a type 1 diabetic and the thyroid had been removed. The medication this participant was taking included humalin NPH (20 units x 2), Humalog (8 units x 2) and Oroxine (200 mcg/day). Another participant had the two genetic mutations of methylenetetrahydrofolate reductase (MTHFR: C677T and A1298C mutations) and had been diagnosed with high blood pressure and cardiovascular disease.

The medication for this participant included Karvea (150 mcg/day) and Norvasc (5 mg) for hypertension. One participant, PO5 was a vegetarian but still consumed fish and seafood while all other participants consumed animal meats on a regular basis.

In regards to the vitamin B12 results, there was some variation among the participants in regards to ranges. The range for the serum vitamin B12 assay is 133 to 680 pmol/L for the Princess Alexandra Hospital. It should be noted that reference ranges vary depending on the laboratory. However, it has been reported that this reference range is not correct, as many people in the population will exhibit neurological symptoms of a deficiency at a much higher concentration compared to 133 pmol/L [370, 348]. Researchers vary in opinion as to the lowest concentration that should be considered. The consensus is that a low value of 221 pmol/L is an acceptable 'normal' bottom line [370].

As such the agreement on the lowest normal range indicates that two of the participants would be considered deficient at baseline (PO1=212 pmol/L and PO8=191 pmol/L). One participant was over range PO5=750 pmol/L. Over the 24 hour period, minor increases in range were reported at 3, 6 and 24 hours with the mean increasing slightly from baseline (348.7 pmpl/L) 4.1 pmol/L at 3 hours, 18.2 pmol/L at 6 hours and 35.3 pmol/L at 24 hours (see Figure 5-2). At the three-week interval, the mean went from 348.7 pmol/L at baseline to 600.7 pmol/L (252 pmol/L difference). This indicates a 58% increase with no participants considered deficient at the conclusion of the trial.

One participant, P05 was the only participant who reported a decrease in B12 from baseline (750 pmol/L at baseline to 570 pmol/L at 3 weeks). This variation may have occurred due to poor compliance, a false reading at baseline (as all other results were around the 570 pmol/L: 750=baseline, 570=3h, 580=6h, 610=24h and 570=3 weeks) or previous unreported vitamin B12 administration prior to the trial. The participant had reported missing a number of doses of the B vitamin over the three-week period but was found to be 73% compliant.

In regards to the participants that were sub-clinically deficient at baseline, both participants reported an increase in vitamin B12. PO2 increased from 212 pmol/L to 657 pmol/L within the 3 weeks. PO08 did not have a dramatic increase in B12 and actually had a further decrease at 24 hours. This participant's range decreased from 191 pmol/L to 177 pmol/L at 24 hours and to 272 pmol/L at 3 weeks. Although this result may indicate poor compliance over the 3-week period, the participant however was found to be 82% compliant.

Participant PO08 had the genetic double MTHFR polymorphisms. These polymorphisms may have an impact on this participant's vitamin B12 status, although this has not been proven [372]. Other possible reasons that may be involved with the slight increase include intrinsic factor antibodies (not tested, awaiting results), stress (the participant was in a particularly high acute stress situation during testing), impaired digestion or inadequate utilisation [371]. These confounding factors may have been involved with the end result with the intrinsic factor antibody status still pending.

5.5 CONCLUSION

The results of this small clinical trial indicate a trend that the B vitamin supplement chosen was adequate for vitamin B12 absorption in healthy volunteers, the supplementation outcome was not significant. Taking into consideration that this supplement will be administered to cancer patients undergoing chemotherapy, there will be a number of confounding factors that may interfere with vitamin B12 absorption. Hence, the few confounders found in this study are reasonable indicators that the supplement may still be absorbed despite certain factors. To expand on this trial, an absorption and interaction trial was developed to ascertain the B vitamin status of other B vitamins and the B vitamin status after chemotherapy administration.

6 CHAPTER 6 – STUDY 2: INTERACTION/ABSORPTION STUDY

6.1 INTRODUCTION

The hypothesis for this thesis incorporated two themes. The first of premise was to substantiate whether patients with low B vitamin status prior to chemotherapy administration may be at an increased risk of developing CIPN and the level of severity experienced. To examine this theme, B vitamin status was assessed during chemotherapy administration to ascertain if chemotherapeutic interventions change a patient's B vitamin status. It is posited that a decrease in B vitamins such as vitamin B1, B6 and B12 from specific chemotherapy agents may predispose a patient diagnosed with cancer to the development of CIPN.

To test this concept, an absorption and interaction pilot clinical trial was developed. A small cohort of six participants undergoing chemotherapy was chosen from the main clinical trial. All subjects undergoing chemotherapy agreed and consented to being part of the absorption and interaction trial. All six subjects were undergoing chemotherapy at the time of testing for the absorption/interaction trial and had been taking the selected B vitamin supplement or placebo [see Chapter 4 for B vitamin complex breakdown on page 154]. The subjects continued to take the given supplement throughout this trial, during the entire chemotherapy regime and three months' post-chemotherapy. To assist in clarifying the B vitamin response, six healthy volunteers with similar demographics to the chemotherapy participants were selected to investigate if there was a difference in the blood B group vitamin status with the same supplementation of B vitamins.

The healthy volunteers were given the B vitamin to ascertain if there was an increase in blood B vitamin status over the seventy-two hours without chemotherapy administration. Therefore, as half of the chemotherapy patients were also taking the B vitamin prior and during the absorption/interaction trial, a comparison could be made if chemotherapy administration made a difference in the B vitamin status of patients. The three chemotherapy patients taking the placebo were selected in order to determine the effects that would be observed in B vitamin status after chemotherapy administration with no B vitamin supplementation.

The three chemotherapy agents that were selected for the main trial (vincristine, oxaliplatin and taxane class) were used in this pilot clinical trial to test the B group vitamin status response to each agent. This decision was made rather than testing one agent as all three were being used in the main clinical trial study. Even though each agent has a different mechanism of action, the general B group vitamin status could still be monitored.

6.2 METHODS

6.2.1 Purpose:

To assess and compare the absorption and status of the B group vitamins in patients undergoing chemotherapy with either vincristine, docetaxel or oxaliplatin who were taking an oral B vitamin supplement or placebo; and further compared to the healthy volunteers who were taking the same B vitamin supplement.

6.2.2 Design

Six participants enrolled in the main clinical trial consented to be part of the pilot clinical trial assessing absorption and interaction. From the six participants who were receiving chemotherapy there were three participants that were taking the B vitamin supplement and three that were taking the placebo (microcrystine cellulose). A blood sample was collected prior to the administration of chemotherapy (baseline value), and then twenty-four and seventy-two hours. Six healthy volunteers who also had blood samples taken at baseline, then post administered one capsule of B group vitamins twice a day (same as the main clinical study). A blood sample was then collected at twenty-four and seventy-two hours after B group vitamin administration.

The blood sample analysis consisted of vitamin B1 (thiamine diphosphate), vitamin B2 (flavin adenine dinucleotide), vitamin B6 (pyridoxal-5-phosphate), red cell folate and vitamin B12 (holotranscobalamin). These samples were analysed by Sullivan Nicolaides Pathology. The technical equipment and assays used are listed in Appendix 6.

6.2.3 Participants

1. Six patients diagnosed with cancer undergoing chemotherapy treatment (mixture of males and females). The selected cancer patients were already enrolled in the main clinical trial and had been randomised to either the B vitamin or placebo arm. Hence, the patients were taking the selected B vitamin complex or placebo prior to testing for the absorption/interaction trial.
2. Six healthy volunteers (mixture of males and females) not taking any B vitamin supplements prior to testing.

6.2.4 Key Inclusion Criteria

For the patients diagnosed with cancer:

1. Newly diagnosed with a neoplastic disease

2. Had been prescribed chemotherapy treatment with oxaliplatin, the taxane class or vincristine and had commenced their chemotherapy regime.

3. Aged 18 years old or older.

4. Had been enrolled in the main clinical trial assessing B vitamin supplementation in the prevention of CIPN and were taking either the B vitamin supplement or placebo.

For the healthy volunteers:

1. Aged 18 years old or older
2. Relatively healthy – no known acute disease. Volunteers who had a chronic illness such as diabetes, cardiovascular disease, arthritis were included unless they were experiencing an acute stage of their illness. No volunteers were enrolled if they had chronic liver, digestive (including bowel resections) or kidney disease.

6.2.5 Key Exclusion Criteria

1. Participants who had been prescribed any other concurrent investigational products in another trial such as vitamins, minerals or pharmaceutical agents (drugs).

2. Currently experienced peripheral neuropathy.

3. Had undergone previous chemotherapy treatment with a neurotoxic agent.

4. Women who were pregnant or breast-feeding

5. Any patient who had established cognitive impairment, diagnosed alcoholism, intellectual disability or severe mental illness.

6. Any patient taking concurrent multivitamins, nutritional and/or herbal supplements or fish oils.

6.2.6 Dose and Duration of Supplements

Cancer patients were randomised to a B group vitamin (1 capsule b.i.d) or placebo (1 capsule b.i.d) after meals in the main clinical trial. Cancer patients were undergoing chemotherapy at the time of testing for this absorption/interaction trial with concurrent administration of the B vitamin supplement or placebo. Healthy volunteers did not undergo chemotherapy treatment and were given a B group vitamin (1 capsule b.i.d). Healthy volunteers were not randomised.

6.2.7 Study Site and Ethics Approval

The study sites were the University of Queensland and the Princess Alexandra Hospital, Brisbane, Australia. The University of Queensland Ethics Committee (UH 2010000749) and the Princess Alexandra Hospital Ethics Committee (HREC/10/QPAH/140) approved ethics for this project.

6.2.8 Statistical Analysis

The data was recorded and statistical analysis conducted using the Excel program. The participant numbers for this trial were too small to use SPSS. The concept of this trial was to observe any change in projection from chemotherapy administration in patients supplemented with B vitamins or placebo. Therefore the most effective way of analysing the data was to assess the difference in projection through a line graph and noting any differences observed. The scholar/researcher carried out and completed the analysis.

6.3 STUDY OUTCOMES

6.3.1 Descriptive Statistics

Data for descriptive statistics of the groups are presented in Table 6-1. The healthy volunteers were equal number of males and females and of similar age to the group that was administered chemotherapy. The average weight and BMI of the healthy volunteers was in the range 23-28 Kg/m^2 (deemed healthy). The chemotherapy group had more females than males (4:2) and was equally divided for each chemotherapy agent with one participant on placebo and one participant on the test intervention. There was only one oxaliplatin participant due to the main clinical trial ceasing recruitment of this chemotherapy agent. The average weight and BMI of the chemotherapy participants was overweight (≥30 Kg/m^2).

Table 6-1: **Descriptive Statistics of Participants in Absorption/Interaction Pilot Trial**

		Chemotherapy Participants	Healthy Volunteers
Gender	Male	2	3
	Female	4	3
Age		38.5 ± 15.3 years Mean ± SD	39 ± 13.7 years Mean ± SD
Weight		99.7 ± 32.65 kg Mean ± SD	68.05 ± 8.48 kg Mean ± SD
BMI		29.9 ± 9.33 kg/m^2 Mean ± SD	23.6 ± 2.09 kg/m^2 Mean ± SD
Chemotherapy Agents			
	Docetaxel	3	
	Oxaliplatin	2	
	Vincristine	1	

6.3.2 B Vitamin Blood Results

6.3.2.1 Vitamin B1

The results for vitamin B1 are mixed in regards to the chemotherapy patients as seen in Table 6-2 and Figure 6-1. The healthy volunteers showed a general increase over the seventy-two hours (18%). The chemotherapy patients who were supplemented with B vitamins in comparison showed a decrease in vitamin B1 status twenty-four hours after chemotherapy (15%) administration was begun and then increased above baseline at seventy-two hours (12%). The placebo arm showed a slight increase twenty-four hours after chemotherapy (8%) then a 40% increase from baseline seventy-two hours after chemotherapy administration.

Table 6-2: **Vitamin B1 Results for Absorption/Interaction Pilot Trial**

Vitamin B1 (ref 66-200 nmol/L)			
Allocation No	Baseline	24 hour	72 hour
AT1 DxS	170	220	220
AT2 SxC	160	180	190
AT3 BxG	130	150	160
AT4 NCB	170	200	220
AT5 NxP	150	170	180
AT6 AxB	160	170	180
Average	156.66	181.66	191.66
SD	15.05	24.83	24.01

Vitamin B1 (ref 66-200 nmol/L)			
Allocation no	Baseline	24 hour	72 hour
PA015AJM - VB	220	220	190
PA009BJS - DB	210	170	
PA043MME-AB	230	170	310
Average B vits	220	186.66	250
SD	10	28.86	84.85
PA011GMB – VP	170	160	290
PA012EBS - OP	140	190	130
PA049EMD – DP	150	150	340
Average Placebo	153.33	166.66	253.33
SD	15.27	20.81	109.69

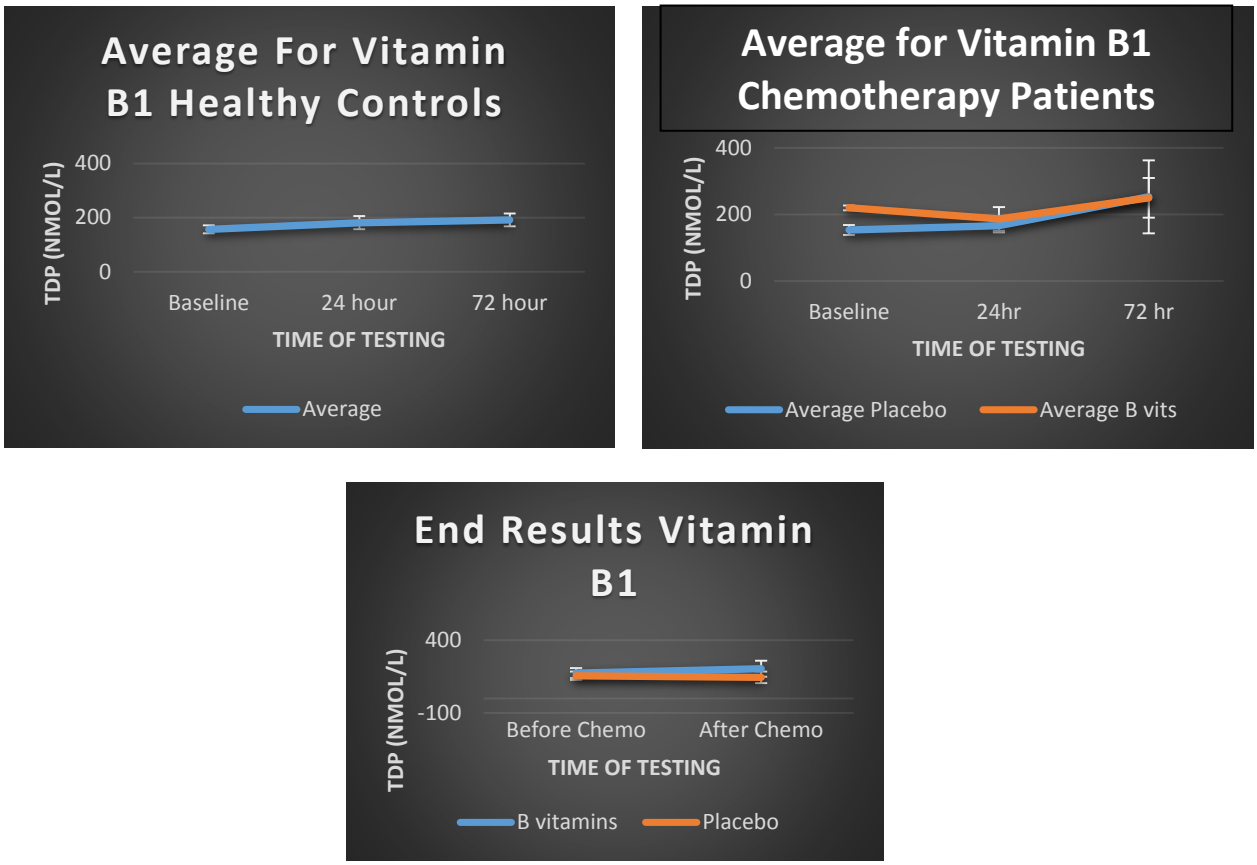


Figure 6-1: **Vitamin B1 Results for Absorption/Interaction Pilot Trial**

6.3.2.2 Vitamin B2

Data are presented in Table 6-3 and Figure 6-2. Vitamin B2 showed a noticable decrease at 24 hours (18%) with the B vitamin supplemented chemotherapy group versus the placebo (4%), however, both groups showed an increase at 72 hours (B vitamin arm=15%; placebo arm= 9%). The healthy controls indicated a normal increase over the 72 hours (11%).

Table 6-3: **Vitamin B2 Results for Absorption/Interaction Pilot Trial**

Vitamin B2 (ref 180-470 nmol/L)			
Allocation No	Baseline	24 hour	72 hour
AT1 DxS	320	350	360
AT2 SxC	340	360	370
AT3 BxG	310	340	340
AT4 NCB	290	310	320
AT5 NxP	250	280	320
AT6 AxB	290	310	320
Average	300	325	338.33
SD	30.98	30.16	22.28

Vitamin B2 (ref 180-470 nmol/L)			
Allocation No	Baseline	24 hours	72 hours
PA015AJM - VB	320	310	290
PA009BJS - DB	350	220	
PA043MME-AB	290	260	330
Average B vits	320	263.33	310
SD	30	45.09	28.28
PA011GMB - VP	280	240	240
PA012EBS - OP	260	280	250
PA049EMD - DP	270	260	370
Average Placebo	270	260	286.66
SD	10	20	72.34

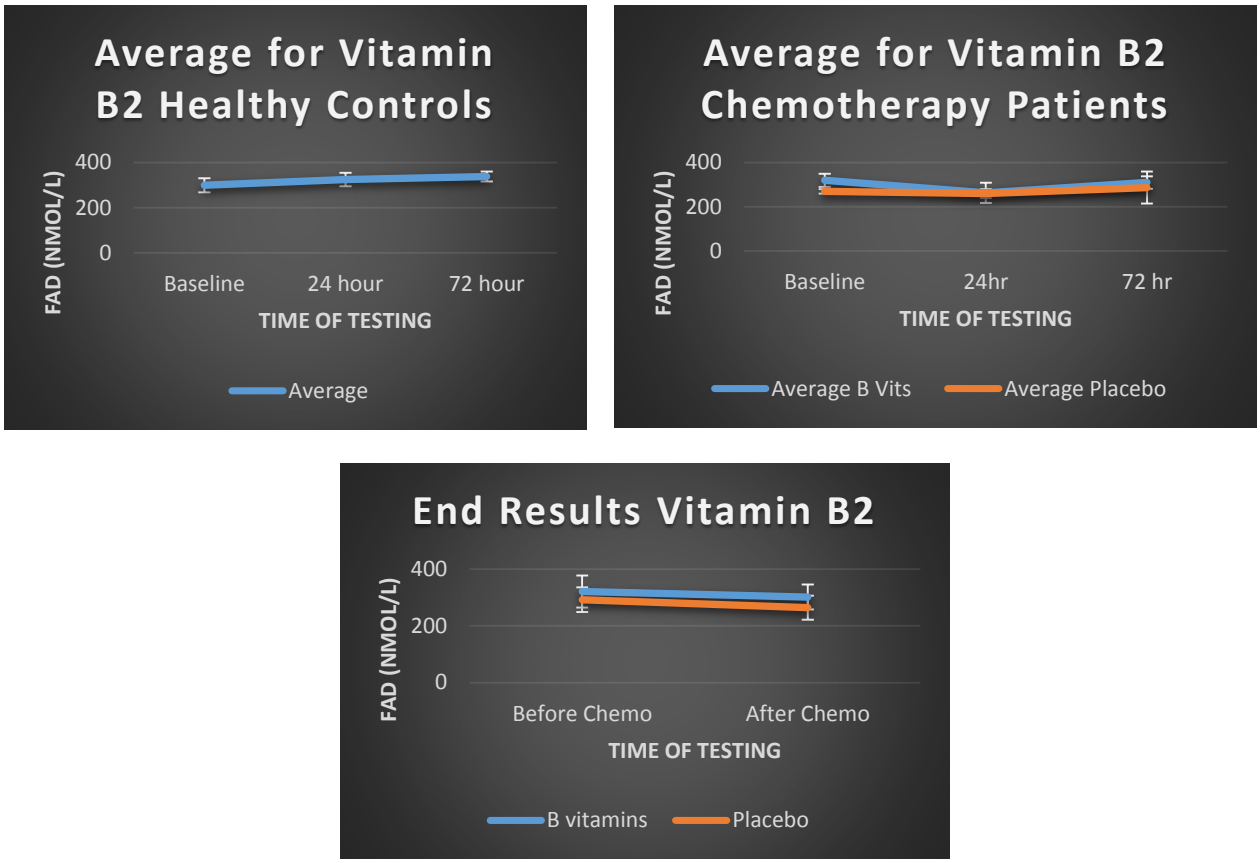


Figure 6-2: **Results of Vitamin B2 in Absorption/Interaction Pilot Trial**

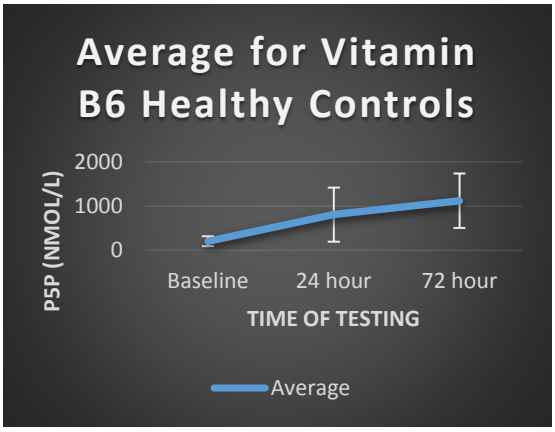
6.3.2.3 Vitamin B6

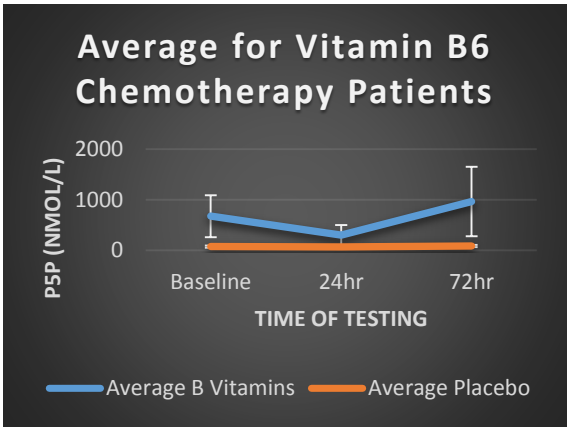
Data for this section are presented in Table 6-4 and Figure 6-3. The results of vitamin B6 demonstrated a marked increase in circulating pyridoxal-5-phospate (P5P) the active form of vitamin B6 with 40 mg of vitamin B6 daily. The healthy individuals all showed an increased response to supplementation, with an average increase between 5 to 12 fold. Whereas only one subject showed a two fold increase. The chemotherapy patients showed that the B vitamin supplement group had a decline (65%) and then a marked increase (79%) over the 24 hour and 72 hour time points respectively. Alternatively the placebo group showed minimal difference over the 72 hours compared to the B vitamin supplemented arm (24 hour decrease 9%; 72 hours increase by 12.6%).

Table 6-4: Vitamin B6 Results in Absorption/Interaction Pilot Trial

Vitamin B6 (ref 35-10 nmol/L)			
Allocation No	Baseline	24 hour	72 hour
AT1 DxS	350	1840	2160
AT2 SxC	200	440	920
AT3 BxG	330	520	690
AT4 NCB	130	370	690
AT5 NxP	85	400	690
AT6 AxB	130	1280	1580
Average	204.16	808.33	1121.66
SD	111.64	610.65	614.47

Vitamin B6 (ref 35-10 nmol/L)			
Allocation no	Baseline	24 hour	72 hour
PA015AJM-VB	950	500	480
PA009BJS - DB	200	100	
PA043MME-AB	880	310	1450
Average B Vits	676.66	303.33	965
SD	414.28	200.08	685.89
PA011GMB - VP	90	85	110
PA012EBS - OP	90	80	65
PA049EMD - DP	50	45	90
Average Placebo	76.66	70	88.33
SD	23.09	21.79	22.54





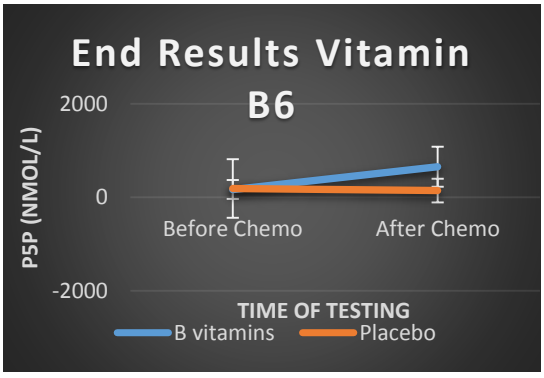


Figure 6-3: Vitamin B6 Results for Absorption/Interaction Pilot Trial

6.3.2.4 Folate

The overall B group vitamin status in blood of all participants including the healthy controls were all within optimal range indicating a generally favourable absorption of folic acid as seen in Table 6-5 and Figure 6-4. This may in part be due to the fortification of foods in Australia [373]. The healthy controls increased their folate status by 9% over the seventy-two hours of supplementation. The B vitamin supplemented chemotherapy group (including the patient on oxaliplatin as they have folinic acid as part of their chemotherapy combination) had a 2% increase over twenty-four hours and went back to baseline at seventy-two hours. The placebo group had a 8.5% decrease in red cell folate seventy-two hours after chemotherapy administration.

Table 6-5: **Folate Results for Absorption/Interaction Pilot Trial**

Folic Acid (ref >900 nmol/L)			
Allocation No	Baseline	24 hour	72 hour
AT1 DxS	2452	1999	2507
AT2 SxC	2146	2667	2374
AT3 BxG	1580	1682	1741
AT4 NCB	1528	1471	1600
AT5 NxP	1478	1498	1442
AT6 AxB	1501	1773	2033
Average	1780.83	1848.33	1949.5
SD	414.26	445.28	429.22

Folic Acid (ref >900 nmol/L)			
Allocation No	Baseline	24 hour	72 hour
PA015AJM-VB	3369	3004	2916
PA009BJS - DB	2968	2600	
PA012EBS - OP	2881	3513	3386
PA043MME-AB	2261	2589	2289
Average B Vits	2869.75	2926.5	2863.66
SD	458.11	436.07	550.36
PA011GMB - VP	2502	2023	2273
PA049EMD - DP	2321	2420	2095
Average Placebo	2411.5	2221.5	2184
SD	127.98	280.72	125.86

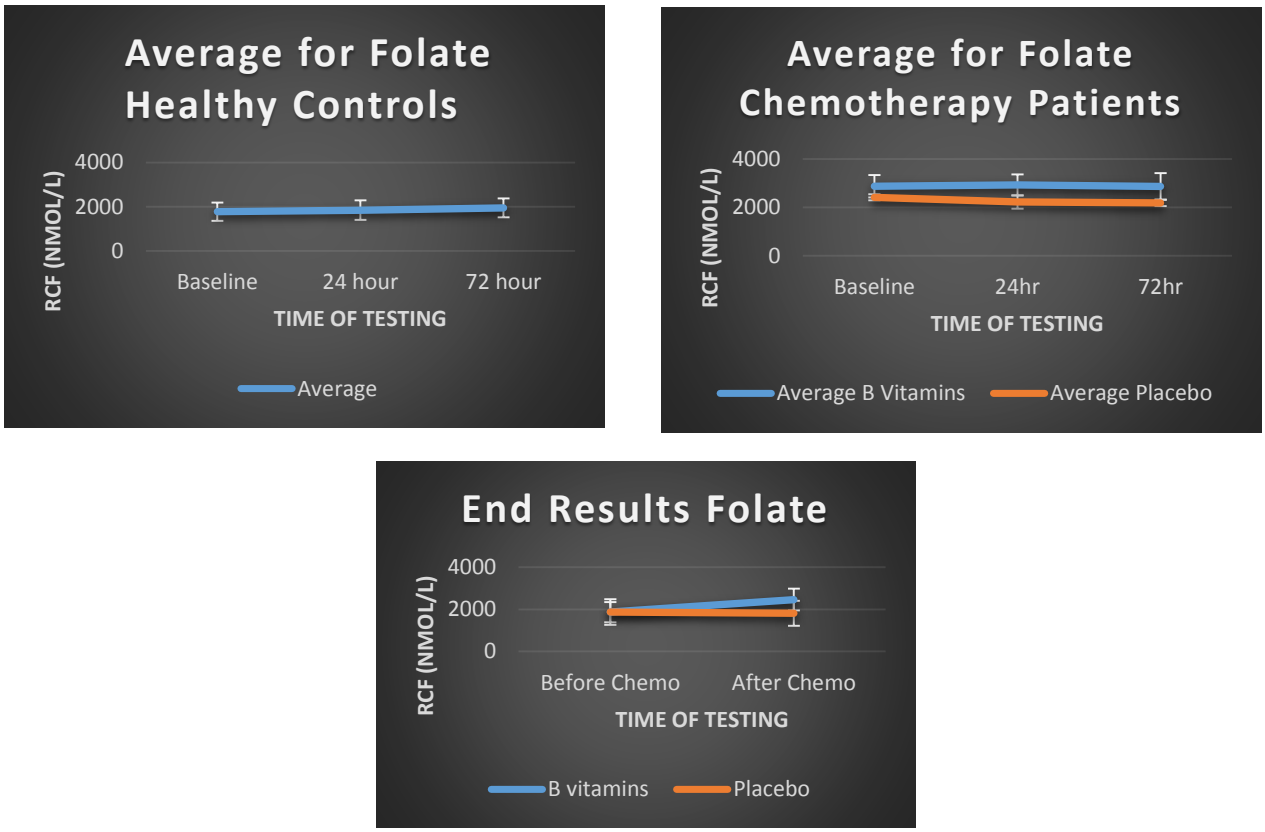


Figure 6-4: **Folate Results for the Absorption/Interaction Pilot Trial**

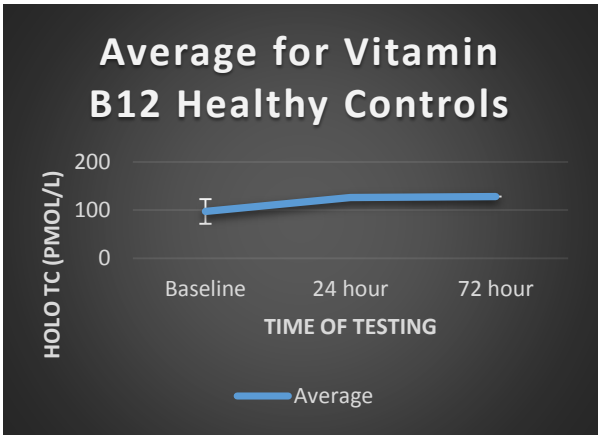
6.3.2.5 Vitamin B12

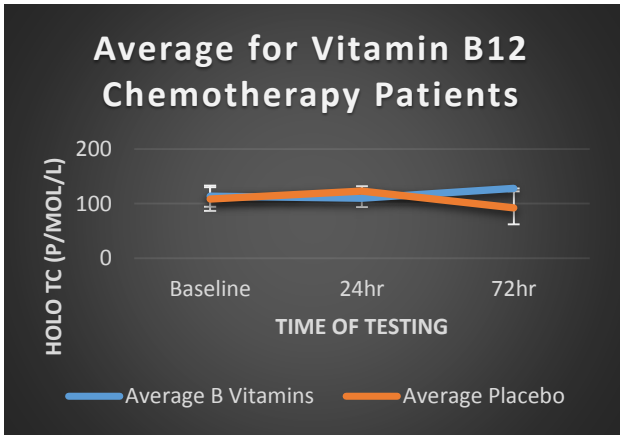
An unknown factor that the test chosen for vitamin B12, the Holo TC is only measured to 128 pmol/L was not discovered until this study was conducted. The company, Sullivan Nicolaides Pathology who conducted the assays did not communicate this fact to us, nor did the researchers ask. So, the results of this study are impaired as the true increase cannot be seen. There are no results higher than 128 pmol/L. Therefore, the healthy controls showed that supplementation over 72 hours reached maximum result capacity as seen in Table 6-6 and Figure 6-5. The chemotherapy participants had mixed results. The B vitamin arm decreased their vitamin B12 status by 3.5% twenty-four hours after chemotherapy and then increased 14% by seventy-two hours. The placebo arm increased their vitamin B12 status twenty-four hours after chemotherapy by 12% and then decreased 25% by seventy-two hours.

Table 6-6: Vitamin B12 Results for Absorption/Interaction Pilot Trial

Vitamin B12 (Holo TC) (ref >35 pmol/L)			
Allocation No	Baseline	24 hour	72 hour
AT1 DxS	128	128	128
AT2 SxC	128	128	128
AT3 BxG	97	128	128
AT4 NCB	76	121	128
AT5 NxP	72	123	128
AT6 AxB	82	128	128
Average	97.16	126	128
SD	25.34	3.16	0

Vitamin B12 (Holo TC) (ref >35 pmol/L)			
Allocation No	Baseline	24 Hours	72 Hours
PA015AJM - VB	128	128	128
PA009BJS - DB	92	96	
PA043MME-AB	122	106	128
Average B Vits	114	110	128
SD	19.28	16.37	0
PA011GMB - VP	128	128	78
PA012EBS - OP	112	128	127
PA049EMD - DP	85	113	72
Average Placebo	108.33	123	92.33
SD	21.73	8.66	30.17





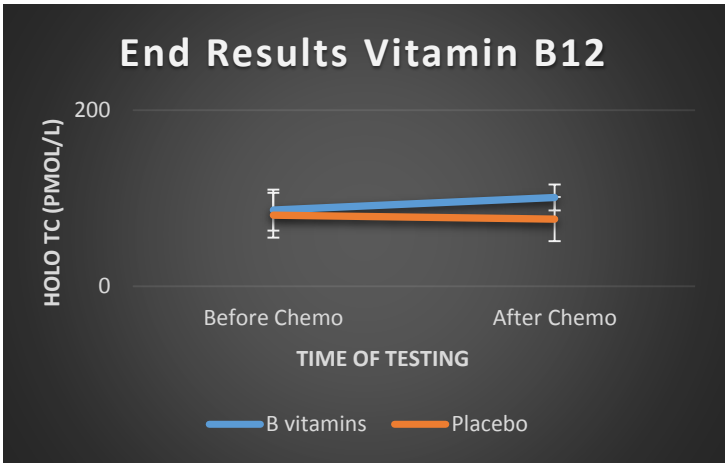


Figure 6-5: Vitamin B12 Results for Absorption/Interaction Trial

6.4 DISCUSSION

The absorption/interaction study before discussing the data does not indicate any results due to the low number of participants and the fact there was no healthy controls on placebo. Thi is a major weakness within the pilot trial and further more substantial trials are required to substantiate any information obtained from this trial. The low number of participants was due to ethic clearance and their concern of further venous puncture on patients who are already receiving numerous blood test and needle interventions. The healthy controls in retrospect should have been divided into two groups, one B vitamin and one placebo to ascertain any differences. The orginal decision for the healthy volunteers to all take the B vitamin oral supplement was due to the low numbers of participants. By having all healthy volunteers taking the B vitamin, it was surmised to give a better median result than only two to three people in one group.

Each B vitamin had varing results. Vitamin B1 blood levels increased over the seventy-two hours for the healthy volunteers (18%) which was expected, however it was hypothesised that the vitamin B1 status would decrease after chemotherapy. This occurred with the B group vitamin arm (decreased 15% by 24 hours) however the placebo arm increased at 24 hours after the chemotherapy administration (8%) and further still at 72 hours (40%). The increase seen in the placebo arm may be due to the glucose release by the liver after chemotherapy administration.

The cofactor of Vitamin B1, TDP, acts as a cofactor for transketolase and for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes as discussed in chapter 1 [374] page 41-42. All of these enzymes play a fundamental role in intracellular glucose metabolism. As seen in diabetic patients, an increased requirement for these enzymes has been found in high blood glucose status [374]. Therefore, this may explain the increase of TDP in the serum in the placebo arm. The body adapted to the higher requirement, wllhereas it is assumed that the B group vitamin arm may have had a higher saturation level in the tissues, therefore the body did not have to increase its production of TDP to adapt to the high blood glucose requirement. The blood tests of the study participants before and after chemotherapy as seen in Figure 6-1 indicates that the placebo group did decrease its thiamine level after chemotherapy thereby depleting the vitamin B1 status, whereas the B vitamin arm increased from baseline.

For vitamin B2, a decrease in vitamin B2 was seen in the B vitamin supplemented chemotheapy arm (18%) compared to placebo (4%) and then both arms increased in blood status over seventy hours (B vitamin=15%; placebo=9%). This slight increase in both placebo and B vitamin groups indicates no changes in this B vitamin after chemotherapy administration.

Comparing the results with the final blood results of the main trial seen in Figure 6-2, both B vitamin and placebo groups showed a decrease in vitamin B2 status from the commencement of chemotherapy to the completion of chemotherapy. It was expected that vitamin B2 levels would have increased with supplementation, however this was not the case. The reason for this may lie with vitamin B6. Vitamin B2 is the main cofactor for the enzyme pyridoxine phosphate oxidase which is the enzyme that converts the inactive vitamin B6 to the active form in the liver [58]. The results of the vitamin B6 assays showed a marked increase of circulatory pyridoxal-5-phosphate (P5P) which his the active form of vitamin B6 [58]. This dramatic increase of vitamin B6 and the use of vitamin B2 to assist in this activation may be why the vitamin B2 levels decreased.

Also, this may explain why the vitamin B2 increased at 72 hours after chemotherapy as it had a higher requirement. In addition, its role in other co-enzymes such as glutathione reductase, thioredoxin reductase, monoamine oxide, and its involvement with the electron transport chain, choline metabolism and 5-methyl tetrahydofolate [58] may also play a key role in the explaination of the B2 status.

Interpreting the high levels of vitamin B6 seen in Table 6-4, perhaps excess circulating vitamin B6 due to supplementation is temporarily decreased, whereas if the circulating levels are only reaching adequate amounts of vitamin B6 as seen in the placebo arm then no difference in circulating P5P (vitamin B6) levels are noted. A number of reasons for this occuring include the chemotherapy agent binding to the circulating P5P or interferance with the molecule in the blood however a plausible explanation for this is beyond the scope of this study at present. An answer may be found with the NMR completion studies that will be conducted in the future. Overall, supplementation throughout chemotherapy and at the completion of chemotherapy has a marked increase in P5P.

For folate status, all participants were found to have adequate amount of circulating folate. All chemotherapy participants had a higher baseline than the general healthy volunteers (B vitamins= 38% higher; placebo=26% higher). The end results of the study seen in Figure 6-4 found that the overall baseline for cancer patients was similar to the healthy controls, therefore it may have been the patients selected for this trial that had a higher baseline folate status. The placebo participants also showed a minor decrease after chemotherapy administration (8.5%) however overall, the final blood B group vitamin results indicated very little difference from baseline to after completion of chemotherapy course. The B vitamin group showed no significant differences during chemotherapy administration although increased in the final blood results by approximately 500 nmol/L. High folate intake has been linked with health concerns such as reduced cognitive function [375] and colon cancer [376, 377]. Continual supplementation with folic acid is not suggested, so considering the high initial

levels of folic acid, patients who consume a multi-vitamin or vitamin B complex regularly should be careful of high folic acid status.

The assay for Vitamin B12 as stated did not exceed 128 pmol/L, which means the true vitamin B12 status of patients and healthy volunteers were unable to be obtained. However, changes in vitamin B12 status were seen in Figure 6-5. According to Vu's [62] study in 1993, it was expected that vitamin B12 would be temporarily decreased with chemotherapy administration. The B vitamin group showed a small decrease after chemotherapy administration (3.5%) than an increase due to supplementation (14%). The placebo group however, showed an increase after chemotherapy (12%) than a marked decrease within 72 hours (25%). This response may be due to the impact on the liver from the chemotherapy. It has been found that liver damage or disease may release stored vitamin B12 therefore showing high vitamin B12 levels [378, 379]. Hence, the increase 24 hours after chemotherapy administration may indicate liver damage than the decrease at 72 hours is the actual status of vitamin B12. Overall, the final blood results do not show any significant changes in vitamin B12 status after chemotherapy administration.

In summary, a slight decrease in vitamin B1, B2 and B12 blood levels and a marked decrease in vitamin B6 were seen over twenty-four hours for patients supplemented with B vitamins undergoing chemotherapy compared to healthy volunteers. For the patients on placebo, a slight decrease was seen in vitamin B6 and folate after chemotherapy with vitamin B1 and B12 status increasing twenty-four hours after chemotherapy administration. Vitamin B1 continued to increase seventy-two hours after chemotherapy administration while vitamin B12 levels dropped for patients on placebo.

Overall, no information of substance can be extracted from the information obtained from this pilot trial. The small number of participants and the small amount of changes noted makes it impossible to interpret correctly and give any substantial findings.

Limitations were noted for this pilot trial and the results may not be a true indication of what occurs for patients B vitamin status after chemotherapy administration as stated above as it may only be a chance finding due to the following limitations identified.

The limitations include:

a. Low number of participants (no power analysis was conducted. The decision on the number is due to ethical clearance.)
b. More than one chemotherapy agent (in retrospect to test only one chemotherapy agent for this pilot clinical study would have been more scientifically meaningful.)
c. The Holo TC as the vitamin B12 pathology test as the assay is stopped at 128. Using serum vitamin B12 would have been a better choice for this study.

d. The healthy volunteers were not randomised. In retrospect, it would have been beneficial to randomise the healthy volunteers to three receiving the B vitamin supplement and three receiving the placebo. Or if ethically approved, an increase number of both chemotherapy patients and the healthy volunteers to be able to conduct statistical analysis on the data collected.

If the researchers could do this study over again, ideally it would have been better to test only one chemotherapy agent with 20 participants at a particular time during their chemotherapy treatment. This may have been at the beginning and at the last chemotherapy administration to see a comparison. As this is not the case, the researchers assessed the results as stated.

The results of this study do not answer the question of the hypothesis regarding B vitamin status during chemotherapy but do give a snap shot as to what may be occurring for some chemotherapy patients. B vitamin status may potentially be compromised by chemotherapy administration. However, further research is required.

6.5 CONCLUSION

In summary, no specific information can be obtained from the data collected from this pilot trial due to the low participant numbers and changes noted. The results show only a snap shot of a small number of chemotherapy patients B vitamin status after one chemotherapy administration. Further information on B vitamin status for chemotherapy patients will have more statistical power in the main trial seen in chapter 7.

7 CHAPTER 7 – STUDY 3: MAIN RESEARCH PROJECT

7.1 INTRODUCTION

The main clinical trial was conducted to assess the efficacy and safety of an oral vitamin B complex formulation over placebo that was posited to reduce the development of CIPN. The aim was to recruit between ninety and one hundred and thirty-five participants however, due to the complexity of recruitment and time restraints of completing a post doctorate this clinical trial was ceased after 71 participants were recruited. The reduced number of participants lowered the power of the study but still gave a result.

7.2 METHODS

7.2.1 Purpose

The purpose of the clinical trial was to evaluate the efficacy and safety of a vitamin B complex to reduce the incidence and severity of oxaliplatin-induced; the taxane class-induced; and vincristine-induced CIPN.

7.2.2 Design

The design of the clinical study was a single-blinded placebo-controlled randomised clinical trial (RCT). The main purpose of the RCT was to assess the prevention of CIPN through oral supplementation of a vitamin B complex. The end-point classification was efficacy and safety. All participants that adhered to the inclusion and exclusion criteria and signed an informed consent were then randomised before the commencement of any chemotherapy cycle. The subjects continued till the completion of the chemotherapy treatment regimens and were then followed three months' post-chemotherapy. The follow up assessment post-chemotherapy was to evaluate the possibility that participants in the clinical study may have developed a delayed CIPN from certain chemotherapy agents (i.e. the Taxane class, vincristine and oxaliplatin) [380]. The complete time of involvement for participants who consented for this clinical trial varied between six to nine months in duration depending on their chemotherapy regimen.

7.2.3 Primary Outcome

The primary outcome for this clinical trial was the occurrence of peripheral sensory and motor neuropathy as defined by changes in the TNS, which is a measurement tool for the development of

CIPN. The TNS measurement tool can be found in Appendix 2. An independent neurologist at the PA hospital, who was blinded to the allocation of the participants, conducted the TNS examination. The same neurologist conducted all neurology tests on all participants.

7.2.4 Secondary Outcomes

There were four secondary outcome measures used, namely the MD Anderson Brief Pain Inventory, EORTC Quality of Life Questionnaire, Patient Neurotoxicity Questionnaire and patient diaries. In addition, blood pathology tests were conducted before and after chemotherapy for each participant and included the serum levels of vitamins B1, B2, B6, folate and vitamin B12. The questionnaires were completed at baseline, three months, six months and nine months depending on the participant's chemotherapy regimen. All subjects completed participant diaries throughout the administration of their chemotherapy cycles, and three months' post-chemotherapy cessation. The patient neurotoxicity questionnaire was completed by the participants and entered into their diaries every second week as shown in Appendix 3. The comparison of results between the two arms addressing the occurrence of CIPN, pain and changes in blood status are the secondary outcomes.

7.2.5 Participants

Following a power and sample size analysis (section 4.5.3 and table 4-2), the aim of the clinical trial was to recruit ninety participants [plus 50% attrition rate]. This equated to one hundred and thirty-five subjects. Participants were a mixture of males and females recruited primarily from the Princess Alexandra Hospital in Brisbane, Australia from selected oncologists, haematology oncologists and cancer care coordinators. The participants were randomised into two groups:

Group 1: Chemotherapy plus oral vitamin B complex

Group 2: Chemotherapy plus placebo (microcrystalline cellulose plus 1% of beta-carotene for colour)

Recruitment was ceased after seventy-one participants had been inducted due to time constraints imposed for completion of a post doctorate degree. The recruitment of patients was slow due to I) the stringent/tight inclusion and exclusion criteria; ii) the number of patients prescribed the selected chemotherapy regimens; iii) the willingness of subject participation in the clinical trial; iv) subjects' transport issues to and from the PA Hospital; v) access to the patients who were prescribed the chemotherapy agents in time to arrange testing for the clinical trial and; vi) the mental state of the prospective participants following the diagnosis of cancer and having to undergo chemotherapy (e.g. feelings of being too overwhelmed).

7.2.6 Key Inclusion Criteria

1. Participants who were newly diagnosed with a neoplastic disease and chemotherapy naïve;
2. Participants who were prescribed chemotherapy treatment with oxaliplatin, the taxane class or vincristine.

7.2.7 Key Exclusion Criteria

1. Participants who had been prescribed any other concurrent investigational products;

2. Participants who currently experienced peripheral neuropathy;

3. Participants who had undergone chemotherapy treatment previously with a neurotoxic agent;

4. Participants who were pregnant or breast feeding;

5. Any participant that had established cognitive impairment, diagnosed alcoholism, intellectual disability or severe mental illness;

6. Any participants that were taking concurrent natural supplements such as multivitamins, nutritional and/or herbal supplements or fish oil formulations.

7.2.8 Dose and Duration of Supplements

Participants were randomised to a B group vitamin [1 capsules x b.i.d] or placebo [1 capsules x b.i.d] taken with or after meals. Duration of the intervention was either six or nine months starting at the commencement of chemotherapy, throughout the participant's chemotherapy regime and three months' post-chemotherapy completion.

7.2.9 Study Site and Ethics Approval

The study sites were the University of Queensland and the Princess Alexandra Hospital, Brisbane, Australia. The University of Queensland Ethics Committee (UH 2010000749) and the Princess Alexandra Hospital Ethics Committee (HREC/10/QPAH/140) granted ethical approval for this project. The trial was registered with the TGA for clinical trial notification (CTN form) and with the Australian New Zealand clinical trial registry (ACTRN12611000078954). Furthermore, the RCT trial was audited twice from the Princess Alexandra hospital ethics governance committee during the course of 2.5 years.

7.3 RESULTS AND OVERVIEW

In total, one hundred and twenty-one participants were screened for eligibility and fifty of the subjects were excluded due to not meeting the inclusion and exclusion criteria, or for being too overwhelmed with diagnosis of a cancer. The full list of details for exclusion have been presented in Table 7-1.

Seventy-one participants were randomised to either the vitamin B complex arm or placebo. Thirty-eight participants were randomised to the vitamin B complex arm and thirty-three to the placebo arm. Of the participants randomised, three were excluded from the vitamin B complex arm and six from the placebo group leaving thirty-five participants to receive the vitamin B complex and twenty-seven to receive placebo; that is a total of sixty-two evaluable subjects.

Additional drop outs were recorded at three-months follow up. At this time point there were twenty-seven participants available for assessment in the vitamin B complex and twenty-three in the placebo arm, for a total of fifty evaluable subjects. Eight subjects were not available due to drop out or other reasons in the vitamin B complex arm and four in the placebo arm. The full list of reasons for drop out can be seen in Table 7-2. By the six-month follow up review, twenty-six participants were available for assessment in the vitamin B complex arm with eleven participants finishing the trial at this time point. Twenty-two participants were available for assessment in the placebo arm with eleven completing the trial. At nine-months there were fourteen participants available for assessment in the vitamin B complex arm and eleven in the placebo arm. Therefore, forty-seven participants (66.2%) completed the clinical trial from randomisation. The final attrition rate was 14%. A diagrammatic representation of the RCT trial is presented in Figure 7-1.

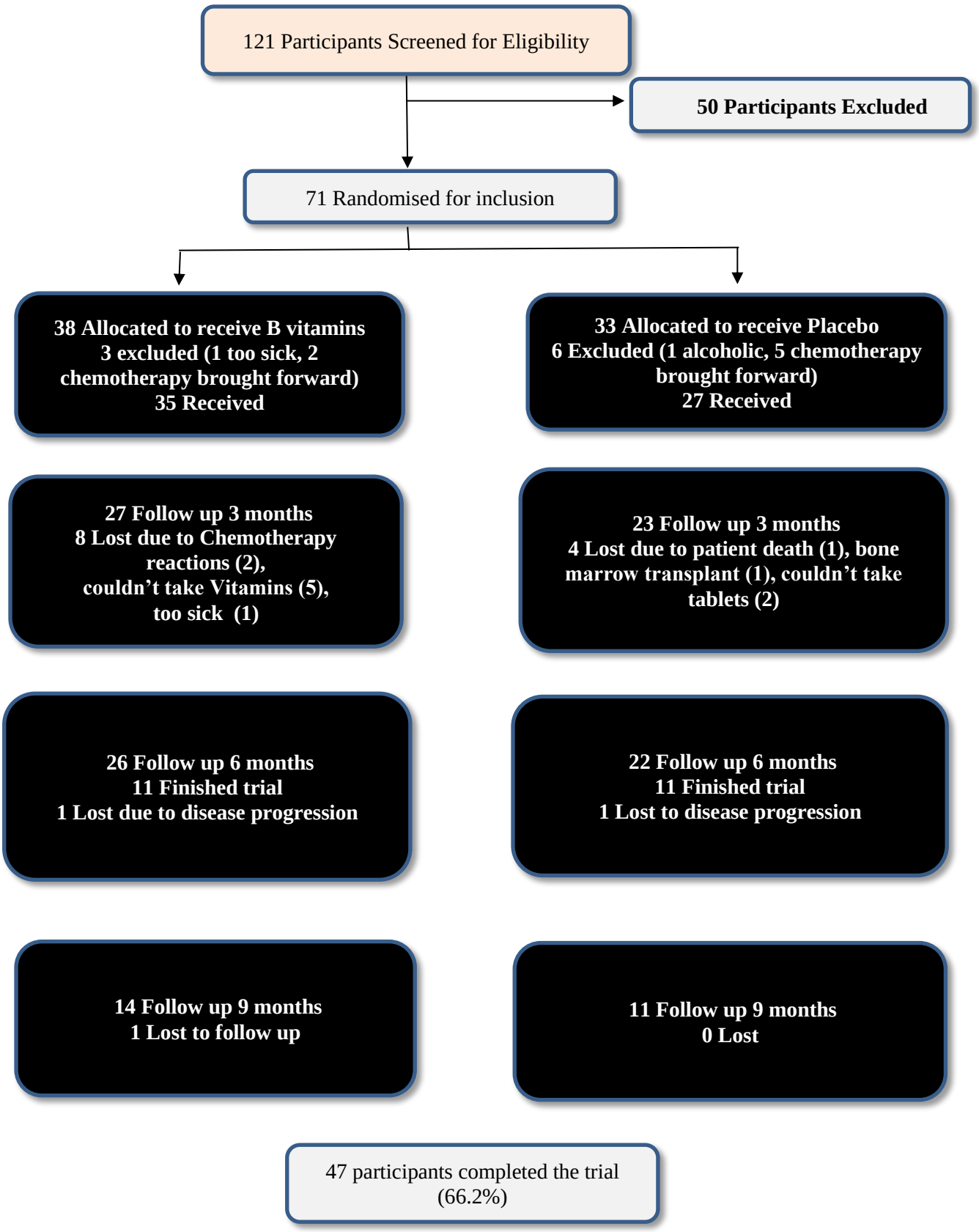


Figure 7-1: Diagrammatic View of the RCT for B Vitamins in the Protection of CIPN.

Table 7-1: Reason for Exclusion

Number	Reason for Exclusion
21	Did not meet inclusion/exclusion criteria • 11 had their chemotherapy brought forward before our testing • 2 declined chemotherapy • 3 had peripheral neuropathy • 1 had chemotherapy before • 2 alcoholics and/or used recreational drugs • 2 in another study – Vitamin D
14	Too overwhelmed with diagnosis
6	Transport issues
4	Said they didn't want to be in the study
3	Poor English – didn't understand trial
2	Didn't turn up for testing

Table 7-2 Reason for Withdrawn or Drop Out

Participants	Reason
9	Excluded (2 due to B12 deficiency, 4 failed Neurology test, 2 too sick to get neurology test completed- in patients, 1 too high in vitamin B6 levels)
7	Withdrew due to not being able to take the capsules
1	Died
2	Disease progression after chemotherapy
2	Reacted to their chemotherapy regime and their chemotherapy were ceased. I.e. Sweet's syndrome and pulmonary artery spasms with increased risk of heart attack
1	Now getting bone marrow transplant
1	Too sick to get to neurology test after chemotherapy completed
1	Lost to follow up for 9 month (last follow-up) – had skin cancer cut out of his face and had developed dementia – couldn't remember his appointments

7.3.1 Descriptive Statistics

The descriptive statistics have been divided up into the total cohort of the trial and compared the results of the vitamin B complex arm to the placebo arm.

7.3.1.1 Total Cohort Demographics

There were more female participants than males (67.6% vs 32.4%) and the taxane class was the most common chemotherapeutic agent administered (66.2%). Due to poor recruitment of colorectal cancer patients administered oxaliplatin (5.6%), recruitment for this demographic group of subjects was ceased after 6 months. This was decided as a statistical significant investigation for oxaliplatin administration in the RCT was deemed not achievable. Therefore, the RCT focused on recruiting breast and lymphoma patients. Lung cancer patient recruitment was ceased after one and half years into the RCT trial due to the poor survival rate experienced by patients inducted into the RCT (note: one patient died during treatment from chemotherapy toxicity and radiation; and two had disease progression after treatment).

The average age of subjects inducted into the study was approximately 55 years of age and the majority of participants were over-weight or obese (average BMI 30). The majority of participants were still very active (67.8% ECOG performance score) with limitations mainly due to recent surgery, particularly breast cancer patients or lymphoma impingement. Two participants with a score of 3 on the Eastern Cooperative Oncology Group (ECOG) performance scale were excluded from the trial. The ECOG[23] performance score is a set of scales and criteria that is used by clinicians and researchers to assess how a patient's disease is progressing or how the disease is affecting the patient's daily living ability [381].

The main race of participants was Caucasian (81.7%), 14% of patients were still continuing to smoke (lung and breast cancer participants), 47.9% consumed alcohol on a weekly basis and just over 90% consumed caffeine daily. No subjects stated that they were vegans on this clinical trial or history of

[23] ECOG – Is the Eastern Cooperative Oncology Group which as presented a set of scales and criteria that is used by doctors and researchers to assess how a patient's disease is progressing or how the disease is affecting their daily living ability.

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.

358. Oken MM., C.R., Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP, *Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group.* Am J Clin Oncol, 1982. **5**:649-655.

being a vegan. One participant was a current vegetarian and one subject stated they had been a vegetarian previously for twenty years but were not now. This data was collected to focus on vitamin B12 as vitamin B12 can only be obtained from animal foods. A long history of being a vegan or a vegetarian indicates the participant could have low stores of vitamin B12 or could be deficient in vitamin B12. The subject who was a current vegetarian was found to have adequate vitamin B12 levels (PA 042 NCB Vitamin B12=58 pmol/L at baseline and >128 pmol/L after chemotherapy with ref >35). This dietary information substantiated that the cohort was consuming adequate amounts of vitamin B12.

PA042 NCB who stated that they were a current vegetarian was randomised to the B vitamin arm and did develop a delayed CIPN. The baseline TNS score was 2, which was due to decreased reflexes, no sensory or motor nerve peripheral neuropathy. After chemotherapy, the participants TNS score was 5, which entailed a score of 1 for sensory peripheral neuropathy (mild) and 4 for reflexes indicating no reflexes. At three months follow up post-chemotherapy, the TNS score for PA042 NCB was 14. This consisted of 1 for sensory peripheral neuropathy, 1 for pin sensibility, 1 for vibration, 4 for reflexes, 4 for sural nerve and 3 for peroneal nerve. Therefore, the neurologist was unable to locate sural nerve activity and found limited nerve activity in the participant's peroneal nerve.

This delayed CIPN cannot be attributed to low vitamin B12 as the participant's B12 levels were in normal range before and after chemotherapy administration. The subject's vitamin B6 status was elevated post-chemotherapy (1880 nmol/L in ref 35-110 nmol/L). Elevated vitamin B6 has been associated with the development of peripheral neuropathy although normally from a high supplemental dose (>500 mg) over nine months to a year [102]. Further discussion on vitamin B6 can be seen on pages 199 and 220.

The full demographics of the total cohort is presented in Table 7-3:

Table 7-3: Descriptive Statistics of Total Cohort

Chemotherapy Patients	Demographics of Total Cohort	
	Variable	**Number (%)**
Age	54 ± 12 years Mean ± SD	
Weight	85 ± 21.5 kg Mean ± SD	
BMI	30 ± 7 kg/m^2 Mean ± SD	
Gender	Males Females	23 (32%) 48 (68%)
Chemotherapy agents	Vincristine Oxaliplatin Taxanes	20 (28%) 4 (6%) 47 (66%)
Types of Cancer	Breast Lymphoma Lung Large Bowel Prostate Endometrial	36 (51%) 20 (28%) 9 (13%) 4 (7%) 1 (1%) 1 (1%)
ECOG	0 1 2 3	46 (65%) 20 (28%) 3 (4%) 2 (3%)
Race	Caucasian NZ Maori Sri Lankan) Italian Indigenous American Samoan Chilean	58 (82%) 5 (7%) 1 (1% 1 (1%) 2 (3%) 1 (1%) 1 (1%)
Smokers	14%	2.7 ± 7.1 / day
Alcohol consumption	48%	3.6 ± 9.5 / week
Caffeine consumption	90%	3.5 ± 2.9 / day
Vegetarians	1.6%	
History of vegetarian	1.6%	
Red meat consumption	97%	3.1 ± 1.8 per week
White meat consumption	97%	2.6 ± 1.4 per week
Fish and seafood consumption	70%	1.4 ± 1.5 per week

7.3.1.2 Demographics/Descriptive Statistics for Group A and B

The two research groups were randomised and appropriately matched at baseline. However, due to early recruitment termination, more participants were randomised to the vitamin B complex group rather than the placebo group. Females comprised 78.7% of the participants' randomised to the vitamin B complex arm of the study compared to 60.6% in the placebo arm. The distribution of chemotherapy agents were well matched with the taxanes being the main chemotherapeutic agent assessed. The vitamin B complex arm versus placebo for taxanes consisted of 68.4% versus 63.6% of the participants respectively. Of those, the majority were docetaxel administrations in both groups (50% versus 36.4% respectively).

For vincristine and oxaliplatin, there were equal numbers for each arm (10 vincristine and 2 oxaliplatin in each arm). Breast cancer was the major type of cancer in both arms (20 vs 16 subjects), lymphoma had equal numbers (10 in each arm), lung cancer consisted of 4 subjects in the vitamin B complex arm and 5 in the placebo arm and there were 2 in each arm for subjects diagnosed with large bowel cancer. Additional participants included a subject diagnosed with prostate cancer and another with endometrial cancer. The prostate cancer patient was included as he received ten administrations of docetaxel and the endometrial cancer patient was included as she received six administrations of docetaxel combined with carboplatin.

Age, weight and BMI were similar in each arm as were smoking habits. Alcohol consumption was found to be much higher in the vitamin B complex group compared to placebo (63.2% vs 30.3% respectively) and caffeine consumption was slightly higher in the vitamin B complex group (94.7% vs 84.9%). These differences may be due to the slightly higher participant numbers in the vitamin B complex arm. As mentioned, there was only one vegetarian so the majority in both arms consumed adequate amounts of animal protein for vitamin B12 consumption.

Table 7-4 provides an overview of the descriptive statistics for group A and B.

Table 7-4: **Descriptive Statistics of Group A and B**

	Group A: B Vitamins N=38			**Group B: Placebo N=33**		
Gender	Female	28	74%	Female	20	61%
	Male	10	26%	Male	13	39%
Chemotherapy Agent	Taxanes	26	68%	Taxanes	21	64%
	a) Docetaxel	19	50%	a) Docetaxel	12	36%
	b) Paclitaxel	7	18%	c) Paclitaxel	9	27%
	Vincristine	10	26%	Vincristine	10	30%
	Oxaliplatin	2	5%	Oxaliplatin	2	6%
Types of cancer	Breast cancer	20	53%	Breast	16	49%
	Lymphoma	10	26%	Lymphoma	10	30%
	Lung	4	11%	Lung	5	15%
	Colon	2	5%	Colon	2	6%
	Prostate	1	3%			
	Endometrial	1	3%			
ECOG	0	24	63%	0	21	63%
	1	12	31%	1	9	27%
	2	2	5%	2	1	3%
	3	0	0%	3	2	6%
Age	53.8 ± 12.6			55.2 ± 12		
Weight	82.6 ± 17.6			87.1 ± 25.4		
BMI	29.4 ± 6.3			31.1 ± 7.2		
Smoking	13.2% 2.8 ± 7.7 per day Median ± SD			15.15% 2.6 ± 6.4 per day Median ± SD		
Alcohol consumption	63.2% 3.3 ± 5.5 per week Median ± SD			30.3% 4 ± 12.8 per week Median ± SD		
Caffeine consumption	94.74% 3.5 ± 3.3 per day Median ± SD			84.8% 3.4 ± 2.4 per day Median ± SD		
Vegetarian	2.6%			0%		
History of vegan	2.6%			3%		
Red meat consumption	97.4% 2.81 ± 1.6 per week Median ± SD			97% 3.33 ± 2.04 per week Median ± SD		
White meat consumption	97.4% 2.44 ± 1.24 per week Median ± SD			94% 2.66 ± 1.53 per week Median ± SD		

Fish and seafood consumption	63.2% 1.13 ± 1.29 per week Median ± SD	78.8% 1.75 ± 1.56 per week Median ± SD

The demographics of the participants in each group were similar with the majority in both groups being Caucasian (81.6% vs 81.8%) and married (60.5% vs 60.6%). There were similar childhood diseases in both arms and allergies were found more prevalent in the vitamin B complex arm compared to placebo (52.1% vs 29.6%). Full descriptions of the history of participants can be seen in Table 7-5.

Table 7-5: **History of Group A and B**

	Criteria	**Group A: B Vitamins N=38**	**Group B: Placebo N=33**
Race	Caucasian	31 (81.6%)	27 (81.8%)
	New Zealand	2 (5.3%)	3 (9.1%)
	Indigenous	2 (5.3%)	0
	Italian	1 (2.6%)	0
	Asian	1 (2.6%)	1 (3%)
	American Samoan	0	1 (3%)
	Chilean	1 (2.6%)	0
Social History	Married	23 (60.5%)	20 (60.6%)
	Divorced	5 (13.2%)	5 (15.2%)
	Widowed	5 (13.2%)	1 (3%)
	Significant Other	3 (7.9%)	2 (6.1%)
	Engaged	1 (2.6%)	0
	Single	1 (2.6%)	5 (15.2%)
Childhood illnesses	Measles	22 (57.9%)	17 (51.5%)
	Mumps	12 (31.6%)	12 (36.4%)
	Chicken pox	20 (52.6%)	18 (54.5%)
	Other	Sandy fly eye infect = 1 TIA = 1 German measles = 2 Fainted = 1	Hepatitis = 2 Tonsillitis = 1 Yellow fever = 1
Allergies	Drugs	20 (52.6%)	15 (45.5%)
	Environment	9 (23.7%)	4 (12.1%)
	Food/Beverages	8 (21.1%)	2 (6.1%)

The participants in this clinical trial had a number of other co-morbidities. The main adult disease that both groups experienced was cardiovascular disease, with approximately 36% in each arm being treated for hypertension. Asthma and respiratory problems had a high prevalence with 36.8% in the vitamin B complex arm and 39.4% in the placebo arm. Patients with diabetes are known to experience peripheral neuropathy [341] and if they have been diagnosed with a cancer they have also been simulataneously administered chemotherapy [382]. Subjects who had been diagnosed with diabetes were included in the RCT if no current or previous peripheral neuropathy was known. Subjects that were assessed by a neurologist and had no neuropathy were then subsequently inducted into the clinical trial if they adhered to all other inclusion and exclusion criteria. Overall, two participants in the vitamin B complex arm and four in the placebo arm had diabetes and three borderline diabetics in the placebo group were allowed to participate in the RCT.

Two of six participants in the clinical trial who were diagnosed with diabetes on inclusion, subsequently developed CIPN. Both were in the vitamin B complex group and a borderline diabetic in the placebo group also developed mild CIPN. Not all other participants with diabetes developed CIPN. Although these were only small subject numbers, it indicated that vitamin B complex did not prevent CIPN in subjects who have been diagnosed with diabetes and were undergoing chemotherapy with a neurotoxic agent. There may be a potential to develop CIPN if a patient with diabetes is prescribed a vitamin B complex.

A potential confounder that could have interfered with the neurology assessment for nerve status was arthritis. In the vitamin B complex arm 36.9% of participants and 39.3% in the placebo arm experienced arthritis, or had accidents leaving physical discomfort. Arthritis particularly in the knee and lower extremities, as well the hands, interfered with ECS scores as the neurologist was unable to detect nerve function due to possible damage from the arthritis. Therefore, baseline for sural nerve activity in particular was difficult, although after chemotherapy and follow up had the same result. Arthritis or damage from accidents does not predispose the patient to CIPN like diabetes, but it does interfere with research quantifying nerve function. Table 7-6 shows the full list of adult diseases that the participants experienced.

Table 7-6: **Adult Illnesses of Group A and B**

Adult Illnesses	Criteria	Group A – B Vitamins N=38	Group B – Placebo N=33
Cardiovascular disease	Hypertension	14 (37%)	12 (36%)
	Hypercholesteremia	6 (16%)	5 (13%)
	Chronic heart disease	4 (10%)	4 (12%)
	Other	7 (18%)	0
Respiratory problems	Asthma	7 (18%)	7 (21%)
	Other	7 (18%)	6 (18%)
Digestive problems	Indigestion/GORD	2 (5%)	5 (13%)
	Other	5 (13%)	3 (9%)
Diabetes	T2DM	2 (5%)	4 (12%)
	Borderline diabetes	0	3 (9%)
Bone problems	Osteoarthritis	6 (16%)	8 (24%)
	Rheumatoid arthritis	2 (5%)	0
	Accidents	1 (3%)	4 (12%)
	Other	5 (13%)	1 (3%)
Other	Acne rosacea	0	1 (3%)
	Alcohol foetal syndrome	0	1 (3%)
	Anaemia	1 (7%)	0
	Arachnoid cysts	0	1 (3%)
	Bladder prolapse	0	1 (3%)
	Breast cancer	2 (5%)	0
	Carpel tunnel syndrome	0	3 (9%)
	Clubbing of fingers	0	1 (3%)
	Conn’s syndrome	0	1 (3%)
	Depression/Anxiety	3 (8%)	3 (9%)
	Endometriosis	1 (3%)	0
	Eye problems	2 (5%)	1 (3%)
	Headaches	1 (3%)	0
	Hernia	0	1 (3%)
	Kidney stones	1 (3%)	0
	Migraines	1 (3%)	3 (9%)

	NAFLD	0	1 (3%)
	Obesity	1 (3%)	3 (9%)
	Restless legs / cramping	1 (3%)	1 (3%)
	Skin problems	4	3 (9.1%)
	SLE	1 (3%)	0
	Sleep apnoea	1 (3%)	1 (3%)
	Stroke/TIAs	1 (3%)	2 (6%)
	Telangiectasia around mouth	0	1 (3%)
	Thyroid problems	4 (10%)	4 (12%)
	Vertigo	0	1 (3%)
	Viruses	18 (47%)	8 (24%)

Similar to adult illness and diseases, this cohort has undergone a number of surgical interventions. For the breast cancer and colorectal participants, they all had tumour removal surgical interventions related to their cancer before chemotherapy was commenced. Female patients who had no children had been encouraged to undergo *in vitro* fertilisation (IVF) to cryopreserve embryos before chemotherapy commencement. Three of the participants inducted into the clinical trial had undergone IVF prior to chemotherapy for this reason.

PA019 MJW who was diagnosed with lung cancer had a history of two previous breast cancer associated lumpectomies. This participant was chemotherapy naïve and as such was included in the clinical trial and inducted as a lung cancer subject with the associated chemotherapy regime for this cancer type. PA015 AJM was a lymphoma participant who had a history of a prostatectomy for prostate cancer and who was also chemotherapy naïve and as such was also inducted into the clinical trial. A complete list of all surgical interventions from both the vitamin B complex and placebo arms has been presented in Table 7-7.

Table 7-7: **Past Surgery of Group A and B**

Surgery	Criteria	Group A: B Vitamins N=38	Group B: Placebo N=33
Breast	Lumpectomy	22 (58%)	7 (21%)
	Mastectomy	10 (26%)	6 (18%)
	Fibrous adenoma	2 (5%)	0

	Breast implants	1 (3%)	0
Gynaecological	Hysterectomy	6 (16%)	1 (3%)
	Cone biopsy	4 (10%)	1 (3%)
	Caesarean	3 (8%)	4 (12%)
	Laparoscopy	2 (5%)	2 (6%)
	Tubal ligation	2 (5%)	1 (3%)
	Egg harvest / IVF	4 (10%)	1 (3%)
	Cysts on ovary	1 (3%)	0
	Fibroid	0	1 (3%)
Male	Prostate removal	1 (3%)	0
	Testacies (orchandectomy)	2 (5%)	2 (6%)
Digestion	Biopsy	0	3 (9%)
	Bowel resection	3 (8%)	1 (3%)
	Lap band	1 (3%)	2 (6%)
	Gall bladder removed	5 (13%)	8 (24%)
	Laparotomy	2 (5%)	0
	Tummy tuck	1 (3%)	0
	Appendectomy	7 (18%)	6 (18%)
	Hernia	3 (8%)	3 (9%)
	Colonoscopy	1 (3%)	0
Skeletal	Accidents	7 (18%)	3 (9%)
	Knee	5 (13%)	2 (6%)
	Upper limbs	3 (8%)	3 (9%)
	Back fusion	2 (5%)	0
	Hips	1 (3%)	0
	Carpel tunnel	0	2 (6%)
CVD	Open heart surgery	0	2 (6%)
	Stents	3 (8%)	0
	Pacemaker	0	1 (3%)
Other	Tonsils	2 (5%)	7 (21%)
	Lipomas	2 (5%)	1 (3%)
	Kidney donation	2 (5%)	0
	Skin cancers	1 (3%)	3 (9%)

	Eye operations	0	4 (12%)
	Veins stripped	0	3 (9%)
	Lung operations	0	3 (9%)
	Ear operations	0	3 (9%)
	Teeth extractions	1 (3%)	1 (3%)
	Cysts	1 (3%)	1 (3%)
	Facial	2 (5%)	0
	Pheochromotcytoma	0	1 (3%)
	Spleen	0	1 (3%)
	Thyroid	0	1 (3%)

The prescription medications documented in this trial detailed in Table 7-8 is representative of the number of adult illnesses and diseases the participants expressed. It was important to identify medications that could interfere with the absorption of an oral vitamin B complex. Medications such as protein pump inhibitors have previously been reported to cause a vitamin B12 and iron deficiency [383]. Metformin has also been associated with a vitamin B12 deficiency [384, 385]. The participants who were diagnosed with diabetes and were participating in this clinical trial had all been prescribed metformin (2 in vitamin B complex arm and 4 in the placebo arm). Research reports have shown that only long-term administration of metformin have been associated with metformin-induced vitamin B12 deficiency [384, 385]. As such, all subjects participating in the clinical trial and who were taking metformin, were found to have an adequate vitamin B12 status.

Table 7-8: **Current Medication of Groups A and B**

Current Medication	Criteria	Group A: B Vitamins N=38	Group B: Placebo N=33
Digestion	Proton pump inhibitor	5	10
	Maxalon	1	0
	Movicol	2	1
	Coloxyl	2	1
	Sulfasalazine	1	
	Nausea	0	2
	Bentyl	0	1
Sleep / Anxiety	Tamazapam	3	4
	Valium	2	1
	Oxazepam	1	0

	Restavit	1	0
Depression	Anti-depressant	5	10
Hypertension	Beta Blocker	3	5
	Calcium channel blocker	1	3
	ACE inhibitor	3	1
	Angiotensin II receptor blocker	4	8
	Anti-angina	2	0
	Vasodilator	0	1
	Aldosterone inhibitor	0	1
	Diuretic	0	2
Respiratory	Bronchodilator	2	7
	Spiriva	1	2
	Israel	0	1
	Salbutamol plus	0	1
	Nasal Spray	1	0
Pain	Endone	1	0
	OxyContin	1	0
	Nurofen	1	0
	Paracetamol	2	5
	Endep	1	0
	Mersyndol	1	0
	Inza	0	1
	Fentanyl	0	1
	CorVel	0	1
	Tramadol	0	1
Cholesterol	Statins	8	6
Infections	Antibiotics	4	1
Ear	Sofradex	1	0
Eye	Glaucoma drops	1	0
Anticoagulants	Aspirin	5	7
	Warfarin	2	0
Thyroid	Oroxine	1	3

	Neomercazole	0	1
Diabetic medication	Metformin	2	4
	Gliclazade	0	1
	Diamicron	0	1
	Glucobay	1	0
	Glycade	1	0
Other	Allopurinol	1	0
	Anti-histamine	0	1
	Epilium	0	1
	Femara	1	0
	Fluticasonel/Salmeterol	1	0
	Golgout	0	1
	Hydrochlorothiazide	1	0
	Imigrine	0	1
	Mirina	0	1
	Paraven	1	0
	Prednisolone	1	2
	Suboxane	0	1

7.3.2 Statistics Results for the Main trial

The statistics for this trial were analysed using SPSS statistics software with the assistance of statistician Ms Helen Gramotnev and A/Prof Luis Vitetta. The statistical analysis was based on an intention to treat analysis. All statistical tests and graphics were generated using SPSS version 17.0 or Excel software. The criterion for significance was set at $p<0.05$. The odds ratio was calculated on both the total incidence of CIPN and the subject's perception (PNQ).

Not all statistical analysis for the primary and secondary outcomes assessing both paired T scores and P score using a Mixed Effects Model (MEM) were statistically significant, see Table 7-9. The only time point that was statistically significant was the differences in time on the P score indicating that there was a significant difference in time for the development of CIPN, a known fact as CIPN is a cumulative side effect [10].

No difference was observed between vitamin B complex and placebo groups in the prevention of CIPN. The TNS sensory and motor neuropathy sections, which were tested by the independent neurologist did not show any statistical differences between the treatment groups except for motor

neuropathy at baseline. This was more probable due to the type of cancer subjects had been diagnosed with, such as a lymphoma, lung cancer or from surgical intervention for a breast cancer. The actual test for neuropathy was assessable post-chemotherapy and three months' post-chemotherapy. Neither sensory nor motor neuropathy through the TNS assessment was statistically significant as summarised in Table 7-10 using Chi-squared and Fisher exact tests. An independent t-test and Mann Whitney test was conducted on the TNS, which was reported in Table 7-11 with no statistical significance noted. Therefore, an oral vitamin B complex was not statistically significant in preventing CIPN in this clinical trial.

Table 7-9: **Statistics for Primary and Secondary Outcomes**

Mixed Effects Model						
Outcomes	Placebo v B vitamins		Differences in Time		Placebo v B vitamins over 3 time points	
Scores	T Score	P score	T score	P score	T score	P score
Primary Outcome						
TNS	1.24	0.22	2.41	**0.02**	-0.35	0.73
Secondary Outcomes						
NPQ Sensory	0.85	0.4	0.61	0.55	0.84	0.4
NPQ Motor	0.73	0.47	0.51	0.61	0.48	0.63
NPQ Other	-0.05	0.96	-1.43	0.16	1.13	0.26
EORTC QoL	0.74	0.46	-0.67	0.5	0.28	0.78
Total Pain Score	-0.12	0.9	-0.35	0.73	0.56	0.58
Total Pain Interference	-0.9	0.37	-1.21	0.23	1.51	0.13

Table 7-10: **Neurologists Assessment of Sensory and Motor Nerve Function through TNS of the B Vitamin Arm compared to the Placebo Arm**

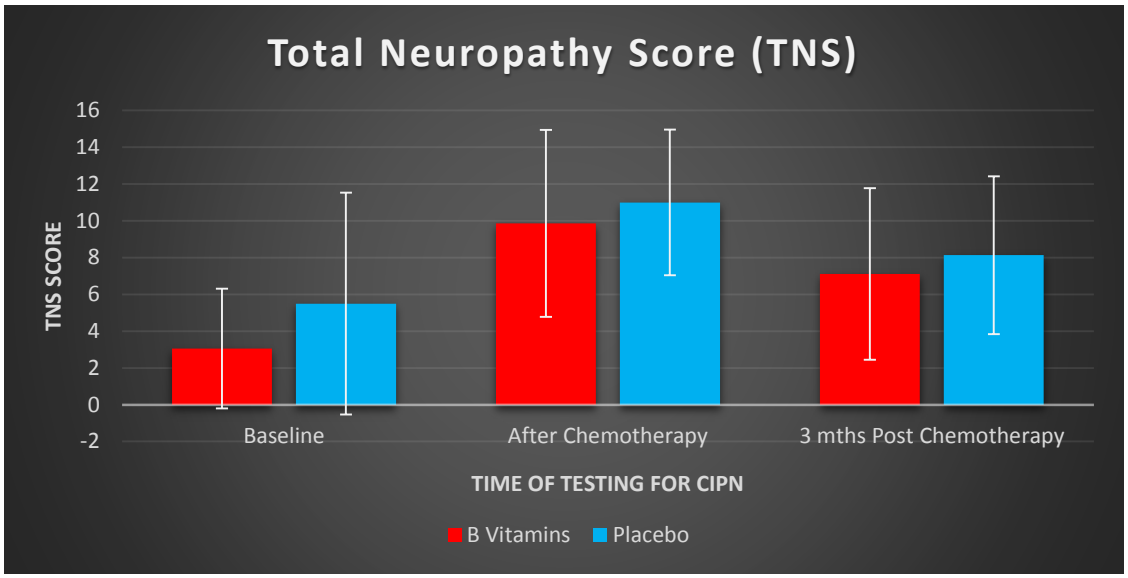
Y/N results: p values			
		Chi-sq	Fisher
Before Chemotherapy	Sensory	0.551	0.42
	Motor	**0.002**	**0.003**
Post-Chemotherapy	Sensory	0.11	0.094
	Motor	0.797	0.508
Three weeks Post-Chemotherapy completion	Sensory	0.961	0.596
	Motor	0.464	0.334

Table 7-11: **Independent Samples T-test and Mann Whitney Test for TNS of the B Vitamin Arm compared to the Placebo Arm**

Independent samples t-test for total TNS scores		
	t-score	p value
Before Chemotherapy	-1.412	0.163
Post-Chemotherapy	-0.893	0.376
Three weeks Post-Chemotherapy	-0.559	0.579
Mann Whitney for TNS scores: p values		
Before Chemotherapy	0.15	
Post-Chemotherapy	0.329	
Three weeks Post-Chemotherapy	0.424	

7.3.2.1 Primary Outcome Results

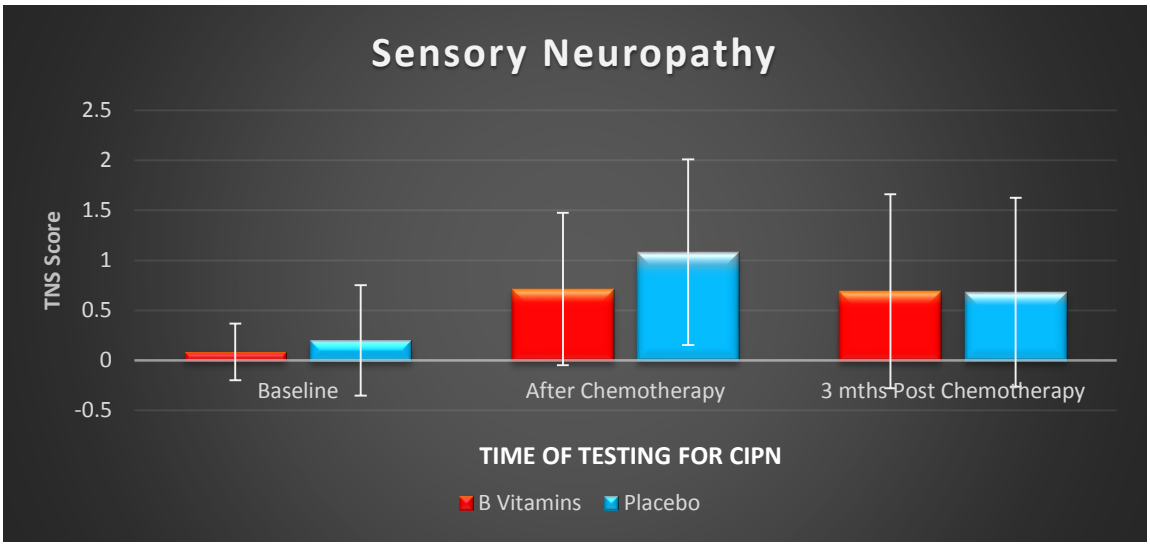
The TNS score before chemotherapy to post-chemotherapy between the B vitamin and placebo arms was the primary outcome for this study. As seen in Figure 7-2, no differences were noted. Although the vitamin B complex group started slightly below the placebo group, both had a similar development of neuropathy and decreased at the three-month follow-up.



Total TNS			
	Baseline	After Chemo	3 mths f/u
B vitamins	3.05 ± 3.25	9.86 ± 5.08	7.12 ± 4.67
Placebo	5.5 ± 6.02	11 ± 3.96	8.14 ± 4.29

Figure 7-2: **Results of the Total TNS score for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.**

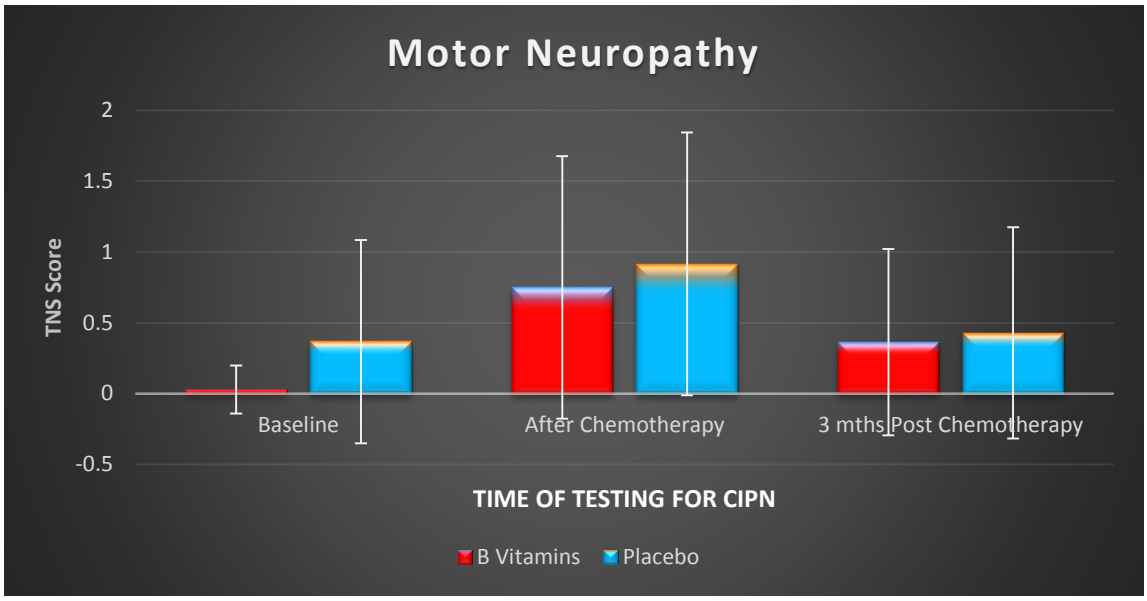
CIPN is represented by both sensory and motor nerve damage, although predominately sensory is reported from neurotoxic chemotherapy agents [386]. Sensory neuropathy is experienced by numbness, tingling, burning or pain in the fingers, and toes and then progresses up the arms or legs [387, 388]. The vitamin B complex showed a very minor trend in decreasing the development of sensory neuropathy during chemotherapy administration, but by the three-month follow-up, both placebo and vitamin B complex arms had exactly the same sensory measure of nerve function as seen in Figure 7-3. Therefore, it was possible that there was some decrease in discomfort during chemotherapy administration but overall it showed no improvement over placebo after completion of chemotherapy cycles.



Sensory			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.08 ± 0.28	0.71 ± 0.76	0.69 ± 0.97
Placebo	0.2 ± 0.55	1.08 ± 0.93	0.68 ± 0.94

Figure 7-3: **Final Sensory Neuropathy Score from the TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.**

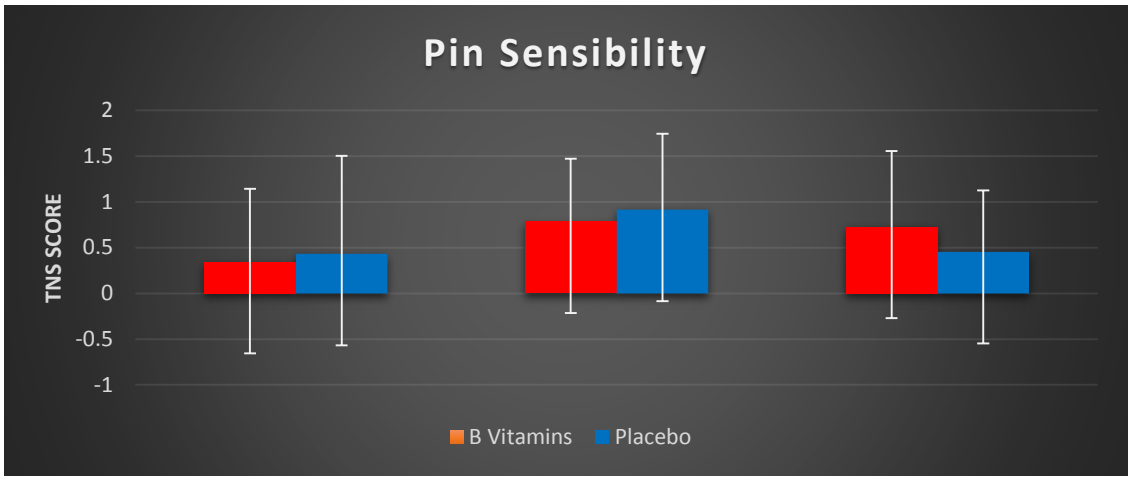
Motor nerve impairment is defined as the inability of a patient to pick up objects or the inability to use their fingers or hands normally. Moreover, it is about strength, so individuals may drop objects or may demonstrate difficulty walking [388]. The motor component started lower in the vitamin B complex arm at baseline compared to the placebo arm. However, the vitamin B complex arm had a slightly higher trajectory during chemotherapy treatment and decreased less at the three-month time point, possibly indicating that a vitamin B complex is of no assistance in decreasing motor nerve damage or motor function as seen in Figure 7-4. The "post-chemotherapy and follow up" results were important for the end-result.



Motor			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.02 ± 0.16	0.75 ± 0.93	0.36 ± 0.65
Placebo	0.37 ± 0.71	0.91 ± 0.93	0.42 ± 0.74

Figure 7-4: **Final Motor Neuropathy Score from TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.**

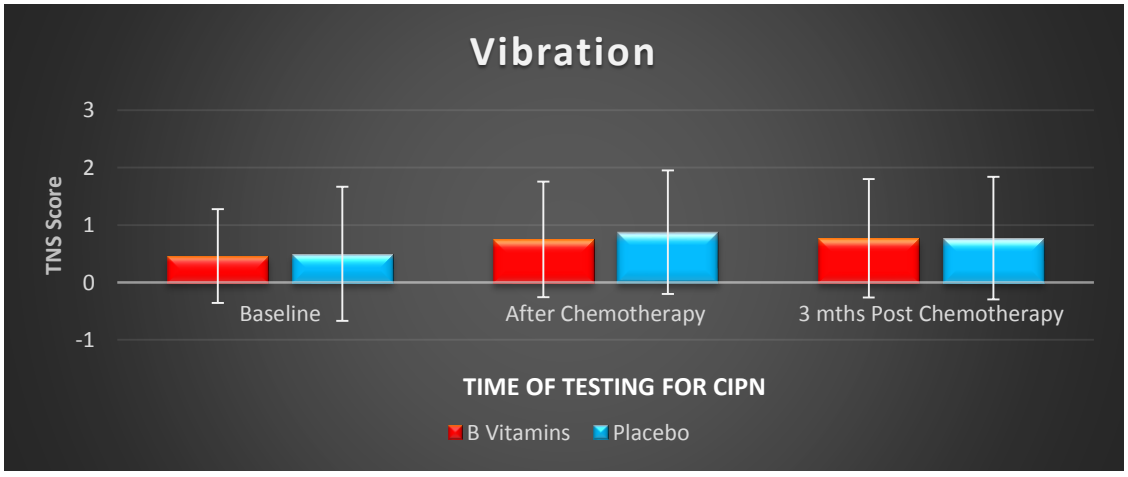
Pin sensibility is a pinprick test on the fingers and the toes, and if the participant cannot feel the pin-prick, the neurologist continues up the hand/arm and feet/leg respectively until the subject responds that a sensation can be felt. Pinprick sensibility has been reported to be a test for small nerve fibre damage [273]. The results of this test showed that a vitamin B complex did not prevent small nerve damage as seen in Figure 7-5. The participants on placebo showed that pin-prick sensation was back to baseline three months' post-chemotherapy administration, the vitamin B complex arm sensation however remained similar to post-chemotherapy. This indicated that a vitamin B complex did not prevent small nerve damage in this clinical trial.



Pin Sensibility			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.34 ± 0.08	0.78 ± 0.68	0.73 ± 0.82
Placebo	0.43 ± 1.07	0.92 ± 0.83	0.45 ± 0.67

Figure 7-5: Pin Sensibility Score from the TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.

The vibration test showed no differences between vitamin complex and placebo as seen in Figure 7-6.

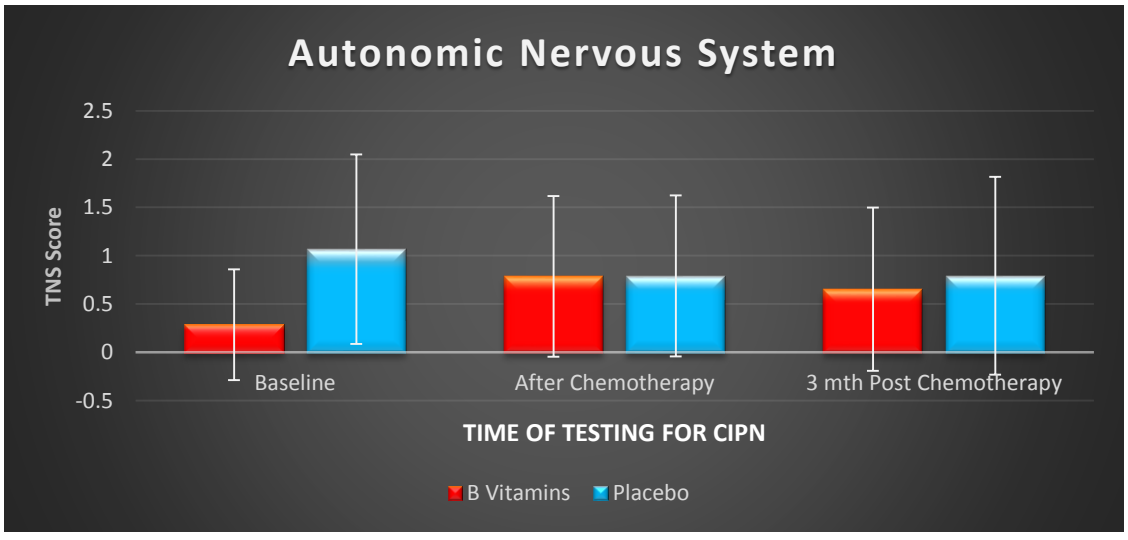


Vibration			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.45 ± 0.81	0.75 ± 1	0.77 ± 1.03
Placebo	0.5 ± 1.17	0.87 ± 1.07	0.77 ± 1.06

Figure 7-6: Vibration Results from TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.

The results from the autonomic nervous system were equivocal. The autonomic nervous system was assessed by the neurologist, by querying the participants regarding symptoms such as sweating and changes in bowel habits (diarrhoea to constipation or vice versa). The neurologist acknowledged the subjective nature of this interpretation of the investigation given that cancer itself causes autonomic NS changes. These included sweating, in addition to chemotherapy causing constipation or diarrhoea depending on the agent administered. All of the breast cancer participants experienced hot flushes after chemotherapy administration due to menopausal symptoms [389]. Lymphoma can also cause sweating [390] and therefore assessing autonomic nervous system activity and its relevance to neuropathy is a challenge.

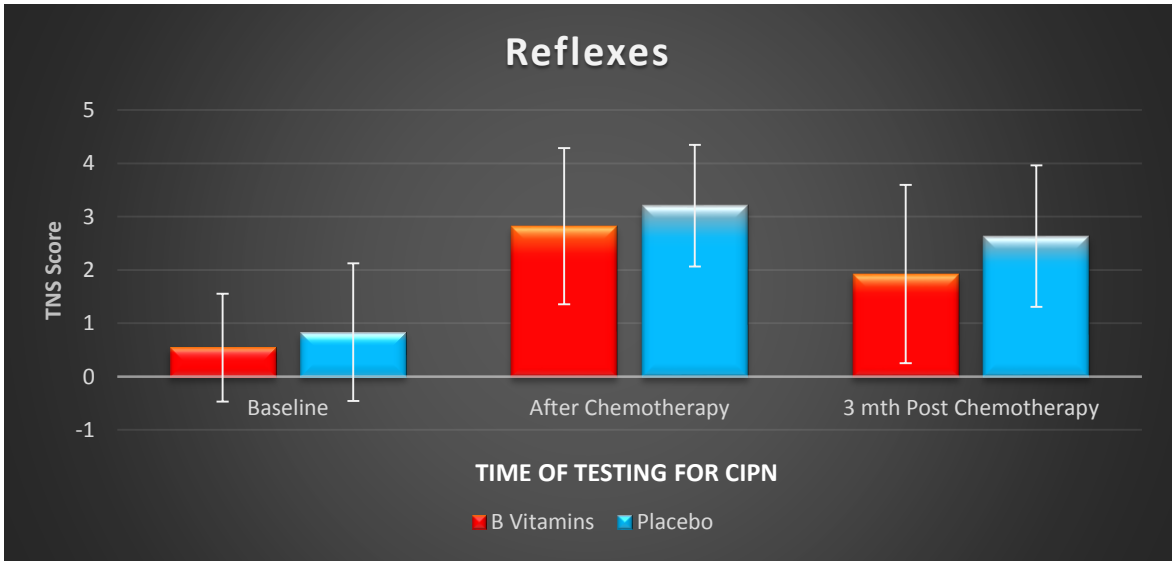
The results indicated that there was no difference between vitamin B complex and placebo as seen in Figure 7-7. The placebo arm was noted to have a higher response than the vitamin B complex arm at baseline or before chemotherapy, which was due to the lymphoma and breast cancer patients' experiencing increased sweating. Therefore, this information was not a true representation of CIPN symptoms.



Autonomic NS			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.28 ± 0.57	0.78 ± 0.83	0.65 ± 0.84
Placebo	1.06 ± 0.98	0.79 ± 0.83	0.79 ± 1.02

Figure 7-7: Autonomic Nervous System Score from TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.

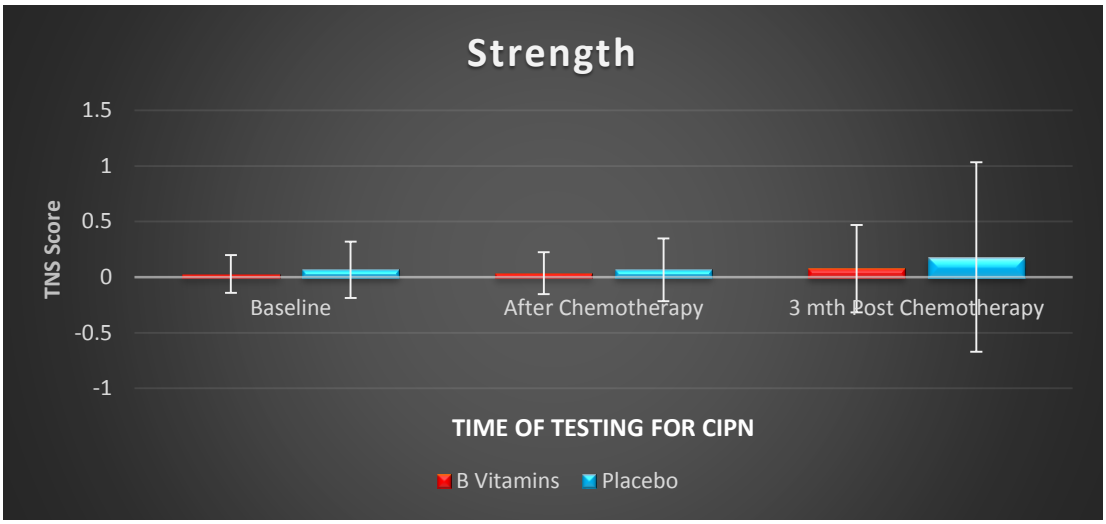
Ninety percent of all of the participants had no reflexes after administration of chemotherapy (85.7% in vitamin B complex arm and 95.8% in the placebo arm) as seen in Figure 7-8. At three months' post-chemotherapy, twenty-five percent of all participants were able to demonstrate reflex ability (34.6% in vitamin B complex arm and 13.6% in the placebo arm). This was related to CIPN and nerve damage reiterating that the vitamin B complex did not prevent CIPN development. However, participants in the vitamin B complex arm recovered their reflexes slightly faster (albeit minimally) than those in the placebo arm after three months (41.3% improvement in vitamin B complex arm compared to 30.9% improvement in placebo arm).



Reflexes			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.54 ± 1	2.82 ± 1.47	1.92 ± 1.67
Placebo	1.53 ± 1.93	3.21 ± 1.14	2.63 ± 1.33

Figure 7-8: Reflex Scores from the TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.

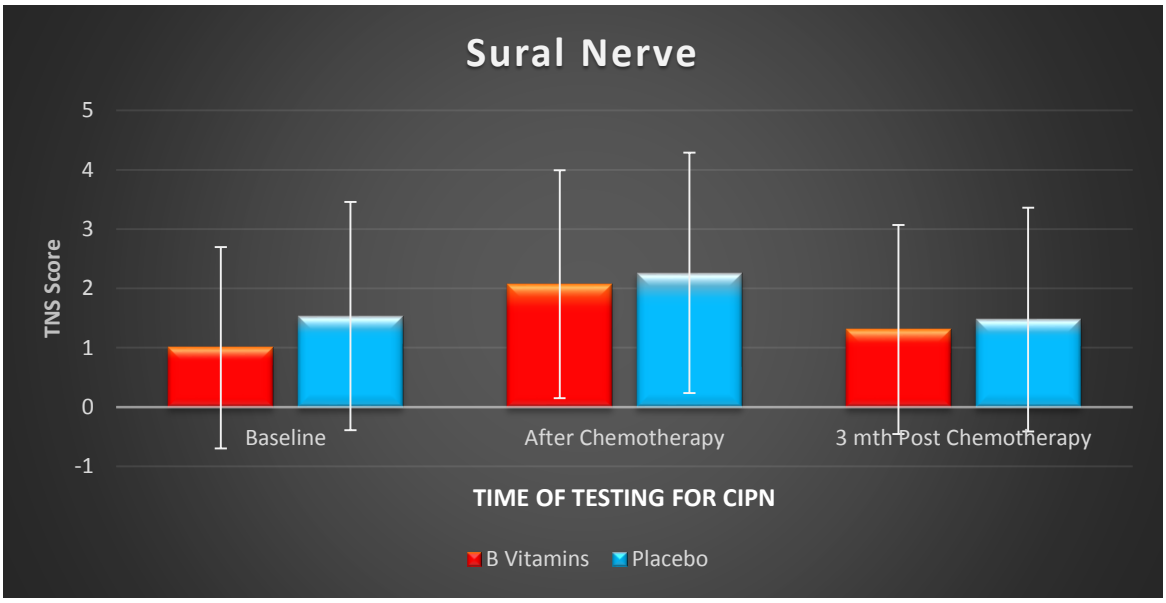
No differences were observed between the vitamin B complex and placebo arms for strength testing post-chemotherapy, nor at the three-month follow up, as depicted in Figure 7-9. This indicates minimal motor nerve damage in participants from both groups, with the exception of outlier data from four participants [see error bars on the graph]. The participants who experienced strength changes also presented with muscle weakness deemed from the effects of chemotherapy and other conditions, such as rheumatoid arthritis or restless leg syndrome.



Strength			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.02 ± 0.16	0.03 ± 0.18	0.07 ± 0.39
Placebo	0.6 ± 0.25	0.07 ± 0.28	0.18 ± 0.85

Figure 7-9: Strength Score from TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.

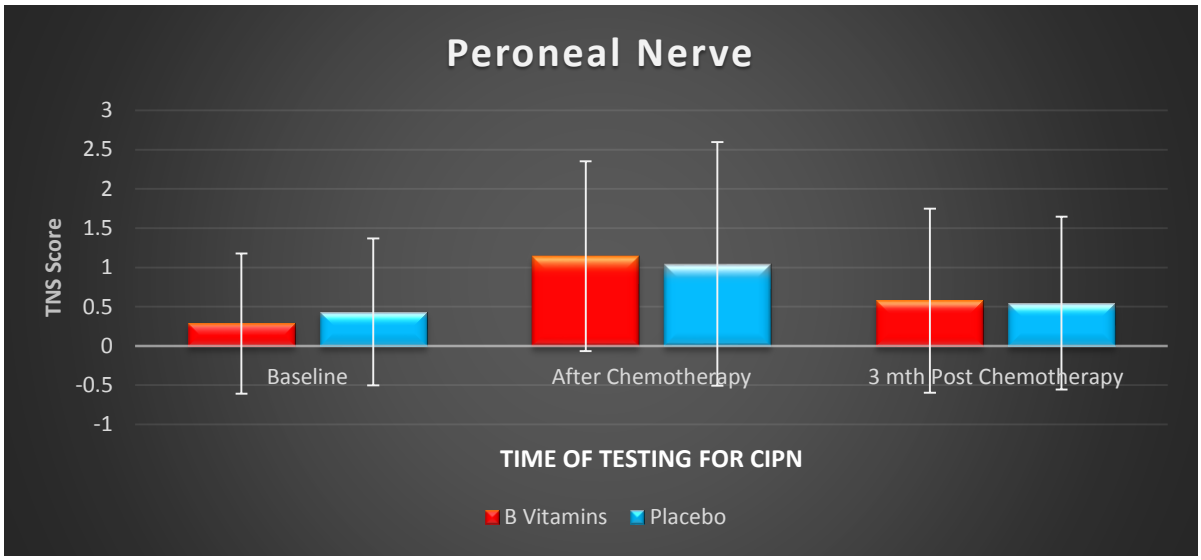
The sural nerve was assessed by the neurologist utilising the ECS. The neurologist noted that in older subjects and those with arthritis present in the lower extremities, the sural nerve was difficult to locate. In addition, a number of participants presented with oedema of the feet and ankles post-chemotherapy (PA027 CxB presented with seeping oedema as the skin had broken, as well as with disease progression post-chemotherapy and is now deceased). The oedema, which is an adverse outcome of chemotherapy administration [368] made it difficult to obtain an accurate sural nerve assessment. This result affected both arms of the study, hence no difference was observed between the two groups in relation to sural nerve scores as presented in Figure 7-10.



Sural Nerve			
	Baseline	After Chemo	3 mths f/u
B vitamins	1 ± 1.7	2.07 ± 1.92	1.31 ± 1.76
Placebo	0.2 ± 0.55	2.26 ± 2.02	1.47 ± 1.89

Figure 7-10: **Sural Nerve Score from TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.**

The neurologist, utilising the ECS, also assessed the peroneal nerve. No difference was observed in the peroneal nerve scores as shown in Figure 7-11. The vitamin B complex arm had a slightly worse projection from baseline; however, minimal difference was observed between the treatment groups.



Peroneal Nerve			
	Baseline	After Chemo	3 mths f/u
B vitamins	0.28 ± 0.89	1.14 ± 1.21	0.57 ± 1.17
Placebo	0.43 ± 0.93	1.04 ± 1.55	0.54 ± 1.1

Figure 7-11: **Peroneal Scores from TNS for Vitamin B Complex and Placebo Arms at Baseline, After Chemotherapy Cessation and Three Months Post-Chemotherapy.**

7.3.2.2 Secondary Outcomes

7.3.2.2.1 Blood Results

All participants in the vitamin B complex arm responded to supplementation with the exception for the vitamin B2 levels, as shown in Table 7-13 and Figure 7-12. The intervention group blood analysis post-chemotherapy demonstrated a significant difference for all the vitamin B complex (see Table 7-14), except vitamin B2. The decrease in vitamin B2 pathology post-chemotherapy for both arms could be due to increased urine output and utilisation within the cells. It can be postulated that vitamin B2 decreased as it is the main co-factor for *pyridoxine phosphate oxidase*, which is the enzyme that converts the different types of vitamin B6 to pyridoxal phosphate [58]. However, vitamin B2 has many functions as described in chapter 1, and with supplementation, the expectation would have been to see it increase in blood serum.

Vitamin B1 blood pathology results increased above the average reference range for the vitamin B complex arm compared to placebo, which decreased by 7% (155.31 to 144.8 nmol/L) from baseline. All standard deviations for each of the B vitamins were in the high range due to the variation of blood results. Blood vitamin B6 levels in particular, was observed to vary extensively. As seen in chapter 6

from the interaction and absorption pilot trial, blood levels of P5P (vitamin B6) was very responsive to supplementation. The same result was observed in the final data seen in Figure 7-12. Four participants in the vitamin B complex arm had blood vitamin B6 results over 1,000 nmol/L (ref 35-110 nmol/L), this was over ten times higher than the reference range shown in Table 7-12. All four participants experienced mild to moderate CIPN.

Given that vitamin B6 toxicity can cause peripheral neuropathy, it is plausible that the increased vitamin B6 blood levels (>1,100 nmol/L) post-chemotherapy may be related to the development of CIPN in these participants. It has been reported that peripheral neuropathy from high dose vitamin B6 supplementation (500-5,000 mg daily for 1-3 years) may give rise to a slow onset peripheral neuropathy [129] compared to the fast onset peripheral neuropathy experienced by chemotherapy. Vitamin B6 has been found to be beneficial for oxaliplatin triggered CIPN [17, 18], cisplatin [78] and vincristine in children [30, 125]. The participants in this RCT trial with high vitamin B6 blood levels post-chemotherapy had been administered taxanes and vincristine. As no research regarding blood vitamin B6 levels post-chemotherapy have been conducted in relation to peripheral neuropathy, and although definitive conclusions are difficult from the data from only four patients, further research is certainly warranted.

Table 7-12: **After Chemotherapy Scores for Participants over 1,000 nmol/L of Vitamin B6**

Participant	Total TNS score		Sensory Neuropathy		Motor Neuropathy		Pin Sensibility		Vitamin B6 nmol/L	
	BC	AC	BC	AC	BC	AC	BC	AC	BC	AC
PA016 GDT	6	11	0	1	0	1	0	1	530	1,290
PA042 NCB	2	14	0	1	0	0	0	0	50	1,880
PA058 MJM	1	17	0	1	0	1	0	2	80	1,560
PA062 GMA	0	21	0	2	0	1	0	1	100	1,080

NB: BC = Before chemotherapy; AC = After chemotherapy

Increasing blood vitamin B12 to reduce the incidence of CIPN was the initial investigative focus for this clinical trial. The blood results post-chemotherapy administration indicated that those participants supplemented with a vitamin B complex increased blood B12 status compared to those randomised to the placebo (Wilcoxon 0.0 vs 0.602; the vitamin B complex arm increased on average by 14 pmol/L and placebo decreased on average by 5 pmol/L). While the vitamin B12 blood levels cannot be

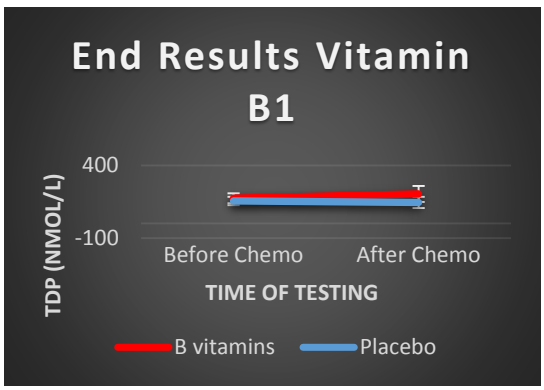
correlated with CIPN development, the result obtained confirms the data from Vu, et al's [62] study which concluded that chemotherapy may cause a temporary deficiency of vitamin B12 in patients undergoing chemotherapy.

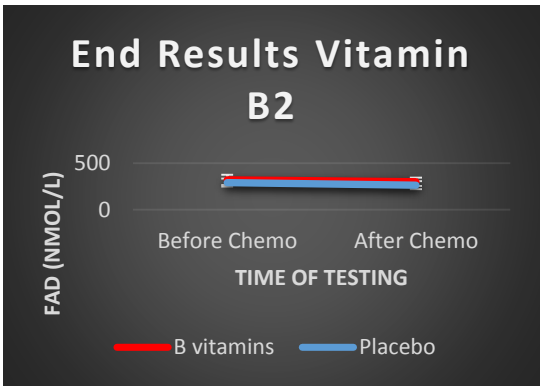
The results for blood folate levels also demonstrated that a vitamin B complex increases red blood cell levels of folate post-chemotherapy compared to placebo, see Figure 7-12. Levels of blood folate in the participants recruited for this clinical trial presented with a blood saturation level of folate prior to chemotherapy administration, which may represent the folate fortification status for foods in Australia [392].

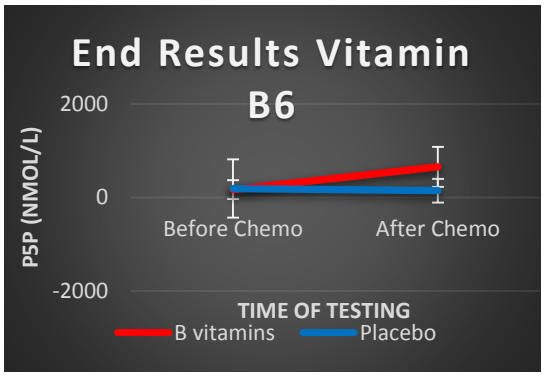
Overall, blood levels for the vitamin B complex showed that supplementation with a vitamin B complex during chemotherapy administration increased blood levels of vitamin B1, B6, B12 and folate compared to placebo.

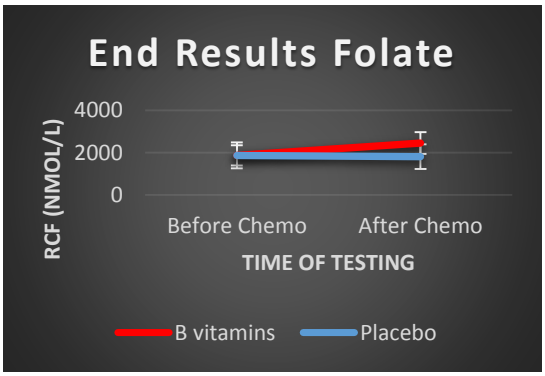
Table 7-13: Pathology Blood Results for All Participants

	B vitamin Arm		Placebo Arm		Ref Range
	Before Chemo	After Chemo	Before Chemo	After Chemo	nmol/L
B1	174.2 ± 34.3	203.9 ± 55.1	155.3 ± 28.4	144.8 ± 38.7	66-200
B2	321 ± 56.5	301.4 ± 43.9	291.9 ± 43.6	264.2 ± 42.3	180-470
B6	167.8 ± 201.9	656.9 ± 428.9	191.1 ± 624	148.1 ± 253.8	35-110
B12	86.8 ± 23.2	101 ± 14.8	80.9 ± 25.5	76.4 ± 25	>35 pmol/L
Folate	1873.3 ± 612.4	2456.6 ± 515.1	1870 ± 477.6	1814.4 ± 588.3	>900









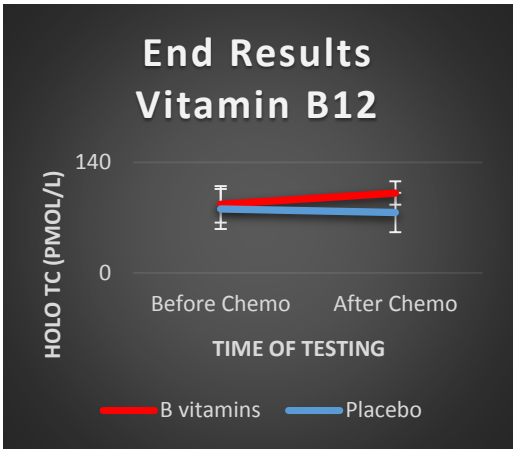


Figure 7-12: Graphic Pictures of Differences in B group Vitamin Results

Table 7-14: Statistics for Blood Pathology Results

Normality Tests: p values		
	Baseline	After Chemo
B12	**0.005**	**0**
B1	**0**	**0**
Folate	0.2	**0.044**
B2	**0.001**	0.095
B6	**0**	**0**

Wilcoxon	**Placebo**		**Treatment**
B12	0.602		**0**
B1	0.129		**0.001**
Folate	0.648		**0**
B2	**0.019**		0.196
B6	0.845		**0**

Mann Whitney U test: p values	
B12	**0.004**
B1	**0.001**
Folate	**0.002**
B2	0.645
B6	**0**

7.3.2.2.2 Comparing Blood Pathology to TNS Results

A comparison between the blood pathology results to the TNS final results was an important outcome measure for this clinical trial. This was to ascertain whether the administration of a vitamin B complex was an efficacious intervention in the prevention or an adverse outcome in promoting CIPN. Vitamin

B12 and B6 data were individually assessed for comparison to the TNS before and after chemotherapy administration. These two B vitamins were chosen due to the pathology results obtained in Table 7-13 and the statistics on the pathology blood levels seen in Table 7-14.

To ascertain normal or non-normal distribution of variables, a Stem-and-Leaf plot was developed for baseline and post-chemotherapy for the variables TNS, vitamin B12 and vitamin B6. The data was found to be not normally distributed as seen in Figure 7-13 to Figure 7-16.

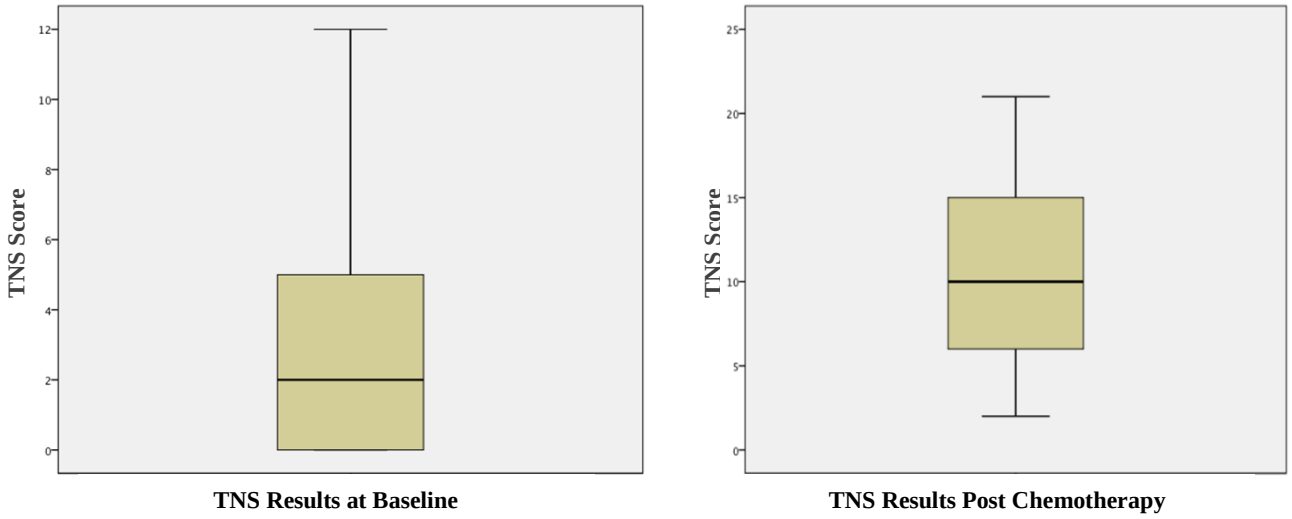


Figure 7-13: Stem-and-Leaf Plot for TNS Final Results at Baseline and Post-Chemotherapy.

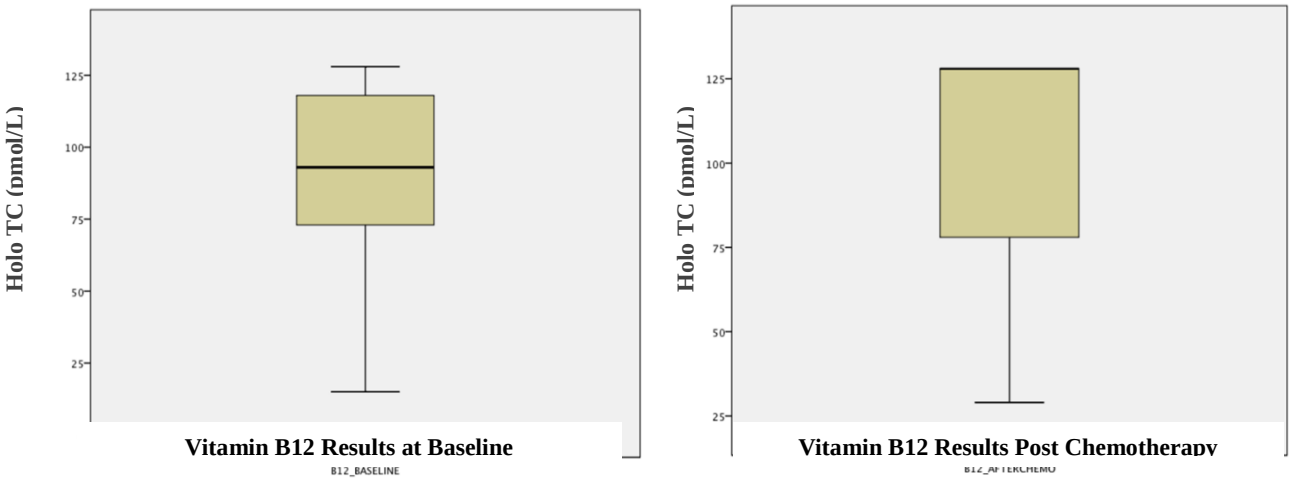
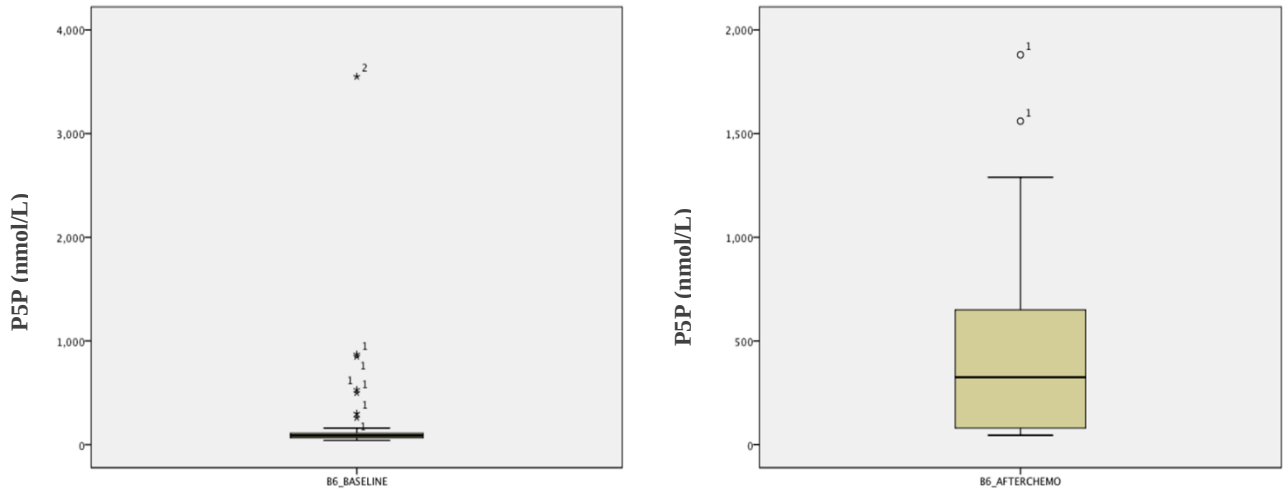


Figure 7-14: Stem-and-Leaf Plot for Vitamin B12 Pathology Results at Baseline and Post-Chemotherapy.



Vitamin B6 Results at Baseline **Vitamin B6 Results Post Chemotherapy**

Figure 7-15: Stem-and-Leaf Plot for Vitamin B6 Pathology Results at Baseline and Post-Chemotherapy.

As the data was not normally distributed, a non-parametric statistical approach was required. A non-parametric Levene's test was used to verify the equality of variances in the samples (from the vitamin B complex and placebo groups), therefore providing a homogeneity of variance (p=>0.05) [370, 371]. The results of the analysis have been presented in Table 7-15 to Table 7-20. The results found that the TNS at baseline and post-chemotherapy were not statistically significant (p=0.760; p=0.300 respectively)..

The vitamin B12 results when compared to the TNS results showed that at baseline there was no statistically significant differences (p=0.770), however the post-chemotherapy result was statistically significant (p=0.024). This data indicates that vitamin B12 statistically correlates to CIPN development. Therefore, it can be surmised that vitamin B12 may have a significant role to play as an important preventative factor in the onset and severity of CIPN development. Vitamin B6 was not found to be statistically significant before or post-chemotherapy (p=0.938; p=0.948 respectively). A possible explanation for this may be due to the high standard deviation found in the blood vitamin B6 results obtained (for the total cohort: baseline SD=513.19; Post-chemotherapy SD=442.29 indicated by normality tests).

From this analysis, administration of vitamin B12 demonstrated a statistically significant impact in decreasing the onset and severity of CIPN development. Vitamin B6 was not found to be statistically significant. The vitamin B12 result was found to be clinically significant for one of the participants who was reported to be deficient in vitamin B12 post-chemotherapy. PA032 PMP who was randomised to the placebo arm presented with moderate to severe CIPN post-chemotherapy and her blood result showed a moderate deficiency in vitamin B12. The participant was supplemented with

vitamin B12 and given an intramuscular injection of vitamin B12 by the oncologist. The CIPN was reduced to a mild deficiency two months' post-supplementation and injection. Further expansion on this participant is presented in Chapter 8.

Table 7-15: TNS at Baseline

TNS at Baseline Descriptive								
Groups	No	Mean	SD	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B vitamin	38	17.48	8.72	1.41	14.61	20.35	2.57	34.57
Placebo	32	16.80	9.76	1.72	13.28	20.32	3.17	30.33
Total	70	17.17	9.15	1.09	14.99	19.35	2.57	34.57

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.014	1	8.014	.094	**.760**
Within Groups	5769.012	68	84.838		
Total	5777.026	69			

Table 7-16: TNS Post-Chemotherapy

TNS Post-Chemotherapy Descriptive								
Groups	No	Mean	SD	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B vitamin	28	13.67	8.56	1.61	10.35	16.99	.89	27.39
Placebo	24	11.39	6.80	1.38	8.52	14.27	2.29	24.71
Total	52	12.62	7.80	1.08	10.44	14.79	.89	27.39

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	66.686	1	66.686	1.096	**.300**
Within Groups	3043.445	50	60.869		
Total	3110.131	51			

Table 7-17: Vitamin B12 at Baseline

Vitamin B12 at Baseline Descriptive								
Groups	N	Mean	SD	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B vitamin	38	17.13	8.75	1.41	14.25	20.00	.12	32.12
Placebo	32	17.78	9.89	1.74	14.21	21.35	.80	31.30
Total	70	17.42	9.22	1.10	15.22	19.62	.12	32.12

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.414	1	7.414	.086	**.770**
Within Groups	5867.525	68	86.287		
Total	5874.939	69			

Table 7-18: Vitamin B12 Post-Chemotherapy

Vitamin B12 Post-Chemotherapy Descriptive								
Groups	N	Mean	SD	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B vitamins	27	6.62	4.93	.95	4.67	8.57	4.26	20.24
Placebo	24	10.60	7.14	1.45	7.58	13.61	.73	21.77
Total	51	8.49	6.33	.88	6.71	10.27	.73	21.77

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	200.779	1	200.779	5.442	**.024**
Within Groups	1807.951	49	36.897		
Total	2008.730	50			

Table 7-19: Vitamin B6 at Baseline

Vitamin B6 Baseline Descriptive								
Groups	N	Mean	SD	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B vitamins	38	17.04	10.00	1.62	13.75	20.33	.80	37.30
Placebo	32	17.22	8.67	1.53	14.09	20.34	.80	39.20
Total	70	17.12	9.35	1.11	14.89	19.35	.80	39.20

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.542	1	.542	.006	**.938**
Within Groups	6035.204	68	88.753		
Total	6035.747	69			

Table 7-20: Vitamin B6 Post-Chemotherapy

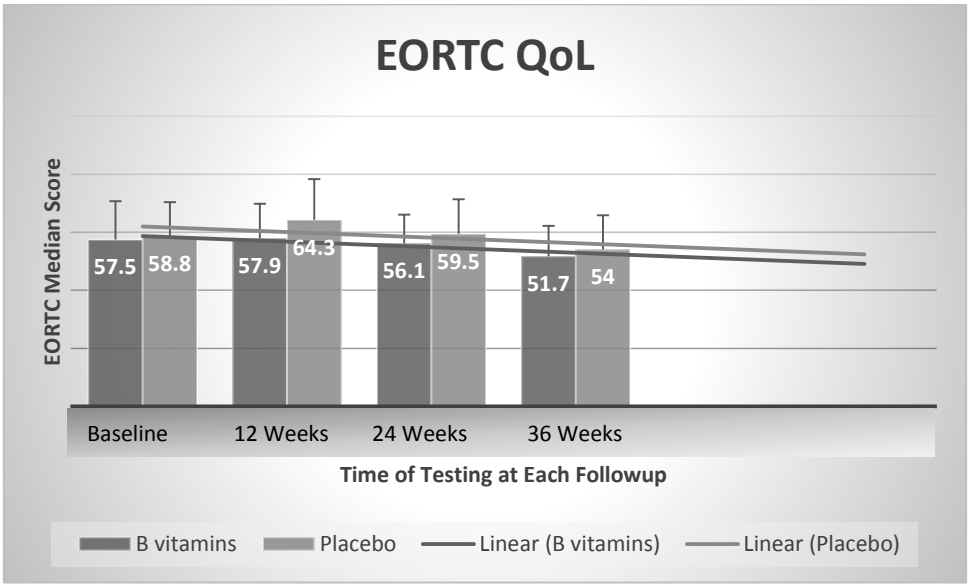
Vitamin B6 Post-Chemotherapy Descriptive								
Groups	N	Mean	SD	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B vitamins	28	8.13	5.63	1.06	5.95	10.31	.61	26.61
Placebo	24	8.01	7.38	1.50	4.89	11.13	.79	34.29
Total	52	8.08	6.43	.89	6.28	9.87	.61	34.29

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.179	1	.179	.004	**.948**
Within Groups	2110.380	50	42.208		
Total	2110.559	51			

7.3.2.2.3 Quality of Life Questionnaire

No differences were observed for quality of life between arms as presented in Figure 7-16. The best obtainable score for this questionnaire was 42 and the worst score was 114, hence the lower the score the better the quality of life experienced. The graph is expressed as the total score for the QoL. Vitamin B complex supplementation has been linked with energy production [58], and could have had an impact on the patient's quality of life but the results of this QoL questionnaire did not support

this result. The projections indicate that the participant's quality of life was similar for each arm and continually improved post-chemotherapy.

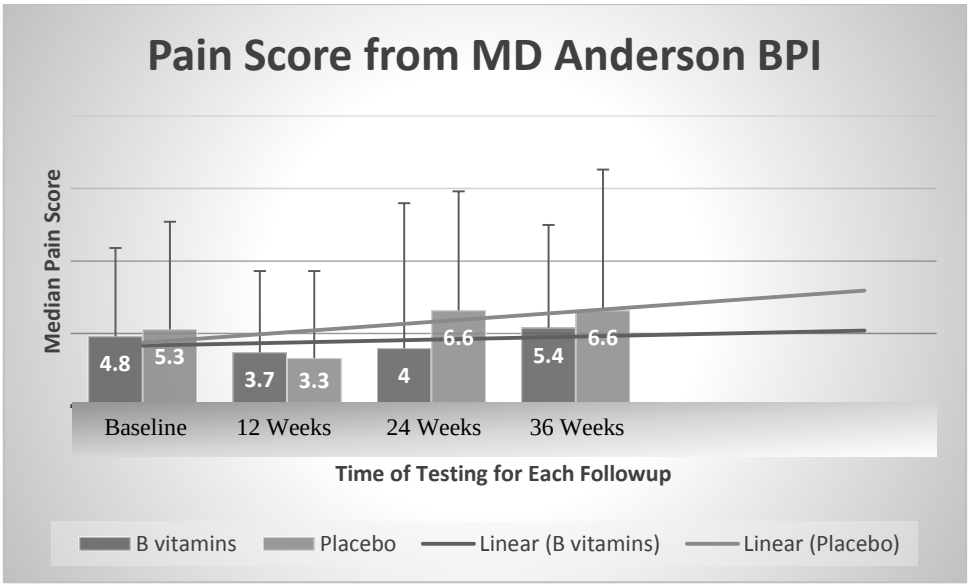


EORTC QoL								
	N	Baseline	N	12 weeks	N	24 weeks	N	36 weeks
B vitamins	38	57.5 ± 13.2	28	57.9 ± 11.9	27	56.1 ± 10	14	51.7 ± 10.5
Placebo	32	58.8 ± 11.6	24	64.3 ± 14	22	59.5 ± 11.9	11	54 ± 11.9

Figure 7-16: Results for the EORTC Quality of Life Questionnaire.

7.3.2.2.4 Pain Score

The pain score was the first section of the MD Anderson Brief Pain Inventory (see Appendix 5) where the participants indicated the pain they had experienced in the last 24 hours. From the results seen in Figure 7-17, the placebo arm experienced slightly more pain, however it was not statistically significant. The projection showed that in both arms the pain was still increased at three months' post-chemotherapy cessation. The pain experienced was not always linked with CIPN, as the pain questionnaire is a general pain score that does not distinguish the origin of pain. The pain documented from participants included general knee or joint pain and those undergoing radiation therapy had radiation associated pain.

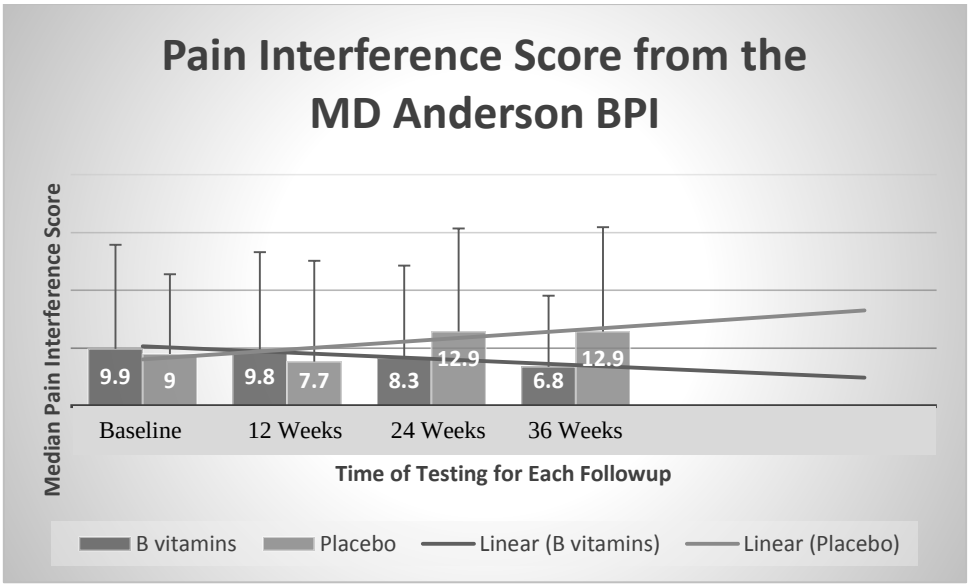


Pain Score								
	N	Baseline	N	12 weeks	N	24 weeks	N	36 weeks
B vitamins	38	4.8 ± 6.1	28	3.7 ± 5.6	27	4. ± 10	14	5.4 ± 7.1
Placebo	32	5.3 ± 7.4	24	3.3 ± 6	22	6.6 ± 8.2	11	6.6 ± 9.7

Figure 7-17: Results for the Pain Scores

7.3.2.2.5 Pain Interference Score

The pain interference score consists of the second part of the MD Anderson Brief Pain Inventory (see Appendix 5). This indicated how pain interfered with a subject's general life and activities over the past 24 hours. The outcome of this analysis presented in Figure 7-18 indicated that the vitamin B complex group had less interference with general activities due to pain compared to the placebo group. The continued projection showed a major difference in direction. Vitamin B complex supplementation cannot be solely attributed to this projection difference but may have played a part in the outcome.



Pain Interference Score								
	N	Baseline	N	12 weeks	N	24 weeks	N	36 weeks
B vitamins	38	9.9 ± 18	28	9.8 ± 16.8	27	8.3 ± 16	14	6.8 ± 12.3
Placebo	32	9 ± 13.8	24	7.7 ± 17.4	22	12.9 ± 17.8	11	12.9 ± 18

Figure 7-18: Results for the Pain Interference Scores

7.3.2.2.6 Patient Neurotoxicity Questionnaire (PNQ)

The statistics for the PNQ was assessed as a risk estimate (odds ratio)and P values using Chi squared and Fischer's exact test, as presented in Table 7-21. The odds ratio was analysed by addressing sensory and motor function at each time point or at an interview. Statistical significance was observed for sensory neuropathy at 24 weeks (OR=5.78, 95% CI = [1.63-20.5]) and at 36 weeks (OR=.8.1, 95% CI = [1.23-53.2]) between the control and the intervention arms. Therefore, there was a statistically significant effect as perceived by the subjects regarding the prevention of sensory neuropathy through supplementation.

This was validated using the Chi-square and Fisher's exact test. Statistical significant was found for sensory peripheral neuropathy in the PNQ (12 weeks p=0.03; 24 weeks p=0.005; 36 weeks p=0.021) as seen in Table 7-21. The motor nerve function was statistically significant at baseline (p=0.004), however, this was due to a participant's cancer site and complications that ensue such as after surgical interventions for breast cancer.

The questionnaire used a linear scale and the participants were asked if they felt any numbness, tingling, burning or pain in their fingers or toes for the sensory perception. Appendix 3 shows the full questionnaire that was utilised at baseline and every second week throughout the clinical trial.

It has been stated that CIPN is generally under reported by clinicians [34], however, these results show a participant's perception under reported sensory neuropathy compared to the neurologist's analysis. The participants' overall felt that sensory neuropathy was not as prevalent for them compared to the neurologist's analysis using the TNS. Therefore, it is important when assessing CIPN to obtain both a clinical diagnosis and the patient's perception for a comparative view and further understanding of the neurotoxicity diagnosed.

Table 7-21: PNQ Assessment of CIPN at Each Time Point.

Y/N results: Patient Neurotoxicity Questionnaire: p values			
		Chi-square	Fisher
Baseline	Sensory	0.077	0.082
	Motor	**0.004**	**0.005**
	Other	0.526	0.389
12 Weeks	Sensory	**0.03**	**0.029**
	Motor	0.477	0.336
	Other	0.53	0.385
24 Weeks	Sensory	**0.005**	**0.005**
	Motor	0.656	0.437
	Other	0.127	0.133
36 Weeks	Sensory	**0.021**	**0.027**
	Motor	0.383	0.325
	Other	0.096	0.183

7.3.3 Results for Each Cancer

7.3.3.1 Breast Cancer

No statistical significance in TNS was found between the control and intervention arms in the subgroup of participants with breast cancer. There was a minor trend in decreased motor function disturbances for the vitamin B complex group over the placebo group, but no differences where observed between the groups for the total TNS or sensory neuropathy. Results have been presented in Figure 7-19.

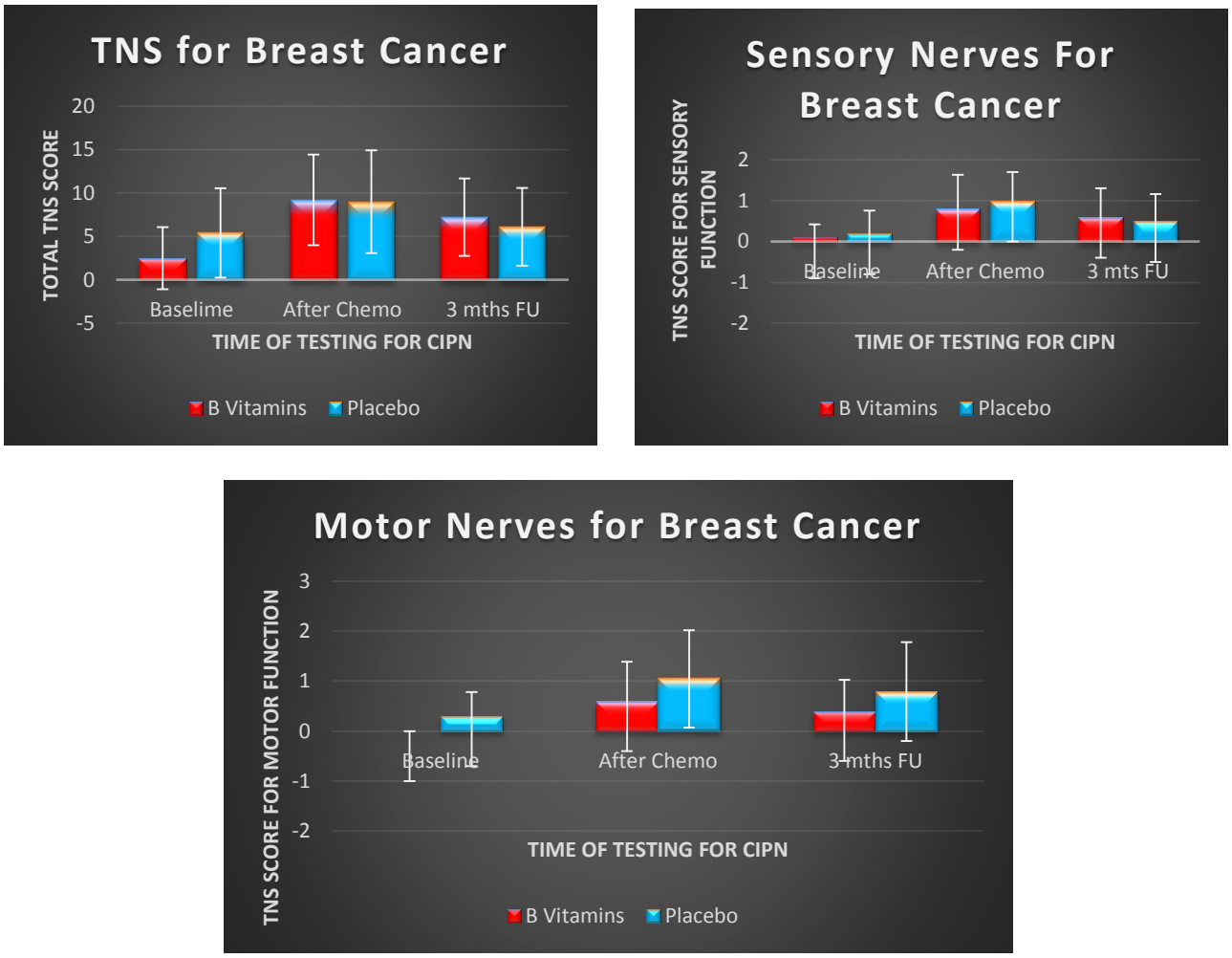


Figure 7-19: Breast Cancer Participant's Outcome from the TNS score

7.3.3.2 Lymphoma

In participants diagnosed with a lymphoma, the total TNS indicated an increase in CIPN development post-chemotherapy in the vitamin B complex arm of the study as compared to the placebo group (trend presented in Figure 7-20). For the participants in this clinical trial with lymphoma diagnosis, the chemotherapy regime wasR-CHOP[24], with the exception of four participants who were

[24] R-CHOP is a chemotherapy regime that involves the administration of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone. 372. Mian M, A.F., Kocher F, Gunsilius E, Willenbacher W, Zabernigg A, Zangerl G, Oexle

administered additional intrathecal methotrexate. These participants received either 6 or 8 cycles administered either two or three weekly. As these results included all participants, a further breakdown can be seen in Figure 7-21, which compares the participants who received 6 cycles versus those that received 8 cycles either two or three weekly.

The intervention arm was found to be statistically significant for the TNS sensory neuropathy compared to placebo post-chemotherapy. By the three months' follow-up, the two arms were very similar in the TNS sensory neuropathy scores. This indicated that the vitamin B complex group may have achieved some protection for the sensory nerves during chemotherapy administration, thereby allowing the participants to complete the chemotherapy regimen. However, the sensory neuropathy three months' post-chemotherapy showed no difference. Considering the results of the total TNS and the motor neuropathy scores for the patients diagnosed with a lymphoma, B vitamin supplementation would need further research to be justified.

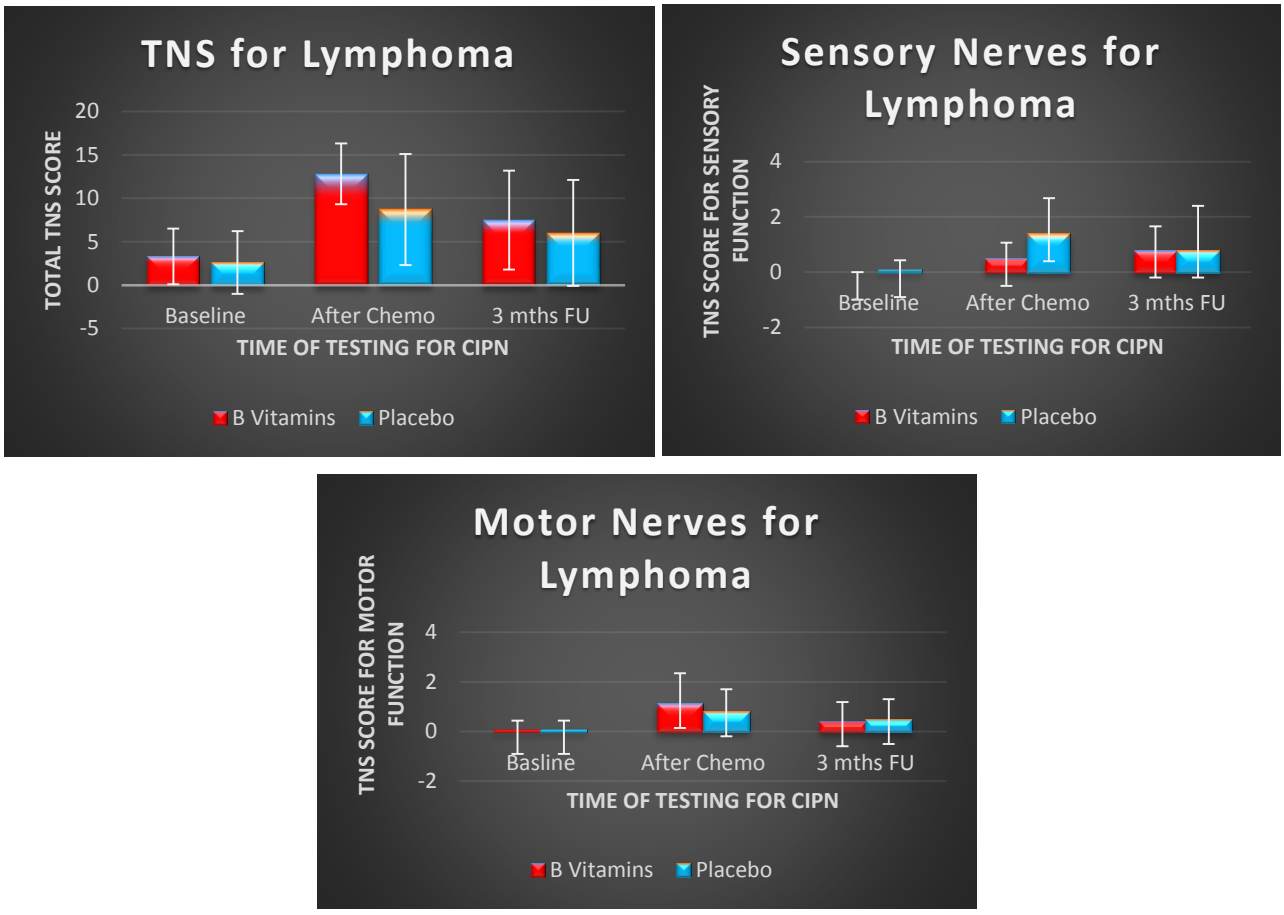


Figure 7-20: Lymphoma Participant Outcomes from the TNS

H, Schreieck S, Schnallinger M, Fiegl M., *A success story: how a single targeted-therapy molecule impacted on treatment and outcome of diffuse large B-cell lymphoma.* Anticancer Res., 2014. **34**(5): p. 2559-64.

Subdividing the patients diagnosed with lymphoma according to the number of cycles administered, how often they received the chemotherapy regimen and the addition of intrathecal methotrexate provided an overview of the impact of dose duration and accumulation. The participants who underwent eight cycles of R-CHOP all received chemotherapy every three weeks. All participants who underwent six cycles of R-CHOP, except for one subject (three weekly) had chemotherapy administered every two weeks. The participants who received the intrathecal methotrexate in addition to R-CHOP were administered chemotherapy every two weeks for six cycles. One participant received two extra weeks of just R-CHOP (participant in vitamin B complex arm).

The participants represented in the Figures 7-21-7.23 consisted of all participants that had completed all TNS evaluations, except one participant who completed baseline and post-chemotherapy (follow up was not continued due to disease progression and kidney failure). Not all subjects who were tested at baseline but did not have any further neurological evaluations were included. Although this trial was an RCT with an intention to treat analysis for the data, the stratification of participants diagnosed with a lymphoma was changed to those that were assessed to ascertain a better observation of results.

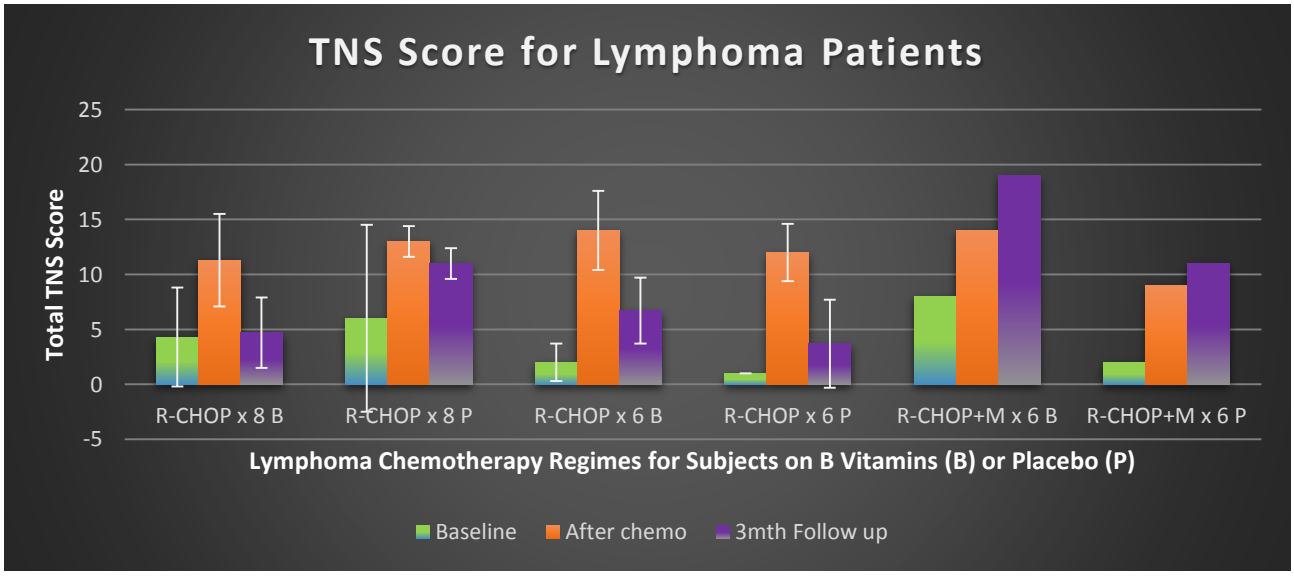
The total TNS score shown in Figure 7-18 demonstrated that all participants experienced CIPN. The participants who received eight cycles of R-CHOP three weekly (R-CHOP x 8) had similar increases in TNS scores post-chemotherapy. However, three months' post-chemotherapy the vitamin B complex arm (3 participants) had reversed their TNS score back to baseline. The placebo arm (2 participants) remained relatively high with little reversal of CIPN development. This indicated that although the vitamin B complex did not prevent the development of CIPN, it may have assisted in the treatment of CIPN for these participants.

The participants randomised to the vitamin B complex arm who received six cycles of R-CHOP two weekly (R-CHOP x 6) all had high TNS scores post-chemotherapy administration. Both arms (3 participants in each arm) improved their nerve function after three months with no statistical difference noted. The participants who received intrathecal methotrexate consisted of one participant per group. Two other participants recruited also received intrathecal methotrexate, however they withdrew from the study due to not being able to consume the capsules (1 placebo, 1 vitamin B complex). Both of the participant's TNS scores increased post-chemotherapy. The participant in the vitamin B complex arm received two extra administrations of R-CHOP two weekly after the initial 6 cycles of R-CHOP+M. This meant that the subject was exposed to a longer duration and accumulation of vincristine.

As presented in Figure 7-18, the R-CHOP+M participant in the vitamin B complex arm had a progressively worse presentation of CIPN post-chemotherapy and at three months' follow-up. This

patient was diagnosed with rheumatoid arthritis post-chemotherapy, which may have impacted his CIPN score. All RCT participants that received intrathecal methotrexate experienced CIPN that progressed post-chemotherapy administration.

Although intrathecal methotrexate does not directly cause peripheral neuropathy, it is well documented for neurotoxicity of diverse clinical manifestations in addition to myelopathy [396]. It is possible that the neurotoxicity from the intrathecal methotrexate was the reason the participants receiving R-CHOP+M experienced moderate CIPN with worsening symptoms post-chemotherapy cessation.



TNS Score		R-CHOP x 8 B	R-CHOP x 8 P	R-CHOP x 6 B	R-CHOP x 6 P	R-CHOP+M x 6 B	R-CHOP+M x 6 P
Baseline	Average	4.3	6	2	1	8	2
	St Dev	4.7	8.5	1.7	0		
After chemo	Average	11.3	13	14	12	14	9
	St Dev	4.2	1.4	3.6	2.6		
3mth Follow up	Average	4.7	11	6.7	3.7	19	11
	St Dev	3.2	1.4	3	4		

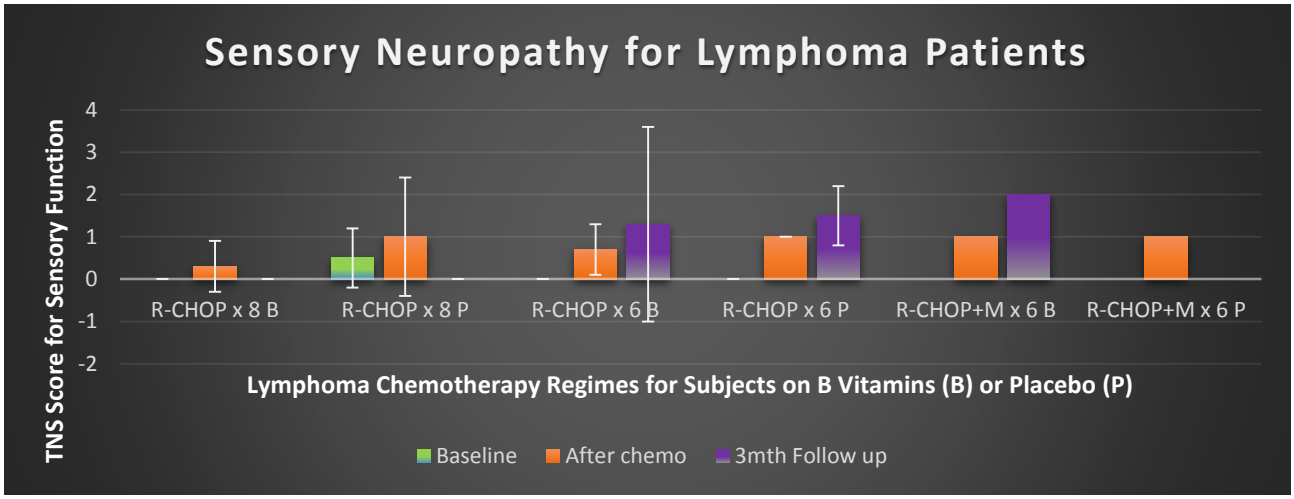
Figure 7-21: The Total TNS Scores for Lymphoma Participants who Received 6 versus 8 Cycles of R-CHOP and the Participants who Received Intrathecal Methotrexate (R-CHOP+M).[25] NB: No standard deviation for R-CHOP+M as only 1 person in each.

[25] Index for figure: 8 = 8 cycles administered three weekly, 6 = 6 cycles administered two weekly, B= B vitamin arm, P= placebo arm

The total cohort of lymphoma patients showed a trend for vitamin B complex supplementation for sensory peripheral neuropathy compared to placebo post-chemotherapy. Breaking the participant data into dose and weekly administration shows a different perspective as presented in Figure 7-22.

The participants that received eight cycles of R-CHOP plus oral vitamin B complex had minimal development of sensory PN with no sensory neuropathy at three months' follow-up. The participants who received R-CHOP plus placebo developed sensory neuropathy post-chemotherapy with minimal reduction of symptoms by three-months follow up. The participants who received six cycles received less exposure to vincristine compared to participants who were administered eight cycles, however the chemotherapy was administered every two weeks compared to every three. This increased dose accumulation could impact CIPN development.

Therefore, it seems that both dose and accumulation play an important role in the development of CIPN for patients administered vincristine. The supplementation of vitamin B complex did not prevent the delayed sensory neuropathy experienced by participants receiving R-CHOP two weekly but may be beneficial for patients receiving R-CHOP three weekly.

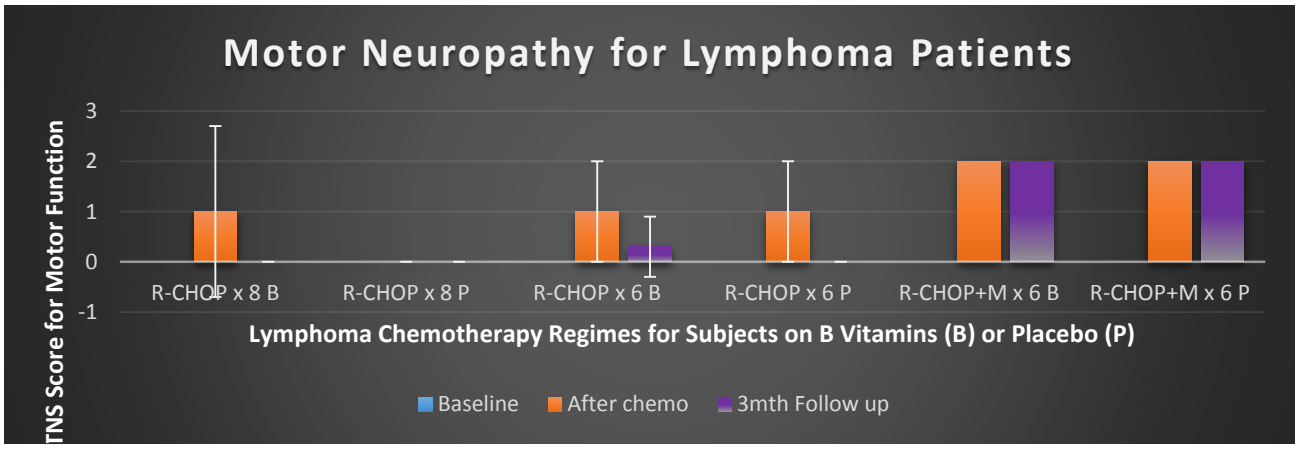


Sensory Nerve Assessment from TNS		R-CHOP x 8 B	R-CHOP x 8 P	R-CHOP x 6 B	R-CHOP x 6 P	R-CHOP+M x 6 B	R-CHOP+M x 6 P
Baseline	Average	0	0.5	0	0	0	0
	St Dev	0	0.7	0	0		
After chemo	Average	0.3	1	0.7	1	1	1
	St Dev	0.6	1.4	0.6	0		
3mth Follow up	Average	0	0	1.3	1.5	2	0
	St Dev	0	0	2.3	0.7		

Figure 7-22: The Sensory Neuropathy Scores from the TNS for Lymphoma Participants who Received 6 versus 8 cycles of R-CHOP and the Participants who Received Intrathecal Methotrexate.

Motor nerve function for the subdivided patients diagnosed with lymphoma shown in Figure 7-23 found that participants who received vitamin B complex and eight cycles of R-CHOP three-weekly developed motor neuropathy during chemotherapy administration. However, this dissipated by three-months follow-up. The participants on placebo and eight cycles of R-CHOP did not develop motor neuropathy at any time during or post-administration of chemotherapy.

All participants who received 6 cycles of R-CHOP two-weekly developed motor neuropathy post-chemotherapy however, the placebo arm's motor neuropathy disappeared completely by three months' follow-up, whereas the vitamin B complex arm still reported minimal motor neuropathy. Both patients who received R-CHOP+M developed moderate motor neuropathy, which did was not reduced after three-months. Similarly, to the sensory neuropathy, the methotrexate could have had an impact on the motor neuropathy development in conjunction with the vincristine. Therefore, a vitamin B complex was not beneficial for preventing motor neuropathy experienced by those patients diagnosed with a lymphoma and may potentially increase the risk of CIPN development. Intrathecal methotrexate may increase the development of motor neuropathy in conjunction with vincristine administration.



Motor Nerves Assessment from the TNS		R-CHOP x 8 B	R-CHOP x 8 P	R-CHOP x 6 B	R-CHOP x 6 P	R-CHOP+M x 6 B	R-CHOP+M x 6 P
Baseline	Average	0	0	0	0	0	0
	St Dev	0	0	0	0		
After chemo	Average	1	0	1	1	2	2
	St Dev	1.7	0	1	1		
3mth Follow up	Average	0	0	0.3	0	2	2
	St Dev	0	0	0.6	0		

Figure 7-23: The Motor Neuropathy Scores from the TNS for Lymphoma Participants who Received 6 versus 8 Cycles of R-CHOP and the Participants who Received Intrathecal Methotrexate.

7.3.3.3 Lung Cancer

Nine patients recruited in this clinical trial had been diagnosed with lung cancer. This small cohort was not representative of a large population of patients with lung cancer, however all participants received weekly carboplatin and paclitaxel in conjunction with radiation. This provided a group of participants who were administered the same treatment regime. The results are presented in Figure 7-24

The results for these participants in this clinical trial showed that a vitamin B complex was beneficial for the prevention of sensory neuropathy. Moreover, it also showed a trend in prevention for total CIPN during chemotherapy, but was noted to have a worse presentation three-months' post-chemotherapy. Two patients randomised to the placebo arm have deceased and one patient randomised to the vitamin B complex had disease progression after chemotherapy and is currently still well.

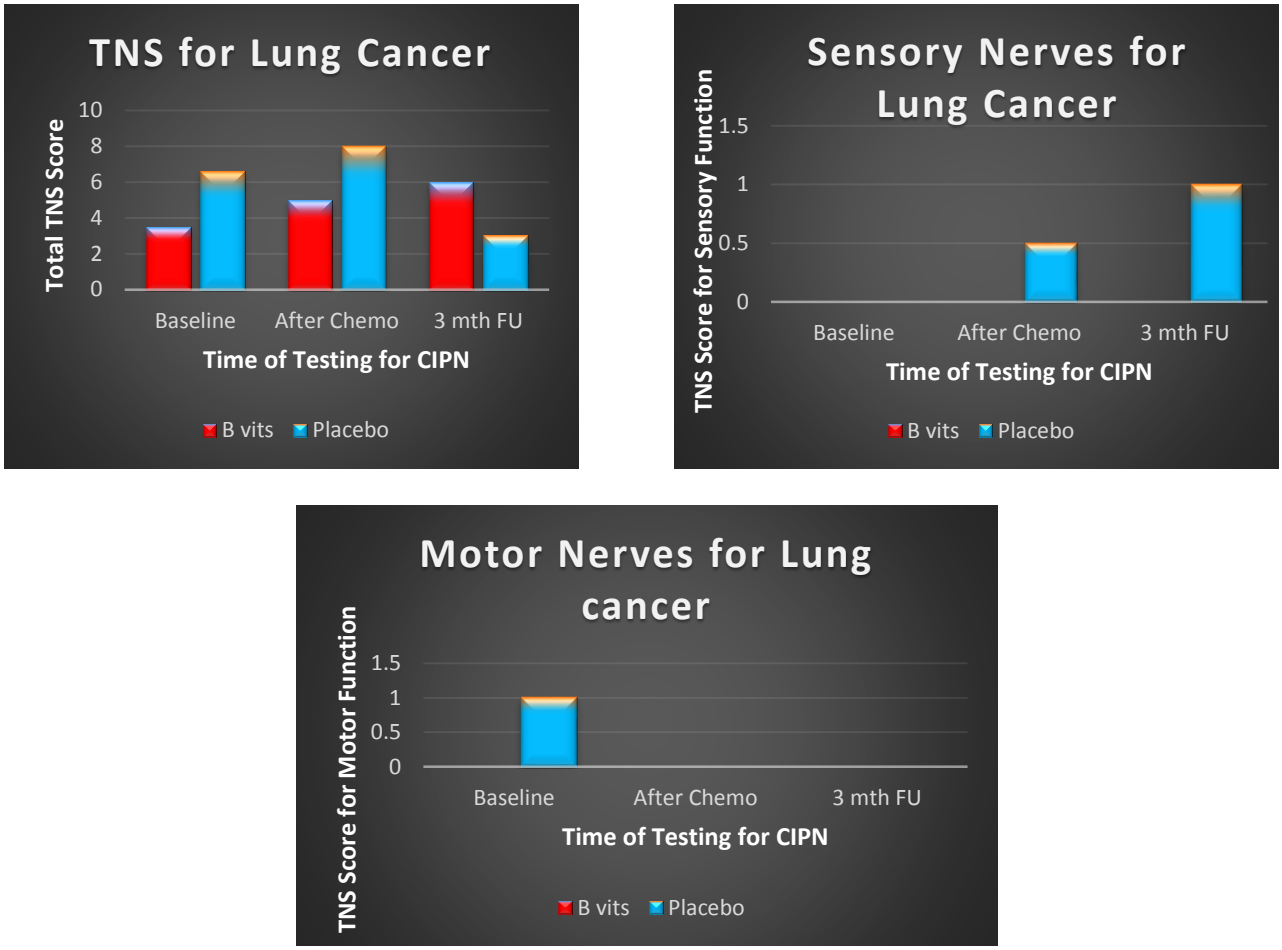


Figure 7-24: Lung Cancer Participant Results from the TNS.

7.4 DISCUSSION

This placebo-controlled randomised clinical trial investigating the efficacy of a vitamin B complex to reduce the incidence of CIPN over placebo recorded a large heterogeneous set of data. In total, one hundred and twenty-one participants were screened for eligibility and fifty of the subjects were excluded. Seventy-one participants were than randomised to either the vitamin B complex arm or placebo. Thirty-eight participants were randomised to the vitamin B complex arm and thirty-three to the placebo arm. Of the participants randomised, three were excluded from the vitamin B complex arm and six from the placebo arm leaving thirty-five participants to receive the vitamin B complex during chemotherapy treatment and twenty-seven to receive placebo for a total of 62 potentially evaluable participants. By completion, forty-seven participants (66.2%) had completed all requirements for this study with an attrition rate of 14%.

The medical treatment for the development of CIPN during chemotherapy administration is to reduce the dose or cease administration of the chemotherapy agent [2]. Four participants in this RCT trial had their neurotoxic chemotherapy dose reduced or ceased due to the development of CIPN. These included from the placebo arm: PA003 RxT who had weekly taxol ceased after nine administrations from the allocated twelve cycles to be administered. PA038 JRW had the vincristine dose reduced by 50% for the fifth administration and then ceased for the sixth cycle. PA055 MJM had the vincristine administration ceased after the fourth administration (6 cycles completed). From the vitamin B complex arm only one participant had a dose reduction. PA070 NLW had the taxol administration decreased by 20% after the second cycle due to CIPN development. This meant that three participants in the placebo had their chemotherapy agent ceased due to CIPN and only one participant in the vitamin B complex arm had a dose reduction of 20%, nevertheless completed the prescribed chemotherapy regimen. These findings build a hypothesis that a vitamin B complex may potentially decrease the onset and severity of CIPN development. Or may assist in the completion of the prescribed chemotherapy regimen, thereby assisting treatment outcomes.

The final-results of the clinical trial reported no statistical significance for the vitamin B complex to prevent CIPN. The primary outcome was based on the TNS scores, which through a Mixed Effects Model (MEM) reported a T score of 0.22 for placebo compared to the vitamin B complex arm with a T score of 1.24. When the placebo arm was compared to the vitamin B complex arm over the three time points of neurological assessments, no statistical significance was found ($p=0.73$; T score = -0.35). A statistical significance from the TNS (primary outcome) was found for the difference in time points of the neurological assessments ($p=0.02$). This indicated that neurological changes were seen from the total cohort between each neurological assessment. CIPN is known to be an accumulative

side effect, which can occur during or after chemotherapy administration from a neurotoxic agent [12]. All other analysis from the MEM scores for secondary outcomes found no statistical significance.

Other statistical analysis of sensory and motor functions from the TNS results using Chi-squared and Fisher's exact tests found no statistical significance (24 weeks: sensory p=0.11 motor p=0.79; 36 weeks: sensory p=0.96 motor p=0.46). Statistical significance was calculated at baseline for motor function (p=0.002), however this has no relevance on the outcome as it was representative of the patients' cancer and cancer related surgical interventions. Independent samples t-test and the Mann Whitney test were also conducted for the TNS with no statistical significance found (Independent samples t-test: 24 weeks p=0.376, t-score=-0.89; 36 weeks p=0.579, t-score=-0.559; Mann Whitney: 24 weeks p=0.329; 36 weeks p=0.424).

A statistically significant result was observed in the patient neurotoxicity questionnaire results for sensory neuropathy (12 weeks p=0.03; 24 weeks p=0.005; 36 weeks p=0.021). In addition, the risk estimate for the PNQ was also statistically significant at both 24 weeks OR=5.78, 95% CI = [1.63-20.5] and 36 weeks OR= 8.1, 95% CI = [1.23-53.2]. Therefore, B vitamin supplementation was found to be statistically significant for the participant's perception of sensory peripheral neuropathy prevention.

Additional relevant results included the small nerve damage encountered with chemotherapy administration that vitamin B supplementation did not reverse. Reflexes were lost in ninety percent of all participants with seventy-five percent still exhibiting no reflexes by three-months of follow-up. Blood pathology results demonstrated that supplementation with a vitamin B complex during administration of chemotherapy increased blood levels of vitamins B1, B6, B12 and folate compared to placebo.

The only B vitamin that was found to be statistically significant in potentially preventing the onset and severity of CIPN was vitamin B12. In addition, a participant (i.e., PA032 PMP) that was found to be deficient in vitamin B12 post-chemotherapy administration presented with moderate to severe CIPN development. After supplementation with vitamin B12, CIPN was significantly reduced. Further expansion on the results of this participant can be found in Chapter 8.

High vitamin B6 blood pathology results were found in four participants in the vitamin B complex arm who presented with mild to moderate CIPN after chemotherapy treatment. It remains to be elucidated if the high vitamin B6 blood levels (over 1,000 nmol/L) encountered was associated with the CIPN development in these participants.

Pain scores did not show a statistical significance and no differences were seen between the two arms except for the projection of pain interference. It was observed that in the vitamin B complex arm of the study, if the projection continued it would have led to significantly less pain interference in these participants than in those randomised to the placebo arm. These results cannot conclusively demonstrate that vitamin B complex supplementation was solely responsible for the reduction of pain and its interference with daily activities.

Examining the data from individual participants regarding cancer types, no benefit was found for breast cancer patients, but possible sensory neuropathy benefits may have been observed for patients diagnosed with a lymphoma undergoing R-CHOP for eight cycles' three weekly and patients diagnosed with a lung cancer undergoing paclitaxel and carboplatin weekly.

7.5 CONCLUSION

Whilst a vitamin B complex was not statistically efficacious when compared to placebo in decreasing the incidence of CIPN, vitamin B12 was observed to correlate in preventing the onset and severity of CIPN development. Participant perception for the prevention of sensory neuropathy with a vitamin B complex was statistically significant, as was the odds ratio for post-chemotherapy and follow up. A vitamin B complex may be beneficial in preventing sensory neuropathy for patients diagnosed with a lymphoma who are to be administered chemotherapy with R-CHOP for eight cycles every three weeks and for those patients diagnosed with lung cancer who receive weekly paclitaxel and carboplatin. A vitamin B complex may play a role in decreasing pain interference for daily activities. One patient who presented with moderate to severe CIPN was found deficient in vitamin B12 post-chemotherapy and benefited from vitamin B12 administration. This was clinically relevant and further information on this participant (i.e., PA032 PMP) is presented in Chapter 8. The overall impression from this clinical study is that patients diagnosed with a cancer who will be administered chemotherapy and who may be at risk of developing moderate to severe CIPN, could benefit from either vitamin B12 or a vitamin B complex, but further research is required. In addition, patients undergoing chemotherapy with a neurotoxic agent should have their vitamin B12 pathologically tested either prior to chemotherapy administration or on presentation of moderate to severe CIPN.

8 CHAPTER 8: CASE STUDIES DOCUMENTING CIPN DUE TO VITAMIN B12 DEFICIENCIES

8.1 INTRODUCTION

CIPN is emerging as a major concern for oncology specialists considering the increasing number of cancer survivors and the lack of standardised prevention strategies and treatments [397]. The incidence of CIPN depends on the chemotherapy agent administered with an estimated prevalence of one third of all patients that undertake a chemotherapy regimen [3]. As previously discussed, patients experiencing moderate to severe CIPN report a reduced quality of life [12], chronic discomfort [2], and disruption of physical abilities for general life activities, which can be temporary or permanent [12]. Currently in clinical oncology practice, CIPN is assessed using the common toxicity scales however, this relies heavily on the patient's subjective reports rather than quantitative testing [398]. CIPN is a potentially reversible side effect although reversibility may be dependent on early detection or identification and modification of chemotherapy treatment as symptoms appear [398]. Permanent CIPN continues to be reported, especially sensory symptoms in the lower extremities among patients treated with oxaliplatin even up to 11 years after treatment [399]. Early differential diagnosis and prevention of permanent CIPN needs to be a priority benefiting the quality of life of cancer patients.

A history of blood vitamin B12 deficiency has been identified as a predisposing factor that may increase the risk of CIPN development [12]. However, patients who have had no previous history of blood vitamin B12 deficits may not be tested before chemotherapy commences in order to assess blood vitamin B12 status. Moreover, a potential blood vitamin B12 deficiency may develop during the chemotherapy treatment [44], therefore potentially subjecting the patient to be predisposed to the development of CIPN and/or the delayed progression of CIPN.

A case report of a cancer patient that participated in this B vitamin clinical trial and who developed CIPN is described below. The participant was found vitamin B12 deficit after the chemotherapy regime had been completed and upon vitamin B12 administration, the severity of CIPN decreased. This allowed the patient more functional ability in daily activities including walking. Another case report has been documented for a participant who was excluded from this clinical trial due to a vitamin B12 deficiency and consented to being followed throughout chemotherapy and after cessation. One other case report provides noteworthy findings from the subject's diaries and has been included to further expand discussions.

8.2 PA032 PMP CASE REPORT ONE

8.2.1 PA032 PMP Details and Results

A 53-year-old female (PA032 PMP) was enrolled in the main clinical trial and was randomised to the placebo group. Initial vitamin B group status pathology bloods, a neurological exam involving the TNS and electro-neurological examination in addition to other history and questionnaires was conducted prior to the commencement of chemotherapy. The same independent neurologist following chemotherapy administration conducted the neurological examination and TNS and blood B group vitamin status was established. This subject's case report details and results are documented in Table 8-1.

Table 8-1: Medical Details of Participant PA032 PMP

PA 032 PMP	Details
DOB	17/06/59
Diagnosed	Breast Cancer in March, 2012
Surgery	PA Hospital 27/3/2012 – right breast lumpectomy and axillary clearance. Previous surgery: Appendectomy, cholecystectomy, basal cell carcinoma on nose (flap repair)
Results	Grade II IDC and DCIS – Oestrogen and Progesterone positive, HER2 negative
Nodes positive/removed:	0/9
Recruited for study	2/6/2012
Race	Caucasian
Marital Status	Married
Other medical considerations	Psoriasis, hypothyroidism, osteoarthritis, reactive arthritis, asthma (late onset), osteopenia, obesity
Medications	Thyroxin 100 mcg daily
Allergies	Codeine, pethidine
Diet history	No history of being a vegan or vegetarian. Normally consumes red meat (beef, lamb, kangaroo) 3-4 times a week, chicken or pork 2-3 times a week and fish or seafood approximately 3-4 times a week. She does drink caffeine, approximately 4-5 cups a day (tea), rarely drinks alcohol, doesn't smoke or take recreational drugs.
Height	162cm
Weight	129kg
BMI	49.2
Chemotherapy regime	Carboplatin and docetaxel (TC) 4 times every three weeks

PA032 PMP is generally healthy, although overweight. The subject has been diagnosed with arthritis with increased difficulty in walking, which adds to this subject's weight issue. This participant works in an office and has a generally sedentary life.

8.2.2 Blood Pathology Results for PA032 PMP

The blood pathology tests for this participant showed that vitamin B12 status had changed and was deficient after chemotherapy administration, as seen in Table 8-2. After supplementation with a B group vitamin complex (equivalent to 1,000 µg/day) and receiving one intramuscular vitamin B12 (dose 1,000 µg), the blood vitamin B12 level reverted back to baseline. All other bloods were similar except for blood levels of vitamin B6, which had increased after supplementation with the oral B vitamin complex. However, this was expected when assessing previous pilot clinical trial results (see Chapter 5). This suggests that vitamin B12 may be a contributing factor linked with peripheral neuropathy presenting in this participant.

Table 8-2: PA032 PMP Blood Pathology Results

Blood Pathology	Baseline 5/6/2012	After Chemo 25/09/2012	Last follow up 8/12/2012	Ref Range
Vitamin B1 (TDP)	140	140	180	66-200 nmol/L
Vitamin B2 (FAD)	280	310	230	180-470 nmol/L
Vitamin B6 (P5P)	95	90	**250 H**	35-110 nmol/L
Red cell folate	2249	2163	2170	>900 nmol/L
Holo TC (Vitamin B12)	107	**29 L**	106	>35 pmol/L

8.2.3 Neurological Testing Results for PA032 PMP

8.2.3.1 Total Neuropathy Score (TNS) used in the Trial

The neurological test results in Table 8-3 showed that the participant's peripheral sensory and motor nerve function decreased after chemotherapy administration. The participant at baseline showed no numbness, tingling or pain in the hands or feet. All motor functions in the hands and feet were preserved, although arthritis did impair some ambulatory walking. The participant had just applied copious amounts of a moisturiser just before arriving at the appointment, and this interfered with the electronic testing therefore showing a mild impairment in the motor function of the test. The sural

nerve was unable to be found due to the arthritis and the moisturiser application. After chemotherapy administration, sensory peripheral nerves and motor nerves were noticeably affected. Level 2 indicates numbness, tingling and neuropathic pain up to the ankle and the wrist.

The pin sensibility is the test for small nerve damage and post-chemotherapy administration was noted past the wrist and ankle in this subject. In addition, there was loss of tendon reflexes, which was observed in many participants in this clinical trial and is also representative of nerve damage. The sural nerve was not unable to be detected after chemotherapy administration, however this time there was not copious amounts of moisturiser applied beforehand. Therefore, peripheral neuropathy was detected affecting the sural nerve function and leading to the inability of the nerve being detected by the electrical testing procedure.

After receiving vitamin B12 intramuscularly (dose 1,000 µg) and orally administering the vitamin B group complex (2 capsules a day equivalent to 1,000 µg a day), retesting was conducted three months later. Sensory nerve function returned to the fingers and toes (level 1). Motor function remained unchanged after chemotherapy administration and pin sensibility was still present to the wrists and ankles. The sural nerve was positively detected in this test, and it is possible that the patients baseline reading could have been similar if the moisturiser hadn't interfered with the test. Alternatively, it is possible that her sural nerve improved with B vitamin complex administration. As the participant was now in menopause, hot flushes were increased in severity indicating autonomic nervous function involvement.

Table 8-3: TNS Results of PA032 PMP

Total Neuropathy Score (TNS)			
Item	**Baseline Score 02/06/2012**	**After Chemotherapy 25/08/2012**	**Three months later 17/11/2012 (2 months after beginning of B12 supplementation)**
Sensory symptoms	0	2	1
Motor symptoms	1 (right hand sweating reduced testing)	2	2
Autonomic symptoms	1 (sweating)	1	2 (menopause post-chemo)
Pin sensibility	0	2	2
Vibration sensibility	0	0	0
Strength	0	0	0
Tendon reflexes	0	4	2
Sural amplitude score	4	4	2
Peroneal amplitude score	0	0	0
Total	7	15	11

8.2.3.2 Neurological Conduction Studies (NCS)

The neurologist conducted extra nerve conduction tests in addition to the tests required for the TNS. These electrical tests assess large nerve activity. The nerve conduction study (NCS) normal values were ascertained from the University of Michigan Medical School [400]. The NCS conducted on PA032 PMP indicate that nerve damage occurred after chemotherapy administration. The main areas affected were the hands (palms), wrists, sural nerve and the peroneal only slightly after chemotherapy. The sural nerve at baseline was unable to be found due to a large amount of moisturiser applied by the patient prior to testing. This makes it difficult to ascertain if damage did occur or if the sural activity was low before chemotherapy administration. Considering the fact that the sural activity had improved by the last neurological test, it can be assumed there may have been some activity prior to chemotherapy. Except for the amplitude of the peroneal after chemotherapy on the 25/8/2012 which is a motor nerve, all nerve damage was sensory. This is congruent with the symptoms displayed by the patient.

Table 8-4: PA032 PMP NCS Study Results

Sensory NCS							
Nerve/Sites	**Rec Site**	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV
		2/6/2012		**25/08/2012**		**17/11/2012**	
R MEDIAN Palm	III	1.30	4.3	2.8	10.2	2.85	4.9
L MEDIAN Palm	III			2.70	12.0	3.30	5.0
L ULNAR – Wrist	Digit V	1.95	19.5				
R ULNAR – Wrist	Dorsum of Hand	1.30	26.1	1.65	1.5	1.85	3.1
L ULNAR – Wrist	Dorsum of Hand	1.65	13.2	1.70	3.6	1.65	3.9
R SURAL – Calf	Lat Malleolus			2.65	6.7	2.35	5.1

NB: First baseline tests were difficult due to moisturiser used by patient

Motor NCS						
Nerve/Sites **Rec Site**	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV
	2/6/2012		**25/08/2012**		**17/11/2012**	
L MEDIAN – APB Wrist	5.10	7.7	4.45	8.0	3.80	7.7
Elbow	9.45	7.8	9.05	7.3	9.35	7.8
R COMM PERONEAL – EDB Ankle	4.05	2.9	3.15	3.3	4.05	2.2
Fib Head	9.65	4.8	10.55	0.7	10.15	2.3
R TIBIAL (KNEE) – AH Ankle	5.05	10.4	3.55	8.3	4.65	3.7

8.2.3.3 Patient Neurotoxicity Questionnaire for PA032 PMP Results

This is a linear scale and indicates the level of CIPN that the patient has experienced in the past week. The results of PA032 PMP's patient neurotoxicity questionnaire can be seen in Table 8-5.

On the 25/8/12, subject PA032 PMP was having difficulties with buttoning clothes, zippers, typing on a keyboard, writing, walking, putting on jewellery, knitting, sewing, working and dialling or using a telephone. By the 17/11/12, all of these issues were resolved and no further difficulties were reported aside from some shortness of breath.

On the 2/10/12 the patient presented at the PA hospital breast cancer clinic following receipt of blood results. Holo TC was reported as 29 (ref >35) indicating a deficit in blood vitamin B12. The oncologist was informed and administered vitamin B12 intramuscularly (dose 1,000 µg). The subject was than provided 3 bottles of the vitamin B complex from the manufactures and advised to take 1 capsule twice daily with food, morning and night. At this visit point, the patient reported that numbness was present up to the hips in both legs and from the wrists to the elbows (considered Grade 3 to 4 CIPN). The subject was referred to a physiotherapist at the PA hospital for the numbness and the lymphoedema that was being experienced.

One week later 9/10/12, the patient reported experiencing a major positive difference. On the 17/11/12, the patient described numbness in the toes to be approximately 50% and on the tips of the fingers.

Table 8-5: PA032 PMP Patient Neurotoxicity Questionnaire Results

Items	**Baseline**	**3 mths**	**6 mths**
	2/6/12	**25/8/12**	**17/11/12**
Numbness, Pain, burning or tingling	0/4	3/4	1/4
Weakness in arms and legs	1/4	3/4	1/4
Difficulty with daily activities	0/4	3/4	0/4

8.2.3.4 Medical Notes for PA032 PMP: Chart from the PA Hospital

As seen in Table 8-6, PA032 PMP still experiences CIPN in the tips of the fingers and toes, which may now be a permanent effect. The peripheral neuropathy still present may represent the actual CIPN the patient developed. It is postulated that had the blood vitamin B12 levels been kept in normal range, the participant would have only experienced the neuropathy in the fingers and toes through the

effect of the chemotherapy. This is in comparison to the severe peripheral neuropathy reported by the patient in the arms and legs.

Table 8-6: PA032 PMP Medical Notes from the PA Hospital Medical Charts

Dates	Medical Notes from patients chart at the PA
29/05/2012	Presented to the breast cancer oncology and radiology clinic, weight 132.6 kg and 165 cm, age 53.
06/06/2012	Commenced chemotherapy After first chemotherapy, cancer care coordinator noted the following: terrible joint and bone pain prescribed endone 10 mg. Diarrhoea bad from Sunday to Monday than settled. Nausea double positive so prescribed pramen. Has rash and possible fistulas and using clindamycin ointment. Has mouth ulcers and continues to use mouth washes.
26/06/2012	Weight: 132.2 kg, bone pain/ myalgia, mouth ulcers, diarrhoea, hot flushes. Prescribed neulasta injection, gastro stop and endone and fentanyl 25 mcg.
17/072012	Bone pain major issues. Fentanyl 25 mcg and endone.
7/08/2012	Weight: 135.6 kg. Pain constant, vomiting, fluid retention, some CIPN, rash. Prescribed Lasix 2 g daily and Zofran (ondansetron) wafers.
18/09/2012	Completed 4 cycles. CIPN noted, muscles sore, referred to physiotherapist. Physiotherapist noted CIPN refer to Appendix 11.
02/10/2012	Holo TC 29 noted by oncologist, prescribed vitamin B12. Given by nurse on the 2/10/12 as prescribed, 1000 mcg IM. CIPN noted as being severe.
9/10/2012	Breast referral clinic: Osteopenia T-score -1.8 in right neck of femur. Rash has improved with antibiotics – seen dermatologist who prescribed hydrozole and advantin cream. Has lethargy, CIPN and taking vitamin B12.
10/12/2012	Referred to neurologist as CIPN still present. BMD: oesteopenic. Prescribed Femara.
22/01/2013	Rash resolved. CIPN improving, occasional partial PN. Arthralgia, myalgia, increase in severity of psoriasis, increase weight gain, non-pitting oedema. BDM: left spine -0.6, right femur -1.8, and left femur -1.5.
16/07/2013	Mammogram, no malignancy. Experiencing bone pain and stiffness, mild CIPN. Lymphedema still in arm and breast.

8.2.3.5 Chemotherapy Regime / Dates for PA032 PMP

The subject was administered the complete dose of her chemotherapy with no reductions or cessations of treatment.

Table 8-7: PA032 PMP Chemotherapy Regime and Administration

Date	**5/6/2012**	**26/6/2012**	**17/7/2012**	**7/8/2012**
Sodium chloride	100 ml IV	100 ml IV	100 ml IV	100 ml IV
Granisetron (3mg)	3 mg IV	3 mg IV	3 mg IV	3 mg IV
Dexamethasone (8mg)	8 mg IV	8 mg IV	8 mg IV	8 mg IV
Docetaxel (75mg/m^2)	184 mg IV	184 mg IV	184 mg IV	184 mg IV
Cyclophosphamide (600mg/m^2)	1480 mg IV	1480 mg IV	1480 mg IV	1480 mg IV
Discharge Medication				
Ondansetron (oral)	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days
Dexamethasone (oral)	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days
Metolopramide (oral)	10-20 mg 4-6 HRLY PRN x 5 days	10-20 mg 4-6 HRLY PRN x 5 days	10-20 mg 4-6 HRLY PRN x 5 days	10-20 mg 4-6 HRLY PRN x 5 days
Dexamethasone (oral) to be taken the day before next chemotherapy	8 mg BD x 1 day	8 mg BD x 1 days	8 mg BD x 1 days	
Pegfilgrastim INJ (6mg) subcutaneous		6 mg ONCE x 1 day	6 mg ONCE x 1 day	6 mg ONCE x 1 day

8.2.3.6 Other Bloods Completed by PA Hospital for PA032 PMP

8.2.3.6.1 Thyroid Function

The participant has had a history of hypothyroidism and is currently taking thyroxin 100 mcg daily. These blood test results as seen in Table 8-8, were to monitor her thyroid function during and after radiation. The thyroid antibodies present still indicate the subject has Hashimoto's disease.

Table 8-8: PA032 PMP Thyroid Function Blood Results

Thyroid	13/6/2012	22/06/2013	Ref Range
TSH	5	4.5	0.20- 4.00 mU/L
Free thyroxine (fT4)	16		15-30 pmol/L
Anti-thyroglobulin Ab		**200**	<60 U/ml
Anti-Thyroid Peroxidase Ab		**280**	<60 U/ml

8.2.3.6.2 Cholesterol

This participant is not on cholesterol medication prior or after chemotherapy administration. The blood results for cholesterol are presented in Table 8-9.

Table 8-9: PA032 PMP Cholesterol Blood Results

Cholesterol	13/6/2012	13/07/2012	22/06/2013	Ref Range
Total Cholesterol	5.4	6.4	6.2	3.9-7.4 mmol/L
Triglycerides	1.4	H 3.3	1.3	<1.5 mmol/L
HDL	1.12			>1.0 mmol/L
LDL	H 3.24			<2.0 mmol/L
Total/HDL ratio	4.8			

8.2.3.6.3 Full Blood Count

PA032 PMP's full blood count is fairly representative of a patient who has undergone chemotherapy with a low white cell count (WCC) and low neutrophils. The cumulative blood test results showed that the patient recovered well from the administration of chemotherapy in regards to red and white blood cell regeneration. The blood pathology results are presented in Table 8-10.

Table 8-10: PA032 PMP Full Blood Count Results

FBC	13/6/2012	13/7/2012	22/06/2013	Ref Range
Hb	141	136	143	115-160 g/L
RCC	4.5	4.5	4.6	3.6-5.2 x10 ˆ12/L
Hct	0.43	0.41	0.42	0.33-0.46
MCV	95	91	93	80-98 fL
MCH	31	30	31	27-35 pg
Platelets	304	172	261	150-450 x10 ˆ9/L
WCC	L 1.5	6.4	5.4	4.0-11.0 x10 ˆ9/L
Neuts	L 0.1	3.1	3.0	2.0-7.5 x10 ˆ9/L
Lymphs	1.3	2.4	1.8	1.1-4.0 x10 ˆ9/L
Monos	L 0.0	0.7	0.4	0.2-1.0 x10 ˆ9/L
Eos	L 0.02	0.06	0.11	0.04-0.40 x10 ˆ9/L
Basos	0.02	0.06	0.05	<0.21 x10 ˆ9/L

8.2.3.6.4 Serum Chemistry

PA032 PMP's full blood chemistry pathology results seen in Table 8-11 shows the participant is experiencing a number of other medical concerns. This includes borderline diabetes (blood glucose of 6), gout (uric acid 0.39 mmol/L), raised liver enzymes which could be from chemotherapy initially but has continued (GGT, ALT, AST, LD) and low calcium in July, 2012.

Table 8-11: PA032 PMP Serum Chemistry Results

Serum Chemistry	13/06/2012 Fasting	13/07/2012 Random	22/06/2013 Fasting	Ref Range
Sodium	138	141	140	137-147 mmol/L
Potassium	4.6	4.0	4.1	3.5-5.0 mmol/L
Chloride	103	106	103	96-109 mmol/L
Bicarbonate	29	27	28	25-33 mmol/L
Other Anions	11	12	13	4-17 mmol/L
Glucose		6.0	6.5	Random (3-6)
Urea	4.6	3.3	4.3	2.5-7.5 mmol/L
Creatinine	59	59	59	50-120 umol/L
eGFR	>90	>90	>90	Over 59 mL/min
Uric acid		H 0.37	H 0.39	0.14-0.35 mmol/L
Total Bilirubin	9	9	9	2-20 umol/L
Alk. Phos	73	83	94	30-115 U/L
Gamma GT	H 69	H 91	H 75	0-45 U/L
ALT	H 56	45	H 60	0-45 U/L
AST	32	33	H 56	0-41 U/L
LD	188	H 277	202	80-250 U/L
Calcium		L 2.24	2.29	2.25-2.65 mmol/L
Adjusted for albumin		2.34	2.33	2.25-2.65 mmol/L
Phosphate		1.1	1.4	0.8-1.5 mmol/L
Total Protein		66	73	60-82 g/L
Albumins	39	39	41	35-50 g/L
Globulins	32	27	32	20-40 g/L

8.2.3.6.5 Cumulative Serum Report

No medical notes were documented as to the reason for testing her digestive enzymes, however results are all in range as seen in Table 8-12.

Table 8-12: PA032 PMP Cumulative Serum Report Results

Serum	13/07/2012	Ref Range
Lipase	130	60-250 U/L
Amylase	41	0-120 U/L

8.2.3.6.6 Vitamin Levels

The serum vitamin B12 as seen in Table 8-13, assists this case report in assessing the participant's vitamin B12 status pre and post clinical trial. PA032 PMP was found deficient in vitamin B12 on the 2/10/2012 (Holo TC 29 pmol/L), which is three months after the serum vitamin B12 test conducted on the 13/7/2012 (>1476). This serum vitamin B12 blood test was taken just before the participant's third administration of chemotherapy (17/07/2012). It has been found that when the liver is damaged, vitamin B12 is released giving a reading of high serum vitamin B12 [401-403], however, tissue reserves are being depleted and the patient can result in a deficiency of vitamin B12 [404].

The blood pathology tests for this participant showed raised liver enzymes and a raised serum vitamin B12 level that could represent the liver releasing stored vitamin B12. The Holo TC is the blood pathology assay for blood levels of vitamin B12 being released from the liver and transported to body tissues [62]. Holo TC blood results have also been found to be raised from liver damage [404], however, it has been reported that it can detect an early deficiency in vitamin B12 compared to serum vitamin B12 levels [62].

PA032 PMP's liver indicated possible damage as noted by raised liver enzymes, so it may be postulated that it was releasing stored vitamin B12 resulting in a vitamin B12 deficiency three-months post serum vitamin B12 test. This participant's vitamin B12 deficiency was symptomatic displayed as peripheral neuropathy and possibly fatigue. The difficulty is differentiating between the patient's symptoms being from chemotherapy and/or a vitamin B12 deficiency.

According to the blood pathology tests, the medical doctors would normally not consider this patient's presentation of CIPN as a possible vitamin B12 deficiency due to her serum vitamin B12 results from July, 2012. Had the subject not participated in this clinical trial, her vitamin B12 most likely would not have been re-tested as her previous tests indicate a saturated state. Therefore, this raises the

question of whether testing vitamin B12 during chemotherapy is worthwhile. Similarly, it is difficult to ascertain whether it gives an accurate reading of the patient's vitamin B12 state.

Testing vitamin B12 during chemotherapy may not give accurate results as the patient may be releasing stored vitamin B12 into the blood from liver damage. However, conducting a one off blood test for vitamin B12 during chemotherapy is different compared to conducting multiple vitamin B12 tests. Adding serum vitamin B12 to blood pathology tests prior to every chemotherapy administration could provide an indication of the patient's vitamin B12 status to the oncologist. If there is an increased risk of peripheral neuropathy from both the administration of chemotherapeutic agents and low vitamin B12, the oncologist would be able to ascertain this early before a vitamin B12 deficiency occurs. Hence, vitamin B12 treatment can be started early **before the patient is in a deficit state that may increase the risk of peripheral neuropathy.**

Is this a reasonable request for all patients undergoing chemotherapy with neurotoxic chemotherapy agents and is it cost effective? When is testing for vitamin B12 conducted if it was not conducted prior to chemotherapy commencement and what do the results indicate? Or should patients take a prophylactic vitamin B12 or B complex during chemotherapy? Patient comfort, quality of life and ability to complete the prescribed chemotherapy regime without dose reduction or cessation are clinically relevant points and important aspects to consider for 'best patient care'.

Intrinsic factor antibodies play an important role in a vitamin B12 deficiency. If a patient is continually found to be low or deficient in vitamin B12 after chemotherapy, then testing for intrinsic antibodies would be indicated. However, if a patient's vitamin B12 level is in normal range without supplementation 6 to 12 months' post-chemotherapy, then testing for intrinsic factor or parental cell antibodies would not be indicated. For PA032 PMP, the serum vitamin B12 was 411 pmol/L seven-months' post-chemotherapy administration which is in the reference range. The patient stated that during the seven months after the end of the B vitamin study, no oral vitamin B12 supplementation had been taken, nor had she been administered intramuscular vitamin B12. Therefore, it can be assumed that this participant does not have intrinsic factor antibodies. The subject was given a pathology request form for intrinsic factor antibody test but it was not performed.

Table 8-13: PA032 PMP Vitamin Level Blood Results

	13/07/2012	22/06/2013	Ref Range
Serum Vitamin B12	H >1476	411	162-811 pmol/L
Red Cell Folate	1265	1290	545-3370 nmol/L
Serum Ferritin	H 743	H 395	15-290 ug/L
Anti-tissue transglutaminase IgA	<4		<4 U/mL

8.2.3.6.7 Isotope Bone Scan

Date: 11/02/2013

History: Recent six weeks of pulsing pain down spine and more constant low back pain radiating through to hips bilaterally. Worse posterior left hip and worse with movement. Previous breast carcinoma.

Findings: Diffuse activity throughout the right breast, noted on recent ultrasound commented on infected sebaceous cyst. The activity is out of proportion however these probably highlighted by the asymmetry and it is assumed that there is not a disuse breast carcinoma. This requires careful correlation with clinical history and clinical evaluation. There was little uptake in knees, ankles and feet which is likely to be degenerative and minimal asymmetry of uptake in the mid to lower lumbar spine is also degenerative. There is no evidence of metastatic disease or other focal bone process of note.

8.2.4.2.8 Auto-antibody, Cancer Markers and Inflammatory Pathology

The blood pathology results seen in Table 8-14 were requested and collected before PA032 PMP's second administration of chemotherapy. Cancer markers and auto-immune markers are not commonly requested at this point of treatment. After the participant's first chemotherapy administration, the cancer care coordinator recorded that the patient had experienced terrible joint and bone pain (not due from neulasta as it had not been administered yet), severe diarrhoea, nausea, mouth ulcers, had a rash and possible fistulas. The subject was prescribed endone for the pain. It is assumed that the blood pathology tests were conducted to elucidate what maybe causing the patient's discomfort. From the blood test results, the subject was experiencing an inflammatory state (CRP = 11 mg/L).

Table 8-14: PA032 PMP Auto-antibody, Cancer Markers and Inflammatory Blood Pathology Results

Pathology	22/06/2013	Ref Range
ANA (HEP-2)	<80	<80
Anti-CCP antibody	<1	<5
CRP	+11	0-6 mg/L
CA 15.3	13	<30 U/ml
CEA	1.1	Up to 4.6 ug/L

8.2.4 Discussion on PA032 PMP

PA032 PMP baseline blood test indicated a normal vitamin B12 level, as measured by Holo TC assay 107 pmol/L ref >35 pmol/L). Prior to the third cycle of chemotherapy, the subject's serum vitamin B12 was found to be elevated >1476 pmol/L (162-811 pmol/L). Subsequently, PA032 PMP's Holo TC assay after completion of the full chemotherapy regime indicated a deficiency in vitamin B12 (29 pmol/L ref >35). No other B vitamin markers were found to be deficient. After two months of vitamin B12 administration (1,000 µg intramuscularly and 1,000 µg taken orally daily), the vitamin B12 levels from the Holo TC assay had risen to 106 pmol/L (ref >35 pmol/L). Seven months after the last blood pathology test in the clinical trial, the subject's vitamin B12 levels were 411 pmol/L (162-811 pmol/L) without vitamin B12 administration indicating normal vitamin B12 absorption and metabolism and possibly no intrinsic factor antibodies.

PA032 PMP's neurology test and patient neurological test showed no neuropathy at baseline, however displayed CIPN (Level 2 to 3) after chemotherapy. After two months of vitamin B12 administration (one 1,000 µg intramuscularly and 1,000 µg taken orally daily) the subjects CIPN was reduced to the tips of her fingers and toes (Level 1). Therefore, a patient experiencing a vitamin B12 deficiency while undergoing chemotherapy may display a severe CIPN. This presentation of peripheral neuropathy when a patient is undergoing chemotherapy may be diagnosed as CIPN by the oncologist without testing for a vitamin B12 deficiency. For this participant, the vitamin B12 status was tested and found to be deficient.

After vitamin B12 administration both intramuscularly and orally, the participant's peripheral neuropathy decreased from severe to mild. Hence, the patient was experiencing severe peripheral neuropathy from both a vitamin B12 deficiency and chemotherapy administration. This diagnosis would not have been elucidated without vitamin B12 blood pathology tests.

This case report indicates that chemotherapy administration can lower vitamin B12 status in certain patients and this may predispose them to the development of peripheral neuropathy, possibly of a severe nature. The development of peripheral neuropathy would then be from both a vitamin B12 deficiency and the neurotoxic chemotherapy administration. The extent of the peripheral neuropathy from the chemotherapy agent cannot be ascertained until the patient's vitamin B12 status has been resurrected into a normal range.

The clinical relevance of this is very important as vitamin B12 is not a common pathology test conducted in patients presenting with CIPN.

A vitamin B12 deficiency is medically acknowledged for causing peripheral neuropathy and has been identified as a potential risk factor for the development of CIPN in differential diagnosis tables [12]. The questions still to be answered are how and when should testing of vitamin B12 be conducted and when to intervene with treatment of vitamin B12. One limitation for cancer patients is the taste and smell of taking an oral B vitamin complex. Therefore, for some patients, taking a prophylactic oral vitamin B complex is not an option for them during chemotherapy. Intramuscular vitamin B12 injections maybe the only option for certain patients who cannot tolerate a dose of an oral vitamin B12, or a B vitamin complex supplement.

A further case report is documented below of a participant who was found deficient of vitamin B12 before chemotherapy administration. The subject was followed throughout chemotherapy and or three-months' post-chemotherapy treatment. Another perspective on the clinical relevance of vitamin B12 pathology testing for cancer patient is discussed.

8.3 PA011 GMB - CASE REPORT 2

The participant PA011 GMB was excluded from the clinical trial due to his baseline blood pathology results indicating 15 pmol/L (ref >35) for Holo TC. The participant was asked to consent to being followed throughout the chemotherapy regime and three-months' post-chemotherapy. Consent was given and the results for this participant are documented below in 8.3.1, Table 8-15.

8.3.1 PA011 GMB Details and Results

PA011 GMB at recruitment had just been retrenched, so was unemployed at presentation at the hospital. The subject had recently separated and had two children under his care. Apart from the lymphoma, the patient was generally healthy.

Table 8-15: PA011 GMB Medical Details and Results

PA011 GMB	Details and Results
DOB	07/06/1968
Diagnosed	Non-Hodgkin's B-Cell Lymphoma in February, 2012
Surgery	PA Hospital: Feb 2012 – lymphoma lump under right arm Previous surgery: Ear to increase hearing (4 yo), twisted testicle (26 yo), car accident (9 yo) for related injuries and prescribed dilantin and tegretol for 6 years
Recruited for study	03/02/2012
Race	Caucasian
Gender	Male
Marital Status	Separated
Other medical considerations	Mild asthma, borderline diabetes, glandular fever (EBV)
Medications	Panadol occasionally and an asthma puffer rarely
Allergies	Bee sting
Diet history	No history of being a vegan or vegetarian. Normally consumes red meat (beef, lamb, kangaroo) 3-4 times a week, chicken or pork 2-3 times a week and fish or seafood rarely. He does drink caffeine, approximately one cup a day, and doesn't drink alcohol, smoke and or take recreational drugs.
Height	187 cm
Weight	117 kg
BMI	33.5
Chemotherapy regime	R-CHOP every three weeks for 6 cycles. (Cylophosphamide, vincristine, doxorubicin, prednisone, rituximab)

8.3.2 Blood Pathology Results for PA011 GMB

On the receipt of PA011 GMB's baseline blood results as seen in Table 8-16, the haematologist team at the PA hospital, Brisbane were informed of his results, noting his low Holo TC levels. An IM vitamin B12 injection (1,000 mg) was prescribed. After three cycles of chemotherapy, PA011 GMB organised further injections of vitamin B12 (three in total of 1,000 mg of vitamin B12 each) with the local general practitioner (GP). The blood taken from this patient for analysis of B vitamins after the cessation of chemotherapy was one week after a vitamin B12 injection. This information was

discovered by the researchers after the subject had the blood taken. The last blood test was taken 2 weeks after receiving another vitamin B12 injection from the GP.

Table 8-16: PA011 GMB Vitamin B Blood Pathology Results

Blood Pathology	Baseline 03/02/2012	After Chemo 17/05/2012	Last follow up 19/10/2012	Ref Range
Vitamin B1 (TDP)	**220 H**	160	**220 H**	66-200 nmol/L
Vitamin B2 (FAD)	340	240	290	180-470 nmol/L
Vitamin B6 (P5P)	70	85	85	35-110 nmol/L
Red cell folate	1430	2502	1808	>900 nmol/L
Holo TC (Vitamin B12)	**15 L**	>128	88	>35 pmol/L

8.3.3 Neurological Testing Results for PA011 GMB

8.3.3.1 Total Neuropathy Score (TNS)

PA011 GMB's results shown in Table 8-17, indicates no peripheral neuropathy at baseline before chemotherapy administration, although the patient presented with sweating due to lymphoma. Testing post-chemotherapy regime, PA011 GMB presented with mild CIPN, with motor nerve function worse than sensory. PA011 GMB experienced a delayed CIPN as the follow-up neurology test conducted three-months' post-chemotherapy cessation found severe (Level 4) sensory neuropathy. Level 4 sensory peripheral neuropathy means numbness, tingling and/or pain above knees and elbows and is functionally debilitating. The sural nerve and tendon reflexes had decreased at three-months' follow-up, however pin sensibility improved. The pinprick test is for small nerve function therefore, indicating the severe sensory neuropathy diagnosed was due to large nerve damage rather than small nerve.

A vitamin B12 deficiency damages large nerve fibres [12] therefore, it could be postulated that the patient's vitamin B12 deficiency found at baseline may have previously damaged the large nerve fibres which the neurotoxic chemotherapy agent further affected.

Table 8-17: PA011 GMB TNS Scores

Total Neuropathy Score (TNS)			
Item	**Baseline Score 04/02/2012**	**After Chemotherapy 02/06/2012**	**Three months later 08/09/2012**
Sensory symptoms	0	1	4
Motor symptoms	0	2	1
Autonomic symptoms	2 (sweating from lymphoma)	3	2
Pin sensibility	0	1	0
Vibration sensibility	0	2	1
Strength	0	0	0
Tendon reflexes	0	4	4
Sural amplitude score	0	0	4
Peroneal amplitude score	0	0	0
Total	2	13	16

8.3.3.2 Neurological Conduction Studies (NCS)

The NCS for PA011 GMB indicates that the patient did experience nerve damage, particularly sensory after chemotherapy administration. The wrist and sural nerve showed nerve damage after chemotherapy, particularly the sural nerve, which was unable to be detected three-months after chemotherapy. The peroneal nerve also showed nerve damage three-months' post-chemotherapy administration. Both the sural and peroneal nerve damage indicates delayed neuropathy from the vincristine. For motor function, the tibial nerve in the ankle showed nerve damage after chemotherapy but was restored to normal function after three months. The patient's median nerve function seems to have been damaged or has low function before chemotherapy on the right hand and then on the left hand after chemotherapy. As the left hand was not tested prior to chemotherapy, it may not have been functioning ideally before chemotherapy. Overall, the patient does display nerve damage from chemotherapy, therefore has CIPN, particularly delayed peripheral neuropathy.

Table 8-18: PA011 GMB Nerve Conduction Study Results

Sensory NCS							
Nerve/Sites	**Rec Site**	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV
		04/02/2012		**02/06/2012**		**08/09/2012**	
R MEDIAN Palm	III					1.75	8.8
L MEDIAN Palm	III					1.70	5.8
R ULNAR – Wrist	Dorsum of Hand					7.30	6.8
R RADIALIS Wrist		1.74	30.4				
L RADIALIS Wrist	Thumb			1.88	18.6	2.05	14.9
L SURAL – Calf	Lat Malleolus	2.42	11.0	3.32	11.8	absent	
R SURAL = Calf	Lat Malleolus	3.00	10.9	3.29	11.3	absent	

Motor NCS						
Nerve/Sites **Rec Site**	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV
	04/02/2012		**02/06/2012**		**08/09/2012**	
R MEDIAN – APB Wrist	3.5	8.1				
Elbow	7.52	6.5				
L MEDIAN – APB Wrist			3.65	8.8		
L RADIAL – EIP Forearm					3.95	7.7
Elbow					9.50	7.1
R COMM PERONEAL – EDB Ankle	5.66	2.5			6.35	3.7
Bl knee-ankle	14.5	0.014			14.60	3.3
L COMM PERONEAL – EDB Ankle	5.37	3.5	4.58	4.4	5.25	3.7
Fib Head					13.10	3.9
R TIBIAL (KNEE) – AH Ankle	5.88	8.6	6.08	5.4	4.95	3.6
L TIBIAL (KNEE) – AH Ankle	3.89	8.2	6.48	4.9	4.15	4.4

8.3.4 Patient Neurotoxicity Questionnaire for PA011 GMB Results

This is a linear scale and indicates the level of CIPN that the patient has experienced in the past week. The results are presented in Table 8-19.

On the 19/04/12 PA011 GMB was diagnosed with grade 2 CIPN from the haematologist oncologist. The patient stated that their fingers felt burnt and were numb. The numbness was present in all fingers but not the palm of the hand and minimal sensation was present in the toes. The patient also experienced difficulty swallowing, buttoning clothes, picking up objects such as a wallet, was unable to work and had shortness of breath.

On the 08/09/2012 PA011 GMB was still experiencing CIPN, however in the patient's opinion it was not as bad and located mainly in the fingers. The subject also stated that the feet felt different, which was affecting daily activity. The patient was still having difficulty with swallowing, experienced asthma and had shortness of breath in addition to anxiety. The neurologist's examination diagnosed level 4 sensory neuropathy at three months' post-chemotherapy administration, therefore a delayed CIPN had occurred (level 1 sensory neuropathy after chemotherapy). From the patient's perspective, the CIPN was worse straight after chemotherapy in the PNQ (3/4) compared to three months' post-chemotherapy administration (1/4) which is completely opposite to the neurologist's examinations.

The difference between clinical examination and patient's perspective highlights the importance of incorporating both aspects in examination and diagnosis of CIPN. The main confounder for chemotherapy patient's perspective is their general feeling of well-being during and straight after chemotherapy administration versus three months' post-chemotherapy. How the patient feels may influence their perspective of CIPN at the time of questioning. Therefore, the examination from an independent neurologist is important for an unbiased, quantitative medical diagnosis.

Table 8-19: PA011 GMB Patient Neurotoxicity Questionnaire Results

Items			
	04/02/12	**19/04/12**	**08/09/2012**
Numbness, Pain, burning or tingling	1/4 (lymphoma under arm)	3/4	1/4
Weakness in arms and legs	0/4	3/4	2/4
Difficulty with daily activities	0/4	2/4	1/4

8.3.5 Discussion on PA011 GMB

PA011 GMB was excluded from the B vitamin clinical trial due to baseline blood vitamin B12 levels being below the reference range (15 pmol/L ref >35). The baseline neurology test indicated no signs of peripheral neuropathy even from a vitamin B12 deficiency. After administration of an IM vitamin B12 (1,000 µg), PA011 GMB consented to being followed throughout chemotherapy and post-chemotherapy. Throughout the patient's chemotherapy regimen, grade 2 CIPN developed mainly sensory neuropathy in fingers and toes. Half way through the chemotherapy regime, further IM vitamin B12 injections from his GP were administered. The researchers were unable to obtain an accurate list of how many IM vitamin B12 injections he had administered, other than the patient's recollection of monthly injections for 3 months.

Immediately after chemotherapy cessation, PA011 GMB's vitamin B12 level was at the highest recorded level (>128 in ref range >35) and the neurology test and patient's perspective (although worse 3/4) indicated grade 2 CIPN. Three-months' post-chemotherapy cessation, PA011 GMB's blood test indicated the vitamin B12 level was in range (88 in ref range >35) however the neurology test from the neurologist identified a delayed severe sensory peripheral neuropathy, level 4.

Results on record suggest that due to the subject's vitamin B12 deficiency at baseline, the liver stores of vitamin B12 may have been depleted leaving no body reserves of vitamin B12. Chemotherapy can cause a temporary deficiency of vitamin B12 [62], which may have predisposed this patient to the development of CIPN. Alternatively, the patient's large nerve fibres may have been damaged by the vitamin B12 deficiency prior to chemotherapy administration, although no peripheral neuropathy was present at baseline from the neurologist's examination. The administration of neurotoxic chemotherapy may have caused further damage thereby increasing the development of CIPN. The vitamin B12 administered IM may not have been adequate in amount to repair the large nerve damage already present from the vitamin B12 deficiency before the neurotoxic chemotherapy agent (vincristine) was administered.

The delayed severe sensory peripheral neuropathy may or may not be attributed to the patient's vitamin B12 deficiency as PA011 GMB's vitamin B12 level was in range. A delayed CIPN can occur with neurotoxic agents and this may be the case for PA011 GMB.

Another participant PA002 KLB was not followed, nor had consented to being followed; however, from discussions with the cancer care coordinator and checking the patient's hospital chart, the original chemotherapy regimen was down-graded from Hyper CVAD to R-CHOP due severe CIPN. Due to no consent, exact details cannot be obtained, therefore these results cannot be substantiated.

8.4 PA016 GDT – CASE REPORT THREE

8.4.1 PA016 GDT Details and Results

PA016 GDT was a breast cancer patient diagnosed with invasive ductal carcinoma (IDC) and was triple positive (oestrogen, progesterone and HER2 positive). The patient underwent a chemotherapy regimen, AC-DT given every three weeks. PA016 GDT was administered four cycles of AC (Adriamycin and cyclophosphamide) and then four cycles of DT (docetaxel and Herceptin). The Herceptin was administered for a year. The patient was randomised to the B vitamin group.

PA016 GDT had documented no CIPN during the chemotherapy regime. After the last administration of DT, the patient became very nauseous and was bed ridden for one week. In that week, the subject

was unable to take the clinical trial capsules and developed moderate CIPN. The CIPN did not dissipate after commencing the capsules again. The patient's bloods and TNS scores are documented below in Table 8-20.

Table 8-20: PA016 GDT Test Results

	Vitamin B12	Total TNS	Vitamin B1	Vitamin B2	Vitamin B6	Folate
Baseline	>128	6	180	380	300	1820
After chemo	>128	11	170	300	530	2790
3 months follow up		12				

PA016 GDT's vitamin B12 levels were at the maximum range both at baseline and after chemotherapy. The neuropathy, particularly sensory, was worse after chemotherapy and this continued at the three-months follow up. This case report has clinical relevance as CIPN development did not occur throughout the patient's chemotherapy regimen until the B vitamin capsules were not taken for a week post last chemotherapy administration. Taking into consideration that her vitamin B12 level was at the maximum level pre and post-chemotherapy, the CIPN development may not be due to a decrease in vitamin B12. Therefore, it may be a consequence of a temporary decrease of other B vitamins or a change in vitamn B serum saturation. One B vitamin that has shown marked decrease and increase in blood levels as expressed in the absorption/interaction study is vitamin B6. In saying this, the patient's vitamin B6 blood status was above reference range before and after chemotherapy.

CIPN's mechanism of action from the chemotherapy agents are still unknown however, it is known that it is an accumulation side effect [10]. It is possible that the patient's development of CIPN was simply an accumulation of the chemotherapy agent. Therefore, vitamin B12 may only play a part in the development of CIPN for certain patients.

8.5 DISCUSSION

The main case report, PA032 PMP who was found deficient in vitamin B12 post-chemotherapy administration exemplifies that vitamin B12 status may be lowered to a point of deficiency during chemotherapy in certain individuals. This vitamin B12 deficiency may present itself as peripheral neuropathy and in conjunction with neurotoxic chemotherapy administration may be seen as severe CIPN when in fact it is partly due to a vitamin B12 deficiency.

This is clinically relevant as patients undergoing chemotherapy with neurotoxic agents who develop severe CIPN may be deficient in vitamin B12. If patients presenting with moderate to severe CIPN are found to be deficient in vitamin B12, it is possible that some of the severe neuropathy would be alleviated by the administration of vitamin B12. This is important for the patient as it decreases pain, discomfort, increases their physical ability to do daily activities and aids their quality of life.

The second case study, PA011 GMB illustrated that a person deficient in vitamin B12 prior to chemotherapy administration can still develop CIPN, despite vitamin B12 administration intramuscularly. This may be due to the large nerves being damaged by the B12 deficiency and the neurotoxic chemotherapy agent, then accumulating and causing further damage that leads to CIPN establishment. Further research is required to substantiate this finding.

The last case report illustrates that vitamin B12 may not be the only B vitamin that could be connected with the development of CIPN. It is possible that vitamin B6 may play more of a major role than previously thought. A journal article describing four children with vincristine induced peripheral neuropathy were treated with vitamin B6 and pyridostigmine with beneficial results [124]. An *in vitro* study with vitamin B6 and oxaliplatin demonstrated that vitamin B6 may protect cells from oxaliplatin-induced peripheral neuropathy without interfering with anti-tumour activity [17]. Further research is required, but the changes seen in vitamin B6 levels after the administration of chemotherapy as seen in chapter 6 (page 149-150) and the rise that occurs after 72 hours with supplementation of 40 mg of vitamin B6 is important to consider. Whether or not vitamin B6 actually provides a protection to nerve cells still needs to be elucidated.

8.6 CONCLUSION

Vitamin status, in particular vitamin B12, can play a role in the development of CIPN in certain patients. Patients experiencing moderate to severe CIPN should have their vitamin B12 levels pathologically tested and if serum vitamin B12 levels are found below 200 pmol/L or holo TC is below 35 pmol/L, then vitamin B12 should be administered. This can be achieved intramuscularly and orally for the duration of the chemotherapy administration and post-chemotherapy for a period of approximately three months, depending on the patient's tolerance for oral supplementation.

A protection from B vitamins, especially vitamin B12 and vitamin B6 may occur for CIPN development. Oral supplementation with a B complex may assist some patients is with the development of CIPN. This is the easiest method of administration, however, not all patients can tolerate the smell of the vitamin B capsule or they may struggle with the ingestion of the supplement. Also the pungent smell of their urine from the B vitamin supplementation is off putting for some

patients and may increase their feeling of being nauseous. As an alternative, intramuscular administration of vitamin B12 may be more appropriate for certain patients.

Monitoring of vitamin B12 levels may also be implemented so early intervention with B12 administration can occur before the patient is deficient. This could be through adding serum vitamin B12 to the blood pathology request forms for patients undergoing chemotherapy with neurotoxic agents before chemotherapy administration and on presentation of CIPN.

These case reports outline the importance that vitamin B12 may play in CIPN development and the clinical relevance for medical practitioners.

9 CHAPTER 9 – DISCUSSION

9.1 GENERAL DISCUSSION

The study upon which this thesis was based assessed a posit that the administration of an oral B vitamin complex formulation could prevent CIPN when vincristine and the taxane class neurotoxic compounds were administered to patients diagnosed with a cancer. Oxaliplatin assessment was ceased due to poor recruitment. The primary outcome of the TNS showed that B vitamins did not statistically decrease the incidence of CIPN ($p=0.22$; t score=1.24). There was however, a trend toward a beneficial effect of B vitamins decreasing the onset and severity of CIPN. The patient's perception that sensory peripheral neuropathy was decreased by B vitamins was statistically significant (12 weeks $p=0.03$; 24 weeks $p=0.005$; 36 weeks $p=0.021$). The risk estimate for patient's perception of sensory neuropathy was also statistically significant for both after chemotherapy cessation and at three-months follow up (OR=5.78, 95% CI = [1.63-20.5] and 36 weeks OR=.8.1, 95% CI=[1.23-53.2]).

Oncologists are aware that CIPN is a common and debilitating side effect from a variety of neurotoxic chemotherapy agents. Moreover, there is awareness of the negative impact CIPN may have on patients who are administered chemotherapy. Important issues to patients and clinicians alike include treatment outcomes, quality of life and psychological wellbeing. To alleviate this debilitating side effect, a number of different agents have been prescribed by clinicians, or have been independently tried by patients, despite the availability of limited research for some of these interventions such as calcium and magnesium infusions, pregablin and gabapentin [249].

The results from the clinical study demonstrated that participants inducted into the clinical trial and allocated to the B complex vitamin arm that developed CIPN, could not reverse the peripheral neuropathy with continued B complex vitamin supplementation. In addition, B complex vitamin supplementation did not protect against small nerve damage or reflex loss. Blood pathology results showed that B complex vitamin status increased (i.e., Vitamin B1, B6, B12 and folate) with supplementation except for vitamin B2. This data was not associated with CIPN prevention when correlated to the TNS results. A high blood level of vitamin B6 was detected in four participants (10.5%) who also had developed mild CIPN (as assessed by the independent neurologist through the TNS and nerve conduction studies). A statistically significant increase ($p=0.001$) in blood vitamin B6 levels were found that indicated a good response to supplementation. This result though, warrants further research to establish whether an increased risk or protection relationship exists for B6 and CIPN.

The pilot study that assessed the absorption and interaction of B complex vitamin administration during chemotherapy, demonstrated that vincristine, docetaxel and oxaliplatin may induce a temporary decrease in vitamins B1, B2, B6 and B12 in patients receiving a B complex vitamin formulation.

The projection of pain interference observed afforded to those participants administered a vitamin B complex supplement experienced significantly ($p<0.05$) less pain interference with daily activity compared to the subjects who were administered a placebo. No other differences were seen for quality of life or pain.

When considering different cancer types, no benefit was found for breast cancer patients. However, a possible sensory neuropathy benefit was found for lymphoma patients undergoing R-CHOP for eight cycles' three weekly and lung cancer patients undergoing paclitaxel and carboplatin weekly. Furthermore, B complex vitamin supplementation did not protect patients with diabetes from the development of CIPN.

The individual case study on PA032 PMP showed that patients that are deficient in vitamin B12 after chemotherapy cessation may present with moderate to severe CIPN. This adverse clinical outcome is due to the administration of neurotoxic chemotherapy as well as a B12 deficiency, which can also present as peripheral neuropathy. Therefore, monitoring a patient's vitamin B12 levels throughout chemotherapy may assist clinicians in preventing moderate to severe development of CIPN that can progress from a vitamin B12 deficiency.

Supplementation with an oral B vitamin may not be appropriate for certain patients due to difficulties in swallowing the capsules and/or the odour of the urine from the B vitamin excretion. When patients that are administered emetogenic chemotherapy regimens, unpleasant odours such as those produced by B vitamins may trigger further unpleasant behaviour that possibly can exacerbate chemotherapy-induced nausea and vomiting. Therefore, the administration of intramuscular vitamin B12 would be the prudent option for patients undergoing chemotherapy and who have been also diagnosed with a blood vitamin B12 deficiency.

If patients present with a blood vitamin B12 deficiency prior to chemotherapy administration, continual intramuscular injections of vitamin B12 may need to be administered throughout the chemotherapy cycles. Consensus of the how often an IM B12 should be administered requires further verification. In a study conducted in 2003 [174] it was suggested that daily IM administrations for 10 days with a dose of 1,000 mcg, then weekly for four weeks and finally monthly was efficacious in maintaining blood B12 levels in those individuals diagnosed with low blood B12 levels. Such B12

dosing regimens could be adopted for patients with pathology verified blood B12 deficiencies prior to the administration of chemotherapy and post completion of treatment.

An additional study that compared IM vitamin B12 to oral vitamin B12 supplementation reported that IM vitamin B12 given on study days 1, 3, 7, 10, 14, 21, 30, 60 and 90 was effective in restoring normal levels of serum cobalamin in all patients by 100% [406]. This study also provided evidence that a B12 supplementation regimen could be implemented for patients who are vitamin B12 deficient prior to chemotherapy administration throughout their entire chemotherapy cycles and three to six months' post-treatment follow-up.

It is important to note that further controlled studies are required to confirm the best vitamin B12 administration for patients found to be deficient in blood vitamin B12 prior to and post-chemotherapy administration.

9.2 CONCLUSION

B vitamin supplementation throughout chemotherapy administration with neurotoxic agents was not superior to placebo ($p>0.05$) for the prevention of CIPN. Patient perception of reduced sensory peripheral neuropathy with B vitamin supplementation over placebo was statistically significant. Although not significant, a trend was observed for the prevention of the onset and severity of CIPN throughout chemotherapy and three-months' post-chemotherapy cessation with B vitamins supplementation over placebo.. Patients with moderate to severe CIPN may have a vitamin B12 deficiency that may lead to a worse symptomatic presentation. Monitoring of blood vitamin B12 throughout chemotherapy administration may prove to be a prudent assessment in order to prevent the development of moderate to severe CIPN due to neurotoxic chemotherapy agents and a blood vitamin B12 deficiency.

9.3 LIMITATIONS OF STUDY

There were a number of limitations identified both during and after the completion of the study. These included:

1. ***Number of participants recruited***: As stated earlier, the aim of the main clinical trial was to obtain ninety participants plus fifty per cent attrition rate, equating to one hundred and thirty-five participants. This was verified after completing a power analysis for sample size. Due to time of recruitment, the main clinical study was stopped following the recruitment of seventy-one patients. Difficulty in recruitment and participant drop-out lowered the number of

participants recruited for the trial and consequently lowered the power of the study and affected achieving statistical significance for the primary outcome.

2. ***Number of agents assessed***: The majority of the clinical trials that have assessed CIPN have focused on one chemotherapy agent, to increase the statistical power of the study and the mechanism of action of that drug. However, due to the time required to complete this study, it was considered that including a variety of chemotherapy agents would increase the number of participants recruited and would allow for completion of the study. This was the case when the type of cancer and chemotherapy agents were separated from the combined results. However, in retrospect, this made the interpretation of the data difficult due to the fact that each chemotherapy agent has a different mechanism of action and each type of cancer has a variety of confounding factors.
3. ***Assessment tools for CIPN***: The assessment tools used for diagnosing CIPN as mentioned is a major limitation in all CIPN trials, as no one assessment tool is used consistently throughout all studies. There is no 'Gold Standard' assessment tool which makes it difficult to compare results to other studies completed. The TNS which was used as the primary outcome is both subjective and qualitative**.** The subjective nature of some of the criteria limits the quality of results from this assessment tool. However, the major benefit for this clinical study was the participation of only one neurologist who volunteered to assess all participants inducted into the study. Therefore, consistency was partly achieved by having an independent neurologist, blinded to treatment allocation assessing all patients at each time point. Extra neurological examinations were conducted to guarantee results.
4. ***Interaction/absorption trial***. The pilot clinical trial investigating blood B vitamin status after chemotherapy administration had several limitations. It may have been more judicious to have focussed on one chemotherapy agent only.
5. ***Backwards power analysis***: Re-analysis of the data calculating a backwards power calculation to assess the power of the completed study may assist in identifying statistical significant differences.
6. ***Multiple statistical comparisons***: A multiple statistical comparison was not completed and it would have allowed for statistical significant results by play of chance.

9.4 CONTRIBUTIONS TO CURRENT KNOWLEDGE

There are a number of provisions to current knowledge that this thesis has contributed. 1) This is the first clinical trial of its kind conducted to assess the efficacy of B complex vitamins administration for the prevention of CIPN. 2) Investigating different agents for the prevention of CIPN has been

identified, as a much needed area of research. 3) Although a not statistically significant result was reported for B complex vitamins over placebo, a trend in favour of the B complex formulation was achieved. This indicated that the study may have been under-powered. Therefore, supplementing with a B complex vitamin was found safe to use during chemotherapy with possible benefits in the prevention of CIPN. The patient's perception of taking a B vitamin supplement was statistically significant in decreasing sensory CIPN. Hence, as no research to date has trialled a B group vitamin during chemotherapy, these results add to the current knowledge base on CIPN. In addition, this study adds to research on vitamin interactions with chemotherapy administration.

No research on blood B vitamin status and chemotherapy agents have been conducted since 1993 where Vu et al. [62] looked at cancer patient's blood vitamin B12 status during and after chemotherapy and radiation treatment. The absorption and interaction pilot trial and the main clinical trial's blood pathology analysis was the first research conducted on vitamin B1, B2, B6, folate and vitamin B12. Although only a very small pilot trial, it provides insight into the fact that chemotherapy agent's vincristine, docetaxel and oxaliplatin may decrease vitamin B1, B2, B6 and B12 in patients taking a B vitamin supplement within twenty-four hours of chemotherapy administration. The results of the main clinical trial indicated that a slight decrease in vitamin B1, B2, B6, folate and B12 were found post-chemotherapy in the placebo arm. Therefore, certain chemotherapy agents may potentially reduce B vitamin status in patients during and post-chemotherapy.

One of the main contributions to current knowledge is the potential for patients with moderate to severe CIPN to be vitamin B12 deficient after chemotherapy administration. A deficiency in vitamin B12 may attribute to the presentation of moderate to severe CIPN. In patients with a vitamin B12 deficiency, B12 supplementation or intramuscular injections of vitamin B12 may assist in decreasing the severity of their CIPN. Therefore, a patient who is found vitamin B12 deficient after chemotherapy may reduce their CIPN presentation from moderate to severe too mild with vitamin B12 administration. Currently, patients presenting with moderate to severe CIPN during treatment have their neurotoxic chemotherapy decreased or ceased. Pathology testing for vitamin B12 is not a traditional test that is conducted by oncologists on presentation of moderate to severe CIPN and the patient is treated for the CIPN with limited success in most cases.

Further research on vitamin B12 status and CIPN development is required to identify trends in clinical presentation, and treatment predictors of a vitamin B12 deficiency that may develop during chemotherapy.

9.5 FURTHER RECOMMENDATIONS

From the pilot trial conducted in this thesis, a number of further recommendations and future trials can be recommended. Firstly, the NMR and mass spectrometry analysis needs to be conducted to ascertain the results from the samples stored. As the NMR gives a wide spectrum analysis any peaks will be analysed to see if they correlate with B vitamin supplementation and CIPN development.

Depending on results found in the NMR analyses, further research into vitamin B6 and its role in peripheral neuropathy and CIPN development or prevention is recommended. Further in vivo pathology tests on patients undergoing chemotherapy and their vitamin B6 status with or without supplementation in combination with neurological examination needs to occur. A focus on oxaliplatin and vitamin B6 supplementation looking at a dose range study and CIPN development would be of benefit.

Introducing regular pathological testing of vitamin B12 for patients undergoing chemotherapy is recommended as prevention is better than treatment for CIPN. It is recommended that hospitals and clinicians set a protocol of incorporating testing vitamin B12 before each chemotherapy administration for all patients undergoing treatment. Therefore, a patient whose blood pathology is indicating a low vitamin B12 status can be administered vitamin B12 early preventing a vitamin B12 deficiency in addition to decreasing the onset and severity of CIPN development.

Lastly, several future trials are recommended incorporating statistically significant results from other agents:

1. A pilot trial assessing B vitamins and omega 3-fatty acids for decreasing the incidence of taxane-induced peripheral neuropathy.
2. A safety and efficacy trial assessing the dose range of vitamin B6 for oxaliplatin-induced peripheral neuropathy.
3. A pilot trial assessing vitamin B12, B6 and omega 3-fatty acids for vincristine-induced peripheral neuropathy.
4. *In vitro* work on Schwann cell cultures and the impact of inflammation on CIPN development.

9.6 FINAL STATEMENT

The study upon which the research of this thesis was based reports that vitamin B complexadministration did not demonstrate efficacy ($p>0.05$) for reducing the total incidence of CIPN development for those patients administered neurotoxic compounds such as the taxanes and vincristine. Notwithstanding, a trend was observed for vitamin B complex administration decreasing the onset and severity of CIPN development. Moreover, patients administered a vitamin B complex supplement reported a reduced onset, severity and incidence of sensory peripheral neuropathy ($p<0.05$).

One case study found that a deficiency in vitamin B12 during or after chemotherapy may also be a contributing factor for moderate to severe CIPN presentation in patients. Further research is warranted however, a prudent investigation prior to commencing chemotherapy administration may be to request a pathology blood test for vitamin B12.

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11 APPENDICES

11.1 APPENDIX 1. MOLECULAR WEIGHT AND BI-PRODUCTS OF B VITAMINS AND CHEMOTHERAPY AGENTS

Molecule	Molecular Weight ($g\ mol^{-1}$)	Pharmacology
Chemotherapy Agents		
Oxaliplatin	397.30[1,7]	**Chemical name**: oxalato(transl-1,2- diaminocyclohexane)[7] **Pharmacology:** dichloro(dach) is the active component.[7] There is no evidence of cytochrome P450-mediated metabolism *in vitro*. Up to 17 platinum-containing derivatives have been observed in plasma ultra-filtrate samples from patients, including several cytotoxic species e.g. monochloro DACH platinum, dichloro DACH platinum, and monoaquo and diaquo DACH platinum in addition to a number of noncytotoxic, conjugated species.[1]
Paclitaxol	853.90[1]	**Chemical name:** Paclitaxol[1] **Pharmacology:** Placlitaxol represents about 5% in faeces, however, it is metabolised in the liver by the cytochrome P450 isozymes CYP2C8 and CYP3A4 primarily to metabolites 6α-hydroxypaclitaxel with minor metabolites, 3'-p-hydroxypaclitaxel and 6α, 3'-p-dihydroxypaclitaxel.[1]
Vincristine	923.04[1]	**Chemical name:** Vincristine sulfate **Pharmacology:** It has been found to be primarily metabolised by hepatic cytochrome P450 isoenzymes in the CYP 3A subfamily. Within 15-30 minutes after injection 90% of vincristine is distributed into tissues from the blood. Excretion is primarily through faeces 80% with 10%-20% in urine. No metabolities have been named.[1]
B Vitamins		
Vitamin B1	300.84[3]	**Chemical name**: Thiamine[8] **Pharmacology**: Combines with adenosine triphosphate (ATP) for form thiamine pyrophosphate (TPP) and thiamine triphosphate (TTP)[8]
Vitamin B2	376.40[2]	**Chemical name:** Riboflavin[8] **Pharmacology:** Phosphorylated to flavin mononucleotide (FMN) in gastrointestinal mucosal cells, erythrocytes and the liver. FMN is than converted to flavin adenine dinucleotide (FAD) which are primarily protein bound.[8]
Vitamin B3	122.13[3]	**Names**: Niacin, Nicotinamide, Nicotinic acid[9] **Chemical name:** Pyridine-3-Carboxyamide[3] **Pharmacology**: Major metabolite NAD++ (nicotinamide adenine dinucleotide.[3] Other metabolites include NADH, NADP and NADPH.[6]
Vitamin B5	476.54[3]	**Chemical name:** Pantothenic acid

		Pharmacology: Converted to major metabolites include CoA and 4'-phosphopantetheine. [9]
Vitamin B6	205.64[3]	**Chemical name:** Pyridoxine, pyridoxal, pyridoxamine **Pharmacology:** Phosphorylated to pyridoxine phosphate, pyridoxal phosphate and pyridoxamine phosphate. In the liver they are than converted to pyridoxal phosphate (PLP). Excreted as pyridoxic acid.[9]
Folate	441.40[6]	**Chemical name:** Pteroylglutamate or pteroylmonoglutamate. Principal pteroylpolyglutamates components found in foods include 5-methyl tetrahydrofolate and 10-formyl tetrahydrofolate. 150 different forms of folate have been reported.[9] **Pharmacology:** Within intestinal cells, folate is converted to dihydrofolate (DHF) and then tetrahydrofolate (THF). Folate is found in the blood as monoglutamate forms such as THF, 5-methy THF and 10-formyl THF plus other minor forms. [9]
Vitamin B12	1355.40[6]	**Chemical name**: Cobalamin. Forms of vitamin B12 include cyanocobalamin, hydroxocobalamin, aquocobalamin, nitritocobalamin, 5'-deoxyadenosylcobalamin and methylcobalamin.[9] **Pharmacology**: In the blood, methylcobalamin comprises of about 60-80% and adenosylcobalamin around 20%. Other minor forms include cyanocobalamin and hydroxocobalamin.[9]
Biotin	244.31[5]	**Chemical name:** Biotin: 5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid.[6,9] **Pharmacology**: Biotin is found in plasma mostly in a free state with small amounts bound to proteins. Its activated form in the body is biotinyl adenosine monophosphate and pyrophosphate. This activated form is converted to Holocarboxylase synthetase which forms the basis for the four biotin-dependant enzymes which include pyruvate carboxylase, acetyl CoA carboxylase, propionyl CoA carboxylase and β-methylcrotonyl CoA carboxylase.[9]
Choline	253.25[6]	**Chemical name**: Choline **Pharmacology**: Choline can be found in the body as part of the neurotransmitter acetylcholine. It can also be found as the phospholipid phosphatidyl choline and spingomyelin.[9]
Inositol	180.16[6]	**Chemical name:** Cyclohexane-1,2,3,4,5,6-hexol[6] **Pharmacology**: *Myo*-inositol can be found in the body as structural basis for a number of secondary messengers such as inositol phosphates, phosphatidylinositol (PI) and phosphatidylinositol phosphate (PIP) lipids.[6]

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7. Alcindor T, Beauger N. Oxaliplatin: a review in the era of molecularly targeted therapy. Curr Oncol 2011 Jan:18(1):18-25
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11.2 APPENDIX 2. TOTAL NEUROPATHY SCORE (TNS)

Item	0	1	2	3	4	Score
Sensory symptoms (numbness, tingling, and neuropathic pain)	None	Limited to fingers or toes	Extension to ankle or wrist	Extension to knee or elbow	Above knees or elbows or functionally disabling	
Motor symptoms	None	Slight difficulty	Moderate difficulty	Assistance required	Paralysis	
Autonomic symptoms	None	One yes	Two yes	Three yes	Four or five yes	
Pin sensibility	Normal	Reduced in fingers or toes	Reduced to wrist or ankle	Reduced to elbow or knee	Reduced above elbow or knee	
Vibration sensibility	Normal	Reduced in fingers or toes	Reduced to wrist or ankle	Reduced to elbow or knee	Reduced above elbow or knee	
Strength	Normal	Mild weakness	Moderate weakness	Severe weakness	Paralysis	
Tendon reflexes	Normal	Ankle reflex reduced	Ankle reflex absent	Ankle reflex absent or others reduced	All reflexes absent	
QST vibration threshold	Normal to < 95 percentile	95–96 percentile	97 percentile	98 percentile	> 99 percentile	
QST thermal threshold	Normal to < 95 percentile	95–96 percentile	97 percentile	98 percentile	> 99 percentile	
Sural amplitude score	Normal or reduced < 5%	76%–95% of LLN	51%–75% of LLN	26%–50% of LLN	0%–25% of LLN	
Peroneal amplitude Score	Normal or reduced < 10%	76%–95% of LLN	51%–75% of LLN	26%–50% of LLN	0%–25% of LLN	
					Total	

LLN—lower limit of normal; QST—quantitative sensory testing
Note. Based on information from Chaudhry et al., 1996, 2002.

11.3 APPENDIX 3. PATIENT'S NEUROTOXICITY QUESTIONNAIRE – LONG VERSION (SHORT VERSION FOR PATIENT DIARIES DOES NOT INCLUDE ITEM 3.)

In relation to chemotherapy induced peripheral neuropathy, indicate with a **vertical line** through the scales below (as shown on the example), the **level of numbness, pain , tingling or weakness** as indicated you have experienced over the **PAST WEEK**.

Item 1: Numbness, pain, burning or tingling in hands or feet and if this affects activities of daily living.

l_____l_____l_____l_____l

None	Mild and no interference	Moderate and no interference	Moderate to severe and interference	Severe and completely prevents you from doing activities

Item 2: Weakness in arms and legs and if this interferes with activities of daily living.

l_____l_____l_____l_____l

None	Mild and no interference	Moderate and no interference	Moderate to severe and interference	Severe and completely prevents you from doing activities

Item 3: Difficulty in swallowing, breathing, drinking or chewing food, or muscle spasms in my mouth/jaws, hands/fingers or feet/toes.

l_____l_____l_____l_____l

None	Mild difficulty	Moderate difficulty	Moderate to severe difficulty	Severe difficulty

If you have indicated a description that states 'moderate to severe' or 'severe' in one or more items above, please indicate by placing an X or writing in the space provided which activity or activities have been interfered with, as a result of therapy.

Button clothes ☐	Use other eating utensils ☐	Operate a remote control ☐
Use a knife ☐	Drive ☐	Put on jewellery ☐
Use a fork ☐	Fasten buckles ☐	Knit ☐
Use a spoon ☐	Sleep ☐	Drinking liquids ☐
Swallowing ☐	Climb stairs ☐	Sew ☐
Open doors ☐	Type on a keyboard ☐	Work ☐
Zippers ☐	Eating/chewing ☐	Tie shoes ☐
Put in or remove contact lenses ☐	Write ☐	Shortness of breath ☐
Work or perform activities Of importance to me, specify ☐	Walk ☐	Dial or use a telephone ☐

11.4 APPENDIX 4. EORTC QUALITY OF LIFE QUESTIONNAIRE

EORTC Quality of Life Questionnaire (QLQ)-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. **There are no "right" or "wrong" answers**. The information that you provide will remain strictly confidential

	Not at All	**A Little**	**Quite a Bit**	**Very Much**
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4
During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4
During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
17. Have you had diarrhoea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Total Score: _____

11.5 APPENDIX 5. BRIEF PAIN INVENTORY

STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #:_____

Brief Pain Inventory (Short Form)

Date: ____/____/____ **Time:** _____

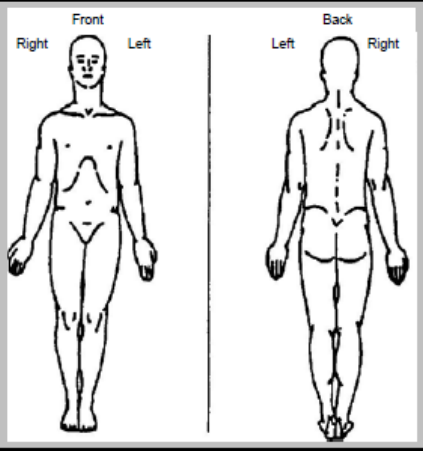
Name: _____ _____ _____

Last First Middle Initial

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its worst in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

4. Please rate your pain by circling the one number that best describes your pain at its least in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

5. Please rate your pain by circling the one number that best describes your pain on the average.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

6. Please rate your pain by circling the one number that tells how much pain you have right now.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

Page 1 of 2

STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #:_____

Date: ___/____/____ **Time:** _____

Name: _____ _____ _____
Last First Middle Initial

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
No Relief — Complete Relief

9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

0 1 2 3 4 5 6 7 8 9 10
Does not Interfere — Completely Interferes

B. Mood

0 1 2 3 4 5 6 7 8 9 10
Does not Interfere — Completely Interferes

C. Walking Ability

0 1 2 3 4 5 6 7 8 9 10
Does not Interfere — Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0 1 2 3 4 5 6 7 8 9 10
Does not Interfere — Completely Interferes

E. Relations with other people

0 1 2 3 4 5 6 7 8 9 10
Does not Interfere — Completely Interferes

F. Sleep

0 1 2 3 4 5 6 7 8 9 10
Does not Interfere — Completely Interferes

G. Enjoyment of life

0 1 2 3 4 5 6 7 8 9 10
Does not Interfere — Completely Interferes

11.6 APPENDIX 6: ASSAYS USED BY SULLIVAN NICOLAIDES FOR B VITAMINS

Test Principle for Sullivan Nicolaides Vitamin B1, B2, B6 Analysis

1.1.1 Vitamin B1[1]

Traditionally, vitamin B1 status was measured using the erythrocyte transketolase test. In that test, the formation of glyceraldehyde-3-phosphate by a TTP-dependent enzyme, transketolase, in haemolysed erythrocytes is taken as an index of patient's thiamine store. Although the test was indicative of only active vitamin (TPP), several interferences could result from certain disease conditions as well as interaction from thiamine-independent enzymes.

The assay described in this procedure is a direct determination of TPP in whole blood via HPLC. It allows rapid measurement of the physiologically active form of vitamin B1 in an isocratic HPLC system with fluorescent detection.

1.1.2 Vitamin B2[2]

The described method allows for the separation and measurement of FAD, FMN and riboflavin in whole blood (though FAD is the only parameter reported at this time). It uses simple protein precipitation and extraction followed by analysis on an Isocratic HPLC system with fluorescent detection.

1.1.3 Vitamin B6[3]

The described method allows for specific determination of vitamin B6 (as pyridoxal-5'-phosphate or P5P). P5P is extracted from whole blood samples. The extracted samples are derivatised to form a stable P5P-fluorescent derivative, then run through an isocratic HPLC with fluorescent detection.

- Chromsystems Reagent kit For The Analysis Of Vitamin B1 In Whole Blood (Chromsystems Part# 35000)
- Chromsystems Reagent kit For The Analysis Of Vitamin B2 In Whole Blood (Chromsystems Part# 37000)
- Chromsystems Reagent kit For The Analysis Of Vitamin B6 In Whole Blood (Chromsystems Part# 31000/WB)

Sent by Sullivan Nicolaides 1st July, 2014

The assay used for Folate is listed below:

ARCHITECT
SYSTEM

en
Folate
REF 1P74
G2-7444/R04
B1P740

Read Highlighted Changes
Revised June 2012

Folate

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	SEPTUM	Septum
IVD	*In Vitro* Diagnostic Medical Device	CONTROL NO.	Control Number
LOT	Lot Number	REPLACEMENT CAPS	Replacement Caps
SN	Serial Number	REAGENT LOT	Reagent Lot
	Expiration Date	REACTION VESSELS	Reaction Vessels
2°C 8°C	Store at 2-8°C	SAMPLE CUPS	Sample Cups
	Caution	CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
	Consult instructions for use	GTIN	Global Trade Item Number
	Manufacturer	PRODUCT OF IRELAND	Product of Ireland

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

- The following warnings and precautions apply to this component:
  - Pre-Treatment Reagent 1

DANGER:	Contains potassium hydroxide.
H314	Causes severe skin burns and eye damage.
H290	May be corrosive to metals.
Prevention	
P234	Keep only in original container.
P260	Do not breathe mist/vapours/spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/ eye protection.
Response	
P301+P330 +P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361 +P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P363	Wash contaminated clothing before reuse.
P310	Immediately call a POISON CENTER or doctor/physician.
P390	Absorb spillage to prevent material damage.
Storage	
P405	Store locked up.
P406	Store in corrosive resistant container with a resistant inner liner.
Disposal	
P501a	This material and its container must be disposed of in a safe way.

- The Microparticles, Assay Specific Diluent and Specimen Diluent contain sodium azide. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagents, calibrators, or controls beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT Folate Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- **Prolonged exposure of Folate Pre-Treatment Reagent 1 to air without septum in place may compromise performance.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, and have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

NOTE: Hereafter, instructions in this package insert that pertain ONLY to the Folate RBC assay are contained in a text box.

- The ARCHITECT Folate Reagent Kit, Folate RBC Lysis Diluent, Folate Manual Diluent, and the Controls must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.

  NOTE: The ARCHITECT Folate Reagent Kit is shipped cold and should be stored at 2-8°C after receipt. Calibrators are shipped frozen and must be stored at -10°C or colder.
- Calibrators and Controls are sensitive to light. **Store bottles in carton to protect from light.**

- Unreconstituted Folate Lysis Reagent (L1) must be stored at 15-30°C. Reconstituted Folate Lysis Reagent (L1) must be stored at 2-8°C. The expiration date is 7 days from the date of reconstitution. Write the expiration date of the reconstituted Folate Lysis Reagent (L1) on the bottle but do not exceed the lot expiration date printed on the bottle.

- The ARCHITECT Folate Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT Folate (1P74) assay files are named "Folate II" and "FolateRBC".
- The ARCHITECT Folate II (assay number 685) and/or FolateRBC (assay number 686) assay file(s) must be installed on the ARCHITECT *i* System before performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be installed on the ARCHITECT *i* System prior to performing the assay. For information on installing and deleting maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 2.
- ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be run at a minimum once every 24 hours. For laboratories processing a higher volume of B12 (List 6C09) and Folate tests on a single module, this procedure must be run more than once in a 24-hour period.
  - If B12 (List 6C09) and Folate are run on a single module and you run > 100 B12 (List 6C09) or > 100 Folate tests in 24 hours, perform the *6041 Daily Maintenance* procedure (version 5 or higher) after every 100 B12 (List 6C09) or 100 Folate tests run.
  - Refer to **LIMITATIONS OF THE PROCEDURE** for additional information.
- If microbial contamination is suspected when running ARCHITECT Folate on the ARCHITECT *i* System due to shifts in results and/or the incidence of calibration failures with the following error codes:
  - 1402 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, calibrators incorrectly loaded
  - 1206 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, concentration too high for Cal A

WARNING:
Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the ARCHITECT Folate assay. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this package insert.

NAME
ARCHITECT Folate

INTENDED USE
The ARCHITECT Folate assay is a chemiluminescent microparticle Folate Binding Protein assay for the quantitative determination of folate in human serum, plasma, and red blood cells on the ARCHITECT *i* System.

SUMMARY AND EXPLANATION OF TEST
Folates are a class of vitamin compounds related to pteroylglutamic acid (PGA), which serve as cofactors in the enzymatic transfer of single carbon units in a variety of metabolic pathways.[1,2] Folate mediated one-carbon metabolism represents one of the most important biochemical reactions that occur in cells. Folates are necessary for nucleic acid and mitochondrial protein synthesis, amino acid metabolism, and other cellular processes that involve single carbon transfers. Folates can serve as carbon donors or acceptors. Since different metabolic pathways require carbon groups with different levels of oxidation, cells contain numerous enzymes that change the oxidation state of carbon groups carried by folates[2] resulting in different metabolically active forms of folate. The predominant form of circulating folate is 5-methyltetrahydrofolic acid (5-mTHF). A methyl group is transferred from 5-mTHF to cobalamin in the pathway that links metabolism of folic acid and vitamin B12.[3]

Folate deficiency can be caused by low dietary intake, malabsorption due to gastrointestinal diseases, inadequate utilization due to enzyme deficiencies or folate antagonist therapy, drugs such as alcohol and oral contraceptives, and excessive folate demand, such as during pregnancy.[4] Because deficiencies of both vitamin B12 and folate can lead to megaloblastic (macrocytic) anemia, appropriate treatment requires differential diagnosis of the deficiency; thus, both vitamin B12 and folate values are needed. Low serum folate levels reflect the first stage of negative folate balance, and precede tissue depletion.[5] Low red-blood-cell folate values reflect the second stage of negative folate balance, and more closely correlate with tissue levels and megaloblastic anemia.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE
The ARCHITECT Folate assay is a two-step assay for the quantitative determination of folate in human serum, plasma, and red blood cells (RBC) using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Two pre-treatment steps mediate the release of folate from endogenous folate binding protein. In Pre-Treatment Step 1, sample and Pre-Treatment Reagent 2 (Dithiothreitol or DTT) are aspirated and dispensed into a reaction vessel (RV). In Pre-Treatment Step 2, an aliquot of sample/Pre-Treatment Reagent 2 mixture is aspirated and dispensed into a second RV. Pre-Treatment Reagent 1 (potassium hydroxide or KOH) is then added. An aliquot of the pre-treated sample is transferred into a third RV, followed by the addition of Folate Binding Protein (FBP) coated paramagnetic microparticles and assay specific diluent. Folate present in the sample binds to the FBP coated microparticles. After washing, pteroic acid-acridinium labeled conjugate is added and binds to unoccupied sites on the FBP-coated microparticles. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of folate in the sample and the RLUs detected by the ARCHITECT *i* optical system.

In the Folate RBC assay, an initial manual pre-treatment step converts RBC-bound folate to measurable folate, after which these samples are processed as described above.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS
Reagent Kit, 100 Tests/500 Tests
NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT Folate Reagent Kit (1P74-25, 1P74-35)
- **MICROPARTICLES** 1 Bottle (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) Anti-Folate Binding Protein (mouse, monoclonal) coupled to microparticles affinity-bound with Folate Binding Protein (bovine), in TRIS buffer with protein stabilizers (human serum albumin and caprine). Minimum concentration: 0.08% solids. Preservatives: sodium azide and antimicrobial agents.
- **CONJUGATE** 1 Bottle (29.0 mL per 100-test bottle/29.0 mL per 500-test bottle) Pteroic Acid (PTA) - acridinium labeled conjugate in MES buffer with protein stabilizer (porcine). Minimum concentration: 4 ng/mL. Preservative: antimicrobial agents.
- **ASSAY SPECIFIC DILUENT** 1 Bottle (5.7 mL per 100-test bottle/25.3 mL per 500-test bottle) Folate Assay Specific Diluent containing borate buffer. Preservatives: sodium azide and antimicrobial agents.
- **PRE-TREATMENT REAGENT 1** 1 Bottle (50.2 mL per 100-test bottle/50.2 mL per 500-test bottle) Folate Pre-Treatment Reagent 1 containing potassium hydroxide.
- **PRE-TREATMENT REAGENT 2** 1 Bottle (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) Folate Pre-Treatment Reagent 2 containing dithiothreitol (DTT) in acetic acid buffer with EDTA.
- **SPECIMEN DILUENT** 1 Bottle (5.5 mL per 100-test bottle/25.9 mL per 500-test bottle) Folate Specimen Diluent containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

Manual Diluent
ARCHITECT Folate Manual Diluent (1P74-50)
- **MANUAL DILUENT** 1 Bottle (4 mL) Folate Manual Diluent containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

Other Reagents
ARCHITECT Folate RBC Lysis Diluent (1P74-40)
- **RBC LYSIS DILUENT** 1 Bottle (12.5 mL) Folate RBC Lysis Diluent (L2) containing citric acid and guanidine hydrochloride. Preservative: antimicrobial agent.

Folate Lysis Reagent (3P21-60)
- **LYSIS REAGENT** 4 Bottles (285-385 mg each) Folate Lysis Reagent (L1) containing ascorbic acid and guanidine hydrochloride.

ARCHITECT *i* Pre-Trigger Solution
- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution
- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer
NOTE: Bottle and volume varies based on order.
- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS
- **IVD**
- For *In Vitro* Diagnostic Use.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions
- **CAUTION**: This product contains human sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[6], Biosafety Level 2[7] or other appropriate biosafety practices[8,9] should be used for materials that contain or are suspected of containing infectious agents.
- The human serum albumin used in the Microparticles and Specimen Diluent has been tested and found to be nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.

- 1120 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, fit response too low for Cal A

the following actions must be taken to protect the integrity of assay results:

- Contact your local customer support representative to schedule the local Abbott Service Representative to perform the *2180 Internal Decontamination* procedure on your ARCHITECT *i* System. If the instrument is connected to an Automatic Reconstitution Module (ARM), the *2182 ARM Decontamination* procedure must also be executed.
- It may be necessary to repeat the decontamination procedure if microbial contamination recurs.

- When configuring the host for the Folate RBC assay, set the appropriate default dilutions:
  - If running whole blood specimens or whole blood controls, configure the default dilution as "RBC DIL".
  - If running controls other than whole blood controls, configure the default dilution as "UNDILUTED".

- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT Folate assay is ng/mL. An alternate result unit, nmol/L, may be selected for reporting results by editing assay parameter "Result concentration units", to nmol/L. The conversion factor used by the ARCHITECT *i* System is 2.265.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen collection tubes listed below were verified for use with the ARCHITECT Folate assays. Other specimen collection tubes have not been tested with these assays.

Folate	Folate RBC
• Serum (glass or plastic tube) • Serum separator (SST) • Lithium heparin plasma • Lithium heparin plasma separator (PST)	• Whole blood dipotassium EDTA (K_2 EDTA) • Whole blood tripotassium EDTA (K_3 EDTA)

- **Do not use human plasma collected in dipotassium or tripotassium EDTA tubes for Folate.**

- **Do not use human whole blood collected in lithium heparin tubes for Folate RBC.**

- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT Folate assays.
- Human serum, plasma, or whole blood specimens to be tested for folate should be protected from light.[10,11]
- Serum or plasma specimens should be collected from fasting individuals. Recent food intake may appreciably increase the folate concentration.[11]
- Do not use hemolyzed specimens. Serum or plasma specimens that are hemolyzed will give falsely elevated folate levels.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - hemolyzed
  - obvious microbial contamination
- Performance has not been established for the use of cadaveric specimens or body fluids other than human serum, plasma, and whole blood.
- For accurate results, serum or plasma specimens should be free of fibrin, red blood cells and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation. Serum or plasma specimens containing red blood cells may give falsely elevated folate levels.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged before testing if
  - they contain fibrin, red blood cells, or other particulate matter, or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.

Storage

- Human serum, plasma, or whole blood specimens to be tested for folate should be protected from light.[10,11]
- Remove serum from clot or separator gel as soon as possible after complete clot formation. If testing will not be performed immediately, serum specimens may be stored either at 2-8°C for up to 7 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Remove plasma from red blood cells as soon as possible upon receipt.[11] If testing will not be performed immediately, plasma specimens may be stored either at 2-8°C for up to 7 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Avoid more than 3 freeze/thaw cycles.

- For red blood cell folate measurements, mix whole blood tube by inverting 10 times to ensure a homogeneous sample. **Determine the hematocrit of each specimen prior to storage.** The hematocrit value will be required in Calculations 1 and 2 beginning on page 6.
- If testing will not be performed immediately, whole blood specimens may be stored at 2-8°C for up to 2 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Avoid more than 1 freeze/thaw cycle.

Shipping

- Before shipping specimens, it is recommended that serum and plasma specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens must be shipped frozen (-10°C or colder) on dry ice and protected from light. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 1P74 ARCHITECT Folate Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT Folate Assay file, may be obtained from:
  - ARCHITECT *i* System e Assay CD-ROM found on www.abbottdiagnostics.com
  - ARCHITECT *i* System Assay CD-ROM
- 1P74-01 ARCHITECT Folate Calibrators
- 1P74-10 ARCHITECT Folate Controls
- 1P74-50 ARCHITECT Folate **MANUAL DILUENT**
- 1P74-40 ARCHITECT Folate **RBC LYSIS DILUENT**
- 3P21-60 Folate **LYSIS REAGENT**
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**

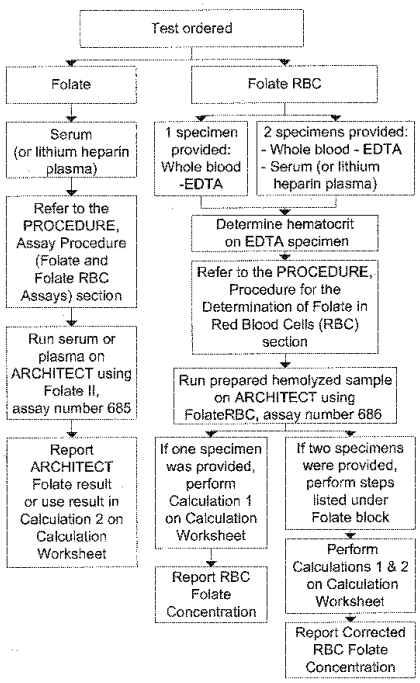
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure Overview

The Folate result is obtained using serum or plasma specimens. The Folate RBC result is obtained using a hemolysate prepared from whole blood. The Folate RBC result includes folate present in the RBCs and in the plasma. In order to obtain the folate concentration only in the RBCs, both specimens are required and a calculation is performed using results from both assays to obtain a Corrected RBC Folate result (if desired). The three paths are shown in the flowchart below based on the specimens provided.

NOTE: The ARCHITECT Folate (1P74) assay files are named "Folate II" and "FolateRBC".



Procedure for the Determination of Folate in Red Blood Cells (RBC)

NOTE: Determine hematocrit of EDTA specimen. This value will be required in Calculations 1 and 2 beginning on page 6.

Part 1: Reconstitution of Folate Lysis Reagent (L1)

- Reconstitute one bottle of the Folate Lysis Reagent (L1) by adding 30 mL distilled or deionized water.
- Cap the reagent bottle and mix by inverting 10 times and let stand for 15 minutes.
- The expiration date is 7 days from the date of reconstitution. Write the expiration date of the reconstituted Folate Lysis Reagent (L1) on the line provided on the bottle label, but do not exceed the lot expiration date printed on the bottle. Store at 2-8°C when not in use.

Part 2: Preparation of Red Blood Cell Hemolysate

NOTE: The assay must be initiated on the final hemolysate within 2 hours.

- Invert the reconstituted Folate Lysis Reagent (L1) an additional 10 times. Pipette 1.0 mL into a suitable sample tube with a cap (example: 2 mL tube).
- Mix whole blood tube by inverting 10 times to ensure a homogeneous sample.
- Add 100 µL of whole blood sample to the sample tube containing the 1.0 mL of the reconstituted Folate Lysis Reagent (L1).
- Cap the tube and mix by inverting 10 times or vortexing and allow to stand at room temperature (15-30°C) for 90 minutes (± 5 minutes). **Protect from light.**
- Pipette 100 µL ARCHITECT Folate RBC Lysis Diluent (L2) into a new sample tube (or ARCHITECT sample cup). Then add 100 µL of hemolyzed sample.
- Mix by swirling or vortexing and initiate assay on this sample within 2 hours.

Assay Procedure (Folate and Folate RBC Assays)

- Before loading the ARCHITECT Folate Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Place a septum on the bottle. For instructions about placing septums on bottles, refer to the Handling Precautions section of this package insert.
- Load the ARCHITECT Folate Reagent Kit on the ARCHITECT *i* System.
  - Verify that all the necessary assay reagents are present.
  - Ensure that septums are present on all reagent bottles. Refer to ARCHITECT Operations Manual, Section 5, for details on how to load reagents.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.

  NOTE: The ARCHITECT Folate (1P74) assay files are named "Folate II" and "FolateRBC".
- Select the appropriate assay protocol.
  - If running a serum or plasma specimen/control, select Folate II (assay number 685, "UNDILUTED").
  - If running an automated dilution on a serum or plasma specimen, select the 1:2 protocol of Folate II (assay number 685, "1:2").
- If running a whole blood specimen or whole blood control, select FolateRBC (assay number 686, "RBC DIL").
- If running controls other than whole blood controls with the FolateRBC assay, select the undiluted protocol of FolateRBC (assay number 686, "UNDILUTED").
  - For additional information on ordering patient specimens, calibrators, and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Order list report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
  - Priority: 85 µL for the first ARCHITECT Folate test plus 35 µL for each additional ARCHITECT Folate test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT Folate test plus 35 µL for each additional ARCHITECT Folate test from the same sample cup.

- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

- Prepare calibrators and controls.
  - Mix the ARCHITECT Folate Calibrators and Controls by gentle inversion (3-5 times) prior to use.
  - Discard ARCHITECT Folate Calibrators after 3 freeze/thaw cycles.
  - To obtain the recommended volume requirements for the ARCHITECT Folate Calibrators and Controls, hold the bottles vertically, and dispense 6 drops of each calibrator or 6 drops of each control into each respective sample cup.

NOTE: It is very important to return the ARCHITECT Folate Calibrators and Controls to their carton and correct storage conditions immediately after use, as follows.
  - **Store ARCHITECT Folate Calibrators at -10°C or colder.**
  - **Store ARCHITECT Folate Controls at 2-8°C.**

  - Load samples. For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures (for folate serum or plasma determinations only)

Specimens with a folate serum or plasma value exceeding 20.0 ng/mL are flagged with the code "> 20.0" and may be diluted using either the Automated Dilution Procedure or the Manual Dilution Procedure.

Automated Dilution Procedure

- If using the Automated Dilution Protocol (assay number 685, 1:2 Protocol), the system performs a 1:2 dilution. The system will use the dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.

Manual Dilution Procedure

- The suggested dilution for ARCHITECT Folate is 1:2. It is recommended dilutions not exceed 1:4.
- For a 1:2 dilution, add 100 µL of the patient specimen to 100 µL of ARCHITECT Folate Manual Diluent (1P74-50). For a 1:4 dilution, add 100 µL of the patient specimen to 300 µL of ARCHITECT Folate Manual Diluent (1P74-50).
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Separate calibrations are required for ARCHITECT Folate II and ARCHITECT FolateRBC assay files.
- To perform a calibration, test ARCHITECT Calibrators A through F in duplicate. A single sample of all levels of ARCHITECT Folate Controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration Range: 0.0 - 20.0 ng/mL.
- Once a calibration is accepted and stored, all subsequent samples may be tested off the appropriate calibration curve without further calibration unless one or more of the following occur:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For best results, establish statistically-based QC ranges to monitor and control the frequency of recalibration.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Folate assay is that a single sample of each control be tested once every 24 hours each day of use. If your laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Folate assay belongs to method group 1.

RESULTS

The ARCHITECT Folate assay uses a 4 Parameter Logistic Curve fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Alternate Result Units

- The default result unit for the ARCHITECT Folate assay is ng/mL. When the alternate result unit, nmol/L, is selected, the conversion factor used by the system is 2.265.
- Conversion Formula: (concentration in ng/mL) x (2.265) = nmol/L
- Formulas and examples indicate ng/mL as the result unit. If the chosen ARCHITECT Folate result is nmol/L, the final result would be in nmol/L.

Calculation of Red Blood Cell Folate Concentration (for Folate RBC Assay only):

Calculation performed by the ARCHITECT *i* System

When the FolateRBC assay is used (assay number 686 utilizing "RBC DIL" protocol), the ARCHITECT *i* System automatically corrects the reported sample result for dilutions that were required during the preparation of the red blood cell hemolysate. This is the ARCHITECT FolateRBC test result. **Do not report this result. Further calculation is required.**

NOTE: A Calculation Worksheet is provided at the end of this package insert to assist with RBC Folate calculations.

Calculations performed by the Operator

Calculation 1

To calculate the RBC Folate concentration from the ARCHITECT FolateRBC test result, use the following formula:

$$\text{RBC Folate Concentration (ng/mL)} = \frac{A}{B} \times 100$$

where:

A = ARCHITECT FolateRBC test result (ng/mL)

B = % Hematocrit (value obtained prior to storage or prior to Procedure for Folate RBC)

Example:

ARCHITECT FolateRBC test result = 64.0 ng/mL

% Hematocrit = 32

$$\text{RBC Folate Conc.} = \frac{64.0 \text{ ng/mL}}{32} \times 100 = 200.0 \text{ ng/mL}$$

Calculation 2
Calculation of Corrected Red Blood Cell Folate Concentration (for Folate RBC Assay only):

Folate concentrations from serum or plasma are very small as compared to RBC folate concentrations, in most cases. It is possible for the serum or plasma folate concentration to be within or above the expected normal range while the RBC folate concentration is below the expected normal range. In these instances, a correction may be needed. The Folate serum (or plasma) result is required for this calculation. The following calculation will correct for serum or plasma folate concentrations:

$$\text{Corrected RBC Folate Conc. (ng/mL)} = C - \left[D \times \left[\frac{100 - B}{B} \right] \right]$$

where:

B = % Hematocrit (value used for B in Calculation 1)

C = RBC Folate Concentration result from Calculation 1 (ng/mL)

D = ARCHITECT Folate Serum (or plasma) result (ng/mL)

Example:

% Hematocrit = 32

RBC Folate Concentration result = 200 ng/mL

ARCHITECT Folate Serum (or plasma) result = 25.0 ng/mL

Corrected RBC Folate Conc.=

$$200.0 \text{ ng/mL} - \left[25.0 \text{ ng/mL} \times \left[\frac{100 - 32}{32} \right] \right] = 146.9 \text{ ng/mL}$$

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measuring Interval

The measuring interval of the ARCHITECT Folate assay is 1.5 ng/mL to 20.0 ng/mL.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes the ARCHITECT Folate assay result should be used in conjunction with other data, *e.g.*, other clinical testing, symptoms, clinical impressions, etc.
- If the folate level is inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.[12,13]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[14] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
- Serum or plasma containing red blood cells may give falsely elevated folate levels. These samples should be centrifuged prior to use. Serum or plasma samples that are hemolyzed will give falsely elevated folate levels.
- Serum and plasma specimens from patients with renal impairment or failure (including dialysis patients) may exhibit varying degrees of falsely depressed folate values.[15] Therefore, to evaluate folate patients with renal impairment or failure, it is recommended that low ARCHITECT Folate values be confirmed by an alternate folate method such as the ARCHITECT Folate RBC assay.
- Methotrexate, aminopterin, and folinic acid (Leucovorin) are chemotherapeutic agents whose molecular structures are similar to folate. These agents cross react with folate binding protein in folate assays.[16]
- Samples to be tested for folate should be protected from light. Light accelerates the degradation of folate.
- Accumulation of denatured protein from the pre-treatment step in the sample probe may impact results of other assays on the ARCHITECT *i* System. ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be run to eliminate this effect. Refer to the **INSTRUMENT PROCEDURE** section for instructions.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal and deficient ranges, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A3.[17] The nutritional status of the specimen donors was unknown. All specimens tested were from fasting, apparently healthy males and non-pregnant females greater than 18 years old from a UK population. Serum and whole blood samples were tested for serum/plasma and red blood cell folate using the ARCHITECT Folate assay. Data from this study are summarized in the following table.

	Expected Values Data Statistics ng/mL (nmol/L)			
	n	Min	Max	Expected Values
Serum/Plasma	155	1.6 (3.6)	19.5 (44.2)	3.1 - 20.5 (7.0 - 46.4)
Whole Blood	168	58.5 (132.5)	733.1 (1660.5)	126.0 - 651.1 (285.4 - 1474.7)

Folate Deficients/Indeterminates

- Folate deficiency is typically associated with serum levels less than 3.5 ng/mL or RBC levels less than 150 ng/mL.[18,21]
- Patients with RBC folate levels ranging from 150 to 250 ng/mL have been associated with megaloblastic erythropoiesis, but folate values in patients with normal erythropoiesis can also fall within this range.[18]
- Often, the diagnosis of folate deficiency cannot be based solely on serum or RBC folate levels, and further testing may be required.[18-20]

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT Folate assay is designed to have a within-laboratory imprecision of:

- ≤ 12% total CV for serum samples from 3.5 ng/mL to 20 ng/mL and ≤ 11% CV for RBC hemolysate between 150 ng/mL and 640 ng/mL.
- a Standard Deviation (SD) ≤ 0.42 for serum samples below 3.5 ng/mL and SD ≤ 16.50 for RBC hemolysate samples below 150 ng/mL.

A study was performed based on guidance from the CLSI document EP5-A2.[22] Three serum panels (S1, S2, and S3) and three hemolysate panels (H1, H2, and H3) were assayed, using 1 instrument, in replicates of 3, at two separate times per day for 20 days, using 2 lots of reagents. Data from this study are summarized in the following table.

ARCHITECT Folate Within-Laboratory Precision

Sample Level	Reagent Lot	Mean ng/mL (nmol/L)	Within-Run SD ng/mL (nmol/L)	Within-Run %CV	Total SD ng/mL (nmol/L)	Total %CV
S1	1	3.5 (7.9)	0.12 (0.27)	3.5	0.14 (0.32)	3.9
	2	3.6 (8.2)	0.14 (0.32)	3.9	0.17 (0.39)	4.7
S2	1	10.8 (24.5)	0.18 (0.41)	1.7	0.41 (0.93)	3.8
	2	11.2 (25.4)	0.21 (0.48)	1.9	0.44 (1.00)	4.0
S3	1	16.8 (38.1)	0.27 (0.61)	1.6	0.53 (1.20)	3.1
	2	17.0 (38.5)	0.24 (0.54)	1.4	0.61 (1.38)	3.6
H1	1	113.2 (256.4)	6.10 (13.82)	5.4	8.82 (19.98)	7.8
	2	118.1 (267.5)	4.69 (10.62)	4.0	6.49 (14.70)	5.5
H2	1	222.9 (504.9)	7.04 (15.95)	3.2	13.29 (30.10)	6.0
	2	221.9 (502.6)	5.59 (12.66)	2.5	12.19 (27.61)	5.5
H3	1	367.2 (831.7)	7.87 (17.83)	2.1	21.60 (48.92)	5.9
	2	359.1 (813.4)	8.90 (20.16)	2.5	22.97 (52.03)	6.4

Autodilution Verification

The ARCHITECT Folate assay was designed to have an absolute mean change in measured concentration of ≤ 20% when comparing manual to autodilution (1:2 dilution). The assay was evaluated for autodilution with the 1:2 autodilution method vs. the 1:2 and 1:4 manual dilution methods using 18 specimens with folate values ranging from 20 to 40 ng/mL. Three replicates each of the autodiluted and manually diluted samples were assayed on one instrument using the ARCHITECT Folate assay. Data from this study are summarized in the following table.

Percent Differences Across Samples

Dilution Comparison	n	Mean/Median %Difference
Auto (1:2) vs. Manual (1:2)	18	0.8
Auto (1:2) vs. Manual (1:4)	18	5.9

Linearity

The ARCHITECT Folate assay was evaluated for linearity by mixing a high (> 20 ng/mL) serum specimen pool in specific ratios with a low (≤ 3.5 ng/mL) serum specimen pool to create 11 mixed sample pools. All pools were tested by the ARCHITECT Folate assay. Based on guidance from CLSI document EP6-A[23], the study demonstrated linearity from 1.6 to 20 ng/mL.

Accuracy to World Health Organization (WHO) Standard

The ARCHITECT Folate assay was evaluated for bias relative to the Folate WHO International Standard 03/178. A minimum of 38 replicates of the WHO Standard was tested on each of 2 instruments. A different reagent lot was used on each instrument and one calibrator lot was used for both instruments.

The Folate assay results were accurate within ± 10% to the 1st International Reference Standard (I.S.) for Serum Folate (03/178). Data from this study are summarized in the following table.

n	Median ng/mL (nmol/L)	Target ng/mL (nmol/L)	Diff.[a] ng/mL (nmol/L)	Two-Sided 95%CL[b] ng/mL (nmol/L)	%Diff.[a]	Two-Sided 95%CL[b] %Diff.[a]
76	5.4 (12.2)	5.3 (12.0)	0.1 (0.2)	0.0, 0.1 (0.0, 0.2)	1.3	-0.6, 1.3

[a] Diff. = Difference

[b] CL = Confidence Limit

Sensitivity

Sensitivity is defined as the Limit of Quantitation (LoQ), which is the lowest amount of analyte in a sample that can be accurately quantitated with a Total Error of ± 39%.[24]

The ARCHITECT Folate assay is designed to have an LoQ of ≤ 3.5 ng/mL.

The Limit of Blank (LoB), Limit of Detection (LoD), and LoQ of the ARCHITECT Folate assay were determined based on guidance from CLSI document EP-17A[25], using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using 1 zero-level (3 replicates) and 5 low-level folate samples (3 replicates each). The following values were determined in this study: LoB = 0.3 ng/mL (0.7 nmol/L), LoD = 0.5 ng/mL (1.1 nmol/L), and LoQ = 1.5 ng/mL (3.4 nmol/L).

Specificity

The specificity of the ARCHITECT Folate assay was evaluated by testing cross-reactivity with aminopterin, folinic acid, and methotrexate in processed human serum containing endogenous folate. Therapeutic levels of these drugs can greatly exceed the levels tested in this study and are expected to interfere with the ARCHITECT Folate assay.[16] A study was performed with the ARCHITECT Folate assay based on guidance from the CLSI document EP7-A2.[26] Aliquots of human serum at two different folate concentrations were supplemented with potential cross-reactants and tested for folate. Data from this study are summarized in the following table.

Interferent	Reference n	Reference Mean/ Median ng/mL	Test n	Test Mean/ Median ng/mL	Diff.[a] ng/mL	%Diff.[b]	%Cross-Reactivity[c]
Aminopterin	40	2.6	40	8.3	5.7	219.2	1.1
≥ 500 ng/mL	40	7.4	39	13.0	5.6	75.7	1.1
Folinic Acid	40	2.9	40	3.4	0.5	17.2	0.5
≥ 100 ng/mL	40	7.9	44	7.3	-0.6	-7.4	-0.6
Methotrexate	45	2.7	40	4.8	2.1	77.8	2.1
≥ 100 ng/mL	40	7.6	40	8.9	1.4	18.2	1.4

[a] Difference = test mean [or median] conc. - reference mean [or median] conc.

[b] % Difference = Difference / reference mean [or median] conc. x 100

[c] % Cross-Reactivity = Difference / interferent conc. x 100

Interference

Potential interference in the ARCHITECT Folate assay from bilirubin, (conjugated and unconjugated), triglycerides, and protein was demonstrated in a study based on guidance from CLSI document EP7-A2.[26] Hemoglobin was not tested due to the high folate content in red blood cells. Refer to the **LIMITATIONS OF THE PROCEDURE** section. Data from this study are summarized in the following table.

Interferent	Reference n	Reference Mean/ Median ng/mL	Test n	Test Mean/ Median ng/mL	Diff.[a] ng/mL	%Diff.[b]
Bilirubin (unconjugated)	40	2.1	40	2.0	-0.1	-4.0
≤ 20 mg/dL	40	7.9	40	7.6	-0.3	-3.8
Bilirubin (conjugated)	40	1.8	40	1.7	-0.1	-5.6
≤ 20 mg/dL	40	7.5	40	7.0	-0.5	-6.7
Protein	40	2.6	40	2.9	0.3	11.5
≤ 12 g/dL	40	8.8	40	9.1	0.3	2.8
Triglycerides	40	2.1	40	2.2	0.1	4.8
≤ 3000 mg/dL	40	7.9	39	8.0	0.1	1.8

[a] Difference = test mean [or median] - reference mean [or median]

[b] % Difference = Difference / reference mean [or median] x 100

Tube Type Matrix Comparison

The following tube types are acceptable for use with the ARCHITECT Folate assay:

- Glass: Serum
- Plastic: Serum, Serum Separator Tube (SST), Lithium Heparin Plasma Tube, and Lithium Heparin Plasma Separator Tube (PST),

On average, the tube types evaluated showed less than a 10% difference when compared to the control tube type (plastic serum). The distribution of the percent differences per tube type is listed in the following table.

Evaluation Tube Type	Distribution of Absolute %Differences[a]	
	< 10%	10% to 20%
Glass, Serum	92.6% (25/27)	7.4% (2/27)
Serum Separator Tube, Plastic (SST)	100.0% (27/27)	0.0% (0/27)
Lithium Heparin Plasma Tube	80.0% (20/25)	20.0% (5/25)
Lithium Heparin Plasma Separator Tube (PST)	92.6% (25/27)	7.4% (2/27)

[a] There were no absolute % difference values > 20%.

The following tube types are acceptable for use with the ARCHITECT Folate RBC assay:

- whole blood dipotassium EDTA (K_2 EDTA)
- whole blood tripotassium EDTA (K_3 EDTA)

All K3 EDTA tubes evaluated (n=27) showed less than 10% difference when compared to matched K2 EDTA tubes.

Method Comparison

Two correlation studies were performed based on guidance from CLSI document EP9-A2[27] using the Passing-Bablok[28] regression method to compare the ARCHITECT Folate assay to the AxSYM Folate assay. One study was performed with serum/plasma specimens and the other with whole blood specimens. The analysis of the results from the serum/plasma study included both the full range of specimens analyzed and a truncated range for the AxSYM Folate assay. The truncated range minimizes any effects due to apparent non-linearity of AxSYM results at the clinically less significant higher folate concentrations. Truncation was unnecessary for whole blood specimens. The tables below summarize the results of these correlation analyses.

Correlation of ARCHITECT Folate to AxSYM Folate

Specimen Type	Conc. Range ng/mL (nmol/L)		r[a]	Intercept ng/mL (nmol/L)	Slope
	ARCHITECT	AxSYM			
Serum/Plasma (n=144)	0.9-28.9 (2.0-65.5)	2.2-33.4 (5.0-75.7)	0.921	-5.85 (-13.25)	1.27
Truncated Serum/Plasma (n=43)	0.9-12.6 (2.0-28.5)	2.2-14.0 (5.0-31.7)	0.963	-1.19 (-2.70)	0.82
Whole Blood (n=123)	145.5-1014.6 (329.6-2298.1)	155.8-1034.5 (352.9-2343.1)	0.895	-33.36 (-75.56)	0.74

[a] r = Correlation Coefficient

Some serum and plasma samples in the upper region of the dynamic range may read lower in the AxSYM Folate assay when compared to the ARCHITECT Folate assay. This can result in a decreased correlation coefficient value over the entire measurement range.

Two correlation studies were performed using the Passing-Bablok regression method to compare the ARCHITECT Folate assay to the ARCHITECT Folate Non-US assay. One study was performed with serum/plasma specimens and the other with whole blood specimens. The table below summarizes the results of these correlation studies.

Correlation of ARCHITECT Folate to ARCHITECT Folate Non-US

Specimen Type	Conc. Range ng/mL (nmol/L)		r[a]	Intercept ng/mL (nmol/L)	Slope
	ARCHITECT	ARCHITECT Non-US			
Serum/Plasma (n=140)	1.4-28.9 (3.2-65.5)	1.0-31.3 (2.3-70.9)	0.997	-0.38 (-0.86)	0.98
Whole Blood (n=131)	145.5-1014.6 (329.6-2298.1)	97.8-900.5 (221.5-2039.6)	0.973	55.67 (126.09)	0.89

[a] r = Correlation Coefficient

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The following US Patents are relevant to the ARCHITECT *i* System or its components. There are other such patents and patent applications in the United States and worldwide.

5 468 646	5 543 524	5 545 739
5 565 570	5 669 819	5 783 699

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CE

June 2012

Calculation Worksheet
(for RBC Folate Calculations)

Sample ID_____
Date_____
Initials_____

Notes: **The ARCHITECT Folate (1P74) assay files are named: "Folate II" and "FolateRBC".**
If only a whole blood specimen was provided for the Folate RBC test, perform Calculation 1.
If the Corrected RBC Folate Concentration result is desired and both a whole blood specimen and a serum or plasma specimen were provided, perform Calculations 1 and 2.
(See flowchart in Assay Procedure Overview section.)

Calculation 1
Calculate the RBC Folate Concentration.

Step 1. ***Record values.***

A = ARCHITECT FolateRBC test result (ng/mL) (value reported by ARCHITECT) _____ (A)

B = % Hematocrit (value obtained in the Storage section on page 4) _____ (B)

Step 2. ***Perform calculation.***

$$\text{RBC Folate Concentration (ng/mL)} = \frac{A}{B} \times 100 = \text{\_\_\_\_\_ (C)}$$

This is the RBC Folate Concentration result. (C)

If the Corrected RBC Folate Concentration result is desired, perform Calculation 2 to correct for the serum (or plasma) Folate concentration. (The Folate serum (or plasma) result is required for this calculation.)

Calculation 2
Calculate the Corrected RBC Folate Concentration.

Step 1. ***Record values.***

B = % Hematocrit (value used for B in Calculation 1) _____ (B)

C = RBC Folate Concentration result from Calculation 1 (ng/mL) _____ (C)

D = ARCHITECT Folate Serum (or plasma) test result (ng/mL) _____ (D)

Step 2. ***Perform calculation by following the steps listed below the equation.***

$$\text{Corrected RBC Folate Conc. (ng/mL)} = C - \left[D \times \left[\frac{100 - B}{B} \right] \right]$$

Subtract B from 100. _____ (E)

Divide result obtained in (E) by B. _____ (F)

Multiply result obtained in (F) by D. _____ (G)

Subtract (G) from C. _____ (H)

This is the Corrected RBC Folate Concentration result (H).

11.7 APPENDIX 7: NHMRC DAILY DOSE AND UPPER LIMIT FOR EACH OF THE B VITAMINS IN THE INTERVENTION.

Activated B Complex vitamin make-up	Dose	Dose we are administering daily	NHMRC Nutrient Guidelines 2006 reference ranges and safety limits Rationale
Biotin	500mcg	1,000mcg	Men RDI = 30mcg/day Female RDI = 25mcg/day Upper level: NHMRC found that there is insufficient evidence of adverse effects in human and animal studies to set an upper level.
Calcium folinate (folinic acid)	500mcg	1,000mcg	Men RDI = 400mcg/day Women RDI = 400mcg/day Upper level: Men and Women = 1,000mcg/day The dose we will be administering is equivalent to the upper level so is considered safe.
Calcium pantothenate (vitamin B5)	163.76mg	327.52mg	Men RDI = 6mg/day Women RDI = 4mg/day Upper Level: NHMRC have set no upper level as there are no reports of adverse effects of oral pantothenic acid in either human or animal studies. Therefore, no upper level was set.
Choline bitartrate	100mg	200mg	Men RDI = 550mg/day Female RDI = 425mg/day Upper level: Adult male and females = 3,500mg/day The amount we will be administering is less than the RDI's for both men and women.
Cyanocobalamin (vitamin B12)	500mcg	1,000mcg	Men RDI = 2.4mcg/day Women RDI = 2.4mcg/day Upper Level: Currently there is no evidence that high amounts of vitamin B12 represents a health risk as no adverse effects have been found with excess vitamin B12 intake or supplementation. Therefore, 1,000mcg a day is a safe level of intake for this study.
Inositol	100mcg	200mcg	Currently inositol is under review by the Therapeutics Goods Act. The NHMRC

			does not mention inositol in its references ranges. A case report in 2010 (Kontoangelos K, eta al. 2010) found that administration of 3g of inositol for 4 years to a 62yo female suffering bipolor disorder for 30 years was effecetive and safe. The administration of inositol is still under review with no negative studies found on supplementation, the dose we are administrating would be considered safe.
Nicotinamide (vitamin B3)	80mg	160mg	Men RDI = 16mg/day Women RDI = 14mg/day <u>Upper Level:</u> For Niacin as nicotinamide Men and Women = 900mg/day The amount administered is below the upper level..
Nicotinic acid (vitamin B3)	20mg	40mg	Men RDI = 16mg/day Women RDI = 14mg/day <u>Upper Level</u>: For Niacin as nicotinic acid Men and Women = 35mg/day The upper level from the NHMRC was based on the flushing reaction. (FNB:10m 1998) The upper level was 50mg/day set on the data from the study of Sebrell & Butler (1938) supported by Spies et al. (1938). An uncertainty factor of 1.5 was selected as the flushing is transient so after rounding, NHMRC decided on 35mg/day for adults. The amount that the patients will receive is just about this at 40mg. It is still under the 50mg/day so patients could possibly experience some flushing, but it is only transient, no longer than 30mins. There are no negative effects from this and it is not considered to be unsafe.
Pyridoxal-5-phosphate (vitamin B6)	10mg	20mg	Men RDI = 1.3mg for 19-50 years old 1.7mg for 50-≥70 years old Women RDI = 1.3mg for 19-50 years old 1.7mg for 50-≥70 years old <u>Upper level:</u> Adult men and women = 50mg/day The upper level was set by the NHMRC using results of studies involving long-term oral administration of pyridoxine at doses
Pyridoxial hydrochloride (vitamin B6)	20mg	40mg	

			less than 1g/day (Berger & Schaumburg 1984, Bernstein & Lobitz 1988, Dalton 1985, Dalton & Dalton 1987, Del Tredici et al. 1985, FNB:IOM 1998, Parry & Bredesen 1985) The no observed adverse effect leve (NOAEL) is 200mg/day identified from the studies of Bernstein & Lobitz (1988) and Del Tredici et al. (1985) These studies had subjects taking supplements for 5-6 months or less, however Dalton and Dalton (1987) has suggested that symptoms might take substantially longer than this to appear. They found that subjects who had reported symptoms had been on supplementation on average for 2.9 years and those reporting no symptoms on supplements on average for 1.9 years. The total amount we will be supplementing is 60mg which is just above the upper level but much lower than the NOAEL of 200mg. They will be receiving this amount for 9 months which is also less than the average 2.9 years reported for subjects experiencing vitamin B6 toxicity side effects such as peripheral neuropathy.
Riboflavin 5-phosphate (vitamin B2)	20mg	40mg	Men RDI = 1.2mg/day Women RDI = 1.1mg/day <u>Upper level:</u> No adverse events have been associated with vitamin B2 consumption in food or supplementation so no upper level can been set by the NHMRC.
Thiamine hydrochloride (vitamin B1)	50mg	100mg	Men RDI = 1.2mg/day Women RDI = 1.2mg/day <u>Upper levels:</u> NHMRC decided that there is no upper level for Thiamin and it does not present a health risk.

References: NHMRC nutrient references value 2006

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11.8 APPENDIX 8. DIET FOR INITIAL ABSORPTION STUDY

Date: _____ Initials: _____ Gender: Male / Female

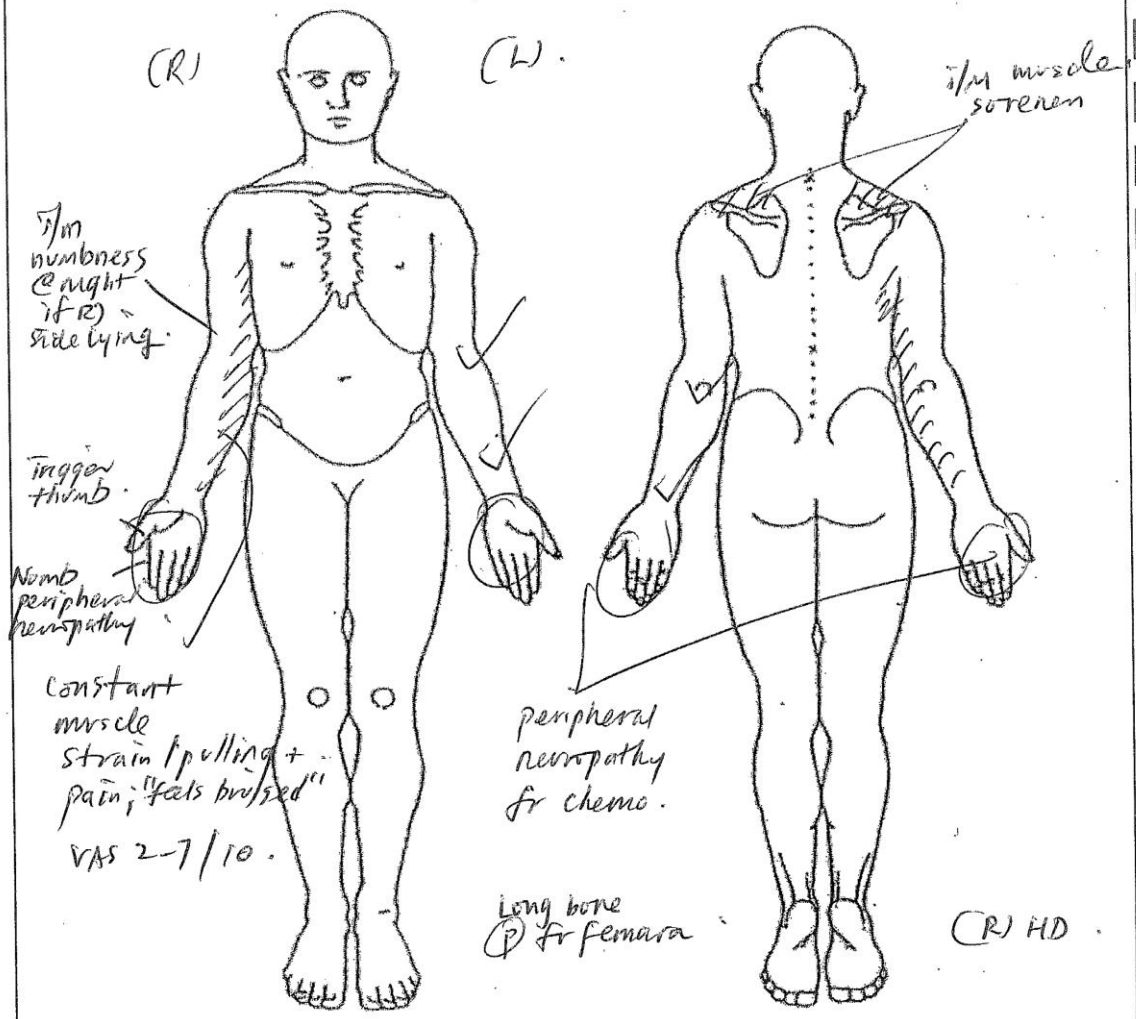
	Foods	Amounts	What was eaten
Breakfast	White bread toasted Butter Jam Banana or Apple	2 -4 slices ½ teaspoon per slice 1 teaspoon per slice 1 medium	
Morning Tea	Grapes or Apple Herbal Tea	1 bunch or medium apple 1-2 cups	
Lunch	Rice cakes Avocado Tomato Cucumber Boiled egg	2-6 cakes 1 tablespoon per cake 2-3 slices per cake 3-4 slices per cake 1-2 medium	
Afternoon tea	Grapes or Apple Or Yoghurt Herbal Tea	1 bunch or medium apple 1 small tub 1-2 cups	
Dinner	Fish – cod grilled White rice or white potato Carrot – raw, baked or boiled Sweet potato – baked or mashed Pumpkin – baked or mashed	1 -2 fillets (120g each) ½ - 1 cup ¼ carrot 2 pieces baked or 1 tablespoon 2 pieces baked or 1 tablespoon	
Supper	Yoghurt	1 small tub	
Beverages	Water Herbal teas	As much as you like As much as you like	

11.9 APPENDIX 11: PHYSIOTHERAPY NOTES ON PA023 PMP

PA017 10M 11/09
10109793

PHYSIOTHERAPY

PRINCESS ALEXANDRA HOSPITAL



Signature ..... Date .....

(YEUNG) PT

03/09/13

DO NOT WRITE IN THIS BINDING MARGIN

PHYSIOTHERAPY

10109871

Queensland Government

PRINCESS ALEXANDRA HOSPITAL

Physiotherapy Progress Notes

Date and Time	Add signature, printed name, staff category, date and time to all entries MAKE ALL NOTES CONCISE AND RELEVANT Leave no gaps between entries
21813	Physio outpatients (Cardiac) Initial:
	Hx noted 54yo R) Dominant c R) Breast ca.
	- wle + Axillary sampling 27/3/13
	- (gz IDC, high + intermediate DCIS)
	- 0/9 nodes +ve
	- Chemo } chart ordered for
	- XRT } more Hx
	- Hormonal therapy }
	c/o + multiple stressors, pt overwhelmed +
	teary at times
	* husband c Alzheimers
	* severe peripheral neuropathy 2° chemo
	* musculoskeletal issues in hands (trigger
	finger) 2° hormones Rx, starting to impact
	on function / job (advice c some heavy lifting)
	* persistent fluctuating R) Breast lymphoedema
	and subjective 'ache' in R) Arm usually
	worse c physical activity, eased c lymphatic
	massage / hydro
	* unable to wear compression sleeve around
	as it's pain * weight gain
	* uses compression Bra + chip bag daily
	o/E/- R) Breast - pink, not warm
	- peau d'orange appearance
	inferiorly
	- firm, pitting inferior 1/2 of breast
	- soft, nil pitting superiorly

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V3.0 12/2012

00011-PA385

Physiotherapy Progress Notes

Queensland Government

PRINCESS ALEXANDRA HOSPITAL

Physiotherapy
Progress Notes

Princess Alexandra Hospital

Date and Time	Add signature, printed name, staff category, date and time to all entries MAKE ALL NOTES CONCISE AND RELEVANT Leave no gaps between entries
12/8/13	→ psoriasis marks inferior to Breast
cont	- R) UL: soft, nil pitting throughout including hand (fingers), ve stemmer
	∞ SOAC = sum of 4 circumferential measures
	R) 20cm AH 203.7cm's
	L) 196.8cm's
	= diff of 6.9cm's R) AH, 20cm 7 L)
	∞ LDEX (=) 7.5
	(chart unavailable to compare to previous)
	∞ maintaining full R) sh ROM = D
	with no "bands" = axillary web syndrome, or cording visible/palpable in R) UL
	∞ fullness visible R) UL but soft, nil pitting
	Rx'd long discussion ± pt/ normalisation of emotional distress around side effects
	2/ KT = horseshoe posterior axillo scapular anastamosis anchored C) Axilla ± 2 strips across to L): advised to remove after 2 days, earlier if signs/symptoms = S+S of skin irritation
	PLAN/weekly Rxs: MLD = manual lymphatic Drainage massage R) Breast, KT, assess compression garments and ? replace although no prescription card
	→ encourage hydro at local pool
	→ ? Refer Lymphoedema clinic for

DO NOT WRITE IN THIS BINDING MARGIN

12 PUBLICATIONS

12.1 NUTRACEUTICALS AND CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY (CIPN): A SYSTEMATIC REVIEW.

Title Page.
Article type : Special article

Nutraceuticals and Chemotherapy Induced Peripheral Neuropathy (CIPN): a Systematic Review.

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Abstract

Chemotherapy-induced peripheral neuropathy [CIPN] is a common significant and debilitating side effect resulting from the administration of neurotoxic chemotherapeutic agents. These pharmaco–chemotherapeutics can include taxanes, vinca alkaloids and others. Moderate to severe CIPN significantly decreases the quality of life and physical abilities of cancer patients and current pharmacotherapy for CIPN, for example, Amifostine and antidepressants have had limited efficacy and may themselves induce adverse side effects. To determine the potential use of nutraceuticals i.e. vitamin E, acetyl-L-carnitine, glutamine, glutathione, vitamin B6, omega-3 fatty acids, magnesium, calcium, alpha lipoic acid and n-acetyl cysteine as adjuvants in cancer treatments a systematic literature review was conducted. Revised clinical studies comprised of randomized clinical trials that investigated the anti-CIPN effect of nutraceuticals as the adjuvant intervention in patients administered chemotherapy. Twenty–four studies were assessed on methodological quality and limitations identified. Studies were mixed in their recommendations for nutraceuticals. Currently no agent has shown solid beneficial evidence to be recommended for the treatment or prophylaxis of CIPN. The standard of care for CIPN includes dose reduction and/or discontinuation of chemotherapy treatment. The management of CIPN remains an important challenge and future studies are warranted before recommendations for the use of supplements can be made.

Key Words: Chemotherapy-induced peripheral neuropathy, Vitamin B6, Glutamine, Glutathione, Acetyl-L-Carnitine, Vitamin E, Alpha Lipoic acid, Magnesium, Calcium and N-Acetyl Cysteine, Omega-3 Fatty Acids, CIPN.

Introduction

Certain neoplastic agents can accumulate in the peripheral nervous system and the neurotoxicity may lead to CIPN. The symptoms of CIPN can be numbness, tingling, burning, decreased touch sensation, decreased strength and movement and sometimes pain in the fingers, toes, hands or feet.[1,2] These neurotoxic chemotherapy agents include the platinum compounds, anti-tubulin agents such as the taxane class and vinca alkaloids, epothilones, thalidomide, proteasome inhibitors and 5-fluorouracil (5FU).[3-8] It is estimated that one third of all patients who undergo chemotherapy experience CIPN and of those, a third can have permanent nerve damage.[3-5] Patients experiencing moderate to severe CIPN report a reduced quality of life,[3] chronic discomfort[4] and disruption of physical abilities for general life activities which can be temporary or permanent.[3] Moreover, CIPN can lead to dose reduction of the chemotherapy agent or possible cessation of treatment which may have an adverse impact on cancer treatment and disease outcomes.[5]

The Aetiology of CIPN

A differential diagnosis of peripheral neuropathy in patients diagnosed with cancer has been reported (Table 1).[2] Requisites for peripheral nervous system neurotoxicity include chemotherapy agent capacity to cross the blood-nerve barrier and nervous system sensitivity to the drug. People with predisposing conditions such as type II diabetes mellitus (T2DM), HIV/AIDS, alcoholism or a vitamin B12 deficiency may be more prone to the agent's adverse effects on the peripheral nervous system thereby increasing the prevalence of CIPN.[2]

Peripheral nerve fibres are composed of small or large fibres. Small nerve fibres are unmyelinated and are comprised primarily of microtubules. They include nerves that sense pain and temperature. Large nerve fibres are myelinated and are composed mainly of neurofilaments that act as a framework for the axon. These fibres sense position and vibration as well as motor control.[2] Both fibres are targeted by neurotoxic chemotherapy agents that may explain why patients experience a variety of symptoms.

Although each neurotoxic chemotherapy agent has a different mechanism of action on the nervous system, all induce a glove and stocking distribution. This means the point most distal from the trunk of the body is affected first (e.g. fingers and toes) and progression is then towards the trunk to hands and feet and then limbs.[2] Each agent has been found to affect one nerve fibre more than others, for example, cisplatin targets large fibres while paclitaxel and vincristine target small fibres.[2]

CIPN can be a temporary side effect which can take up to two years for full recovery. In approximately one third of cases it can be a permanent consequence of the drug neurotoxicity. Symptoms may occur within hours, days or weeks after the introduction of the chemotherapy agent, with cumulative doses increasing the severity and length of time the patient experiences this side effect.[2] Cisplatin differs to other neurotoxic agents as it can induce a delayed CIPN several months after the drug has been administered rather than a more immediate response.[4]

Mechanism of Action of Neurotoxic Chemotherapy Agents

The neurotoxic chemotherapy agents can be divided into four main categories, alkylating and anti-tubulin agents, thalidomide and proteasome inhibitors. A common feature of these drugs is that they are unable to cross the blood-brain barrier thereby protecting the central nervous system. The peripheral nervous system has no protective barrier making it susceptible to neurotoxicity[3] and therefore neurotoxic chemotherapy agents can accumulate and target different regions of the neuron (Figure 1).[6]

Methods

A systematic search of the literature was conducted using PubMed, the Cochrane Library, Science Direct, Scopus, EMBASE, MEDLINE and CINAHL.

Search Terms

Articles were identified using the search terms, "chemotherapy" OR "Cisplatin" OR "Taxanes" OR "Paclitaxel" OR "Docetaxel" OR "Oxaliplatin" OR "Carboplatin" OR "Platinum compounds" OR "Proteasome inhibitors" AND "induced peripheral neuropathy" OR "CIPN" AND "nutrient" OR "vitamin" OR "mineral" OR "Acetyl-L-Carnitine" OR "Glutamine" OR "Vitamin E" OR "Alpha Lipoic acid" OR "Magnesium" OR "Calcium" OR "Vitamin B6" OR "B vitamins" OR "Omega-3 fatty acids".

The Inclusion criteria for this review were: 1) An RCT and/or cross-over clinical trial that used either a placebo comparator or current anti–CIPN treatment as a control; 2) Human participants diagnosed with a cancer and administered chemotherapy; 3) The use of a nutraceutical supplement as the main intervention and specifically investigating its effects on reducing the primary outcome i.e., CIPN; and 4) The clinical study was published in English.

The overall body of evidence (based on a summary of the individual studies) (See Supplemental Information: Clinical Studies Investigating the Efficacy of Selected Nutraceuticals for the Prevention of CIPN) evaluated within this review was assessed using a separate tool, the Australian

National health and Medical Research Council's (NHMRC) body of clinical evidence assessment matrix. This is an assessment tool that assigns a level/grade (Level I: strongest evidence to level IV: weakest evidence) based on the strength of the published study.[9]

Results

Nutraceuticals and Peripheral Neuropathy

The search strategy identified twenty-four studies (Supplemental Information) that provided Level II or III evidence and all had a positive quality rating. All studies included in this review were analysed for common scientific characteristics / attributes consistent with rigorous methodologies, randomised group allocation and clear inclusion and/or exclusion criteria, major findings, and potential limitations.

12.1.1

12.1.2 Study characteristics

The studies selected for this review included randomised controlled trials, open label trials and one retrospective study. The randomised controlled trials identified are divided into eleven double blind placebo controlled trials, five randomised controlled trials including a randomised open label with a blind assessment plus two non-randomised controlled trials. Four open label trials were identified which did not contain a placebo component.

The results of the included studies were mixed. Several nutraceuticals showed initial positive results in reducing CIPN however further studies found that they were either not significant or indicated possible interference with the chemotherapy agents response rate. No nutraceutical currently has been found to significantly show protection or treatment for CIPN.

Magnesium and Calcium

Magnesium and calcium infusions with oxaliplatin also showed initial positive results in a retrospective study.[10] Three clinical trials[11-13] reported that efficacy was not enhanced with one trial (n=174) being terminated due to the treatment group reporting a significant lower response rate compared to placebo.[11]

Vitamin E

Vitamin E demonstrated positive results in three RCT's showing a significant reduction in the relative risk of cisplatin induced PN, especially ototoxicity.[14-18] However, a phase III multi-centre trial combining vitamin E with taxanes, cisplatin, carboplatin, oxaliplatin or combination concluded that vitamin E did not appear to reduce the incidence of sensory neuropathy.[18] Vitamin E efficacy was

limited to those patients administered cisplatin (only 8 from the 207 cohort) during the phase III study. Previous studies have indicated that vitamin E seems to be more protective for cisplatin ototoxicity compared to other neurotoxic agents.[19]

Lipoic acid

One open label study was conducted with alpha lipoic acid (n=15) co-administered with oxaliplatin.[20] This treatment combination showed a trend toward a reduction in the severity of oxaliplatin induced CIPN in 8 out of 15 patients but the trend was not statistically significant.[20]

N-Acetyl Cysteine

N-acetyl cysteine (NAC) administration to cancer patients has been based on the assumption that NAC can increase glutathione production, an effect that may decrease cyto-toxicity.[21] In a further study conducted with NAC no significant changes on electrophysiological testing on both sensory and motor nerves were found between the two groups.[21]

Glutathione

Glutathione has been reported to show positive results in reducing CIPN.[22] Intravenous glutathione administered before cisplatin administration reported promising outcomes for the prevention of cisplatin induced PN without reducing the anti-tumour activity of the chemotherapeutic agent.[23-25] A positive result was also found in two studies with oxaliplatin.[26,27] Both studies reported a significant reduction in oxaliplatin induced PN, mainly relevant to sural sensory nerve reduction. These results present clinical data that indicates that glutathione administration may aid in the prevention of CIPN. However, additional higher quality studies are required, as these trials were limited due to a high participant dropout rate and without long-term follow–up.

Glutamine

Two almost identical clinical studies[28,29] that have been published on the administration of glutamine with paclitaxel are the results from the early study[25] that was merged / extended into a second trial.[26] Both studies were conducted at the same institution over the same period of time but with slightly different numbers of participants and authors, presenting similar results. These studies reported that the patients on glutamine tended to have fewer symptoms than those on placebo however the trend in the nerve conduction studies was not statistically significant. There was a drift in the results that indicated that glutamine decreased the severity of dysesthesias in the fingers and toes. An additional study[30] with glutamine and oxaliplatin co-administration reported that glutamine may reduce the

incidence and severity of oxaliplatin induced CIPN.[30] No significant differences were found between the two groups.[30]

Acetyl–L–Carnitine

Two open label studies reported that acetyl–L–carnitine (ALC) may be a treatment option for paclitaxel and cisplatin induced CIPN.[31,32] A phase III trial demonstrated that ALC did not provide a positive benefit for the prevention of CIPN.[33] The study concluded that patients should be discouraged from using ALC during treatment with taxane therapy. Notwithstanding this recommendation it may be an option for the treatment of CIPN rather than prevention in patients that already may be experiencing CIPN.

Vitamin B6

One DBRCT with vitamin B6 found that it significantly reduced CIPN from cisplatin and hexamtheylmelamin administration.[34] Furthermore the results indicated that the high dose vitamin B6 (100 mg) administered may affect response duration and that it requires further investigation.[34]

12.1.3 Omega–3 Fatty Acids

A recent DBRCT investigating the effect of omega–3 fatty acids on paclitaxel induced peripheral neuropathy found that it significantly reduced the incidence of CIPN from paclitaxel by 70%.[35] The sural nerve conduction test was also significant for omega–3 fatty acids compared to placebo. Further testing in larger randomised clinical trials is required but omega–3 fatty acids do show promise for protection against paclitaxel induced peripheral neuropathy.[35]

12.1.4

12.1.5 Adverse Events and Adherence

The nutraceuticals trialled were generally well tolerated according to the clinical trials reported. Hence, there were limited adverse events noted from the nutraceuticals trialled for the prevention of CIPN. Adverse events were reported for the use of acetyl-l-carnitine only. These included two patients that reported mild nausea associated with acetyl-l-carnitine administration[29] and one patient reported insomnia following supplementation with acetyl-l-carnitine.[30] One additional study described two participants who stopped their vitamin E supplementation after one month.[18] No interpretation of the result was given as to why the participants had stopped taking the vitamin E.

Confounding Factors

The main confounding factors reported in the clinical studies investigated for this review are presented in Table 2. All studies from the supplemental information table indicated that confounding

factors were controlled in the study but were not individually listed in the clinical trials examined. They were itemised under why patients did not complete the trial, why they withdrew their participation or experienced chemotherapy administration toxicity. The confounding factors mentioned may importantly influence the decisions to administer nutraceuticals to cancer patients who are about to undergo chemotherapy treatments. Such decisions may be dependent on those factors that could affect drug absorption, metabolism and or compliance. One study reported the difficulty in reproducing or quantifying peripheral neuropathy as a confounding factor.[25]

Possible Drug-Interactions with High Doses of Nutraceuticals

Three studies have reported that there could be possible drug interactions with the administration of high dose nutraceuticals.[11,25,32] The intravenous administration of magnesium and calcium trial with FOLFOX regime was terminated due to the treatment group reporting a significant lower response rate compared to placebo.[11] The study concluded that a possible drug interaction had occurred between the chemotherapy regime and the magnesium and calcium infusions however no further investigations were continued to determine the mechanism of action. A further study with glutamine[25] indicated that there could have been a protective effect on the tumour from the administered glutamine thereby leading to reduced cytotoxicity from the paclitaxel administered. Moreover, no decrease in response rate was reported in the trial and no further testing was undertaken to ascertain the mechanism of action.[25] The third clinical study that administered vitamin B6 showed a significant decrease in the response rate with cisplatin and HMM.[32] The study concluded that this effect was directly due to vitamin B6 supplementation as it occurred in all participants administered the high dose of vitamin B6. However, it also was noted that vitamin B6 reduced response rate. No investigations were undertaken to determine how this occurred or the possible mechanism of action. Further investigations on the vitamin B6 effect are warranted.

12.1.6

12.1.7 Clinical Implications

The current scientific literature demonstrates that there is limited evidence for the concurrent administration of nutraceuticals with neurotoxic agents in the prevention or treatment of CIPN. Nutraceuticals that may be considered include oral vitamin E with cisplatin administration, omega–3 fatty acids with paclitaxel and possibly the intravenous administration of glutathione with oxaliplatin. Although unvaryingly these may not prevent the development of CIPN they may assist in some cases depending on the severity of the diagnosed cancer and the commencement of medical treatment.

Vitamin B6 also warrants further investigation as a potential protective agent of CIPN. A recent *in vitro* experiment with vitamin B6 and oxaliplatin reported that vitamin B6 showed protection against oxaliplatin induced peripheral neuropathy without compromising drug anti-tumour efficacy.[36]

Review Limitations

The exclusion of unpublished literature may significantly affect this review by introducing a publication bias. In addition, several restraints have been identified with this review namely within the selected studies. The low participant numbers in most studies was a significant limitation. The retrospective study[10] was included because it allowed the formulation of a biologically plausible hypothesis for the efficacy of nutraceuticals to prevent CIPN, even though it presented as a low–level evidence study. Furthermore, no objective markers were used in the assessment of the signs and symptoms of CIPN.

Studies reported using a variety of assessment tools to quantify CIPN development and severity. These included the National Cancer Institute Common Toxicity Criteria (NCI-CTC), total neuropathy score (TNS), nerve conduction studies and/or electrophysiological investigations and quality of life questionnaires. The NCI-CTC is a clinician-based grading scale whereby the assessor may mix impairment, disability and quality of life measures which can lead to different interpretations of results and demonstrates observer objectivity and possible bias.[37] The NCI-CTC is the most commonly used assessment tool in the selected studies. The TNS is stated to be the most comprehensive assessment tool available at this time[37,38] with only two studies using this assessment tool as their main measurement of results.[17,35] The nerve conduction studies and electrophysiological investigations are the other main assessment tool used within these studies. These provide information on compound action potential amplitude and conduction velocity but do not provide information regarding ion channel function or resting membrane potential and are still dependent on the skill of the neurologist conducting the assessment.[39] Hence assessment of CIPN was not uniform among the clinical studies examined. This makes comparisons difficult to evaluate when comparing study results. Moreover, it is therefore possible that researchers may have underestimated the nutraceutical efficacy to prevent CIPN, resulting in significant study design bias.

An additional secondary study limitation involves the assessment of the activities of daily living. Activities of daily living is an important factor in CIPN as it may provide a marker of the level of disability experienced from symptoms by interference of daily activity. Hence activities of daily living are generally subjective and signs on a physical examination may not always be predictive of

whether activities of daily living is affected or if it is important for the participant entering a CIPN clinical trial.

Measurement of CIPN

The measurement/grading or assessment of CIPN is critical for accurate clinical and instrumental monitoring. Currently there is no accurate measurement tool that has been used in either clinical trials or in a clinical setting that monitors CIPN.[40] The lack of standardization and reporting mechanisms has resulted in contentious reporting of CIPN data and consequently CIPN has become an under-diagnosed problem.[41]

CIPN is most commonly graded by clinicians by using common toxicity scales such as the National Cancer Institute – Common Toxicity Criteria (NCI-CTC). However, these scales rarely provide a detailed profile on clinical and pathological aspects of peripheral neuropathy that is a requisite for inclusion into clinical trial methodology. However, other measurement tools such as the Total Neuropathy Score [TNS] provide more detailed information pertaining to motor, sensory and autonomic signs and symptoms, determination of vibration perception thresholds and electrophysiological examination.[37,42]

Of the studies evaluated, there were a variety of measurement tools used that makes comparing results difficult. The majority of studies n=19 used the NCI-CTC as their measurement tool.[10-14,21-31,33-34] Of the studies that used the NCI-CTC six also included electrophysiological tests such as nerve conduction tests,[14,23,26,28,29,31] two included a self-reported perception of peripheral neuropathy[18,33] and one included a quality of life questionnaire.[25] Two studies used the TNS as their measurement tool,[17,35] two used modified versions of the Neurologic symptom score (NSS) and the Neurologic Disability Score (NDS) in addition to patient symptom experience[15,16] and one used the World Health Organisation (WHO) toxicity grading list.[32]

Given that the measurement of CIPN remains difficult due to the use of a number of different measurement tools the ideal recommendation is that a variety of standardized tools be used that include a peripheral neuropathy scale i.e. [TNS], quality of life questionnaire, pain scale and patient's perspective questionnaire.[37,42,43] This however, is impractical due to the very time consuming nature of the combined interventions for clinicians, therefore a more realistic recommendation would be the use the NCI-CTC. Hence when patients report experiencing grade 2 or higher neuropathy occurrence during or after chemotherapy they can then be referred to a neurologist for further testing.

Discussion

Currently there are no established neuroprotective nutraceuticals for the prevention or treatment of CIPN. Results are inconsistent requiring further clinical investigations to confirm efficacy and safety or obtained from relatively small sample sizes. Several nutraceuticals have shown promise for selective neurotoxic chemotherapy agents such as vitamin E (dose 300-600mg) with cisplatin, intravenous glutathione (dose 1.5gm/m^2) for oxaliplatin administration, vitamin B6 (dose 100mg TID) with HMM and cisplatin although interference with response rate was reported, and omega–3 fatty acids (dose 640mg TID) for paclitaxel. Acetyl–L–carnitine (dose 3 grams) has also demonstrated potential for efficacy as a treatment option for CIPN. Further research with large scale randomised controlled trials are warranted.

Conclusion

The overall survival from cancer, free from disease progression has increased in cancer patients, making quality of life an important factor for cancer survivors. CIPN is a major side effect that can interfere with a patient's quality of life, daily activities and also with medical treatments. The disruption of medical therapy for cancer patients that is due to the development of CIPN can affect chemotherapy dose and continuation of treatment that is of clinical importance. Investigating agents that could assist with CIPN prevention and treatment has significant scientific merit. Scientific evidence for nutraceuticals in the prevention and treatment of CIPN is limited. The analysis of the evidence presented according to the NHMRC grading scale of clinical evidence indicates that at the present time there can be no explicit recommendations that can be given for the prevention or treatment of chemotherapy induced peripheral neuropathy. Future clinical studies investigating natural compounds as neuro–protective agents should constitute a priority for this clinically relevant side effect.

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Conflict of Interest None other declared.

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Tables

Figures

Supplemental Information

Table 1: A differential diagnosis of peripheral neuropathy in patients with cancer.[2]

Cause	Neurotoxic Effect on Peripheral Nerves
Vitamin B12 deficiency	Large fibre injury, Dorsal root ganglia damage
Cachexia	Diffuse weakness and muscle wastage
Chemotherapy	Small and/or large fibre injury
Charcot–Marie–Tooth Disease	Large fibre injury
Diabetes Mellitus Type 2	Small fibre injury, slow development
Atherosclerotic Ischemic Disease	Sensory neuropathy in lower extremities
Para–Neoplastic Syndrome	Distal sensory or sensori-motor deficit
Thyroid Dysfunction	Proximal and distal weakness; carpal tunnel syndrome
Alcoholic Neuropathy	Numbness, parasthesias

Table 2: Reported Confounding Factors.

Confounding Factors	n
Disease progression	5
Grade 3 or 4 nausea and vomiting	4
Metastasis (liver, abdomen, peritoneum, lymph nodes, lung, other)	4
Sudden patient death	4
Grade 3 or 4 diarrhoea	3
Patients refused any further treatment	3
Heamatological toxicity (including transient hepatic failure)	2
Severe neutropenia	2
Stomatitis	2
If the treatment was first, second or third line treatment	2
Lethal toxicity from the chemotherapy agent	2
Cardiac and/or neurocerebellar adverse events	2
Grade 3 or 4 allergic reactions to the chemotherapy drugs	2
Thrombocytopenia	1
If the patient had resectable or unresectable lesions	1
Wrong diagnosis	1
Change to treatment protocol	1
Use of Alternative treatments	1

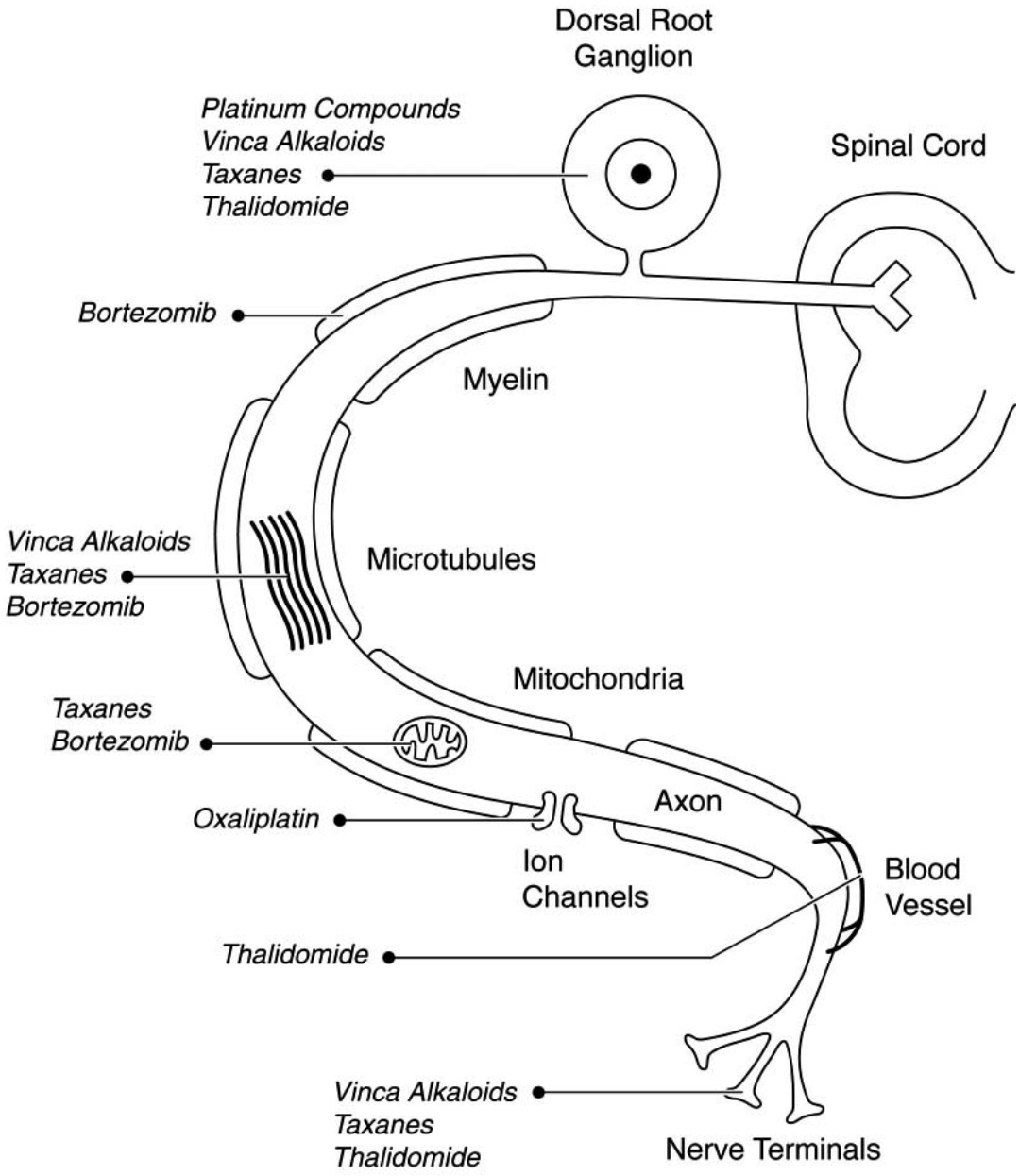
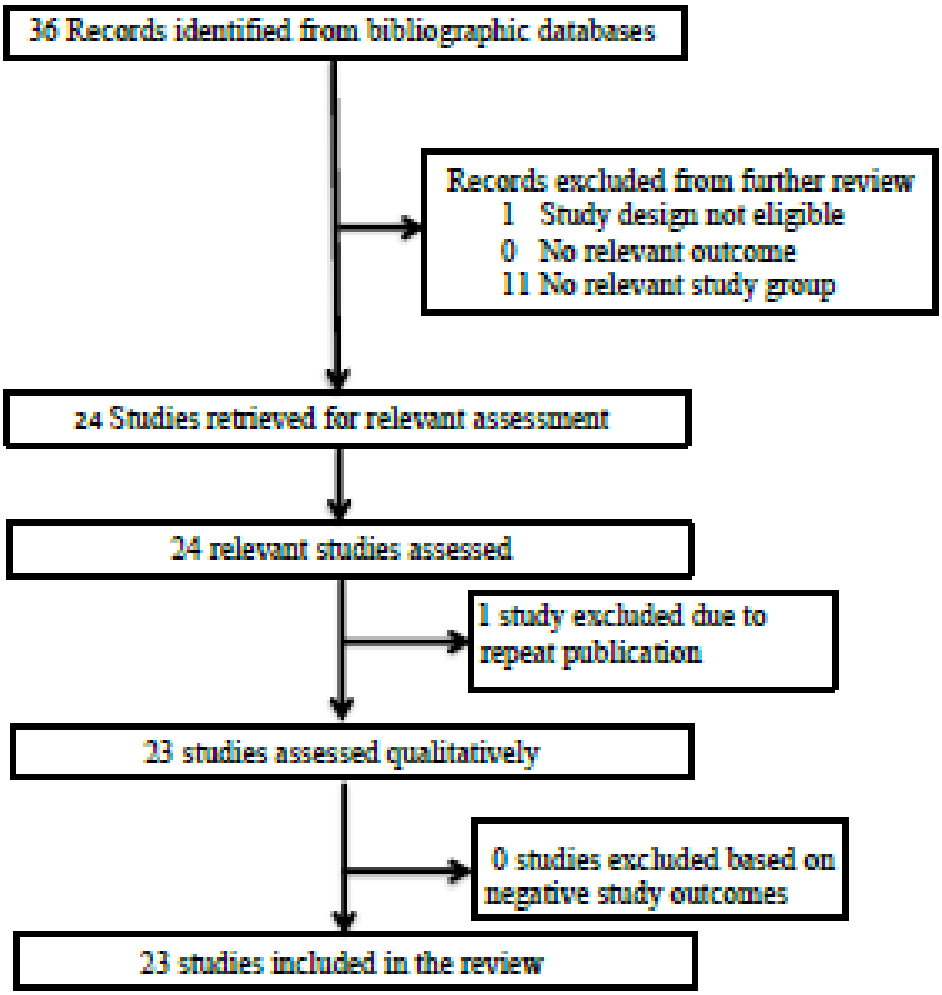


Figure 1: Site of chemotherapy-induced neurotoxicity.[7]

Figure 2. Flow of information for systematic review



Supplemental Information:

Clinical Studies Investigating the Efficacy of Selected Nutraceuticals for the Prevention of CIPN.

Nutraceuticals	Study Type / Level of Evidence [No. Participants]	Duration	Results
Magnesium / Calcium	Retrospective study[10] Level III-2 [161 participants]	3 cycles of FOLFOX (oxaliplatin) given every 2 weeks	— 96 participants received ca gluconate and mg sulphate (1g) before and after oxaliplatin. 65 participants did not — 20% of Ca/Mg group had CIPN versus 45% in control group with tumour response rates similar — Participants with grade 3 or 4 in the Ca/Mg group recovered significantly quicker than the controls — 4% in Ca/Mg group withdrew due to neurotoxicity compared to 31% in control group
	DBRCT[11] Level II [174 participants]	8 cycles of FOLFOX plus bevacizumab given every two weeks	— First 140 participants were randomised to Ca/Mg before and after oxaliplatin — Trial was terminated due to the findings that the Ca/Mg group reported a significantly lower response rate than placebo
	Open label[12] Level III-3 [14 participants]	FOLFOX 12 cycles given every two weeks	— All participants received an infusion of Ca/Mg before and after oxaliplatin — CIPN occurred in 8 participants (57.1%) with no neurotoxicity with functional impairment — Limited sensory neuropathy before the fourth cycle.
	DBRCT[13] Level II [35 participants]	FOLFOX6 12 cycles given every two weeks	— Ca/Mg group n=17 and placebo n=16 given before and after oxaliplatin — The incidence of CIPN after 6 cycles was not significant between the two groups — Blood platinum and the area under the curve were also not significantly different between the two groups
Vitamin E	DBRCT[14] Level II	Cisplatin 6 cycles and 3 months after	— 27 participants completed the 6 cycles of cisplatin — N=13 were randomised to vitamin E (300 mg/d)

	[47 participants]	chemotherapy completion	— The incidence of CIPN was significantly lower in vitamin E group (30.7%) compared to control group (85.7%) using a comprehensive neurotoxicity score based on clinical and neurophysiological parameters — No differences were observed in tumour weight inhibition, tumour growth delay or life span.
	RCT[15] Randomised open label with blind assessment Level III-1 [40 participants]	6 cycles of cisplatin, paclitaxel or combination plus 3 months after chemotherapy cessation	— 31 participants finished 6 cycles of chemotherapy — Vitamin E group (n=16) received 600mg/d compared to a control group (n=15) — CIPN developed in 4/16 (25%) of vitamin E group compared to 11/15 (73.3%) of control group.
	RCT[16] Randomised open label with blind assessment Level III-1 [35 participants]	6 cycles of cumulative cisplatin based regimes	— 30 participants finished 6 cycles of chemotherapy — Vitamin E group (n=16) received 600mg/d compared to a control group (n=19) — CIPN developed in 3/14 (21.4%) of participants assigned to the vitamin E group compared to 11/16 (68.5%) of controls — This study includes the final results of the published 2005 paper from Argyriou[6]. Therefore the study above involves the results incorporated into this journal article as disclosed by the authors.
	DBRCT[17] Level II [108 participants]	Cisplatin – intervention given before chemotherapy (from 3 days before), during and 3 months after chemotherapy cessation	— Vitamin E group (n=54) were given 400mg/d compared to placebo group (n=54) — If participants received <300mg cumulative dose they were excluded which left vitamin E group (n=17) and placebo group (n=24) — The incidence of CIPN was significantly lower in vitamin E group (5.9%) compared to placebo group (41.9%) as measured by TNS. — Authors stated that these results provided Class II evidence that vitamin E supplementation significantly reduces the relative risk of developing signs for symptoms of neurotoxicity (relative risk _0.14)(95% confidence interval_0.02-1.00,p_0.05)
	DBRCT[18] Multi-centre	Administered within 4 days of chemotherapy,	— N=189 evaluable cases, oral vitamin E 300mg/d bd (n=96) compared to oral placebo (n=93)

	Level II [207 participants]	given during and 1 month after cessation of chemotherapy	— Cytotoxic agents included taxanes (n=109), cisplation (n=8), carboplatin (n=2), oxaliplatin (n=50) or combination (n=20) — No difference was found in the incidence of grade 2+ sensory neuropathy between the two arms (vitamin E 34% compared to placebo 29%; P=0.43) — No significant differences was found between treatment arms for time to onset of neuropathy (P=0.58), chemotherapy dose reduction due to neuropathy (P=0.21) or secondary endpoints derived from patient-reported neuropathy symptom assessment. — Concluded vitamin E did not appear to reduce the incidence of sensory neuropathy in this studied group
Alpha Lipoic Acid	Open label[20] Level III-3 [15 participants]	Administered after 6 cycles of oxaliplatin in participants with ≥grade 2 CIPN till full recovery of neurological symptoms or for a maximum duration of 6 months	— N=12 participants had grade 2 CIPN, n=3 participants had grade 3 CIPN — Administration included 600mg IV alpha lipoic acid was given once a week for 3-5 weeks followed by 600mg TDS (total=1800mg/d) orally until full recovery or maximum duration of 6 months. — Neurologic symptoms improved by at least 1 grade in 7 participants with grade 2 CIPN and in 1 patient with grade 3. — Medium time to respond was 4 weeks with median duration of treatment being 2 months (range 1-4 months) — Seven participants did not respond — Concluded that alpha lipoic acid reduced the severity of oxaliplatin CIPN in 8 out of 15 participants (53%)
N-acetyl cysteine (NAC)	RCT[21] Level III-1 [14 participants]	Oxaliplatin (FOLFOX) 12 cycles administered every 2 weeks – monitored until 2 weeks after last administration	— N=5 (1200mg NAC) versus N=9 placebo given 1 ½ hours before each oxaliplatin administration — After 4 cycles 2 participants (40%) in NAC group compared to 7 participants (77.8%) in placebo had clinical grade 1 CIPN — After 8 cycles 3 participants (60%) in NAC group had grade 1 CIPN compared to 4 participants (44.4%) with grade 1 and 5 participants (55.6%) with grade 2-4 CIPN. — After 12 cycles, 3 participants (60%) had grade 1 CIPN and 1 patient (20%) had grade 2-4 CIPN in NAC group compared to 1 patient (11.1%) with grade 1 and 8 participants (88.9%) with grade 2-4 CIPN in placebo group

			— No significant change on electrophysiological testing including sensory and motor nerve studies after 4,8 and 12 cycles were found between the two groups.
Glutathione	DBRCT[23] Level II [50 participants]	Weekly cisplatin for 15 weeks	— Glutathione was administered immediately before cisplatin at a dose of 1.5g/m^2 in 100ml of saline solution over 15 minutes and after at a dose of 600mg by intramuscular injection on days 2 and 5. — Normal saline solution was administered to placebo-randomised participants — Clinical neurologic evaluation and electrophysiologic investigations were performed at baseline, after 9 cycles and 15 cycles Results: At 9th week – no participants showed clinically evident CIPN in glutathione arm compared to 16 participants in placebo arm At 15th week 4/24 participants in glutathione arm displayed CIPN compared to 16/18 in placebo arm (P=0.0001) — Reduced haemotransfusions occurred in glutathione arm (32 v 62) — Treatment delay was reduced in glutathione arm (55 v 94 weeks) — Response rate was 76% (20% complete response) in glutathione group and 52% (12% complete response) in placebo arm — Concluded that glutathione is a promising and effective drug for the prevention of cisplatin induced neuropathy and does not reduce the clinical activity.
	RCT[24] Level III-1 [33 participants]	Weekly cisplatin for 9 weeks	— High risk CIPN participants with relapsed ovarian cancer who had at least a one year disease free interval and prior cisplatin administration — Administration was 50mg/m^2 of cisplatin weekly with +/- 2.5g of glutathione — Response rate in 31 participants was 9/15 in glutathione group and 12/16 in control group including 4/15 vs 7/16 complete responses. — Administered dose intensity was higher in the glutathione arm (100% dose intensity was received by 56% vs 27%) — A trend in neuro-protection was detected in the glutathione treated group with no major differences in other observed toxicities.

			— Possible benefits of concomitant glutathione administration with cisplatin can be expected in high risk neurotoxicity participants without decreasing the anti-tumour activity
	DBRCT[25] Level II [151 participants]	Cisplatin every 3 weeks for 6 cycles	— Cisplatin alone (n=77) versus cisplatin + glutathione (n=74) — Administration of 3g/m^2 of either glutathione or saline was given for 20 minutes before cisplatin administration — CIPN was observed in 42% of cisplatin alone and 39% of cisplatin + glutathione arms which is not significant — 58% of glutathione arm vs 39% of cisplatin alone (P=0.04) were able to receive 6 cycles of cisplatin at any dose — A significant difference was found between the reduction in creatinine clearance for the glutathione treated arm compared to control (74% vs 62%; P=0.006) — QoL scores demonstrated that participants in the glutathione arm were statistically significant for improvement in depression, emesis, peripheral neurotoxicity, hair loss, shortness of breath and difficulty concentrating. They were found to be able to undertake housekeeping and shopping more easily.
	DBRCT[26] Level II [52 participants]	Oxaliplatin every 2 weeks for 12 cycles	— Administration of either glutathione 1,500mg/m^2 (N=21) or saline (N=19) was given before oxaliplatin over a 15 minute infusion period. — Clinical neurologic evaluation and electrophysiologic investigations were performed at baseline, after 4, 8 and 12 cycles of oxaliplatin — After 4^{th} cycle, 7 glutathione and 11 placebo participants showed clinically evident neuropathy — After 8^{th} cycle, 9/21 glutathione and 15/19 placebo showed CIPN — After 12^{th} cycle, grade 2-4 NCIC-TC was observed in 3 glutathione and 8 placebo arm (P=0.004) — The neurophysiologic investigations showed a statistically significant reduction in the sural sensory nerve conduction in the placebo arm but not in the glutathione arm. — Response rate was 26.9% in glutathione arm and 23.1% in placebo arm indicating no reduction in activity of oxaliplatin.

	RCT[27] Level III-1 [27 participants]	Oxaliplatin every 2 weeks for 12 cycles	— Administration of either glutathione 1,500mg/m^2 (n=14) or saline (n=13) was given before oxaliplatin — Evaluations were conducted at 5, 9 and 12 cycles of oxaliplatin — After 12 cycles 7/14 (50%) had grade 1 CIPN and 7/14 (50%) had grade 2 CIPN in gluthione group. — After 12 cycles 9/13 (69%) had grade 2 CIPN and 4/13 (31%) had grade 3 CIPN in placebo group. — At the end of all cycles the glutathione arm showed a statistically significant reduction of neurotoxicity (P=0.0037) compared to placebo arm. — No significant differences were noted in the main pharmacokinetic parameters between the two arms except a lower area under the plasma concentration-time curve and a smaller apparent steady-state volume of distribution when glutathione was co-administered.
Glutamine	NRCT[28,29] Level III-2 [47 participants]	Given orally for 4 days starting 24 hours after paclitaxel completion	— Stage 4 breast cancer participants undergoing high-dose chemotherapy with stem cell support - In intensification high dose paclitaxel (825mg/m^2) was administered continuously IV over 24 hours on day 24 prior to stem cell infusion — N= 33 non-glutamine, n=12 glutamine (10g orally 3 times a day for 4 days) with neurological examinations completed at baseline and 2 weeks after paclitaxel administration — Results: participants who received glutamine had fewer symptoms than those who did not — 8% versus 40% of participants receiving glutamine compared to those who did not had moderate to severe dysesthesias in fingers or toes — The frequency of moderate to severe numbness was observed less often in the glutamine group to non-glutamine group for both fingers and toes (P=0.016 and 0.009 respectively) — Less loss of vibration (P=0.04), reflexes and motor weakness was observed in the glutamine group although not significant except vibration — All signs and symptoms in both groups were reversible — Nerve conduction studies were not statistically significant

	RCT[30] Level III-1 [88 participants]	6 cycles of Oxaliplatin given in FOLFOX regime over 6 months	— N=44 received glutamine and N=44 were control — Dose of glutamine was 15g bd orally for 7 consecutive days every 2 weeks starting on the day of oxaliplatin administration — 16.7% glutamine group versus 38.6% control displayed grade 1-2 CIPN after 2 cycles — 4.8% glutamine group versus 18.2% control displayed grade 3-4 CIPN and 11.9% versus 31.8% after 6 cycles — The glutamine group also had less interference with daily activities (16.7% versus 40.9%) — Need for oxaliplatin dose reduction was lower (7.1% versus 27.3%). — No significant differences between the groups in response to chemotherapy, electrophysiological abnormalities and grade 3-4 non-neurological toxicities or survival. — Glutamine may reduce the incidence and severity of CIPN with oxaliplatin
Acetyl-L-Carntine (ALC)	Open label[31] Level III-2 [25 participants]	8 weeks	— Participants with CIPN ≥3 (Common toxicity criteria – CTC) during paclitaxel or cisplatin therapy or grade ≥2 persisting for at least 3 months after discontinuing drugs were selected — 1gram tid of ALC orally was administered for 8 weeks — All participants except 1 reported symptomatic relief — 2 reported grade 1 nausea — Sensory neuropathy improved in 15 participants (60%) — Motor neuropathy in 11out of 14 participants (79%) and total neuropathy score (TNS) improved in 23 participants (92%) — Symptomatic improvement persisted in 12 of 13 participants 13 months after ALC administration — ALC may be a treatment option for paclitaxel and cisplatin CIPN

	Open label[32] Level III-2 [26 participants]	10 days	— CIPN participants were enrolled: N=5 cisplatin, N=11 paclitaxel, N=11 combination of both cisplatin and paclitaxel — ALC 1gram IV infusion over 1-2 hours was administered for at least 10 days — A decrease in at least 1 WHO grade improvement in CIPN severity was noted in 73% of participants — 1 case of insomnia related to ALC treatment was reported — ALC may be an effective and well tolerated agent for the treatment of CIPN but results need to be confirmed by a DBRTC
	DBRCT[33] multi-centre Level II [409 participants]	24 weeks	— N=208 ALC participants (1000mg TID) versus N=201 placebo participants in women with stage I-III breast cancer undergoing adjuvant taxane therapy — At 12 weeks, the mean FACT-NTX score was 5.2 points lower in ALC arm versus 4.5 points lower in placebo group. — At 12 weeks in a linear regression adjusting for baseline score, planned treatment and age, score were 0.9 points lower in the ALC arm versus placebo (95%CI:-2.2 to 0.4, p=0.17) — At 24 weeks the mean FACT-NTX score was lower for ALC arm (5.3 vs 3.6) compared to placebo arm — At 24 weeks the multivariate models were 1.8 points lower in the ALC arm (95%CI: -3.2 to 0.4, p=0.01), indicating more self-reported CIPN — At 24 week more Grade 3 to 4 CIPN was found in the ALC arm (p=0.04), in addition to 38% in ALC arm having a >5 point decrease score compared to 28% in placebo group (OR=1.57, p=0.05) and the FACT-TOI scores were 3.5 points lower in the ALC (95%CI: -6.5 to -0.4, p=0.03) — No differences between arms were observed for fatigue or other toxicities — Concluded that ALC has no evidence of a positive impact at 12 weeks of treatment and may increase CIPN by 24 weeks of treatment. — Correlative studies are being conducted to explain this unexpected result and participants should be discouraged from using ALC.

Vitamin B6	DBRCT[34] Level II [248 participants] Multicentre	Between 1-70 months (median duration of response was 8.3 months)	— Participants with stages III-IV ovarian epithelial cancer (114 with and 134 without prior chemotherapy) were randomized to 4 groups: Group A: Cisplatin (DDP) 37.5mg/m^2 day 1 Hexamethylmelamin (HMM) 200mg/m^2 day 8-21 repeat on 21 day cycle Group B: DDP 37.5mg/m^2 day 1HMM (HMM) 200mg/m^2 day 8-21 repeat on 21 day cycle Vitamin B6 100mg TID day 1-21 repeat Group C: Cisplatin (DDP) 75mg/m^2 day 1 Hexamethylmelamin (HMM) 200mg/m^2 day 8-21 repeat 21 day cycle Group D: DDP 75mg/m^2 day 1 HMM 200mg/m^2 day 8-21 repeat on 21 day cycle Vitamin B6 100mg TID day 1-21 repeat — Participants with higher dose cisplatin experienced more severe nausea, vomiting and CIPN — Study demonstrated that the combination of cisplatin and HMM was an effective regimen for advanced ovarian carcinoma — B6 was found to significantly reduce CIPN however it was found to adversely affect response duration. The mechanism by which vitamin B6 may unfavourably affect response duration requires further investigation.
Omega 3 Fatty Acids (Omega 3FA)	DBRCT[35] Level II [69 participants]	During chemotherapy of paclitaxel (dose 175 mg/m^2) IV every 3 week for 4 cycles and one month after	— Breast cancer participants randomized to N=35 allocated to receive Omega 3FA (dose 640mg t.i.d) and n=34 placebo (sunflower oil t.i.d) with 57 participants finishing the trial — Primary outcome was based on rTNS evaluated pre-chemotherapy and on month after the end of chemotherapy — Omega 3FA group: 21 participants (70%) did not develop CIPN compared to 11 participants (40.7%) in the placebo group. — In the Omega 3FA group 9 participants (30%) had CIPN with 4 participants with mild CIPN (13.3%) and 5 participants with moderate CIPN (16.7%) with no severe CIPN compared to 16 participants (59.3%) had CIPN with 10 participants with mild CIPN (37%) , 5 participants (18.5%) with moderate CIPN and 1 patient (3.7%) with severe CIPN. — A statistically significant difference was seen in the incidence of the Omega 3 FA group compared to the placebo [OR=0.3, 0.95% C = (0.10-0.88), p = 0.029] therefore the omega 3 FA group had a 70% lower risk of developing CIPN.

			— A statistically significant difference was also seen in the nerve conduction test for sural a-SAP for omega 3FA group (p=0.015) — No other significant differences found

DBRCT: Double Blind Randomized Controlled Trial; SBRCT: Single Blinded Randomized Controlled Trial; RCT: Randomized Controlled Trial; NRCT: Non Randomised Clinical Trial; CS: Case Study; TNS: Total Neuropathy Score

12.2 CANCER CHEMOTHERAPEUTICS: CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY (CIPN) AND B GROUP VITAMINS.

Title Page

Review: Chemotherapy Induced Peripheral Neuropathy (CIPN) and B vitamins.

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Tables and Figures

Table 1: A differential diagnosis of peripheral neuropathy in patients with cancer
Table 2: Neurotoxic Chemotherapy agents

Figure 1: Site of chemotherapy-induced neurotoxicity.

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Abstract

Chemotherapy-induced peripheral neuropathy [CIPN] is a significant and debilitating side effect resulting from the administration of neurotoxic chemotherapeutic agents. Moderate to severe CIPN significantly decreases the quality of life and physical abilities of patients diagnosed with cancer and current pharmacotherapy for CIPN with agents such as Amifostine, anticonvulsants, and antidepressants have had limited efficacy and may themselves induce additional adverse side effects. Each class of chemotherapeutic agent has a particular mechanism of action in cancer treatment and the neuropathy that develops from these drugs is treatment and dose duration dependent. Currently, there are two studies that have investigated vitamin B6 and CIPN with no research data available on B vitamin complex administration and CIPN. Furthermore, a B vitamin deficiency or temporary deficiency may predispose a patient to the development of CIPN as deficits in this vitamin group can target the same focal points in a peripheral neuron. We hypothesize that B group vitamin administration has the potential to assist in the prevention and treatment of CIPN.

Introduction

Particular neoplastic agents can accumulate in the peripheral nervous system and the neurotoxicity may lead to chemotherapy induced peripheral neuropathy [CIPN]. The symptoms of CIPN can be numbness, tingling, burning, decreased touch sensation, decreased strength and movement and sometimes pain in the fingers, toes, hands or feet (1, 2). These neurotoxic chemotherapy agents include the platinum compounds, anti-tubulin agents such as the taxane class and vinca alkaloids, epothilones, thalidomide, proteasome inhibitors and 5-fluorouracil (5FU) (3-8). It is estimated that one third of all patients who undergo chemotherapy experience CIPN and of those, a third can have permanent nerve damage (3-5). Patients experiencing moderate to severe CIPN report a reduced quality of life (3), chronic discomfort (5) and disruption of physical abilities for general life activities which can be temporary or permanent (3). Moreover, CIPN can lead to dose reduction of the chemotherapy agent or possible cessation of treatment which may have an adverse impact on cancer treatment and disease outcomes (4).

Aetiology of CIPN

Requisites for peripheral nervous system neurotoxicity include chemotherapy agent capacity to cross the blood-nerve barrier and nervous system sensitivity to the drug. People with predisposing conditions such as type II diabetes mellitus (T2DM), HIV/AIDS, alcoholism or a vitamin B12 deficiency may be more prone to the agent's adverse effects on the peripheral nervous system thereby increasing the prevalence of CIPN (2). Given that cancer is multi-factorial, a differential diagnosis of the types of peripheral neuropathies experienced by cancer patients is necessary to ascertain peripheral neuropathy from chemotherapy agents [Table 1].

Peripheral nerve fibres are composed of small or large fibres. Small nerve fibres are unmyelinated and are comprised primarily of microtubules. They include nerves that sense pain and temperature. Large nerve fibres are myelinated and are composed mainly of neurofilaments that act as a framework for the axon. These fibres sense position and vibration as well as motor control (2). Both fibres are targeted by neurotoxic chemotherapy agents that may explain why patients experience a variety of symptoms.

Although each neurotoxic chemotherapy agent has a different mechanism of action on the nervous system, all induce a glove and stocking distribution. This means the point most distal

from the trunk of the body is affected first (e.g. fingers and toes) and progression is then towards the trunk to hands and feet and then limbs (2). Each agent has been found to affect one nerve fibre more than others, for example, cisplatin targets large fibres while paclitaxel and vincristine target small fibres (2).

CIPN can be a temporary side effect which can take up to two years for full recovery. In approximately one third of cases it can be a permanent consequence of the drug neurotioxicity. Symptoms may occur within hours, days or weeks after the introduction of the chemotherapy agent, with cumulative doses increasing the severity and length of time the patient experiences this side effect (2). Cisplatin differs to other neurotoxic agents as it can induce a delayed CIPN several months after the drug has been administered rather than a more immediate response (5).

Mechanism of action of Neurotoxic Chemotherapy Agents

The neurotoxic chemotherapy agents can be divided into four main categories, alkylating and anti-tubulin agents, thalidomide and proteasome inhibitors [Table 2]. A common feature of these drugs is that they are unable to cross the blood-brain barrier thereby protecting the central nervous system however the peripheral nervous system has no protective barrier making it susceptible to neurotoxicity (3) and neurotoxic chemotherapy agents can accumulate and target different regions of the neuron (6) [Figure 1].

Mechanism of Action of B vitamins

The vitamin B complex [vitamins B1, 2, 3, 5, 6, 12, folate, choline and biotin] function as coenzymes in several intermediary metabolic pathways for energy generation, blood cell formation and metabolism. Deficiencies in one or more of these B group vitamins may promote nerve dysfunction and nerve damage that can lead to peripheral neuropathy (20). Research has demonstrated that administration of B vitamins can assist in regenerating nerve fibres and myelination (3, 5, 21-25).

To date, no research on B group vitamins and CIPN has been undertaken. However, two studies using only vitamin B6 and the development of CIPN have been reported (26, 27). Research on vitamin B6 and CIPN has been conducted in vitro with oxaliplatin (26) and in vivo with cisplatin (27). Garg MB and Ackland SP (2010) administered vitamin B6 to a range

of cell lines with induced cytotoxicity from oxaliplatin. It was demonstrated that vitamin B6 did not affect the oxaliplatin's anti-cancer effect. Although no direct testing on peripheral neuropathy induced from oxaliplatin was conducted, this research demonstrated safety information for the use of vitamin B6 with oxaliplatin administration as it is unlikely that it will reverse the anti-tumour effect of the drug (26).

Wiernik RH et al. (1992) conducted a clinical trial on Hexamethylmelamine and low or moderate dose cisplatin with or without vitamin B6. Two hundred and forty-eight patients with stage III-IV ovarian epithelial cancer were randomised into four groups. Results found that vitamin B6 administration reduced neurologic toxicity from cisplatin administration however, it was also found to have an adverse effect on response duration. The dose administered was 100mg three times a day (total 300mg) of vitamin B6 from day 1-21 (27). Considering the CIPN was reduced, further studies with a lower dose of vitamin B6 are warranted to confirm if the response duration result is vitamin B6 dose dependent.

Furthermore, research has demonstrated that certain chemotherapeutic agents can induce a temporary deficiency in vitamin B12 which may predispose a patient to CIPN development (28). Additional studies on 5 Fluorouracil (5FU), an agent that also exhibits neurotoxicity was shown to induce Wernicke's encephalopathy, an acute neuropsychiatric syndrome that results from a vitamin B1 deficiency (8, 29-33). Hence, it is possible to hypothesize that specific chemotherapy agents may induce a temporary B vitamin deficiency which could then be a major causal contributor to the development of CIPN in patients undergoing treatment with neurotoxic chemotherapy agents. Moreover, deficits in B group vitamins can biochemically affect the health of nerve fibres and ganglia that neurotoxic chemotherapy agents can adversely modulate.

The mechanism of action of how the B vitamins may prevent CIPN is still to be elicited. However, from research, we can surmise certain actions which may assist in preventing CIPN with B vitamins. Firstly, a vitamin B1 deficiency has been found to cause an axonal degeneration that is comparable to that seen from vincristine (34). As a result of axonal degeneration from a vitamin B1 deficiency, chromatolysis of dorsal root ganglia neurons and anterior horn cell neurons can occur which again is very similar to that seen from vincristine administration (35,36). Active regeneration of peripheral nerves was found in patients with a vitamin B1 deficiency receiving thiamine supplementation (34). Therefore it is possible that vitamin B1 may play a key role in the prevention of CIPN from vincristine administration

through decreasing axonal degeneration, chromatolysis of dorsal root ganglia neurons and regeneration of peripheral nerves.

Research has also demonstrated that vitamin B1 administration suppresses thermal hyperalgesia [increased sensitivity to pain] by reducing hyper-excitability and lessening alterations of sodium currents in injured dorsal root ganglia neurons in rats (24). This maybe correlated to the injury incurred by oxaliplatin in the dorsal root ganglia as it affects voltage-gate sodium currents and causes axonal degeneration without evidence of primary demyelination (3, 5). Therefore thiamine administration potentially regenerates axons, reduces pain and aids in balancing sodium currents in cancer patients receiving oxaliplatin.

Vitamin B12 may also aide in preventing CIPN although no direct connection has yet been reported. Research has found that a deficiency in vitamin B12 results in an imbalance of cytokines and growth factors that directly affect glial cells and nerve myelination. Abnormally high levels of TNF-α and abnormally low levels of epidermal growth factor (EGF) have been found in vitamin B12 deficiencies which can cause demyelination resulting in nerve damage (39,40). Other cytokines and end products reported from vitamin B12 deficiencies include increased NF-κB, accumulation of N^{ϵ}-Carboxymethyllysine (CML) in sural nerves and activation of the receptors for advanced glycation end products (RAGE) pathway (38). These are all related to an inflammatory response (38).

Furthermore, an in-vitro study found that neuroblastoma cells that were deficient in vitamin B12 showed slower proliferation but faster differentiation. This was through interacting signalling pathways related to an increased expression of catalytic protein phosphatase 2A (PP2A), pro-nerve growth factor (proNGF) and two tumor necrosis factor alpha converting enzymes (TACE) (41). Nerve regeneration, myelinogenesis, reduction in inflammatory cytokines such as TNF-α and up-regulation of neural IGF-1 gene expression has been shown to reverse or markedly improve peripheral neuropathy by administration of vitamin B12 (21, 22, 37, 42-47). Hence, vitamin B12 has the potential in conjunction with other B vitamins to assist in preventing CIPN development.

Discussion

CIPN can affect chemotherapy dose and continuation of treatment which is of clinical importance. Investigating agents that can assist with CIPN treatment and prevention has

significant scientific merit. Several different substances hypothesised for pharmacological neuroprotection (e.g. Amifostine) have been trialled however neither prophylactic strategies nor symptomatic treatments have proven useful at this stage (5, 48).

B group vitamins have the potential to decrease the onset and severity of CIPN as a deficiency of B vitamins biochemically affects the health of nerve fibres and ganglia that neurotoxic chemotherapy agents can further adversely modulate. Reducing this chemotherapy induced side effect is clinically relevant for disease outcome and quality of life.

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Notes

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Table 1: A differential diagnosis of peripheral neuropathy in patients with cancer [2]

Cause	Neurotoxic Effect on Peripheral Nerves
Vitamin B12 deficiency	Large fibre injury, Dorsal root ganglia damage
Cachexia	Diffuse weakness and muscle wastage
Chemotherapy	Small and/or large fibre injury
Charcot-Marie-Tooth disease	Large fibre injury
Diabetes Mellitus	Small fibre injury, slow development
Atherosclerotic ischemic disease	Sensory neuropathy in lower extremities
Paraneoplastic syndrome	Distal sensory or sensori-motor deficit
Thyroid dysfunction	Proximal and distal weakness; carpal tunnel syndrome
Alcoholic neuropathy	Numbness, parasthesias

Table 2: Neurotoxic Chemotherapy agents

Chemotherapy Category	Mechanism of Action	Chemotherapeutic Drug	Incidence of Peripheral Neuropathy	Cancers Treated	Sensory Symptoms	Motor Symptoms	Other common side effects
Alkylating Agents	Bind and cross link DNA strands, impairing cell division and inducing apoptosis. Neurotoxicity mechanism due to intracytoplasmatic protein binding and ion channel interactions inducing neuronal apoptosis of the DRG.	**PLATINUM COMPOUNDS:** Cisplatin (Platinol®) Carboplatin (Paraplatin®) Oxaliplatin (Eloxatin®)	59-92% [2,10,11] 25% [4] Acute 80-90 % Chronic 15-25% [2,4,8]	Breast Colon Lung Testicles Bladder Ovaries [4]	Mild to moderate numbness and tingling of hands and feet can occur after prolonged (4-6 months) of therapy and may develop weeks after last dose. Symptoms can become severe with high cumulative doses. Reduced or absent Achilles tendon reflex. [2,4,8]	Weakness is rare but can occur with high doses of Cisplatin and Oxaliplatin. [2,4]	Ototoxicity, vestibular toxicity, anemia, neutropenia, leukocytopenia, thrombocytopenia, nausea, mucositis, vomiting, granulocytopenia, hypochromia [11,12]

Chemotherapy Category	Mechanism of Action	Chemotherapeutic Drug	Incidence of Peripheral Neuropathy	Cancers Treated	Sensory Symptoms	Motor Symptoms	Other common side effects
Anti-Tubulin Agents	Microtubule depolymerisation which inhibits mitosis resulting in apoptosis in rapidly dividing cells. Soluble tubulin exists in a cell as a heterodimer. During polymerisation, these heterodimers link together to form protofilaments which form a hollow cylinder that makes up the backbone of the microtubule. Anti-tubulin agents bind to the tubulin and	**TAXANE CLASS:** Paclitaxel (Taxol®) Docetaxel (Taxotere®) Abraxane™	60% [3,8,14] 50% [3,8] 71% [15]	Breast Ovaries Prostate Non-small cell lung cancers [13]	Mild to moderate numbness, tingling, burning/stabbing pain of hands and feet are common which can become severe with increased doses. Reduced or absent achilles tendon reflex [2,13]	Weakness of distal muscles has been documented with high cumulative doses of *paclitaxel and docetaxel.* [2,13]	Neutropenia, anemia, myalgia/arthralgia, nausea, alopecia, hypersensitivity reactions (allergic reactions) [16]
		VINCA ALKALOID CLASS: Vincristine (Onkovin®) Vinorelbine (Navelbine®) Vindesine Vinorelbine.	75% [3,4,17] 25% [3,4] 25% [3,4] 25% [3,4]	Lymphomas Leukaemia Neuroblastoma Rhabdomyo-sarcoma Ewing's sarcoma Wilm's tumour Multiple myeloma [18]	Mild to moderate numbness, tingling, burning/stabbing pain of hands and feet are common and can become severe with increased doses. Reduced or absent Achilles tendon reflex [2,4]	Weakness of distal muscles, decreased deep tendon reflexes, and foot drop have been noted with high doses [2,4]	Granulocytopenia, leukcytopenia, anemia, fatigue, nausea, alopecia, constipation, mouth ulcers [18]

	interfere with the heterdimerisation disrupting the microtubule dynamics (failure of alignment of the daughter chromosomes and their bipolar attachment to mitotic spindles)	**Epothilones:** Ixabepilone Patupilone MBS-310705 Epothilone D (KOS-862) Sagopilone (ZK-EPO)	60-71% [2,13]	Breast Gastric Soft tissue sarcomas Prostate Ovaries Colon Lymphomas [13]	Mild to moderate numbness, tingling, burning/stabbing pain of hands and feet are common which can become severe with increased doses. Reduced or absent achilles tendon reflex [2,13]	Weakness of distal muscles with high doses [2,13]	Neutropenia, leukopenia, nausea, stomatitis, pharyngitis, diarrohea, fatigue. [19]
Proteasome inhibitors	Inhibition of protein degradation through the ubiquitin-proteasome pathway which carries out the regulated degradation of damaged cellular proteins.	Bortezomib	35-50% [5,6]	Multiple myeloma Mantle-cell lymphoma [6]	Can induce severe pain. Small-fibre, axonal, sensory neuropathy manifesting as burning, hyper- or hypo-esthesia and paresthesias affecting both hands and feet. [6]	Severe CIPN results in vibration sense and ankle jerks. Muscle weakness is uncommon. [6]	Tiredness, diarrhoea, rashes, skin discolouration, sore mouth, loss of appetite, decreased blood counts, swelling, fluid retention [20]

Chemotherapy Category	Mechanism of Action	Chemotherapeutic Drug	Incidence of Peripheral Neuropathy	Cancers Treated	Sensory Symptoms	Motor Symptoms	Other common side effects
Thalidomide	A glutamic acid derivative that induces the production of interferon-γ and interleukin-2, and inhibits tumour necrosis factor-α production and angiogenesis. It also inhibits the migration of both immune and phagocytic cells, reduces tumour-associated macrophage infiltration and suppresses the proliferation of human myeloma cells	**Thalidomide**	50-83% [6]	Multiple myeloma Myeloma [2,6]	Mild to moderate numbness, tingling, burning hands and feet are common which can become severe with increased doses. Pain is uncommon [2,6]	None noted	Nausea, vomiting, blood clots, birth defects, fatigue, loss of appetite, dizziness, rashes, ankle swelling, fluid retention [6]

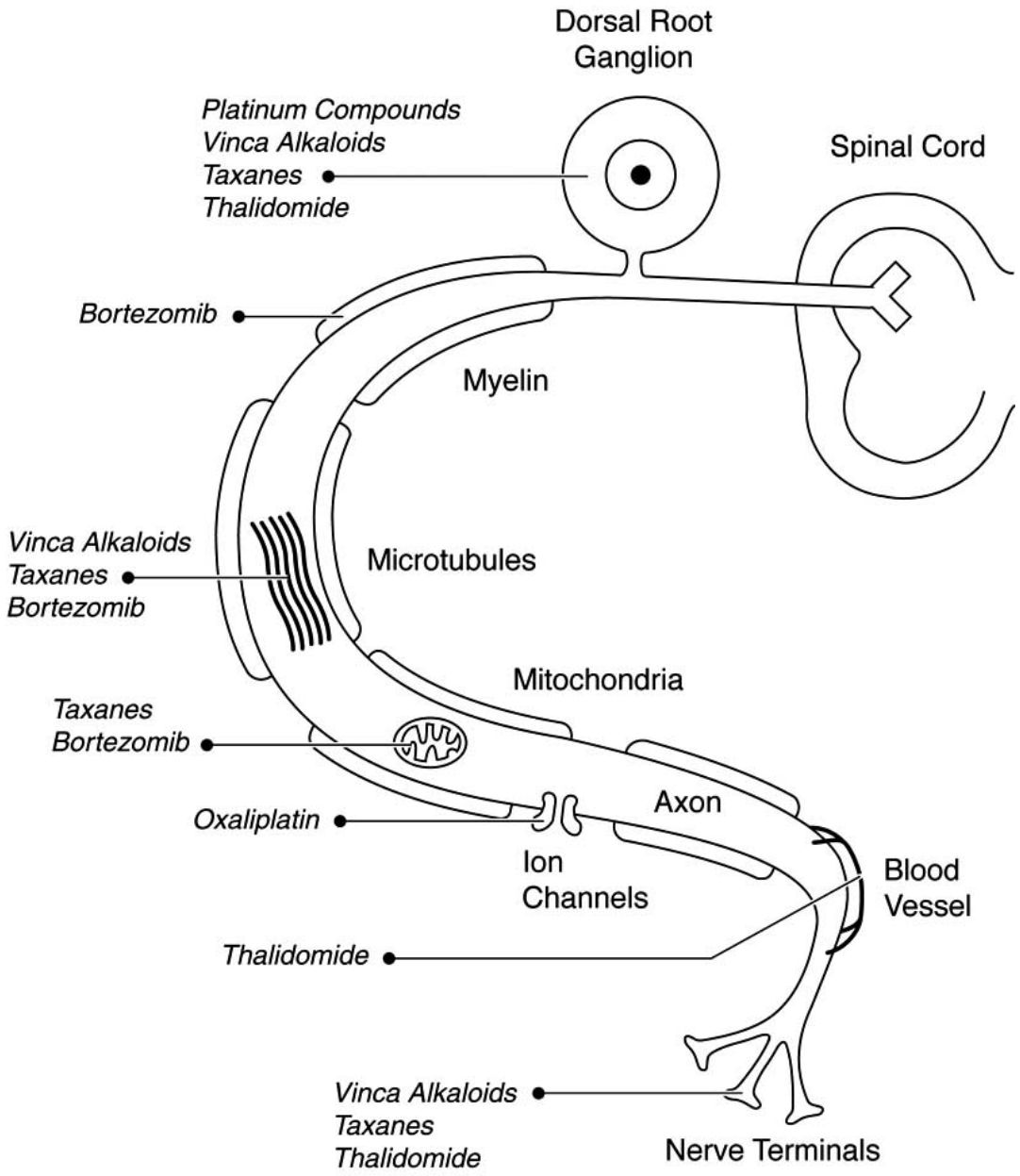

Figure 1: Site of chemotherapy-induced neurotoxicity. [6]

12.3 HERBAL MEDICINE AND CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY (ACCEPTED BY CLINICAL REVIEWS IN FOOD SCIENCE AND NUTRITION IN JANUARY 2014 – AWAITING PUBLICATION)

Herbal Medicines and Chemotherapy Induced Peripheral Neuropathy (CIPN): a Critical Literature Review.

Abstract

Background: Chemotherapy-induced peripheral neuropathy [CIPN] is a common significant and debilitating side effect resulting from the administration of neurotoxic chemotherapeutic agents. These pharmaco–chemotherapeutics can include taxanes, vinca alkaloids, platinum analogues and others. Moderate to severe CIPN significantly decreases the quality of life and physical abilities of cancer patients and current pharmacotherapy for CIPN (e.g. Amifostine and antidepressants) have had limited efficacy and may themselves induce adverse side effects.

Methods: To determine the potential use of herbal medicines as adjuvants in cancer treatments a critical literature review was conducted by electronic and manual search on nine databases. These include PubMed, the Cochrane Library, Science Direct, Scopus, EMBASE, MEDLINE, Google Scholar and two Chinese databases CNKI and CINAHL. Thirty-four studies were selected from 5614 studies assessed and comprised of animal studies, case reports, retrospective studies and minimal randomized clinical trials investigating the anti-CIPN effect of herbal medicines as the adjuvant intervention in patients administered chemotherapy. The thirty-four studies were assessed on methodological quality and limitations identified.

Results: Studies were mixed in their recommendations for herbal medicines as an adjuvant treatment for CIPN.

Conclusion: Currently no agent has shown solid beneficial evidence to be recommended for the treatment or prophylaxis of CIPN. Given that the number of cancer survivors is increasing, the long-term side effects of cancer treatment, is of major importance.

Key Words: Chemotherapy-induced peripheral neuropathy, herbal medicines, peripheral neuropathy, herbs.

Abbreviations

AC	*Acorus calamus*
CIPN	Chemotherapy-Induced Peripheral Neuropathy
CAPEOX	Chemotherapy regime comprising of: Capecitabine and Oxaliplatin
DBRCT	Double Blind Randomised Controlled Trial
FOLFOX	Chemotherapy regime comprising of: Folinic acid (leucovorin), Fluorouracil and Oxaliplatin
GB	*Ginkgo biloba*
GJG	*Gohsajinkigan*
HIV	Human Immunodeficiency virus
IPN	Induced Peripheral Neuropathy
IV	Intravenous
MC	*Matricaria chamomilla*
NHMRC	National Health and Medical Research Council
PN	Peripheral Neuropathy
PNQ	Peripheral Neuropathy Questionnaire
RCT	Randomised Controlled Trial
SO	*Salvia officinalis*
TCM	Traditional Chinese Medicine

Herbal Medicines and CIPN

Introduction

Chemotherapy-induced peripheral neuropathy [CIPN] is major dose limiting side effect of certain chemotherapy agents including taxanes, platinum compounds, epothilones, vinca alkaloids, thalidamide and newer agents such as bortezomib and lenolidamide [Wolf et al., 2008]. The incidence of CIPN varies depending on the chemotherapy agent but can range from 20% to 75% [Caveletti et al., 2011]. The symptoms of CIPN can be numbness, tingling, burning, decreased touch sensation, decreased strength and movement and sometimes pain in the fingers, toes, hands or feet [Armstrong et al., 2005; Visovsky et al., 2008].

Mechanism of Action for CIPN

Currently, the exact mechanism of action underlying the neurotoxic activity of the chemotherapy agents that can cause CIPN is largely incomplete and data is frequently based on poorly supported assumptions. There are similarities between the cytotoxicity mechanism of action of the chemotherapy agent and their action on the peripheral nervous system cells. However, if the amount of DNA damage or cellular damage exceeds the cells ability to repair, the cell will undergo apopotosis or cell death. This damage does not explain the sensory, possible motor involvement and pain associated with CIPN [Caveletti et al., 2011]. These neoplastic agents have been found to accumulate in the peripheral nervous system and its this accumulation that is said to cause the neurotoxicity leading to CIPN however, the mechanism of action is still relatively unknown. [Bhagra et al, 2007; Cavaletti et al., 2008; Cavaletti et al, 2004; Park et al., 2008; Pirzada et al., 2000; Schiff et al., 2009].

Justification for conducting a literature review on herbal medicine and CIPN

It is estimated that one third of all patients who undergo chemotherapy experience CIPN and of those, a third can have permanent nerve damage [Bhagra et al, 2007; Cavaletti et al., 2008; Cavaletti et al., 2004]. Patients experiencing moderate to severe CIPN report a reduced quality of life [Cavaletti et al., 2008], chronic discomfort [Cavaletti et al., 2004] and disruption of physical abilities for general life activities, which can be temporary or permanent [Cavaletti et al., 2008]. Moreover, CIPN can lead to dose reduction of the chemotherapy agent or possible cessation of treatment, which may have an adverse impact on cancer treatment and disease outcomes [Bhagra et al., 2007].

Currently no pharmaceutal or neutraceutical agent has shown solid beneficial evidence to be recommended for the treatment or prophylaxis of CIPN. Given that the number of cancer survivors is increasing, the long-term side effects of cancer treatment, is of major importance. Herbal medicine

may have the potential to provide prevention or treatment for CIPN. Hence, examining the current literature to assess studies conducted on herbal medicine and CIPN may provide information to its possible benefit.

Herbal treatments may offer a different aspect to assisting CIPN however the main problem with research involving herbal extracts or medicinal herbs is the understanding of the mechanism of actions and the fact that the herbs contain a number of active compounds. Moreover, Asian herbal therapies contain a combination of multiple herbs, which adds to the complexity of study analysis, data interpretation and an assessment of benefit. Isolation of one extract which is common in scientific research is unable to be conducted with herbal medicine research in most situations as the combination of compounds or combination of herbs such as Asian herbal therapies are seen to work in synergy.

The Aetiology of CIPN

A differential diagnosis of peripheral neuropathy in patients diagnosed with cancer includes a vitamin B12 deficiency, cachexia, chemotherapy, Charcot-Marie-Tooth disease, diabetes mellitus type II, atherosclerotic ischemic disease, para-neoplastic syndrome, thyroid dysfunction and alcoholic neuropathy [Armstrong et al., 2005]. Requisites for peripheral nervous system neurotoxicity include chemotherapy agent capacity to cross the blood-nerve barrier and nervous system sensitivity to the drug. People with predisposing conditions such as type II diabetes mellitus (T2DM), HIV/AIDS, alcoholism or a vitamin B12 deficiency may be more prone to the agent's adverse effects on the peripheral nervous system thereby increasing the prevalence of CIPN [Armstrong et al., 2005].

Peripheral nerve fibres are composed of small or large fibres. Small nerve fibres are unmyelinated and are comprised primarily of microtubules. They include nerves that sense pain and temperature. Large nerve fibres are myelinated and are composed mainly of neurofilaments that act as a framework for the axon. These fibres sense position and vibration as well as motor control [Armstrong et al., 2005]. Both fibres are targeted by neurotoxic chemotherapy agents, and that may explain why patients experience a variety of symptoms.

Although each neurotoxic chemotherapy agent has a different mechanism of action on the nervous system, all induce a glove and stocking distribution. This means the point most distal from the trunk of the body is affected first (e.g. fingers and toes) and progression is then towards the trunk to hands and feet and then limbs [Armstrong et al., 2005]. Each agent has been found to affect one nerve fibre

more than others, for example, cisplatin targets large fibres while paclitaxel and vincristine target small fibres [Armstrong et al., 2005].

CIPN can be a temporary side effect, which can take up to two years for full recovery. In approximately one third of cases it can be a permanent side effect of the drug's neurotoxicity action. Symptoms may occur within hours, days or weeks after the introduction of the chemotherapy agent, with cumulative doses increasing the severity and length of time the patient experiences this side effect [Armstrong et al., 2005]. Cisplatin differs to other neurotoxic agents as it can induce a delayed CIPN several months after the drug has been administered rather than a more immediate response [Cavaletti et al., 2004].

Mechanism of Action of Neurotoxic Chemotherapy Agents

The neurotoxic chemotherapy agents can be divided into four main categories, alkylating and anti-tubulin agents, thalidomide and proteasome inhibitors. A common feature of these drugs is that they are unable to cross the blood-brain barrier thereby protecting the central nervous system. The peripheral nervous system has no protective barrier making it susceptible to neurotoxicity [Cavaletti et al., 2008] and therefore neurotoxic chemotherapy agents can accumulate and target different regions of the neuron [Ferrier et al., 2013].

Methodology

Selection Criteria

The Inclusion criteria for this review were:

1) Any type of human trial e.g. RCT, retrospective, case study;
2) Animal studies;
3) The use of a herb or combination of herbs as the main intervention and specifically investigating its effects on reducing the primary outcome i.e. CIPN; and
4) The journal article or abstract must be written in English (a number of Asian journal articles had the abstract in English but the journal article in their language i.e. Japanese or Chinese.)

Databases

The following databases were used to retrieve journal articles: PubMed, the Cochrane Library, Science Direct, Scopus, EMBASE, MEDLINE, and Google Scholar.

Chinese databases included CNKI and CINAHL.

Search Terms

Electronic databases were searched using the following search terms, "chemotherapy-induced peripheral neuropathy" OR "Cisplatin" OR "Taxanes" OR "Paclitaxel" OR "Docetaxel" OR "Oxaliplatin" OR "Carboplatin" OR "Platinum compounds" OR "Proteasome inhibitors" AND "peripheral neuropathy" OR "CIPN" AND "herb" OR "cannabis" OR "chamomile" OR "ginkgo" OR "sweet bee venom" OR "turmeric" OR "sage" OR "hypericum" OR "herbal medicines" OR "Chinese herbal medicines" OR "ayurvedic herbal medicines".

The overall body of evidence (based on a summary of the individual studies: See Table 1 and 2) evaluated within this review was primarily assessed using a separate tool, the Australian National Health and Medical Research Council's (NHMRC) body of clinical evidence assessment matrix. This is an assessment tool that assigns a level/grade (Level I: strongest evidence to level IV: weakest evidence) based on the strength of the published study [NHMRC, 2009]. Supportive evidence was obtained from animal studies.

Risk bias assessment

The risk bias of both animal and human studies was assessed using the Cochrane Risk of Bias Assessment tool (http://handbook.cochrane.org/ , part 2, Chapter 8). All studies were reviewed by two reviewers (JS, LV).

Data Synthesis

All human clinical trial data (excluding case studies) was analysed using RevMan version 5.2.7 to quantify and compare the efficacy outcomes of the intervention versus control.

Results

A total of 5614 journal articles were identified. These were retrieved through electronic search and examination of references in reviews. Dissemination of the articles and abstracts decreased the total of articles from 5614 to 34 relevant journal articles. These results found 6 single herbs [Abad et al., 2011a, 2011b; Al Moundhri et al., 2013; Cakil, 2012; Huang et al., 2007; Lim et al., 2013; Marshall et al., 2004; Muthuraman A et al., 2011; Ozturk et al., 2004; Park et al., 2011; Xu O, et al., 2004; Yoon et al., 2012;], one extract [Xu F, et al., 2011], one receptor agonist [Rahn et al., 2007; Rahn et al., 2008] and 8 combinations of herbs [Bahar et al., 2013; Deng and Zou, 2007; Fujii et al., 2004; Hashimoto et al., 2004, 2006; Hidaka et al., 2009; Hosokawa et al., 2012; Kaku et al., 2012; Kono et

al., 2011, 2013; Nishioka et al., 2011; Pan et al., 2012; Shindo et al., 2008; Sima et al., 2009; Sun et al., 2008; Tatsumi et al., 2009; Ushio et al., 2012; Yamada et al., 2012; Yamamoto et al., 2009;].

All studies included in this review were analysed for common scientific characteristics however lower levels of evidence was used as rigorous randomised clinical trials were limited. The flow chart of study selection can be seen on figure 1. Of the journal articles identified n=18 were animal studies (see table 1) with human clinical studies consisting of one multi-centre, randomised double-blind placebo-controlled trial, six randomised trials, six retrospective studies, one uncontrolled study and three case reports found, [n=17] (see Table 2).

Animal Studies (*See table 1)*

Single Herbal Medicines in Animal Studies and CIPN

1. *Acorus calamus rhizome*

Acorus calamus (AC) is an Ayurvedic herb traditionally used to treat or manage pain and inflammation. This study investigated if AC had protective effects for vincristine-induced painful neuropathy in rats. *Acorus calamus* was compared to pregabalin (Lyrica) and was found to be comparable as both attenuated the vincristine-induced painful neuropathy [Muthuraman et al., 2011].

2. *Curcumin longa*

Curcumin, an extract from turmeric has been reported to have strong anti-inflammatory, anti-cancer and antioxidant activity [Al Moundhri et al., 2013]. This study looked at curcumin's potential protective effect on cisplatin and oxaliplatin induced behavioural, biochemical and histopathological changes in rats. Rats were randomly divided into five groups (six rats per group): 1) IV glucose and distilled water; 2) IV oxaliplatin and distilled water; 3) IV oxaliplatin and curcumin in water; 4) IV cisplatin and distilled water; 5) IV cisplatin and curcumin in water. Results found that oral curcumin reversed the alterations in the plasma neurotensin and sciatic nerve platinum concentrations and markedly improved sciatic nerve histology. This study doesn't provide complete evidence for neuroprotection however it does give evidence warranting further research.

3. *Ginkgo biloba (EGb 761)*

Ginkgo biloba (GB) has been found in in-vivo and in-vitro studies to have potential as a neuroprotective agent [Mills, 1991]. Four studies on animals have been conducted to test GB as a

neuroprotective agent against cisplatin-induced peripheral neuropathy particularly focusing on ototoxicity [Cakil et al., 2012; Huang et al., 2007; Ozturk et al., 2004; Xu O, et al., 2004].

Cakil B, et al (2012) tested twenty rats with normal hearing to cisplatin exposure with one group receiving 100mg/kg of GB for 10 days. Results indicated that ginkgo could protect against inner ear cisplatin-induced ototoxicity. Huang X, et al. (2007) also tested GB on rats to investigate its protective effect for ototoxicity by cisplatin administration. Results from this investigation also found GB to be protective against cisplatin-induced ototoxicity.

Xu O, et al. (2004) investigated the combination of GB and deferoxamin (DFO, a chelating agent) on guinea pigs again focusing on the ototoxicity induced by cisplatin. They concluded that the combined use of GB and DFO reduced cisplatin-induced ototoxicity and that the combination was better than using just GB alone. The fourth animal study was conducted on mice by Ozturk G, et al. (2004) who investigated the neuroprotective effects of GB for cisplatin-PN, not just ototoxicity. They evaluated the neuropathy by conducing nerve conduction velocity (NCV) tests on the mice. Results indicated that GB was effective in preventing some functional and morphological peripheral nerve deteriorations induced by cisplatin administration.

4. *Matricaria chamomilla (chamomile)*

Matricaria chamomilla (MC) is traditionally used for sedation, pain management, spasms, inflammation and wound healing [Berry, 1995; Hosokawa et al., 2012]. This study's aim was to investigate the effects of MC hydro-alcoholic extract on cisplatin-induced peripheral neuropathy compared to morphine in mice. Mice were divided into 6 groups which received: 1) normal saline; 2) MC hydro-alcoholic extract; 3) cisplatin; 4) MC hydro-alcoholic extract and cisplatin; 5) morphine; 6) morphine and cisplatin. The results found that cisplatin caused significant pain ($P<0.05$) and that MC given before cisplatin decreased the pain response significantly ($P<0.05$) in the first and second phase. In comparison to morphine, morphine had analgesic effects in the first phase while MC had anti-inflammatory effects in the second phase [Abad et al., 2011a].

5. *Salvia officinalis*

Salvia officinalis (SO) or sage is a traditional culinary herb that has been found to have analgesic and anti-inflammatory activity [Howes et al., 2003, Wang et al., 2001]. One study investigated an SO hydro-alcoholic extract on vincristine-induced peripheral neuropathy in mice compared to morphine [Abad et al., 2011b]. Sixty mice were divided into six groups similar to the chamomile study [Abad

et al., 2011a]. Results found that SO could be effective as a treatment for vincristine-induced peripheral neuropathy [Abad et al., 2011b].

6. *Sweet bee venom (pharmacopuncture)*

Sweet bee venom treatment is a part of a normal oriental medical practice in Korea for the treatment of various pain and neurological symptoms [Yoon et al., 2012]. Pharmacopuncture is a treatment by which pharmaceuticals and acupuncture are joined by injecting pharmaceutical derivatives into acupuncture points chosen for each patient's diagnosis and symptoms based on TCM [Yoon et al., 2012].

A recent article looked at bee venom acupuncture for cold allodynia induced from oxaliplatin in rat [Lim et al., 2013]. It found that it was effective in alleviating the oxaliplatin-induced cold allodynia in rats which was partly due to the activation of the noradrenergic system.

Herbal Medicine Extracts in Animal Studies and CIPN

1. *Verticinone (Extract from Bulbus Fritillaria*)

Verticinone is an isosteroidal alkaloid, which has been extracted from the *Bulbus* Fritillaria [Xu F, et al., 2011]. It has been used in China for more than 2000 years as an antitussive, expectorant and anti-asthmatic [Lee et al., 2006]. Recently, verticinone has demonstrated in pharmacological studies more diverse bioactivities such as inhibiting angiotensin I converting enzyme [Li et al., 2006], antagonizing M-receptor (the muscarinic acetylcholine receptor) activity and significantly elevating cAMP concentrations in HEK cells [Oh et al., 2003].

This study aimed to investigate verticinone's effect on paclitaxel induced neuropathic pain compared to morphine. The authors concluded that verticinone seemed to exert good analgesic effects for the inflammatory pain and the CIPN pain. The hypothesised this could be through both the central and peripheral nervous system and that further studies are warranted [Xu F et al., 2011].

Herbal Medicines with Receptor Agonist Activity in Animal Studies and CIPN

1. *Cannabis (Cannabis sativa L.)*

Cannabinoids have been tested for nerve suppression and two studies have examined the cannabinoids suppression potential for CIPN from vincristine [Rhan et al., 2007] and paclitaxel [Rahn et al., 2008]

in rats. The two main cannabinoids tested include 1) CB1/CB2 receptor agonist WIN55,212-2 and; 2) receptor-inactive enantiomer WIN55,212-3, a CB2-selective agonist (R,S)-AM1241 [Rahn et al., 2007].

For the vincristine experiment, WIN55,212-2 was administered intrathecally (in the spine) or locally into the hind paw of rats. Results found that WIN55,212-2 not WIN55,212-3 suppressed vincristine evoked mechanical allodynia. The authors concluded that cannabinoids can suppress the maintenance of vincristine-induced mechanical allodynia through the activation of CB1 and CB2 receptors and this was mediated partly at the spinal cord level [Rahn et al., 2007]. For the paclitaxel experiment, they concluded that cannabinoid CB(2) receptors maybe a potential treatment for paclitaxel CIPN [Rahn et al., 2008].

Combination Herbal Medicines in Animal Studies and CIPN

1. *Geramii herba plus Aconiti radix*

The combination of *Geramii herba* and *Aconiti radix* herbs was used as an external application in a rat model of oxaliplatin CIPN. The results showed that mechanical allodynia and thermal hyperalgesia were alleviated, nerve growth factor (NGF) was increased and substance P was decreased in the treated rat group compared to oxaliplatin alone [Sima et al., 2009].

2. *Goshajinkigan (Japan), Niu Che Sen Qi Wan (Chinese), Pilula renales plantaginis et achyranthis (Lat.)*

Nine trials on both animals and humans have been conducted on this formula [Bahar et al., 2013; Hashimoto et al., 2004, 2006; Kaku et al., 2012; Kono et al., 2011, 2013; Nishioka et al., 2011; Shindo et al., 2008; Ushio et al., 2012; Yamamoto et al., 2009]. This formula contains ten herbs: *Rehmannia viride radix, Achyranthis bidentatae radix, Corni fructus, Dioscorea opposite rhizome, Plantaginis semen, Alismatis rhizome, Moutan cortex, Cinnamomi cortex, Aconiti lateralis praeparata tuber,* and *Poria alba* [Japan Society of Oriental Medicine, 2005]. A rat study tested this formula (GJG) on paclitaxel-IPN in three different studies.

The first trial found no neuroregeneration in histological examination [Hashimoto et al., 2004] however the second trial found that it showed a positive effect on cold allodynia [Hashimoto et al., 2006]. A recent trial on mice found that GJG was effective for mechanical allodynia from paclitaxel but not for tumour allodynia [Akbar Bahar et al., 2013]. Another rat study tested GJG on oxaliplatin-IPN and found that it prevented cold hyperalgesia but not mechanical allodynia or axonal degeneration in rat sciatic nerves. However, after CIPN had developed, a single administration of GJG reduced both cold hyperalgesia and mechanical allodynia [Ushio et al., 2012].

3. *Shakuyakukanzoto (Japanese), Shao Yao Gan Cao Tang (Chinese), Formula glycyrrhizae et paeonia (Lat), Peony and Licorice Decoction (English)*

This herbal formula is a combination of Peony and Licorice in a decoction. Three studies have been conducted on this herbal combination, one mouse study [Hidaka et al., 2009], a retrospective case analysis [Fujii et al., 2004] and a retrospective clinical trial comparison [Hosokawa et al., 2012]. The mouse study invested paclitaxel-IPN and found that this combination significantly relieved the allodynia and hyperalgesia induced by paclitaxel [Hidaka et al., 2009].

Human Studies on CIPN and Herbal Medicine *(see Table 2)*

Single Herbal Medicines on Human Studies and CIPN

1. *Ginkgo biloba (EGb 761)*

One retrospective study has been conducted on human beings administered oxaliplatin [Marshall et al. 2004]. The retrospective analysis was conducted on 17 colorectal patients who were being treated with either FOLFOX or CAPEOX chemotherapy regimes for either adjuvant or metastatic treatment [Marshall et al., 2004]. GB 120mg b.i.d was given orally either after cycle 1 or 2 of treatment. They concluded that GB appeared to decrease the intensity and duration of the acute dysethesias caused by oxaliplatin and have initiated a phase II RCT to confirm results. To date, no results have been published from this phase II trial which apparently started in 2004. [Marshall et al., 2004]

2. *Sweet bee venom (pharmacopuncture)*

Two journal articles outlining case studies using sweet bee venom for CIPN have been found with the agent used being identified as melittin, an extracted active ingredient from the bee venom that has the allergens removed such as phospholipase A_2 (PLA_2), hyaluronidase and histamine. Melittin is a low molecular weight peptide and is reported to have analgesic, anti-inflammatory and anti-cancer effects [Choi et al., 2006; Kwon et al., 2006; Wang et al., 2001].

The first article outlined 5 case reports receiving a one week course of treatment with sweet bee venom pharmacopuncture. No side effects were experienced and results indicated clinical improvement of CIPN [Park et al., 2011]. Another case series on 11 patients were treated for three weeks with six sweet bee venom pharmacopuncture treatments. Results showed a reduction in the WHO CIPN grade and PNQ score indicating a reduction in CIPN [Yoon et al., 2012]. Further studies are required to confirm their findings.

Combination Herbal Studies on Human Studies and CIPN

1. *Bu Yang Huan Wu (Chinese)*

This formula consists of *Astragulus membranaceus radix, Angelica sinesis radix, Prunus persicae semen, Paeoniae rubra radix, Ligustici chuanxiong rhizome, Lumbricus terrestris, Spatholobi caulis, Curcuma radix, Chaenomeles lagenaria fructus* and *Achyranthes bidentatae radix.* In traditional Chinese medicine (TCM) it is said to tonify the yang and restore the five-tenths decoction [Zhou et al., 2006]. This decoction was used in an RCT of 84 participants (intervention n=44, control n=40)

for the treatment of CIPN after oxaliplatin administration. Results indicated that Bu Yang Huan Wu reduced the development of CIPN in the treatment group tested by standardized clinical tests [Sun et al., 2008].

2. *Modified Bu yang Huan Wu (Chinese)*

The modified formula used was *Bu Yang Huan Wu* plus *Liuwei Di Huang* which contains the herbs: *Astragulus membranaceus radix, Ligustrum lucidum fructus, Paeoniae rubra radix, Lumbricus terrestris, Prunus persicae semen, Rehmanniae viride radix, Corni officinalis fructus, Dioscorea opposite radix, Alismatis rhizome, Poria alba, Spatholobi caulis, Scolopendra, Mori fructus, Glycyrrhizae radix, Dipsaci fructus, Lycii fructus, Coicis semen, Atractylodis Rhizome, Phellodendri cortex, Scorpio, Moi ramulus* and *Cyathula officinalis.* A RCT was conducted using this decoction on 32 patients with existing CIPN from various chemotherapy agents. The treatment was compared to 32 patients who were treated daily with 2500μg of vitamin B1 orally in addition to intramuscular injections of 100mg of vitamin B1. The Chinese herbal formula was found to be significantly more effective compared to the vitamin B1 treatment ($P<0.05$) [Deng et al., 2007].

3. *Modified Chai Hu Long Gu Mu Li Wan (Chinese)*

This modified Chinese oral combination of herbs consists of *Psuedostellaria heterophylla,Pinelliae rhizome, Glycyrrhizae radix, Scutellaria baicalensis radix, Bupleuri radix, Fossiliaossis mastoid, Ostreae concha, Rubia cordifolia radix, Scutellariae barbatae herba* and *Fritillariae thunbergia bulbi.* This combination was used in a RCT of 48 patients with ovarian cancer undergoing paclitaxel administration. They were divided into a control group of paclitaxel only or a treatment group of paclitaxel plus a combination of the oral Chinese herbal decoction and an external washing of the feet with Chinese herbs (*Astragulus membranaceus radix, Angelica sinensis radix, Paeoniae radix, lumbricus terrestris, Ligustici chuanxiong Rhizome, Prunus persicae semen* and *Carthami flos*.) Results indicated that the incidence rate of CIPN in the treatment group was nearly half compared to the paclitaxel only group. Therefore this Chinese formula may help in preventing paclitaxel CIPN [Pan et al., 2012].

4. *Geramii herba plus Aconiti radix*

A RCT was conducted on 58 patients experiencing CIPN from oxalipatin, taxol or capecitabine. 30 patients were assigned to the treatment group and 28 to the control group. After one week of treatment, the external application of the two herbs was found to reduce pain, paraesthesia and swelling. The authors concluded that *Geramii herba* plus *Aconiti radix* may relieve neuropathy and

improve quality of life. No species of these two herbs were mentioned in the abstract [Sima et al., 2009].

5. *Goshajinkigan (Japan), Niu Che Sen Qi Wan (Chinese), Pilula renales plantaginis et achyranthis (Lat.)*

Six human trials have been conducted on Goshajinkigan [GJG].. Firstly, GJG was invested in a non-controlled trail on 14 patients receiving oxaliplatin. GJG was administered every day after the first oxaliplatin infusion and results indicated that it seemed to prevent acute oxaliplatin-IPN [Shindo et al., 2008]. Two retrospective studies were conducted on FOLFOX (oxaliplatin) and GJG [Kono et al., 2011; Nishioka et al., 2011]. The first trial was conducted on 45 patients with 22 patients receiving GJG with their FOLFOX regime compared to 23 who did not receive GJG. They found that the incidence of grade 3 CIPN in the GJG group was significantly lower than in the control group ($P<0.01$, log-rank test) [Nishioka et al., 2011].

The second study investigated 90 patients undergoing FOLFOX for metastatic colorectal cancer. There were four groups: 1) FOLFOX plus GJG; 2) FOLFOX plus GJG plus Ca^{2+}/Mg^{2+}; 3) FOLFOX plus Ca^{2+}/Mg^{2+}; 4) FOLFOX only. Results included the incidence rate for each group which were 91% for FOLFOX only, 100% in FOLFOX plus Ca^{2+}/Mg^{2+}, 79% in FOLFOX plus GJG plus Ca^{2+}/Mg^{2+} and 50% in FOLFOX plus GJG. The authors concluded that GJG reduced the neurotoxicity of oxaliplatin without affecting the response rate [Kono et al., 2011].

In another retrospective trial, GJG was investigated for paclitaxel-IPN in 82 breast and gynaecological cancer patients. The investigators concluded that GJG was possibly effective for the treatment and the prevention of paclitaxel-IPN was seemed more effective if administered at the beginning of chemotherapy treatment [Yamamoto et al., 2009]. Another human trial on GJG involved a prospective RCT on paclitaxel/carboplatin treatment for 29 ovarian or endometrial cancer patients. They were divided into two groups; 1) 14 patients received vitamin B12; 2) 15 patients received vitamin B12 and GJG and were given treatment for six weeks. Grade 3 CIPN was observed in 2/14 patients receiving vitamin B12 compared to 0/15 receiving vitamin B12/GJG. It was concluded that GJG inhibits the progression of CIPN but further trials are warranted [Kaku et al., 2012].

A recent phase II multi-centre, randomised, double-blind, placebo-controlled trial was conducted and published on GJG and oxaliplatin-induced PN [Kono et al., 2013]. In this trial, patients undergoing FOLFOX for colorectal cancer was randomised to either receive oral GJG (7.5g) or matching placebo daily. The severity of CIPN was assessed using the common toxicity criteria for adverse events every two weeks until the 8th cycle, and then every 4 weeks thereafter. The primary endpoint was the incidence of grade 2 CIPN or greater before the 8th cycle. The incidence of grade 2 or greater CIPN was 39% in the GJG arm and 51% in the placebo arm. The authors concluded that GJG shows promise in delaying the onset of grade 2 or greater CIPN without impairing FOLFOX efficacy [Kono et al., 2013].

6. *Keishikajutsubuto (Jananese), Gui Zhi Jia Shu Fu Tang (Chinese), Decoctum ramulorum cassia cum atractylodis macrocephae et aconite (Lat)*

This oriental formula contains *Cinamomi cortex, Aconiti lateralis praeparata tuber, Zingiberis rhizome, Jujubae fructus, Glycyrrhizae radix* and *Atracylodis macrocephalae rhizome.* A non-controlled trial was conducted investigating this herbal formula on 11 patients with metastatic colorectal cancer undergoing FOLFOX administration. A reduction of CIPN was observed in 5 cases (45.5%) after cessation of chemotherapy [Yamada et al., 2012].

7. *Ogikeishigomotsuto (Japanese), Huang Qi Wu Wu Tang (Chinese), Decotum quinque medicamentorum cum astragalo (Lat.), Astragulus and Cinnamon Five herb combination (English)*

This oriental herbal combination contains *Astragalus membranaceus radix, cinnamomi cortex, Paeonia alba radix, Jujubae fructus* and *Zingiberis 12-371hizome*. A single case study was published using this formula for neuropathic pain induced by oxaliplatin. It showed a positive effect in reducing the pain and the patient was allowed to continue chemotherapy treatment with oxaliplatin [Tatsumi et al., 2009].

8. *Shakuyakukanzoto (Japanese), Shao Yao Gan Cao Tang (Chinese), Formula glycyrrhizae et paeonia (Lat), Peony and Licorice Decoction (English)*

Two studies human studies have been conducted on this formula, a retrospective case analysis [Fujii et al., 2004] and a retrospective clinical trial comparison [Hosokawa et al., 2012].

The retrospective case analysis investigated 23 patients with paclitaxel-IPN and observed that this combination had a positive effect on the neuropathic pain experienced by these ovarian cancer patients [Fujii et al. 2004]. Lastly the retrospective clinical trial compared Shakuyakukanzoto (peony and licorice) to Goshajinkigan (GJG). This was a preventive study and investigated 20 metastatic colorectal cancer patients administered Shakuyakukanzoto in conjunction with FOLFOX compared to 24 patients administered GJG. The Shakuyakukanzoto group was found to prevent 65% CIPN compared to 50% in the GJG group. It was concluded that both formulas may prevent oxaliplatin-IPN [Hosokawa et al., 2012].

Discussion

Current improvements in detection and treatment strongly correlate with increases survival rates of those patients diagnosed with cancer [Coleman et al., 2011]. With an increased survival rate, long-term side effects from chemotherapy and other medical treatments has raised significant awareness as it can affect quality of life and clinical outcomes [Bhagra et al., 2007; Cavaletti et al., 2008; Takenaka et al., 2012]. CIPN is an important side effect that can affect quality of life and can be a permanent consequence of treatment [Bhagra et al., 2007; Caveletti et al., 2004, 2008]. Currently, there are no standard recommended treatments or prophylactic options that employ pharmacological, nutraceutical or herbal medicines. Agents that have shown promise such as duloxetine [Takenaka et al., 2012; Smith et al., 2013], vitamin E [Argyriou et al., 2005, 2011; Pace et al., 2003, 2010], omega 3 fatty acids [Ghoreishi et al., 2012], and Asian herbal medicines in particular Goshajinkigan [Bahar et al., 2013; Hashimoto et al., 2004, 2006; Kaku et al., 2012; Kono et al., 2011, 2013; Nishioka et al., 2011; Shindo et al., 2008; Ushio et al., 2012; Yamamoto et al., 2009] require further research, however, in order to assess the strength of efficacy.

Traditional scientific research is based on a single agent or active compound; herbal medicines though can be comprised of numerous active compounds with synergistic efficacy. For example in the use of Asian herbal medicines, a combination of herbs can be employed [Kono et al., 2013]. This combination may give a multi-targeted approach that complicates the identification and elucidation of the active compound and the mechanism of action. Nevertheless warranted research on single herbal extracts and compounds through validated clinical studies can still provide useful data.

Investigations carried out with herbal medicines for CIPN found no neuroprotection or treatment when a single compound or herb was studied. Animal models provide basic research for further studies but do not guarantee that the herb or compound will be efficacious in human clinical trials.

Herbal medicines such as *Ginkgo biloba* [Marshall et al., 2004] and *curcumin* [Al Moundhri et al., 2013] warrant further research as they have reported a positive clinical likelihood from animal studies.

An important and common mechanism of action within the identified medicinal herbs trialled for CIPN is their anti-inflammatory activity [Al Moundhri MS et al., 2013; Berry, 1995; Choi et al., 2006; Ghoreishi et al., 2012; Howes et al., 2003; Kwon et al., 2006; Lee et al., 2006; Mills, 1991; Muthuraman et al., 2011; Wang et al., 2001]. The anti-inflammatory activity of the herbs may be a plausible mechanism of action that assists with the protection and treatment of CIPN. Medical treatment of peripheral neuropathy has involved non-steroidal anti-inflammatory drugs for the inflammation and pain associated with this condition [Kaley and Deangelis, 2009]. This potential mechanism of action warrants further research which may attribute to other herbal remedies, nutrients or pharmaceuticals being trialled for CIPN.

The Asian herbal combination remedies investigated for CIPN were difficult to analysis as a number of the journal articles were written in their Asian language rather than English. Therefore the information obtained for twelve out of the nineteen herbal combinations journal articles was extracted from abstracts. From these Asian herbal combinations, Goshajinkigan (GJG) is the herbal medicine that has been trialled extensively through animal and human clinical trials [Bahar et al., 2013; Hashimoto et al., 2004, 2006; Kaku et al., 2012; Kono et al., 2011, 2013; Nishioka et al., 2011; Shindo et al., 2008; Ushio et al., 2012; Yamamoto et al., 2009]. Of the clinical trials, three were retrospective studies using controls for Folfox and paclitaxel administration [Kono et al., 2011; Nishioka et al., 2011; Yamamoto et al., 2009], one RCT [Kaku et al., 2013] comparing vitamin B12 administration with the herbal combination and a recent phase II multi-centre, randomised, double-blind, placebo-controlled trial conducted as an adjuvant treatment with FOLFOX [Kono et al., 2013]. All studies concluded that this herbal combination may provide neuroprotection however, as this is a Japanese combination and has been trialled in Japan, it may be difficult to transfer into other countries depending on their National regulatory body. Another limitation with GJG is that not all details of its mechanism of action have been clearly identified and for certain herbs, their effects are unknown [Schroder et al., 2013].

All other trials with the Asian combination herbs were animal models, case studies, retrospective studies and limited RCT's so the quality of evidence is very low. Without further randomised clinical trials that are written in English and well established, they are not recommended for use.

Two herbal treatments have been investigated for peripheral neuropathy that may be considered for use in CIPN. These include St John's wort and capsaicin cream. St John's Wort (*Hypericum perforaturn)* was selected to trial for PN as tricyclic antidepressant medication is used medically for its treatment. In a crossover DBRCT, 54 patients with or without diabetes were investigated for treatment with St John's Wort (SJW) for diabetic PN. Participants were randomly assigned to either SJW (900mcg hypericin/tablet) or placebo for five weeks. They were then crossed over to the other treatment for an additional five weeks. No statistical significant was found in pain indexes however a trend was noted for an improved overall pain score [Sindrup et al., 2001].

Capsaicin has been investigated as a topical application for diabetic [Biesbroeck et al., 1995; Chad et al., 1990; Low, et al., 1995; No authors listed, 1991; Tandan et al., 1992] and HIV peripheral neuropathy [Brown et al., 2013; Clifford et al., 2012; Simpson et al., 2103]. The majority of studies have been conducted on diabetes PN with most resulting in statistical significance [Biesbroeck et al., 1995; Chad et al., 1990; Low, et al., 1995; No authors listed, 1991; Tandan et al., 1992]. The results indicate that capsaicin cream may provide relief for chronic, intractable pain and reduce dependence on opioids however the main side effect of burning at the site of application may be of concern [No authors listed, 1991]. It is recommended that it is not used as a monotherapy but in combination with oral medication to assist this condition.

Clinical Relevance

We have previously reported in a systematic review, that there was limited evidence for the administration of nutraceuticals in the treatment or prevention of CIPN [Schloss, et al., 2013]. Herewith we again report that the current scientific literature demonstrates that there is limited evidence for the concurrent administration of herbal remedies as adjuvants to neurotoxic agents for the prevention or treatment of CIPN. The clinical studies conducted with single or combination herbal medicines identified in this review do not provide a clear recommendation for clinical use. *Goshajinkigan* has shown the most promise in preventing severe CIPN but maybe limited in use depending on national governing body guidelines. Other herbs that may be considered after further research include *Ginkgo biloba*, curcumin and capsaicin cream.

Conclusions

Investigations into the research on herbal medicine and CIPN have not yet yielded clinical evidence to support the standard use of herbal medicine as a prevention or treatment of CIPN. However, data does suggest that herbal medicine may provide potential benefits with further research required. More

extensive scientific research into the mechanism of action of not only the herbal extracts but the neurotoxic activity of the chemotherapeutic agents may provide a key in identifying remedies that may help prevent or treat this side effect.

Tables and Figures:

1. **Figure 1:** Flow chart of study selection
2. **Table 1:** Animal Studies with Herbal Medicines for the Prevention and or Treatment of CIPN
3. **Table 2:** Human Clinical Studies with Herbal Medicines for the Treatment and or Prevention of CIPN

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Table 1: Animal Studies with Herbal Medicines for the Prevention and or Treatment of CIPN

Medicinal Herb	Study Type	Chemotherapy Agent	Results
Single Medicinal Herbs			
Acorus calamus rhizome	Rat model (Muthuraman et al., 2011)	Vincristine	Improvement in neuropathic pain
Curcumin	Mouse model (Al Moundhri et al., 2013)	Oxaliplatin and cisplatin	Possible neuroprotection
Gingko biloba	Rat model (Cakil et al., 2012)	Cisplatin	Protection of ototoxicity
	Rat model (Huang et al., 2007)	Cisplatin	Protection of ototoxicity
	Mice model (Ozturk et al., 2004)	Cisplatin	Some functional and morphological protection against CIPN
	Guinea pigs (Xu O, et al., 2004)	Cisplatin	Protection of ototoxicity
Matricaria chamomilla (chamomile)	Mouse model (Abad et al., 2011)	Cisplatin	Improvement of neuropathic pain
Salvia officinalis	Mouse model (Abad et al., 2011)	Vincristine	Improvement in neuropathic pain
Sweet Bee Venom	Rat model (Lim et al., 2013)	Oxaliplatin	Alleviated cold related pain
Extract			
Verticinone (Extract from *Fritillaria bulbus*)	Mouse and rat model (Xu F et al., 2011)	Paclitaxel	Decreased inflammation and neuropathic pain
Receptor agonist			

Cannabis-Receptor agonists	Rat model (Rahn et al., 2008)	Paclitaxel	Reduced pain and sensitivity to stimuli
	Rat model (Rahn et al., 2007)	Vincristine	Reduced pain and sensitivity to stimuli
Combination Herbal Studies			
Geramii herba plus *Aconiti radix*	Rat model (Sima et al., 2009)	Oxaliplatin, taxol or capecitabine	Reduced neuropathic pain and paraesthesia
Goshajinkigan (Japan)	Mice model (Bahar et al., 2013)	Paclitaxel	Prevents paclitaxel-IPN without interfering with the anti-cancer action of paclitaxel.
	Rat model (Hashimoto et al., 2004)	Paclitaxel	Improvement in neuropathic pain
	Rat model (Hasmimoto et al., 2006)	Paclitaxel	Improves paclitaxel-IPN without affecting anti-tumour efficacy
	Rat/mouse model (Ushio et al., 2012)	Oxaliplatin	Improvement in neuropathic pain, no neuroregeneration
Shakuyakukanzoto (Japanese)	Mouse model (Hidaka et al., 2009)	Paclitaxel	Reduced neuropathic pain and hyperalgesia

DBRCT: Double Blind Randomized Controlled Trial; SBRCT: Single Blinded Randomized Controlled Trial; RCT: Randomized Controlled Trial; NRCT: Non Randomised Clinical Trial; CS: Case Study; TNS: Total Neuropathy Score

Table 2: Human Clinical Studies with Herbal Medicines for the Treatment and or Prevention of CIPN

Medicinal Herb	Study Type	No of Pts[26]	T[27]	C[28]	NHMRC Rating	Chemotherapy Agent	Results
Single Medicinal Herbs							
Ginkgo biloba	RS (Marshall et al., 2004)	17	N/A[29]	N/A	Level IIIb	Oxaliplatin	Possible neuroprotection
Sweet bee venom (pharmacopuncture)	Case series (Park et al., 2011)	5	N/A	N/A	Level IV	Taxol, carbo/taxol[30]	Injecting into the acupoint decreased pain and neuropathy (treatment)
	Case series (Yoon et al., 2012)	11	N/A	N/A	Level IV	Taxol, carbo/taxol	Injecting into the acupoint decreased pain and neuropathy (treatment)
Combination Herbal Studies							
Bu Yang Huan Wu (Chinese)	RCT (Sun et al., 2008)	84	44	40	Level II	Oxaliplatin	Reduced development of CIPN
Modified Bu yang *Huan Wu* (Chinese)	RCT (Deng, et al, 2007)	64	32	32	Level IIIa	Different chemotherapies	Reduced development of CIPN

[26] Pts: Participants
[27] T: Treatment group
C: Control group or placebo
[29] N/A: Not applicable
[30] Carbo/Taxol: Carboplatin and Paclitaxol chemotherapy combination

Modified *Chai Hu Long Gu Mu Li Wan* (Chinese)	RCT (Pan et al., 2012)	48	N/A	N/A	Level II	Paclitaxel	Possible neuroprotection
Geramii herba plus *Aconiti radix*	RCT prospective (Sima et al., 2009)	58	30	28	Level II	Oxaliplatin	Reduced neuropathic pain
Goshajinkigan (Japan)	NRCT (Shindo et al., 2008)	14	N/A	N/A	Level IIIb	Oxaliplatin	Reduced acute neurotoxicity
	RS (Nishioka et al., 2011)	45	22	23	Level IIIa	Folfox	Possible neuroprotection
	RS (Kono et al., 2011)	90	11 Plus CaMg [31] = 21	44 CaMg = 14	Level IIIa	Folfox	Possible neuroprotection, no change of anticancer activity
	RS (Yamamoto et al., 2009)	82	N/A	N/A	Level IIIb	Paclitaxel	Possible neuroprotection, better when administered early
	RCT prospective study (Kaku et al., 2012)	29	Vit B12 + I = 15	Vit B12 = 14	Level IIIa	Carbo/Taxol	Less severe neurotoxicity, better in combined group
	DBRCT (Kono et al., 2013)	89	44	45	Level II	Folfox	Acceptable safety margin and indicates a delay in onset of grade 2 or higher CIPN without impairing FOLFOX efficacy.
Keishikajutsubuto (Jananese)	Uncontrolled study (Yamada et al., 2012)	11	N/A	N/A	Level IIIb	Folfox	76.6% mean improvement

Ogikeishigomotsuto (Japanese)	Case report (Tatsumi et al., 2009)	1	N/A	N/A	Level IV	Oxaliplatin	Reduced neuropathic pain
Shakuyakukanzoto (Japanese) Peony and Licorice Decoction (English)	Retrospective case analysis (Fujii et al., 2004)	23	N/A	N/A	Level IV	Paclitaxel	Reduced neuropathic pain
	RS comparison (Hosokawa et al., 2012)	44	20 (SKK)	24 (GJG)	Level IIIa	Folfox	50% response in Shakuyu-kanzoto and 65% in Goshajinkigan on prevention of neurotoxicity.

DBRCT: Double Blind Randomized Controlled Trial; SBRCT: Single Blinded Randomized Controlled Trial; RCT: Randomized Controlled Trial; NRCT: Non Randomised Clinical Trial; CS: Case Study; TNS: Total Neuropathy Score; RS: Retrospective Study

[31] CaMg: Calcium and magnesium infusion

12.4 CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY (CIPN) AND VITAMIN B12 DEFICIENCY: PUBLISHED

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Chemotherapy-Induced Peripheral Neuropathy (CIPN) and Vitamin B12 Deficiency.

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Dear Editor,

We are submitting this interesting case report as a letter to the editor to identify an important issue in supportive cancer care.

Abstract

Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) is a common significant and debilitating side effect resulting from the administration of neurotoxic chemotherapeutic agents. Moderate to severe CIPN can significantly decrease the quality of life and physical abilities of cancer patients. Nervous system dysfunction can include sensory, sensorimotor and autonomic nervous system damage and a third of all patients who experience this side effect have permanent consequences. Currently, early termination of treatment or dose reduction of the chemotherapy agent is the standard treatment.

Case Report: A clinical report on a cancer patient who underwent chemotherapy administration and was diagnosed with severe grade 3 CIPN is presented. The patient was a participant in a clinical trial testing B vitamins for CIPN.

Results: The patient was found to be vitamin B12 deficient after chemotherapy administration. Within months of oral vitamin B administration and a vitamin B12 intramuscular (IM) injection, the patient's CIPN was significantly reduced to grade 1.

Conclusion: Cancer patients who experience severe CIPN may have their chemotherapy dose reduced or terminated due to this side effect. Treatment may involve anti-depressants, gabapentin or pregabalin for CIPN pain. Differential diagnosis needs to include vitamin B12 testing involving holotranscobalamin (active B12) as an early marker of a vitamin B12 deficiency. Treatment with vitamin B12 in CIPN patients found to be low or deficient in vitamin B12 may reduce the severity of CIPN experienced and allow the continuation of chemotherapy treatment if required which can benefit patient outcomes. Quality of life may also improve in addition to physical abilities.

Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) continues to be a major concern for oncological practise considering the increasing number of cancer survivors and the lack of standardised prevention or treatment [1]. The incidence of CIPN depends on the chemotherapy agent administered but is estimated to occur in one third of all patients undergoing chemotherapy [2,3]. The prevalence of CIPN has been estimated to be 68.1% the first month after the administration of

neurotoxic chemotherapy agents and 60% three months post chemotherapy treatment. Patients were found to still have 30% prevalence six months or more after chemotherapy according to the results published in a systematic review conducted in 2014 [4]. Patients experiencing moderate to severe CIPN report a reduced quality of life [5], chronic discomfort [6], and disruption of physical abilities for general life activities which can be temporary or permanent [5].

Currently in clinical practise, CIPN is assessed using the common toxicity scales however, these rely heavily on the patient's subjective reports rather than quantitative testing [7]. CIPN is a potentially rescindable side effect although reversibility may be dependent on early detection or identification and modification of chemotherapy treatment [7]. Permanent CIPN has still been reported, especially sensory symptoms in the lower extremities among patients treated with oxaliplatin up to 11 years after treatment [8]. Early differential diagnosis and prevention of permanent CIPN needs to be a priority for extending the quality of life of cancer patients.

Patients with a previous history of a vitamin B12 deficiency has been identified as a predisposing condition that may increase the risk of developing CIPN [5]. However, patients who have had no previous history of a vitamin B12 deficiency may not be tested before chemotherapy commences for vitamin B12 status. Moreover, a potential vitamin B12 deficiency may develop during chemotherapy administration [9] that can therefore potentially predispose the patient to developing and/or delaying the development of CIPN.

We present a clinical case of a cancer patient who developed CIPN and was found to be vitamin B12 deficient after completing a chemotherapy regimen. Upon vitamin B12 administration, the severity of CIPN decreased that allowed the patient increased functional ability in daily activities including the ability to walk.

Case Study

A 53 year old female was enrolled in a randomised clinical trial and was randomised to the placebo arm. Initial B group vitamin status pathology bloods, a neurological exam involving the Total Neurology Score (TNS) and electro-neurological examination in addition to other history and questionnaires was conducted prior to the commencement of chemotherapy. The same independent neurologist following chemotherapy administration conducted the neurological examination and TNS. Blood vitamin B group pathology and questionnaires were assessed at post chemotherapy and at a three-month follow up visit. This patient's case report details and results are documented in Table 1.

The patient was overweight and generally healthy. The patient had been diagnosed with osteoarthritis with increased difficulty in walking, adding to this patient's weight issue. The patient worked in an office and had a generally sedentary lifestyle.

Blood Pathology Results

The blood pathology tests for this patient before chemotherapy was in the reference range, 107 pmol/L (ref range >35 pmol/L) presented in Table 2. After chemotherapy administration (Table 3 documents the chemotherapy regime) the vitamin B12 status decreased and was deemed as deficient as presented in Table 2 and Figure 1. After supplementation with a B group vitamin complex (equivalent to 1,000 µg/day of vitamin B12) and receiving one intramuscular vitamin B12 injection (dose 1,000 µg), the blood vitamin B12 level reverted back to baseline (106 pmol/L). Other blood pathology conducted for B vitamin status including vitamin B1, B2, B6 and folate were found to be equivalent to baseline blood levels. The exception was vitamin B6, which had increased after supplementation with the oral B vitamin complex (95 nmol/L at baseline to 250 nmol/L reference range 35-110 nmol/l). Therefore as vitamin B12 was the only B vitamin found deficient after chemotherapy administration, changes in the patient's neuropathy symptoms were correlated by administration of vitamin B12.

Results of the Neurological Testing

Total Neuropathy Score (TNS) used in the Clinical Trial

The neurological test results presented in Table 4 showed that the patient's peripheral sensory and motor nerve function had decreased after chemotherapy administration compared to baseline. The neurologist's examination of the patient found no numbness, tingling or pain in the hands or feet at baseline. There was however noted interference to the electrical testing for the patient at baseline that occurred due to the application of copious amounts of a moisturiser just prior to testing. This interference with the electronic testing showed a mild impairment in motor function and interference with the baseline assessment of the sural nerve. The neurologist noted through assessment that despite the application of the moisturiser and impairment to ambulatory walking due to osteoarthritis, the motor nerves examined for the patient's hands and feet were all functioning within the normal reference ranges.

Following chemotherapy administration, the neurologist's examination and electronic testing of sensory and motor peripheral nerves reported a notable reduction in function. The patient was identified as exhibiting Level 2 CIPN that confirmed numbness, tingling and neuropathic pain up to the ankle and the wrist of her lower and upper extremities respectively. The pin sensibility is the test from the TNS that examines small nerve damage, and the patient's score after chemotherapy

administration indicated that nerve damage was evident proximal to the wrist and ankle. Furthermore there was loss of tendon reflexes a hallmark representative of nerve damage. The sural nerve was not detectable by electronic testing at baseline or after chemotherapy administration.

Following the administration of an intramuscular injection of vitamin B12 (dose 1,000 µg) with a concomitant oral administration of a vitamin B group complex (equivalent to 1,000 µg per day of vitamin B12), sensory nerve function was restored to the fingers and toes (level 1 CIPN) after 60 days. Motor function remained unchanged and pin sensibility still indicated nerve damage in the wrists and ankles. The sural nerve was positively detected. The patient's menopause triggered severe hot flushes complicated the clinical neurological picture; this being indicative of autonomic nerve function involvement.

Neurological Conduction Studies (NCS)

The neurologist conducted extra nerve conduction tests in addition to the tests required for the TNS. These electrical tests assessed large nerve activity. The nerve conduction study (NCS) normal values used were ascertained from the University of Michigan Medical School [10]. The NCS conducted on this patient indicated that nerve damage occurred after chemotherapy administration, of which the data is presented in Table 5. The main areas affected post chemotherapy administration were the hands (palms), wrists, sural nerve and in part the peroneal nerve. The sural nerve at baseline as discussed was unable to be found due to a large amount of moisturiser applied by the patient prior to testing. This made it difficult to ascertain if damage had occurred or if the sural activity was low before chemotherapy administration. Considering the fact that the activity of the sural nerve had improved by the last neurological test, it can be postulated that there may have been some activity prior to chemotherapy.

The main nerves affected as demonstrated by the NCS tests were sensory nerves. The amplitude of the peroneal nerve was the only motor nerve identified as demonstrating decreased nerve function after chemotherapy administration. This is congruent with the symptoms displayed by the patient.

Results of Patient Neurotoxicity Questionnaire

Throughout the clinical trial, patients were asked to complete a patient neurotoxicity questionnaire (PNQ). This is a linear scale and indicates the level of CIPN that the patient has experienced in the past week. The results of this patient's PNQ were 0 out of 4 at baseline for any numbness, pain, burning or tingling as well as difficulties with daily activities. At the three-month follow-up visit, the patient registered 3 out of 4 for numbness, pain, burning or tingling in the arms and legs. Weakness in the arms and legs was 1 out of 4 at baseline and 3 out of 4 at the three-month follow-up visit.

Two months after the intervention with an IM injection of B12 and B vitamin supplement the numbness, pain, burning, tingling and weakness of the arms and legs was ranked 1 out of 4 by the patient. The inference with daily activities was ranked 0 out of 4.

The results documented post chemotherapy administration (25th August 2012) indicated that the patient had difficulties with buttoning clothes, zippers, typing on a keyboard, writing, walking, putting on jewellery, knitting, sewing, working and dialling or using a telephone. At the six month follow up (17th November 2012) which is three months post administration of B12 and B group vitamins, all of the identified daily issues were resolved and no further difficulties were reported aside from some shortness of breath.

On the 2nd October, 2012 the patient presented at the Princess Alexandra hospital breast cancer clinic following receipt of blood results. The patient's Holo TC (vitamin B12) result was reported as 29 (ref >35 results reported on 25/9/2012) indicating a deficiency in blood vitamin B12. The patient's oncologist was informed and requested administration of vitamin B12 intramuscularly (dose 1,000 µg). The patient was also provided with 3 bottles of a vitamin B complex from the manufactures that supplied the active supplement for the clinical trial that the patient had been enrolled in. The patient was advised to take 1 capsule twice daily with food, morning and night. At the oncology appointment on the 2nd October, 2012 the patient reported that numbness was present up to the hips in both legs and from the wrists to the elbows (Grade 3 to 4 CIPN on the NCI-CTC scales as assessed by the oncologist). The patient was referred to a physiotherapist for the numbness (CIPN) and lymphoedema.

The patient reported one week later on the 9th October, 2012 significant improvement in CIPN symptoms which had reduced dramatically to the feet and hands. On the 17th November, 2012 which was the patients six month follow up, the patient described numbness in the toes and on the tips of the fingers to be approximately 50%.

Other Medical Notes

The patient presented to the breast cancer oncology and radiation public clinic at the Princess Alexandra Hospital in Brisbane, Australia on 29th May, 2012. After the first administration of the chemotherapy regime on the 6th June, 2012 the cancer care coordinator reported the patient had experienced terrible joint and bone pain, severe diarrhoea and nausea. In addition, the patient had a rash and possible fistulas and mouth ulcers. Throughout the chemotherapy administration regimen, the patient reported severe joint and muscle pain; nausea and vomiting; fluid retention; CIPN; diarrhoea and a rash.

The patient had a history of hypothyroidism, which was medicated with 100 mcg of thyroxin daily as documented in Table 1. The patient's thyroid function was monitored throughout the chemotherapy administration regimen (TSH = 5 and 4.5 mU/L). The full blood count and serum chemistry was representative of a patient undergoing chemotherapy with a low white cell count (WCC) and was neutrophilic, which recovered after chemotherapy cessation (WCC = 1.5 to 5.4 ref range 4-11 x10ˆ9/L, Neutrophils 0.1 to 3 ref range 2-7.5 x10ˆ9/L). The patient throughout chemotherapy regimen was borderline type 2 diabetic (blood glucose = 6), experienced gout (uric acid 0.39 mmol/L ref range 0.14-0.35 mmol/L), had raised liver enzymes (GGT=75 U/L, ALT= 60 U/L, AST=56 U/L) and borderline calcium (2.24 ref range 2.24-2.65 mmol/L).

Discussion

The serum vitamin B12 as seen in Table 2 assists this case report in assessing the participant's vitamin B12 status pre- and post- chemotherapy administration. The patient was found deficient in vitamin B12 on the 2nd October, 2012 (Holo TC 29 pmol/L) which is three months after the serum vitamin B12 test conducted on the 13th July, 2012 (>1476). This serum vitamin B12 blood test was taken the day before the patient's third administration of chemotherapy (17th July, 2012). The raised serum vitamin B12 level found could be representative of liver damage. It has been found that when the liver is damaged, vitamin B12 is released giving a reading of high serum vitamin B12 [11-12] however, tissue reserves are being depleted and the patient can result in a deficiency of vitamin B12 [11].

The blood pathology tests for this patient showed raised liver enzymes and a raised serum vitamin B12 level which could represent the liver releasing stored vitamin B12. The Holo TC is the active vitamin B12 blood pathology assay for blood levels of vitamin B12 being released from the liver and transported to body tissues [9]. Holo TC blood results have also been found to be raised from liver damage [11] however, it has been reported that it can detect an early deficiency in vitamin B12 compared to serum vitamin B12 levels [9].

The patients liver results indicated possible damage as noted by raised liver enzymes so it may be postulated that it was releasing stored vitamin B12 resulting in a vitamin B12 deficiency three months post serum vitamin B12 test. This patient's vitamin B12 deficiency was symptomatic as displayed by the peripheral neuropathy and fatigue. Therefore the differential diagnosis of the patient's symptoms included chemotherapy and a vitamin B12 deficiency.

According to the blood pathology tests, clinicians would normally not consider this patient's presentation of CIPN as a possible vitamin B12 deficiency in addition to the neuropathy from the chemotherapy due to the serum vitamin B12 results from July, 2012. Had the patient not participated

in a clinical trial assessing B vitamin status, the vitamin B12 most likely would not have been re-tested as the previous tests indicated a saturated state. Therefore, this raises the question of whether testing vitamin B12 during chemotherapy is worthwhile. Similarly, it is difficult to ascertain whether it gives an accurate reading of the patient's vitamin B12 state.

Intrinsic factor antibodies play an important role in a vitamin B12 deficiency. If a patient is continually found to be low or deficient in vitamin B12 after chemotherapy, then testing for intrinsic antibodies would be indicated. However, if a patient's vitamin B12 level is in normal range without supplementation 6 to 12 months post chemotherapy, then testing for intrinsic factor or parental cell antibodies would not be indicated. For this patient, the serum vitamin B12 was 411 pmol/L seven months post chemotherapy administration, which is in the reference range. The patient stated that during the seven months post last neurological exam, no oral vitamin B12 supplementation was consumed nor had an intramuscular vitamin B12 injection been administered. Therefore, it can be assumed that this participant does not have intrinsic factor antibodies. The patient was given a pathology request form for intrinsic factor antibody test but it was not performed.

The patient's baseline blood test indicated a normal vitamin B12 level as measured by Holo TC assay 107 pmol/L ref >35 pmol/L). Prior to the third cycle of chemotherapy, the patient's serum vitamin B12 was reported as elevated >1476 pmol/L (162-811 pmol/L). Subsequently, the patient's Holo TC assay after completion of the full chemotherapy regime indicated a deficiency in vitamin B12 (29 pmol/L ref >35). No other B vitamin markers were found to be deficient. After two months of vitamin B12 administration (1,000 µg intramuscularly and 1,000 µg taken orally daily) the vitamin B12 levels from the Holo TC assay had risen to 106 pmol/L (ref >35 pmol/L). Seven months post last blood pathology test, the patients's vitamin B12 levels was 411 pmol/L (162-811 pmol/L) without vitamin B12 administration indicating normal vitamin B12 absorption and metabolism and possibly no intrinsic factor antibodies.

The patient's neurological test showed no neuropathy at baseline, however displayed CIPN (Grade 2 to 3 on NCI-CTC scales) after chemotherapy. After two months of vitamin B12 administration (1,000 µg intramuscularly and 1,000 µg taken orally daily) the patient's CIPN was reduced to the tips of the fingers and toes (Grade 1 on NCI-CTC scales). Therefore a patient experiencing a vitamin B12 deficiency while undergoing chemotherapy may display a severe CIPN. This presentation of severe CIPN is normally attributed to the administration of the neurotoxic chemotherapy agent and vitamin B12 may not be tested. For this patient, the vitamin B12 status was tested and found to be deficient therefore the severe CIPN presentation was due to both the administration of the neurotoxic chemotherapy agent and a vitamin B12 deficiency. This diagnosis / presentation would have gone un-noticed without a request for vitamin B12 pathology blood test.

This case report indicates that chemotherapy administration may lower vitamin B12 status in certain patients and that this may predispose them to the development of peripheral neuropathy, possibly of increased severity. The development of peripheral neuropathy would then be causal due to both the neurotoxicity of chemotherapy administration and a concomitant vitamin B12 deficiency. The extent of the peripheral neuropathy from the chemotherapy agent cannot be ascertained until the patient's vitamin B12 status has been recovered toward a normal range.

The clinical relevance of this case study is of significant importance to clinicians as vitamin B12 is not a common pathology test requested in those patients presenting with moderate to severe CIPN due to Vitamin B12 deficits following the administration of certain chemotherapeutic agents.

A vitamin B12 deficiency is medically acknowledged as causal for peripheral neuropathy and has been identified as a potential risk factor for differentially diagnosing the development of CIPN [3]. This then questions as to how and when should a request for testing the blood level of vitamin B12 be conducted and when to intervene with treatment for those diagnosed with a deficiency of vitamin B12. One of the confounding factors for cancer patients is the taste and smell of taking an oral B vitamin complex. For some patients, taking a prophylactic oral vitamin B complex is not an option during chemotherapy administration therefore intramuscular vitamin B12 injections may be the only option for certain patients.

One option for clinical application may be to request vitamin B12 blood levels with every pathology test conducted before the administration of neurotoxic chemotherapy agents. Identifying those patients who are at increased risk of a vitamin B12 deficiency may lead to better patient management by reducing the risk of developing CIPN during chemotherapy administration with neurotoxic agents. The blood pathology level of serum vitamin B12 or Holo TC at which vitamin B12 treatment would be implemented would need to be determined. Neurological symptoms can be experienced by patients not undergoing chemotherapy at 200 pmol/L [11] hence the intervention range of vitamin B12 maybe 250 pmol/L for serum vitamin B12 and 35 pmol/L for Holo TC. Therefore prevention of neurological side effects from a vitamin B12 deficiency would be avoided and the development of CIPN would only be from the neurotoxic chemotherapy agent.

The cost analysis of conducting vitamin B12 pathology tests for each patient being administered a neurotoxic chemotherapy agent is prohibitive. Hence, it is proposed that a vitamin B12 pathology test be conducted before chemotherapy administration and when the patient presents with moderate to severe CIPN. Patients may be advised to take an oral B vitamin complex or vitamin B12 supplement prophylactically through chemotherapy administration. Alternatively, a prophylactic IM injection of

vitamin B12 may be administered to those patients deemed at risk of a vitamin B12 deficiency through chemotherapy administration.

Conclusion

Vitamin status, in particular vitamin B12, can play a role in the development of CIPN in certain patients. Patients experiencing moderate to severe CIPN should have their vitamin B12 levels pathologically tested and if serum vitamin B12 levels are found below 200 pmol/L or holo TC is below 35 pmol/L, then vitamin B12 should be administered intramuscularly and orally for the duration of the chemotherapy administration. Administration post chemotherapy for a period of approximately three months should be continued depending on the patient's tolerance for oral supplementation.

A protection from vitamin B12 may be inferred for CIPN development. Oral supplementation with a B vitamin complex or vitamin B12 supplement may reduce the risk of CIPN development in certain patients. However, some patients may not tolerate the smell of the vitamin B capsule or may have difficulty with ingestion of the supplement. Also the pungent smell of their urine from the B vitamin supplementation can be off putting for some patients and may increase their feeling of nauseousness. As an alternative, intramuscular administration of vitamin B12 may be more appropriate for certain patients.

Monitoring of vitamin B12 levels may also be implemented so early intervention with B12 administration can occur before the patient is found deficient. This could be through adding serum vitamin B12 to the blood pathology request forms for patients that will undergo chemotherapy with neurotoxic agents before chemotherapy cycles commence and at presentation of moderate to severe CIPN.

This case report outlines the importance vitamin B12 may play in CIPN development and the clinical relevance for medical practitioners.

Further clinical trials looking at vitamin B12 levels in patients with neurotoxic agents will be undertaken. The next study which is being designed is a clinical trial aimed at taxol agents only. One arm will be a control monitoring CIPN and vitamin B12 status with no intervention and the other arm will be administered intramuscular vitamin B12 monthly during chemotherapy and three months post-chemotherapy. This clinical trial is aimed at gathering further information pertaining to vitamin B12 levels during chemotherapy administration and possible prevention of CIPN.

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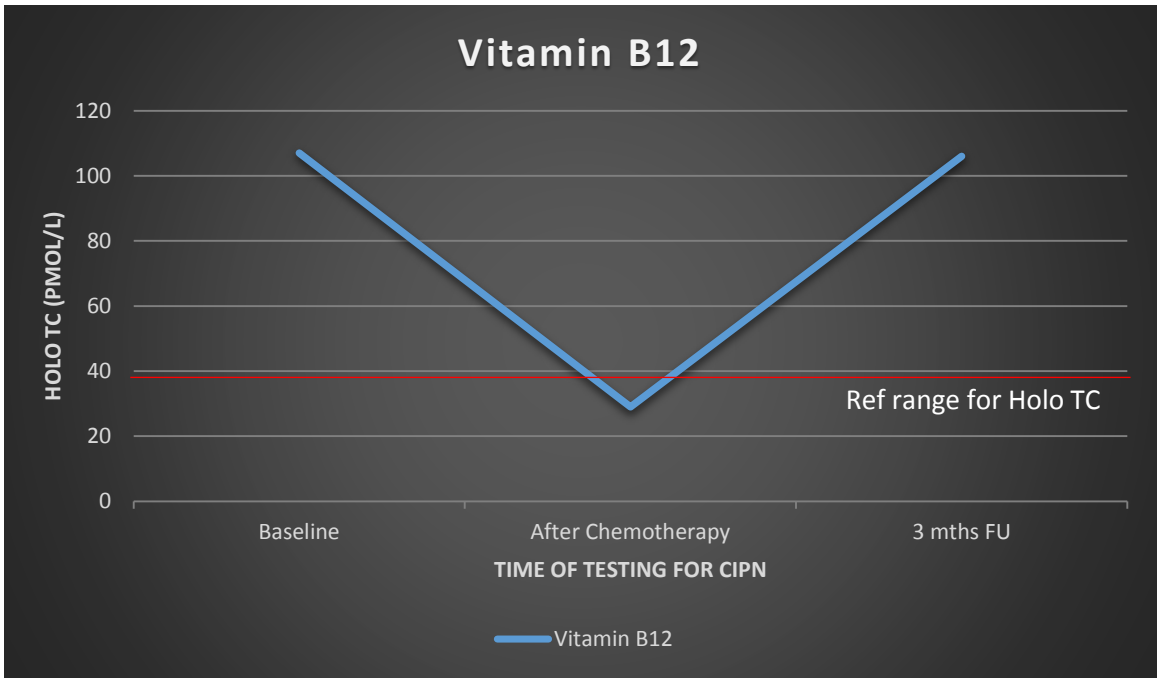

Figure 1: Vitamin B12 results (Holo TC) for Case study before and after chemotherapy

Table 1: Medical Details of Participant PMP

PA 032 PMP	**Details**
DOB	17/06/59
Diagnosed	Breast Cancer in March, 2012
Surgery	PA Hospital 27/3/2012 – right breast lumpectomy and axillary clearance. Previous surgery: Appendectomy, cholecystectomy, basal cell carcinoma on nose (flap repair)
Results	Grade II IDC and DCIS – Oestrogen and Progesterone positive, HER2 negative
Nodes positive / removed:	0/9
Recruited for study	2/6/2012
Race	Caucasian
Marital Status	Married
Other medical considerations	Psoriasis, hypothyroidism, osteoarthritis, reactive arthritis, asthma (late onset), osteopenia, obesity

Medications	Thyroxin 100 mcg daily
Allergies	Codeine, pethidine
Diet history	No history of being a vegan or vegetarian. Normally consumes red meat (beef, lamb, kangaroo) 3-4 times a week, chicken or pork 2-3 times a week and fish or seafood approximately 3-4 times a week. She does drink caffeine, approximately 4-5 cups a day (tea), rarely drinks alcohol, doesn't smoke or take recreational drugs.
Height	162cm
Weight	129kg
BMI	49.2
Chemotherapy regime	Carboplatin and docetaxel (TC) 4 times every three weeks

Table 2: Blood Pathology Results

Blood Pathology from Clinical Trial	**Baseline 5/6/2012**	**After Chemo 25/09/2012**	**Last follow up 8/12/2012**	**Ref Range**
Vitamin B1 (TDP)	140	140	180	66-200 nmol/L
Vitamin B2 (FAD)	280	310	230	180-470 nmol/L
Vitamin B6 (P5P)	95	90	**250 H**	35-110 nmol/L
Red cell folate	2249	2163	2170	>900 nmol/L
Holo TC (Vitamin B12)	107	**29 L**	106	>35 pmol/L
Results from the PA Hospital		**13/07/2012**	**22/06/2013**	**Ref Range**
Serum Vitamin B12		H >1476	411	162-811 pmol/L
Red Cell Folate		1265	1290	545-3370 nmol/L
Serum Ferritin		H 743	H 395	15-290 ug/L

Table 3: Chemotherapy Regime and Administration

Date	**5/6/2012**	**26/6/2012**	**17/7/2012**	**7/8/2012**
Sodium chloride	100 ml IV	100 ml IV	100 ml IV	100 ml IV
Granisetron (3mg)	3 mg IV	3 mg IV	3 mg IV	3 mg IV
Dexamethasone (8mg)	8 mg IV	8 mg IV	8 mg IV	8 mg IV
Docetaxel (75mg/m^2)	184 mg IV	184 mg IV	184 mg IV	184 mg IV
Cyclophosphamide (600mg/m^2)	1480 mg IV	1480 mg IV	1480 mg IV	1480 mg IV
Discharge Medication				
Ondansetron (oral)	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days
Dexamethasone (oral)	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days	8 mg BD x 2 days
Metolopramide (oral)	10-20 mg 4-6 HRLY PRN x 5 days	10-20 mg 4-6 HRLY PRN x 5 days	10-20 mg 4-6 HRLY PRN x 5 days	10-20 mg 4-6 HRLY PRN x 5 days
Dexamethasone (oral) to be taken the day before next chemotherapy	8 mg BD x 1 day	8 mg BD x 1 days	8 mg BD x 1 days	
Pegfilgrastim INJ (6mg) subcutaneous		6 mg ONCE x 1 day	6 mg ONCE x 1 day	6 mg ONCE x 1 day

Table 4: Total Neuropathy Score Results

Total Neuropathy Score (TNS)			
Item	**Baseline Score 02/06/2012**	**After Chemotherapy 25/08/2012**	**Three months later 17/11/2012 (2 months after beginning of B12 supplementation)**
Sensory symptoms	0	2	1
Motor symptoms	1 (right hand sweating reduced testing)	2	2
Autonomic symptoms	1 (sweating)	1	2 (menopause post chemo)
Pin sensibility	0	2	2
Vibration sensibility	0	0	0
Strength	0	0	0
Tendon reflexes	0	4	2
Sural amplitude score	4	4	2
Peroneal amplitude score	0	0	0
Total	7	15	11

Table 5: NCS Study Results

Sensory NCS							
Nerve/Sites	**Rec Site**	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV
		2/6/2012		**25/08/2012**		**17/11/2012**	
R MEDIAN Palm	III	1.30	4.3	2.8	10.2	2.85	4.9

L MEDIAN Palm	III			2.70	12.0	3.30	5.0
L ULNAR – Wrist	Digit V	1.95	19.5				
R ULNAR – Wrist	Dorsum of Hand	1.30	26.1	1.65	1.5	1.85	3.1
L ULNAR – Wrist	Dorsum of Hand	1.65	13.2	1.70	3.6	1.65	3.9
R SURAL – Calf	Lat Malleolus			2.65	6.7	2.35	5.1

NB: First baseline tests were difficult due to moisturiser used by patient

Motor NCS						
Nerve/Sites Rec Site	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV	Latency Ms	Peak Ampl µV
	2/6/2012		**25/08/2012**		**17/11/2012**	
L MEDIAN – APB Wrist	5.10	7.7	4.45	8.0	3.80	7.7
Elbow	9.45	7.8	9.05	7.3	9.35	7.8
R COMM PERONEAL – EDB Ankle	4.05	2.9	3.15	3.3	4.05	2.2
Fib Head	9.65	4.8	10.55	0.7	10.15	2.3
R TIBIAL (KNEE) – AH Ankle	5.05	10.4	3.55	8.3	4.65	3.7