Cape Peninsula University of Technology

ULTRASOUND EVALUATION OF THE EXTRACRANIAL CEREBROSPINAL VENOUS SYSTEM AND CAROTID ARTERIES IN PATIENTS WITH MULTIPLE SCLEROSIS

By

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Thesis submitted in fulfilment of the requirements for the degree

Master of Technology: Radiography

in the Faculty of Health and Wellness Sciences

at the Cape Peninsula University of Technology

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Bellville

September 2013

Gloria in excelsis Deo

DECLARATION

I, Merlisa Claudia Nelson, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Multiple Sclerosis (MS) is characterised by demyelination within the central nervous system (CNS), which may result in neurological disabilities over time, causing considerable hardship to patients and their families, in addition to being costly to treat. Recent studies have linked MS to impaired cerebral blood flow, called chronic cerebrospinal venous insufficiency (CCSVI). Anecdotal evidence has suggested that surgical correction thereof results in improvement of symptoms experienced by MS patients. To my knowledge, no information is available in the literature on carotid artery disease in MS. The USA National MS Society has therefore called for more research to be done in this area.

This cross-sectional observational sub-study will determine, by ultrasound (B-Mode, Colour and Pulsed-wave Doppler), the prevalence of chronic venous insufficiency (CCSVI) and carotid artery disease in the selected sample of MS patients within the region of the Western Cape, South Africa.

Biochemical data; lifestyle factors such as physical activity and smoking; and nutritional status of MS patients were determined from the main study entitled: *"The development of a comprehensive gene-based, pathology supported intervention program for improved quality of life in patients diagnosed with multiple sclerosis"* (Division of Chemical Pathology, NHLS, Tygerberg Hospital, and University of Stellenbosch).

Twenty-nine (29) patients were aged between 28-64years and they suffered from MS for 0.83-27years. A larger proximal and mid cross-sectional diameter (CSD) of the right IJV compared to the left (differences significant, P= 0.026 and P=0.023) was demonstrated. Increased intima media thickness (IMT) was present in 13.33% of the non-smoking MS group and 20% in the smoking MS group. IJV reflux was evident in 13.33% of the MS group.

A significant reduction of cross-sectional diameters of the IJV's was evident in smoking MS patients; suggesting that smoking is not only a risk factor for atherosclerotic disease but could also be related to narrowing of the major neck veins. This study also supports findings of other studies viz that there's no significant correlation between extracranial venous abnormalities and MS.

Early carotid artery disease was noted in smoking and non-smoking MS patients, however the findings were non-significant.

ACKNOWLEDGEMENTS

I wish to thank:

- My God and loving Saviour for being my source of strength and for never failing me.
- My supervisor, Ms Ferial Isaacs, for always having an open door when I needed advice; having faith in me when I was in doubt; encouraging and guiding me throughout my Master's degree.
- My co-supervisor, Prof. Susan J. Van Rensburg, for guidance and support; encouraging me and being a great teacher.
- Prof. Martin Kidd (statistician) for determining the statistical data and always being available to assist me with my statistical queries.
- Dr Bergman, Ross and partners Radiologists for allowing me to carry out my research within the ultrasound department.
- Mr Shafick Hassan (Nursing and Radiography Department, CPUT) for funding part of the study.
- University Research Fund of CPUT for funding part of the study
- My work colleagues for being a source of encouragement and always believing in me in times when I doubted myself.
- Yolandi for agreeing to be a model in demonstrating the ultrasound techniques.
- My family and friends for their constant encouragement, support and unconditional love.
- My mom and late grandmother for always being my rock.
- Annette for her patience with me during my studies and assisting with proof-reading.
- Deon for helping me with the corrections and references.

DEDICATION

To my son,

Joshua

GLOSSARY

ADEM	Acute Disseminated Encephalomyelitis
BBB	Blood-brain barrier
CPUT	Cape Peninsula University of Technology
CNS	Central nervous system
CSF	Cerebrospinal Fluid
CCSVI	Chronic Cerebrospinal Venous insufficiency
CCA	Common carotid artery
CSA	Cross-sectional area
CSD	Cross-sectional diameter
DVT	Deep Vein Thrombosis
EDV	End diastolic velocity
EP	Evoked potential
ECA	External Carotid Artery
FRC	Faculty Research Committee
Hcy	Homocysteine
HHcy	Hyperhomocysteinemia
ICA	Internal carotid artery
IJV	Internal jugular vein
IJVs	Internal jugular veins
IMT	Intima media thickness
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
NASCET	North American Symptomatic Carotid Endarterectomy Trials
PSV	Peak systolic velocity
PACS	Picture Archives and Communication System
PW	Pulsed-wave
VA	Vertebral artery
VV	Vertebral veins
VEP	Visual Evoked Potential

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CHAPTER ONE

INTRODUCTION

Multiple Sclerosis (MS) is characterised by demyelination within the central nervous system (CNS), which may result in neurological disabilities over time, causing considerable hardship to patients and their families, in addition to being costly to treat. Standard medication for MS does not improve disease outcome, although it may reduce the relapse rate and slow disease progression in about 50% of patients (Axtell et al, 2011). Genetic studies have linked HLA II genes to disease risk in MS, however no genetic factors have been exclusively found that predict disease outcome (Ramagopalan et al, 2008). This suggests that a combination of both genetic and environmental factors play a role in disease aetiology.

Recent studies have linked impaired cerebral venous outflow with MS and surgical correction thereof has shown rapid improvement of MS symptoms (Zamboni, 2006).

To my knowledge, no information is available in the literature on carotid artery disease in MS. In view of this, research in this field is warranted. The USA National MS society has also called for more research to be done in the MS field.

MS has been linked to a dysregulation of iron and homocysteine metabolism (Van Rensburg et al. 2006). Parallels have been found between MS and iron-dependent inflammatory vascular disease (Zamboni 2006). Patients with MS display altered blood coagulation (Steinman et al. 2008) which under normal conditions prevents blood loss from a ruptured blood vessel by forming a clot; however, such clots can result in the occlusion of the vessel. More than 20 distinct factors, both procoagulant and anticoagulant, interact in an extremely complex manner involving protein-protein and protein-ion reactions, which include positive and negative feed-back mechanisms controlling the kinetics of clotting. Vascular homeostasis and cerebral microcirculation are regulated by neurons and astrocytes which are closely associated with the endothelial cells of the Blood Brain Barrier (BBB). Disruptions to this homeostatic balance can contribute to nervous system pathologies as well as neurodegenerative diseases such as MS (Adams, 1988).

Recently Singh and Zamboni (2009) described altered cerebral venous drainage in patients with MS, as a condition called chronic cerebrospinal venous insufficiency (CCSVI). Their research suggested that this condition may be a major risk factor for MS. The original investigation, by Zamboni et al (2009), in a group of 65 patients and 235 controls showed CCSVI to be associated strongly with an increased risk factor of MS by 43 fold. The insufficient blood flow may lead to iron deposition in the brain of MS patients, since erythrocytes cross the BBB.

Subsequent studies have not consistently confirmed Zamboni's original findings (Zivadinov et al. 2011), and have found the prevalence of venous occlusions to differ in different populations. Blood-brain barrier (BBB) insufficiency in MS, such as fibrinogen leakage into the brain, has also been reported (McQuaid et al. 2009). Fibrinogen is a blood plasma protein involved in blood clotting. With the catalytic action of thrombin, fibrinogen is converted into molecules of the insoluble protein fibrin, which link together to form clots. When these clots are enzymatically digested D-dimers are produced. According to Aksungar et al. (2008), there is a significant correlation between D-dimer and homocysteine levels in MS patients.

Handel et al (2010) states that attempts should be made to understand the pathway of causes of CCSVI in MS and further research into the role of CCSVI in MS is encouraged.

This cross-sectional observational substudy has determined, by ultrasound, the prevalence of abnormal extracranial venous outflow patterns and carotid artery disease in the selected sample of MS patients within the region of the Western Cape, South Africa.

Biochemical data; lifestyle factors such as physical activity and smoking; and nutritional status of MS patients were determined from the main study entitled: *"The development of a comprehensive gene-based, pathology supported intervention program for improved quality of life in patients diagnosed with multiple sclerosis"* (Division of Chemical Pathology, NHLS, Tygerberg Hospital, and University of Stellenbosch).

Ultrasound technology has become an important tool for healthcare professionals in South Africa as it offers many advantages compared to other imaging modalities. It is cost effective, easily available, and does not use ionizing radiation.

When impaired blood flow in the major neck vessels is demonstrated by ultrasound; and a significant correlation between the ultrasound findings and biochemical data as well as lifestyle factors are illustrated; then preventative measures can be taken by advising lifestyle and diet changes for improved quality of life in these patients.

CHAPTER TWO

LITERATURE REVIEW

Multiple Sclerosis (MS) was first described by Jean-Martin Charcot in 1868 (Patti et al, 2012). In 1862 he was nominated as “Medicin de Hoptitaux” at “la Salpêtrière” and in 1872 was appointed as Professor of Pathological Anatomy at the University of Paris. Charcot saw 30 cases of MS in his career and in 1868 he clearly delineated disseminated sclerosis and described the main neuro-pathological findings of the disease based on clinical observations and autopsy findings (Clanet, 2008).

MS affects 6 in 10 000 persons (20 -50 years of age) and the incidence is increased with a positive family history and the frequency of the disease being higher in cooler climates (Dähnert, 2007:310). In 1967, Geoffrey Dean, reported results of a survey on the annual incidence, prevalence and mortality of MS in the White South African population. The incidence, prevalence and mortality of the disease was high amongst the White South Africans with it being higher in English-speaking and lower in Afrikaans-speaking White South Africans. The disease has been reported to be less common amongst the Coloured and Asian South Africans, and rare amongst Black Africans (Dean, G.,1967). Further studies on MS in South Africa by Modi et al (2008) states that there are approximately 5 000 persons suffering from MS in South Africa with Gauteng and the Western Cape having the most respondents. The study concludes that MS is widely diagnosed in the White South African population and affects females more than males (F:M=3:1).

2.1 WHAT IS MULTIPLE SCLEROSIS (MS)?

MS is stated to be one of the most common chronic disabling neurological disease of young adults that attacks the central nervous system (brain, spinal cord and optic nerves) (Mateo Paz Soldan & Rodriquez, 2002:S248; National MS Society, 2010).

Barnett and Sutton (2006) described MS as a venocentric focal inflammatory demyelinating disease of the central nervous system (CNS). According to Loveday,(1991:209), this disease causes patchy destruction of myelin in the nerve sheaths. Myelin is described as a fatty substance that surrounds and protects the nerve fibres in the CNS. When myelin is damaged, scar tissue forms, which is termed sclerosis. A damaged myelin sheath or nerve fibre will cause nerve impulses travelling to and from the brain to be distorted or interrupted which in turn produces a variety of symptoms in MS patients (National MS Society, 2010).

Four clinical forms of MS were standardised in 1996: relapsing remitting, secondary progressive, primary progressive and relapsing progressive (Van Rensburg & van Toorn, 2010). However, it remains difficult to diagnose patients into subtypes due to the heterogeneity of the disease. Some patients also present with a benign disease subtype.

2.2 PATHOGENESIS OF MULTIPLE SCLEROSIS

The pathogenesis of MS is unknown (Zamboni et al, 2009:392). However, scientists believe that a combination of several factors (immunological, environmental, infectious and genetic) may be involved (National MS Society, 2010). Sir Austin Bradford Hill (1965) posed the question concerning environment and disease: association or causation? He states that diseases may have more than one cause. As an example, the temporary relationship of association raises the question of whether a particular diet leads to disease or do the early stages of the disease lead to peculiar dietetic habits. Van Rensburg et al (2006) found that some MS patients present with low blood iron parameters and these patients will benefit from iron supplementation. Iron and a functional folate-vitamin B12-methylation pathway are needed for regeneration of myelin. In a pilot study MS patients followed a programme of nutritional supplements designed to promote the regeneration of myelin as well as life style modification. Patients partaking in the study also had blood tests including iron parameters, and genetic tests. The results of the study indicated that the patients on the regimen displayed significant neurological improvement (Van Rensburg et al. 2006).

Increased levels of fibrinogen and homocysteine are associated with vascular damage (Van Rensburg, 2006 & Reinhart, 2003).

2.2.1 Vascular Parameters and Blood flow in MS

Studies have revealed that dysfunction of the blood-brain barrier (BBB – defence mechanism that prevents many substances that circulate in the bloodstream from invading the brain) plays a vital role in MS. BBB leakage occurs in MS, including fibrinogen leakage into the brain (McQuaid et al, 2009).

2.2.1.1 Fibrinogen

Plasma fibrinogen is an important part of the blood clotting process. Blood clots are made up of arrays of cross-linked fibrin that form an insoluble fibrous network. Fibrinogen is made from fibrin through a proteolytic reaction catalysed by the serine protease thrombin (Voet & Voet, 1990:1087).

The blood clotting cascade in humans can be seen in Figure 2.1.

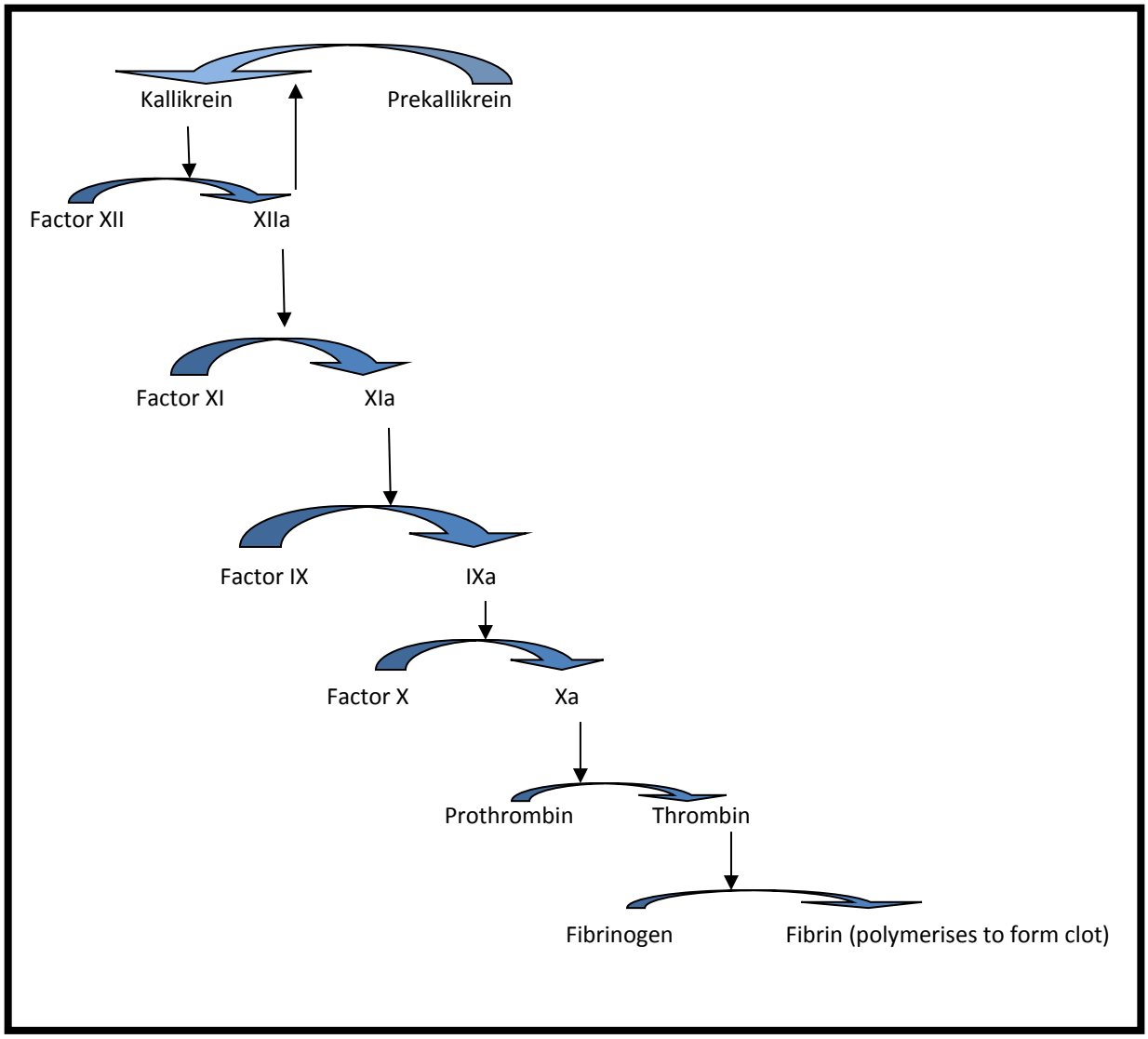

Figure 2.1: Blood clotting cascade in humans
Each of the curved arrows represents a proteolytic reaction, in which a protein is cleaved at one or more specific sites. With the exception of fibrinogen, the substrate in each reaction is an inactive zymogen. Each product is an active protease that proceeds to cleave another member in the series, except for fibrin. When thrombin cleaves fibrinogen at several points, the trimmed protein (fibrin) polymerises to form a clot (Adapted from Zubay, G, 1993: 256).

Fibrinogen is a soluble glycoprotein dimer found in the plasma with a molecular weight of 340kDa and is composed of 3 pairs of non-identical polypeptide chains (alpha, beta and gamma chains) (Reinhart, 2003; Kamath & Lip, 2003). This glycoprotein comprises 2 to 3% of plasma protein (Voet & Voet, 1990:1087).

According to Oswald et al (1983) the normal range of plasma fibrinogen is 145-348 mg/dl. The increase in fibrinogen levels correlates with the severity of peripheral arterial disease (Kerlin et al, 2004:1728). Increased fibrinogen concentration results in an increase in blood viscosity which in turn is inversely related to blood flow. Viscosity refers to the thickness of

blood, thus if the viscosity is increased the blood flow rate will be reduced and the blood flow shear stress increased. An increase in blood flow shear stress can activate platelets and endothelial cells (Postlethwaite, 1976; Kamath & Lip, 2003 & Lominadze et al, 2004).

2.2.1.2 Effect of Fibrinogen on Intima Media Thickness

Increased intima media thickness (IMT) of the common carotid artery (CCA) is regarded as a marker for early atherosclerotic disease. In a study conducted by Grebe et al (2010), a positive association between plasma fibrinogen levels and CCA-IMT was demonstrated. An increase in fibrinogen levels are indicative of increased CCA-IMT which in turn correlates with early atherosclerotic changes of the carotid artery. Reinhardt (2003:212) states that fibrinogen accumulates in atherosclerotic plaques and early lesions contain primarily fibrinogen and fibrin. It is suggested from these findings that fibrinogen is a contributing factor to atherosclerosis.

2.2.1.3 Homocysteine

Homocysteine (Hcy) is a sulfydryl amino acid which is structurally similar to cysteine with an additional methylene group. The normal homocysteine level in blood plasma is 5-8µmol/l (Kumar et al, 2008). This amino acid is formed during the metabolic conversion of methionine to cysteine, which is illustrated in Figure 2.2 (De Koning et al, 2003:431-432).

The risk for the development of atherosclerotic vascular disease is increased with mild to moderate elevation of Hcy (Guo et al, 2009). Increased levels of Hcy are associated with endothelial dysfunction in blood vessels (Woo et al, 2002). Hyperhomocysteinemia (HHcy) can cause injury to the endothelium of the blood vessels, activate the platelets and enhance the production of fibrinogen. This results in the proliferation of smooth muscle cells, which can cause narrowing (stenosis) of the lumen of blood vessels.

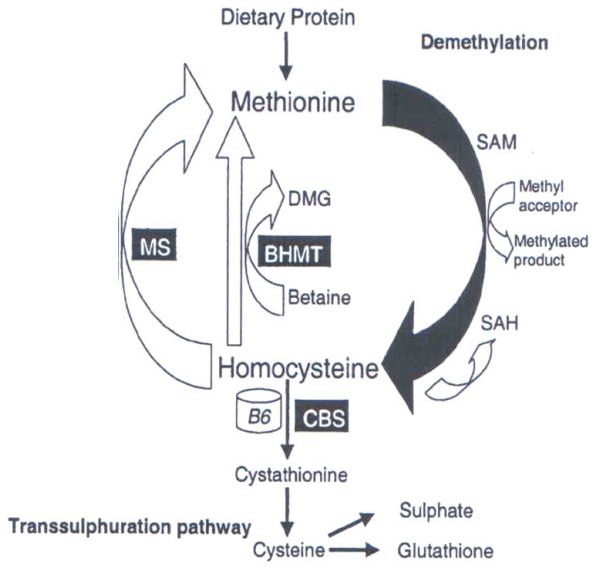

Figure 2.3: Metabolism of Homocysteine
Dietary methionine is converted to the methyl donor S-adeosylmethionine (SAM) and is demethylated to S-adenosylhomocysteine (SAH), which is subsequently cleaved into adenosine and homocysteine. Through the transulphuration pathway, homocysteine is converted to cystathionine and cysteine by the enzyme cystathionine β-synthate (CBS) and the cofactor vitamin B6 (methylcobalamin). In liver and kidney, homocysteine is also remethylated by the enzyme betaine homocysteine methyltransferase (BHMT), which transfers a methyl group to homocysteine via demethylation of betaine to dimethylglycine (DMG) (Adapted from De Koning et al, 2003:432).
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The causes for increased homocysteine (hyperhomocysteinemia) are as follows:

- Genetic (inherited enzyme deficiencies)
- Physiological
- Lifestyle factors
- Vitamin deficiencies
- Systemic disorders, and
- Drugs

(Guthikonda & Haynes, 2006).

De Koning (2003) estimates that 5 to 7% of the general population has mild to moderate hyperhomocysteinemia. Also, increased Hcy levels in MS patients are associated with vascular damage as described by (Van Rensburg et al, 2006).

A study conducted by Selhub et al (1995) demonstrated a prevalence of ≥25% carotid artery stenosis in 43 percent of men and 34 percent of women with Hcy of ≥14.4μmol per litre.

Mueller et al (2001) also states that high Hcy levels are associated with an increased risk of carotid artery disease and is an independent risk factor for a ≥ 50% stenosis of the internal carotid artery (ICA) in patients with peripheral vascular disease.

Elevated Hcy levels are also a risk factor for increased common carotid artery wall thickness (intima media thickness), which in turn is an indicator of early systemic atherosclerosis and of future stroke and myocardial infarction (Adachi et al, 2002 & Linnebank et al, 2006). Increased carotid artery intima media thickness is strongly associated with coronary artery disease as described by Baptista et al (2008:44). Baptista (2008) and his colleagues also stated that approximately 40% of patients with early premature coronary artery disease, peripheral vascular disease and deep vein thrombosis (DVT) present with HHcy.

HHcy can be treated through vitamin supplementation; folic acid, vitamin B6 and B12 being the core supplements (Guthikonda and Haynes, 2006). Long term use of folic acid improves arterial endothelial function and has potential of preventing atherosclerosis in persons with hyperhomocysteinemia (Guo et al, 2009).

2.2.1.4 Chronic cerebrospinal venous insufficiency (CCSVI)

Decreased arterial flow to the brain may cause a decreased cerebral venous outflow in MS patients (Sundstrőm et al, 2010). The internal jugular veins (IJVs) are considered to be the main outflow pathway for intracranial venous blood (Schreiber et al, 2003:1802). Postural changes cause alterations of venous outflow from the brain (Doepp et al, 2004:568). Flow in the IJV is more dominant in the supine position and in the erect position a natural increase in flow in the vertebral veins (VV) is noted (Schreiber et al, 2003:1802).

Zamboni (2006) conducted a study on iron dependent inflammation in chronic venous disease and discovered an unusual phenomenon of reflux in the internal jugular vein (IJV) in MS patients during a carotid Duplex examination in Italian MS patients as seen in Figures 2.3 and 2.4. Further research also demonstrated stenosis (Figure 2.5) and absent flow in the IJV and/or vertebral veins (VV) in patients with MS diagnosed according to the revised M^{c}Donald criteria (Zamboni et al, 2009:23).

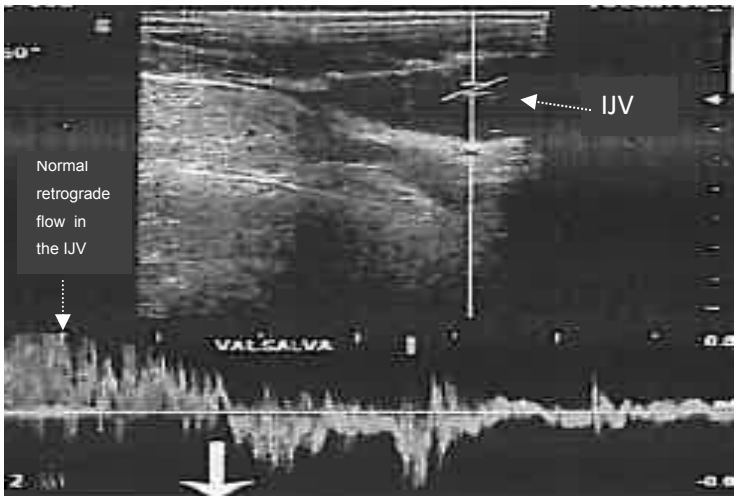

Figure 2.4: Reflux in the IJV under valsalva.
Reflux in the IJV under valsalva in a MS patient. Solid arrow indicates reversed flow from chest to head (Adapted from Zamboni, 2006:590).
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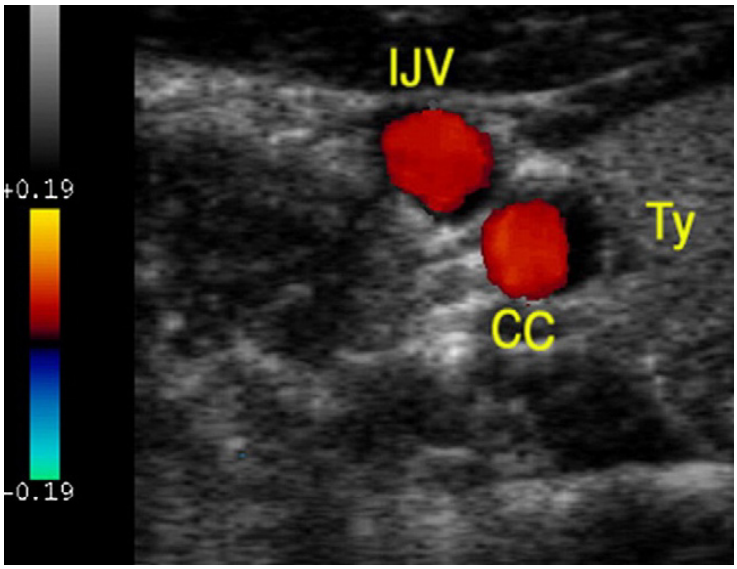

Figure 2.5: Reflux in the IJV.
(Depicted from Zamboni, Menegatti et al 2009:23).
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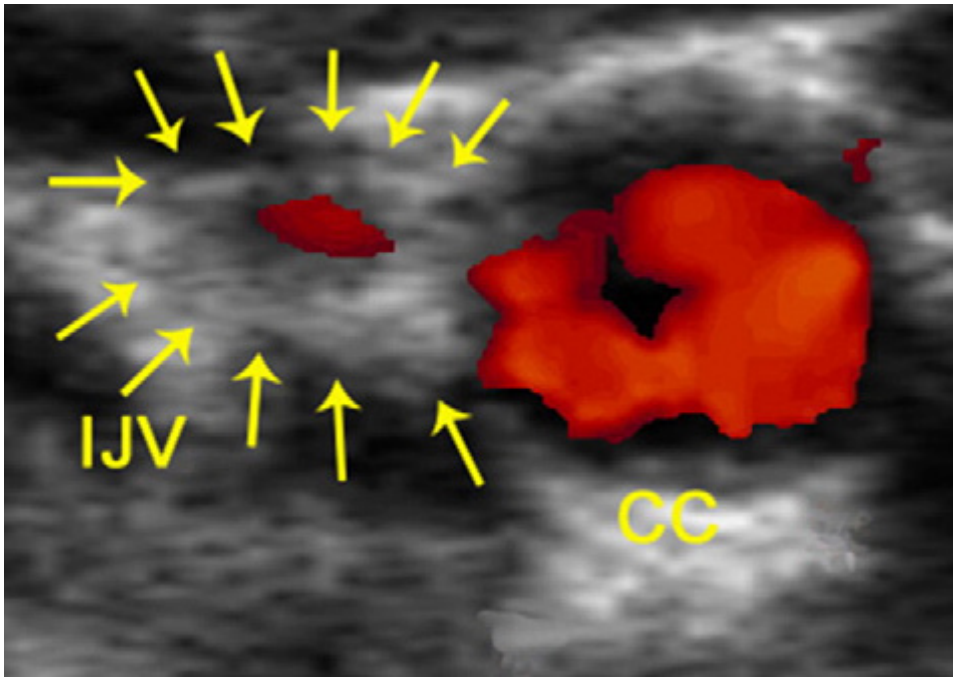

Figure 2.6: Stenosis of the IJV.
Stenosis of the IJV (yellow arrows) with severe lumen reduction. Flow direction in the IJV is the same as that of the common carotid artery (CC) towards the brain (Depicted from Zamboni , Menegatti et al, 2009:24).
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Zamboni et al (2009:395) defined the abnormal cerebrospinal venous drainage in patients with MS as chronic cerebrospinal venous insufficiency (CCSVI). He also discovered that reflux in the extracranial veins occurred in any body position without the need for valsalva suggesting that reflux was not due to valve incompetence but rather due to focal stenosis which was not associated with postural or respiratory mechanism.

The diagnosis of CCSVI is based on sonographic criteria (Valdueza et al, 2013:657).

Zamboni et al (2011) states that the presence of any 2 of the criteria as described below, indicate CCVSI:

Criterion 1: Reflux in the IJV and/or VV

I) Bidirectional flow in one or both of the IJVs in both supine and erect positions.

II) Reversal or bidirectional flow in one or both of VVs in supine and erect positions.

Criterion 2: Bidirectional flow (or reflux) in the intracranial veins and sinuses.

Criterion 3: IJV stenosis

I) Severe reduction of the cross-sectional area (CSA) of the IJV in the supine position <0.3cm^2 which does not increase with valsalva.

II) Intraluminal defects such as webs, septa or malformed valves with changes in blood flow patterns

Criterion 4: Absence of detectable flow in the IJV and/or VV

I) Absence of a Doppler signal in the IJV and/or the VV in supine and erect positions or

II) In one posture with bidirectional flow detected in the other position.

Criterion 5: Abnormal changes of the IJV CSA with change in position (change of hydrostatic pressure)

The anatomy of the cerebrospinal venous system with areas of venous narrowing associated with CCSVI is depicted in Figure 2.6.

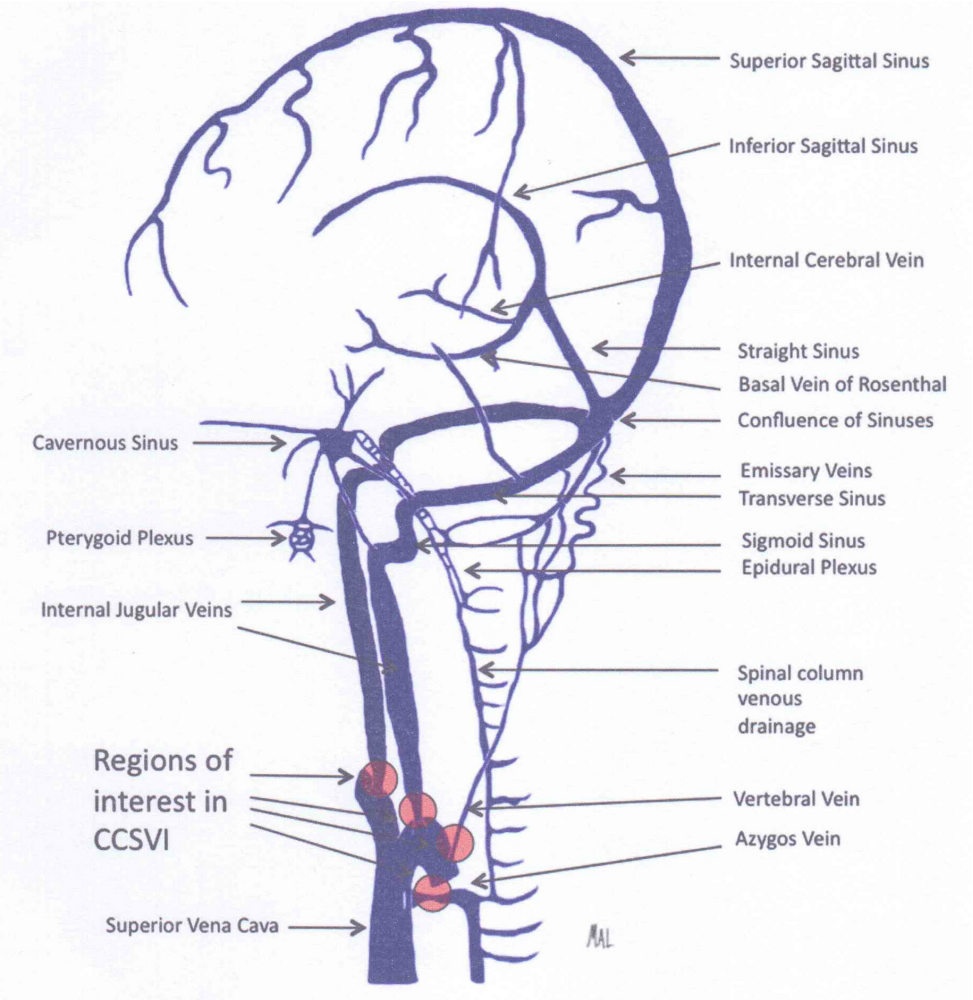

Figure 2.7: Anatomy of the cerebrospinal venous system.
Illustration of the predominant veins and sinuses involved in the craniocervical venous outflow. The areas in red depict the locations of interest of venous narrowing associated with chronic cerebrospinal venous insufficiency (Figure as originally published in Lazzero, M. A., Zaidat, O. O., Mueller-Kronast, N., Taqi, M. A. & Woo, D. (2011) Endovascular therapy for chronic cerebrospinal venous insufficiency in multiple sclerosis Front. Neur. 2:44 doi10.3389/fneur.2011.00044)

Zamboni's hypothesis has generated much interest from many scientists and MS patients as well as criticism from clinicians (Reekers et al. 2011). A note of caution was suggested by the National MS Society of America until more data became available.

Ghezzi, Comi and Federico (2011) suggested that appropriate epidemiological studies be performed to define the possible relationship between CCSVI and MS. Al-Omari and Rousan (2010:115), suggested that there may be a strong association between haemodynamic abnormalities and morphological changes of the IJV and MS.

In a study conducted by Simka et al (2010) on extracranial Doppler in MS patients it was found that the abnormalities of the extracranial veins can be of different combinations. The most common pathology noted was an inverted valve at the junction of the IJV with the brachiocephalic vein.

Zivadinov et al (2011) conducted a study to determine the prevalence, sensitivity and specificity of chronic cerebrospinal venous insufficiency (CCSVI) in a large group of 289 MS patients by means of transcranial and extracranial echo-colour Doppler. The findings indicated an increase in prevalence of CCSVI in MS, however it pointed against CCSVI being one of the primary contributing factors in the development of MS. Further research performed by Zivadinov et al (2012) demonstrated, by brain magnetic resonance imaging (MRI), no association with more severe lesion burden or brain atrophy in patients with MS or in healthy controls.

Mayer et al (2011) concluded that the findings based on the ultrasound assessment of the cerebral and cervical veins do not provide supportive evidence for the presence of CCSVI in MS patients.

According to Reekers et al (2011) the abnormal venous drainage, characterised by ultrasound, causes intracerebral flow disturbances which leads to periventricular deposits. Zamboni encourages balloon angioplasty as a form of treatment of venous outflow problems. He suggested that as a result of the balloon dilatation of the stenosis, CCSVI can be cured and at the same time alleviate MS symptoms.

MS patients who underwent the procedure stated that their personal quality of life had improved after treatment (Reekers et al, 2011).

However, Fragoso (2011) states that Zamboni's theory is not yet proven and cannot be recommended as a definitive treatment for MS.

Morovic and Zamboni (2012) conducted a study entitled: "CCSVI associated with multiple sclerosis", and found that there is a great variability on prevalence of CCSVI in MS patients

which could be a result of different methodologies used in venous ultrasound assessment. However, according to these authors, CCSVI can be regarded as one of the multiple factors involved in MS pathogenesis.

The diagnostic value of Doppler sonography for the assessment of internal jugular vein (IJV) abnormalities was evaluated by Simka et al (2013). Their research showed that the currently used sonographic criteria of the extracranial vessels for detection of obstructive venous abnormalities in the IJVs are of limited diagnostic value. It was suggested that catheter venography be used to diagnose this vascular pathology.

According to Baracchini et al (2012), no part of the CCSVI theory has solid supportive scientific evidence and no proven (based on stern scientific methodology and evidence-based medicine) therapeutic effect of balloon angioplasty procedure has been shown to date.

Leone et al (2013) states: “CCSVI was not associated with MS itself nor its severity. We cannot rule out the possibility that CCSVI is a consequence of MS or of ageing”.

2.3 SYMPTOMS OF MULTIPLE SCLEROSIS

The most common symptoms of MS are:

- Fatigue
- Numbness
- Burning sensation
- Walking, balance and co-ordination problems
- Dysesthesia
- Signs of brain neoplasm: headaches, seizures, dizziness, nausea, weakness, altered mental status
- Diplopia
- Bladder dysfunction
- Bowel dysfunction
- Sexual dysfunction
- Emotional changes
- Depression
- Spasticity

(Dähnert, 2007 & National MS Society, 2010).

2.4 DIAGNOSIS OF MULTIPLE SCLEROSIS

The criteria for diagnosis of MS were updated by the International Panel on the Diagnosis of Multiple Sclerosis in 2001, which included specific guidelines. These criteria are referred to as McDonald Criteria (National MS Society, 2010)

Table 2.1 illustrates the Diagnostic criteria based on clinical presentation.

Table 2.1: Diagnostic criteria for Multiple Sclerosis

Clinical Presentation	Additional Data Needed for MS Diagnosis
Two or more attacks; objective clinical evidence of 2 or more lesions	None[a]
Two or more; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *or* Await further clinical attack implicating a different site
One attack; objective clinical evidence of 2 or more lesions	Dissemination in time, demonstrated by MRI[d] *or* Second clinical attack
One attack; objective clinical evidence of 1 lesion (mono-symptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *and* Dissemination in time, demonstrated by MRI[d] *or* Second clinical attack
Insidious neurological progression suggestive of MS	Positive CSF[c] *and* Dissemination in space, demonstrated by 1) Nine or more T2 lesions in brain *or* 2) or more lesions in spinal cord, *or* 3) 4-8 brain plus 1 spinal cord lesion *or* abnormal VEP[e] associated with 4-8 brain lesions, or with fewer than 4 brain lesions plus 1 spinal cord lesion demonstrated by MRI *and* Dissemination in time, demonstrated by MRI[d] *or* Continued progression for 1 year

If criteria indicated are fulfilled, the diagnosis is multiple sclerosis (MS); if the criteria are not completely met, the diagnosis is "possible MS"; if the criteria are fully explored and not met, the diagnosis is "not MS".

[a]No additional tests are required however, if tests magnetic resonance imaging (MRI), cerebral spinal fluid (CSF) are undertaken and are *negative*, extreme caution should be taken before making a diagnosis of MS. Alternative diagnoses must be considered.
[b]MRI demonstration of space dissemination must fulfil the criteria (Table 2.2)
[c]Positive CSF determined by oligoclonal bands detected by established methods (preferably isoelectric focusing) different from any such bands in serum or by a raised IgG index.
[d]MRI demonstration of time dissemination must fulfil the criteria in Table 2.3.
[e]Abnormal visual evoked potential of the type seen in MS (delay with a well-preserved wave form)
(Adapted from Mc Donald et al, 2001:124).

Table 2.2: Magnetic Resonance Imaging Criteria for brain abnormality

Three of four of the following
1. One gadolinium-enhancing lesion or nine T2-hyperintense lesions if there is no gadolinium enhancing lesion
2. At least one infratentorial lesion
3. At least one juxtacortical lesion
4. At least three periventricular lesions

(Adapted from McDonald et al, 2001:123)

Table 2.3: Magnetic Resonance Imaging Criteria for Dissemination of lesions in time

1. If a first scan occurs 3 months or more after the onset of the clinical event, the presence of a gadolinium-enhancing lesion is sufficient to demonstrate dissemination in time, provided that it is not at the site implicated in the original clinical event. If there is no enhancing lesion at this time, a follow-up scan is required. The timing of this follow-up is not crucial, but 3 months is recommended. A new T2- or gadolinium-enhancing lesion at this time then fulfills the criterion for dissemination in time.
2. If the first scan is performed less than 3 months after the onset of the clinical event, a second scan done 3 months or more after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan not less than 3 months after the first scan that shows a new T2 lesion or enhancing lesion will suffice.

(Adapted from McDonald, 2001:123)

2.5 TOOLS FOR MAKING A DIAGNOSIS

- Medical History and Neurological exam (National MS Society, 2010)
- Magnetic resonance imaging (MRI):

  This is the modality of choice with 95-99% specificity. Presence of MS plaques (lesions) in different parts of the central nervous system can be detected. It can also differentiate between old, new and active lesions (Dähnert, 2007 & National MS Society, 2010).
- Visual Evoked Potential (VEP):

  These tests are recordings of the nervous system's electrical responses to the stimulation of specific sensory pathways. Evoked potential (EP) tests can sometimes provide evidence of scarring along the nerve pathways which does not show up during the neurological exam (National MS Society, 2010)
- Cerebrospinal Fluid (CSF) Analysis:

  Analysis of CSF obtained from a spinal tap can detect levels of certain immune system proteins and presence of oligoclonal bands. These bands are found in the spinal fluid of approximately 90-95% of persons with MS and indicate immune

response within the CSF. However oligoclonal bands cannot be used as positive proof of MS because they are present in other diseases as well (National MS Society, 2010).

- Blood tests (National MS Society, 2010)

2.6 CURRENT TREATMENT

There is no one specific treatment option for MS, however the use of steroids provoke a rapid decrease in size of the MS lesions and loss of enhancement (Dähnert, 2007:311).

2.6.1 Management of acute attacks

Methylprednisolone

2.6.2 Disease-modifying treatments

As of April 2013, eight disease-modifying treatments have been approved by regulatory agencies of different countries:

1. Interferon beta-1a (Avonex, Rebif, CinnoVex, ReciGen) injected 1x or 3x/wk
2. Interferon beta-1b (Betaseron) injected every second day
3. Glatiramer acetate (Copaxone) injected daily
4. Mitoxantrone (Novantrone) intravenous infusion every three months
5. Natalizumab (Tysabri) intravenous infusion at monthly intervals
6. Fingolimod (Gilenya) daily single oral dose
7. Teriflunomide (Aubagio) daily single oral dose
8. Dimethyl fumarate (BG12, Tecfidera) twice daily oral dose

2.6.3 Outcomes of Disease-modifying treatments:

- Reduced number of relapses
- Reduced number of brain lesions
- Reduced rate of disability progression

2.6.4 Problems with Disease-modifying treatments:

- Expensive
- Adverse Effects
- Don’t attenuate or reverse disability progression

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

A cross-sectional observational study was done on patients with multiple sclerosis (MS) within the Western Cape region. This was primarily to determine whether there are abnormal blood flow patterns in the extracranial cerebrospinal venous system (internal jugular and vertebral veins) and carotid arteries in the selected sample of participants using ultrasound imaging.

This ultrasound study was a sub-study of an ethically approved collaborative study between the Cape Peninsula University of Technology, the National Health Laboratory Service and the University of Stellenbosch entitled: *"The development of a comprehensive gene-based, pathology supported intervention program for improved quality of life in patients diagnosed with multiple sclerosis"* (Division of Chemical Pathology, NHLS, Tygerberg Hospital, and University of Stellenbosch). Multiple Sclerosis (MS) patients were recruited by the principal investigator of the main study as well as via electronic media and word of mouth. Ultrasound examinations of these patients were performed at a private Radiology practice within the Western Cape, South Africa.

Data collection, including the ultrasound examinations of the participants took place during the period December 2011 and December 2012.

3.1 RESEARCH QUESTION

What is the prevalence of abnormal blood flow, demonstrated by ultrasound, in the extracranial cerebrospinal venous system and carotid arteries in patients with multiple sclerosis (MS)?

3.2 RESEARCH OBJECTIVES

The main objectives of the present study were to establish with ultrasound:

- The prevalence of thrombus, reflux, and stenosis of the proximal, mid and distal segments of the right and left internal jugular vein (IJV).
- The prevalence of stenosis, occlusion, plaque formation, and increased intima media thickness of the right and left common carotid artery (CCA).
- The prevalence of stenosis, occlusion and plaque formation of the right and left internal carotid artery (ICA) and external carotid artery (ECA).
- The prevalence of stenosis, occlusion, plaque formation and reversed flow of the right and left vertebral artery.

- A significant difference in the ultrasound findings of MS patients on the nutritional programme (Appendix A) versus those MS patients not on the nutritional programme.
- Correlation between biochemical markers (homocysteine and fibrinogen) and ultrasound findings.
- Effects of lifestyle factors (smoking and physical activity) on the Internal Jugular Veins and Carotid arteries in MS patients.

3.3 SAMPLE

3.3.1 Sample size

A minimum of 25 patients, as calculated using specified formula, were required to demonstrate significant results, however 29 patients were included in the study.

The formula used to calculate the minimum sample (n) was:

$n = 4(p)(1-p)/\text{interval}^2$

$n = 4(0.5)(1-0.5)/(0.2)^2$

$=4(0.5)(0.5)/0.04$

$=1/0.04$

$=25$

n – sample size

p – observed population (0.5=50%)

confidence interval – 0.2 (20%)

3.3.2 Inclusion criteria

Patients diagnosed with MS, according to the criteria of McDonald et al (2001), by their neurologists were included in this study. Written and signed informed consent (Appendix B) was obtained from participating subjects. Male and female participants from all race groups between 20 and 65 years of age were included in the study.

3.3.3 Exclusion criteria

Patients diagnosed with other neurological diseases namely neuromyelitis optica and Acute Disseminated Encephalomyelitis (ADEM) were excluded from the study as well as patients previously diagnosed with carotid artery and extracranial venous disease.

3.4 ULTRASOUND AND IMAGING EQUIPMENT

The GE Logiq 9 and GE Logiq E9 ultrasound machines with Doppler software (B-mode imaging, Colour Doppler, Power Doppler and pulsed-wave Doppler) and 9-12 multifrequency linear transducer was used to image the carotid arteries and extracranial venous system. A high frequency linear ultrasound transducer was used to optimally visualise the superficial vessels, namely the carotid arteries, internal jugular veins and vertebral veins. B-mode imaging was used to interrogate the major neck vessels for tortuosity, anatomical variation, plaque formation in the carotid arteries, measure intima media thickness of the common carotid artery and cross-sectional diameter of the internal jugular veins. Colour Doppler was used to assess the vessels for patency, direction of blood flow within the vessel and detect an occlusion if present. Power Doppler is used to detect trickle or slow flow if a carotid occlusion was suspected, however Power Doppler was not used in this study. Pulsed-wave Doppler was used to detect carotid stenosis by measuring the speed of blood flow in systole and diastole within the carotid vessel being sampled. The Pulsed Repetition Frequency for this study was set as 150cm/s for assessment of the carotid vessels. Waveforms of the carotid vessels were also illustrated using pulsed-wave Doppler, and an increase in velocity measurements results in dampening of the waveform.

The captured ultrasound images were stored onto the ultrasound system's hard drive and hard copies of the images were printed on A3 paper using the Xerox (Phaser 7760) Colour Laser printer.

3.5 ULTRASOUND EXAMINATION

The As Low As Reasonably Achievable (ALARA) Doppler ultrasound thermal index of <100mW/cm^2 was maintained at all times to prevent the possible adverse biological effects of Doppler ultrasound which may include heating and cavitations of tissues (Kremkau, 2006: 352).

3.5.1 Carotid Ultrasound protocol

The carotid and vertebral arteries were imaged using B-mode ultrasound, colour Doppler and pulsed-wave Doppler. The vessels were assessed for plaque formation, patency and stenosis.

The carotid ultrasound findings for each participant were captured onto a template (See Appendix C).

Table 3.1 illustrates the documented ultrasound findings with the normal and abnormal values.

Table 3.1: Carotid Ultrasound findings

DOCUMENTED ULTRASOUND FINDING	NORMAL and ABNORMAL VALUES
Intima media thickness (IMT) of CCA	IMT >0.8 is regarded as abnormal (Carroll, 2005:946)
ICA (internal carotid artery)	Peak systolic velocity (PSV) should be <125cm/s with a high end diastolic velocity (EDV) (Myers & Clough, 2004:104)
CCA (common carotid artery)	The CCA is regarded as 50% stenotic if the PSV is increased more than twofold in comparison with the proximal velocity (Myers & Clough, 2004:107)
ECA (external carotid artery)	The PSV should not exceed 200cm/s (Carroll, 2005:959)
Peak systolic velocity ratios of ICA/CCA	A ratio of <2 is associated with a 0-49% ICA stenosis. A ratio that exceeds 3.5 corresponds to >75% stenosis (Carroll, 2005:959 & NASCET, 1991).
End diastolic velocity of ICA	An end-diastolic velocity of <40cm/s is indicative of 0-49% ICA stenosis (NASCET, 1991)
Plaque formation (location, size and morphology)	
Location and degree of stenosis if present	
Occlusion/s if present	

The North American Symptomatic Carotid Endarterectomy Trials (NASCET) criteria was used to grade internal carotid artery stenosis, where a stenosis ≥ 70% is regarded as significant (NASCET, 1991) (see Appendix D).

3.5.1.1 Scanning technique of the carotid artery:

The examination is performed with the patient in the supine position using a linear multifrequency (9-12MHz) ultrasound transducer and coupling gel (Figure 3.1).

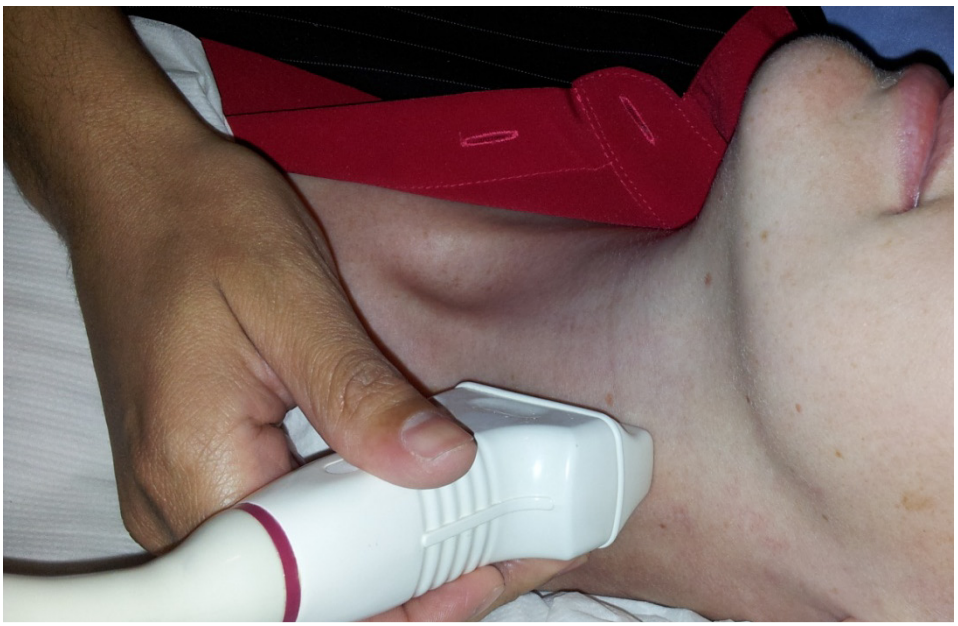

Figure 3.1: Carotid Ultrasound Technique.
Patient in supine position with transducer in longitudinal plane assessing the CCA.

3.5.1.2 B-mode imaging of the carotid artery:

Using B-mode imaging the CCA is assessed in the transverse and longitudinal planes from the supraclavicular notch up to its bifurcation, and along the ICA and ECA as high distally as can be seen as seen in Figure 3.2. This assessment is performed to identify any anatomical variation, tortuosity and plaque formation.

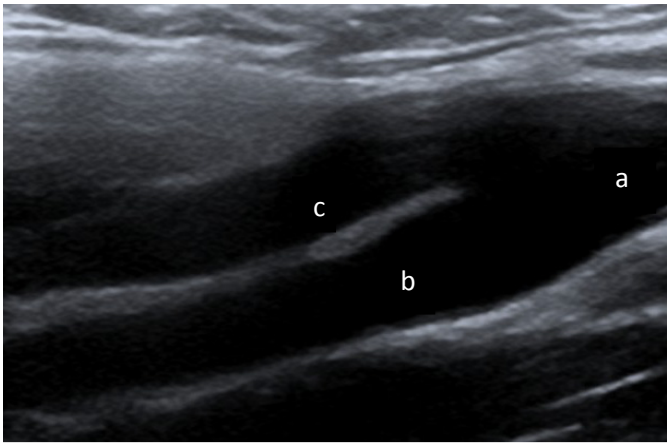

Figure 3.2: B-mode image of a normal Carotid Artery in the longitudinal plane.
Carotid bulb**-** a, Internal Carotid Artery – b, External Carotid Artery – c (Permission granted by patient #26)

An average IMT over a 2cm segment of the mid CCA is measured in the longitudinal plane (Figure 3.3). An IMT of 0.8mm is regarded as upper limit of normal. The ultrasound machine has a built-in software programme which calculates the average IMT of the segment sampled.

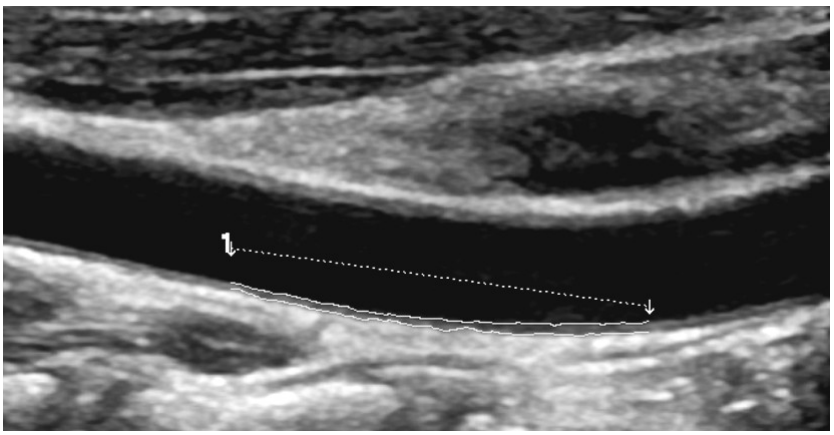

Figure 3.3: B-mode image of a normal Right Common Carotid Artery (CCA) in the longitudinal plane.
Average IMT=0.42mm (upper limit of normal = 0.8mm)
(Permission granted by patient #7).

3.5.1.3 Colour Doppler of the carotid artery:

Colour Doppler is useful in assessing patency of the vessel, direction of blood flow in the vessel as well as detecting areas of abnormal flow within the vessel.

Whilst scanning in the longitudinal plane, colour Doppler was activated and the CCA, ICA, ECA and vertebral artery was assessed. The gain should be adjusted until the colour is displayed only within the vessel lumen. The colour box should be parallel to the vessel being sampled. If no colour is detected within a vessel using the optimised colour Doppler, then the power Doppler should be activated to determine whether the vessel is occluded or trickle flow is present.

3.5.1.4 Pulsed-wave (PW) Doppler of the carotid artery:

After the B-mode and Colour Doppler imaging, PW Doppler is used to measure the peak systolic velocity (PSV) and end diastolic velocity (EDV) of the CCA, ICA, ECA and VA.

A Doppler angle of 60° and a sample volume (SV) of 2mm is used to reproduce an accurate velocity measurement. The angle cursor should be parallel to the wall of the vessel segment being sampled as depicted in Figure 3.4. At least 3 waveforms of each vessel should be captured and recorded.

A spectral waveform of the mid CCA (Figure 3.4) approximately 3cm distal to the carotid bulb is recorded and the proximal and mid segments of the ICA (Figure 3.5) and ECA (Figure 3.7) if optimally visualised. If the carotid bifurcation is at the level of the angle of mandible then only the proximal ICA and ECA can be optimally assessed. The PSV and EDV of the mid VA (Figure 3.6) are recorded.

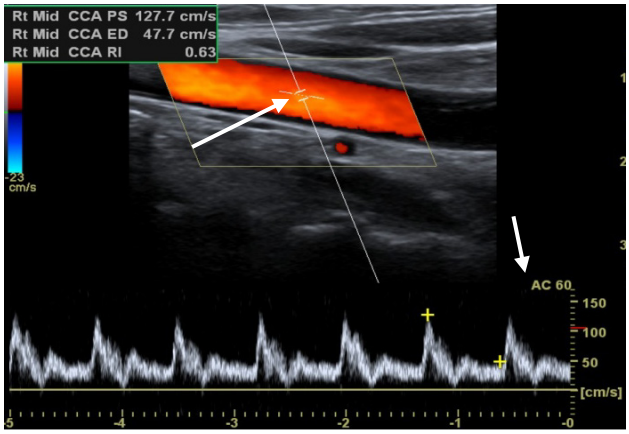

Figure 3.4: Colour Doppler and Spectral analysis of a normal Right Common Carotid Artery (CCA) at a Doppler angle of 60 degrees (arrow).
The Peak Systolic Velocity = 127.7cm/s, End Diastolic Velocity = 47.7cm/s. (Permission granted by patient #17)

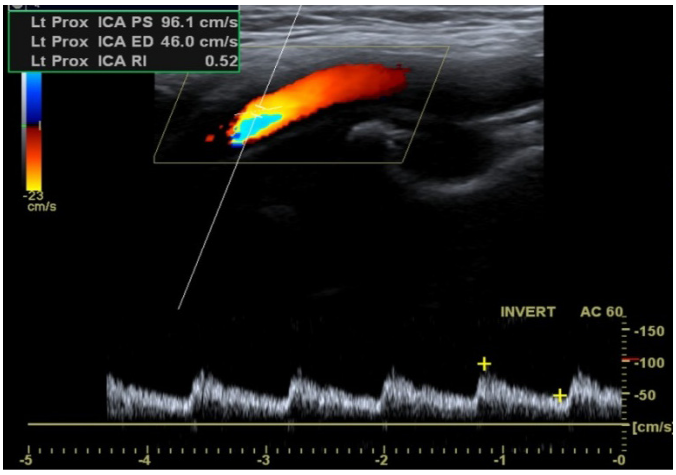

Figure 3.5: Colour Doppler and Spectral analysis of a normal Left Internal Carotid Artery (ICA).
Peak Systolic Velocity (PSV) = 96.1cm/s, End Diastolic Velocity (EDV) = 46cm/s. Mosaic colours depict aliasing due to tortuosity of vessel. (Permission granted by patient #20)

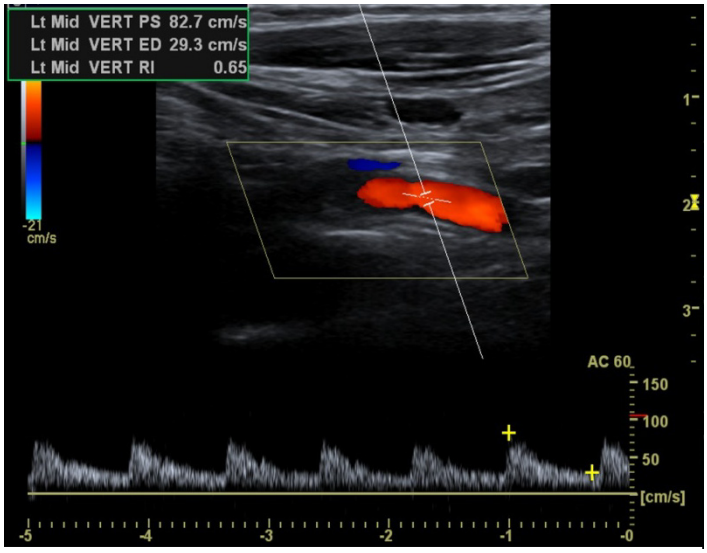

Figure 3.6: Colour Doppler and Spectral analysis of a normal Left Vertebral Artery (VA).
PSV = 82.7cm/s, EDV=29.3cm/s (Permission granted by patient #15)

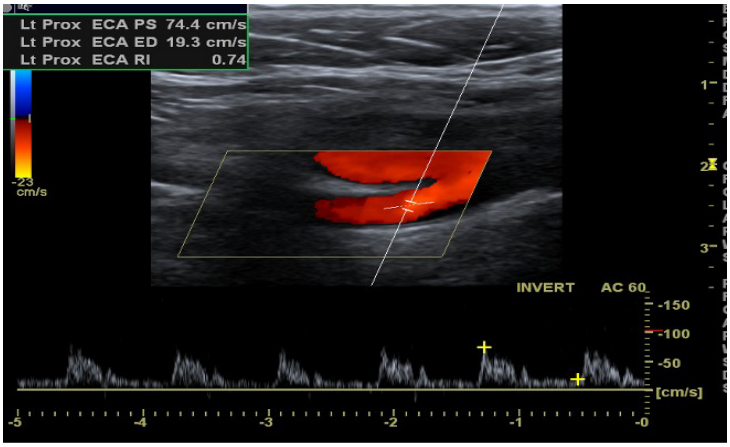

Figure 3.7: Spectral Analysis of a normal Left proximal External Carotid Artery (ECA).
PSV=74.4cm/s, EDV=19.3cm/s (Permission granted by patient #1)

3.5.2 Extracranial cerebrospinal venous ultrasound protocol

For this examination the right and left internal jugular and vertebral veins were imaged using B-mode ultrasound, colour Doppler and pulsed-wave Doppler.

The internal jugular vein was examined with the patient in the supine position (Figure 3.8) as this is the dominant outflow pathway with the patient in this position and can thus be easily visualised.

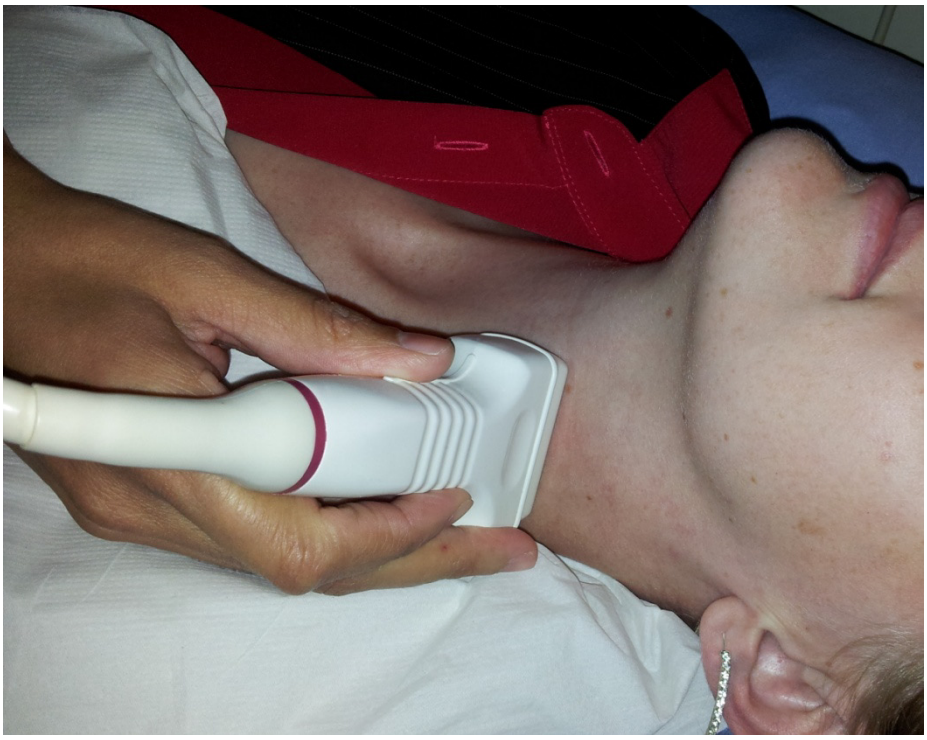

Figure 3.8: Ultrasound examination of the IJV (Internal Jugular Vein). Patient in supine position with transducer in transverse plane assessing the right IJV.

The vertebral vein was examined with the patient in the semi-erect position (Figure 3.9) as it is the dominant outflow pathway with patient sitting and because the vein is more prominent in this position it can thus be easily seen and imaged.

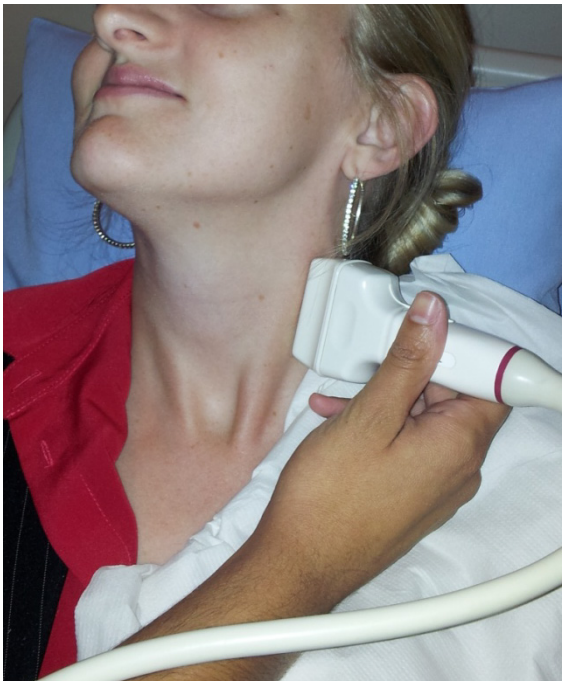

Figure 3.9: Ultrasound examination of the Vertebral Vein.
Patient in semi-erect position with transducer in longitudinal plane assessing the VV for patency.

The IJV was assessed for patency, stenosis and reflux, whilst the VV was assessed for patency and reflux.

The following cerebrospinal venous ultrasound findings of each participant were captured onto a template. (See Appendix E):

- Patency of the proximal, mid and distal right and left internal jugular veins. Patency of the right and left vertebral veins.
- Presence of non-occlusive and occlusive thrombus within the internal jugular and vertebral veins.
- Reflux in the proximal, mid and distal right and left internal jugular veins. Reflux > 0.88s was regarded as abnormal (Zamboni et al, 2009:393).

- Cross-sectional diameters of the proximal, mid and distal right and left internal jugular veins to assess for stenosis. The mean cross-sectional diameter of the right IJV in men is 1.48cm and left IJV is 1.37cm, whilst in women the mean cross-sectional diameter (CSD) of the right IJV is 1.27cm and the left IJV 1.17cm (Khatri et al, 2001:286).

3.5.3 Scanning technique of the internal jugular vein:

A linear multifrequency (9-12MHz) ultrasound transducer is used with coupling gel.

3.5.3.1 B-mode imaging of the internal jugular vein:

The IJV is assessed with the patient in the supine position and the head slightly extended. The entire IJV from the supraclavicular notch to the angle of mandible is interrogated in longitudinal and transverse planes, keeping probe pressure on the vessel to a minimum. Any thrombus, stenoses or valve abnormalities are captured and documented.

The cross-sectional diameter (antero-posterior) of the IJV at the level of the supraclavicular notch, midway between the supraclavicular notch and angle of mandible, and at the level of the angle of mandible is measured from wall to wall and recorded (See Figure 3.10).

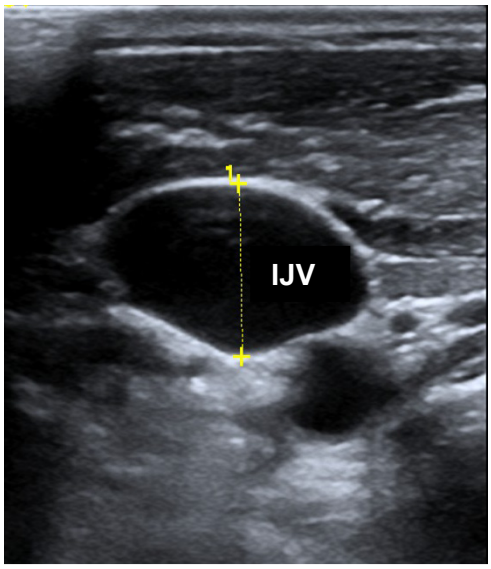

Figure 3.10: Cross-sectional Diameter (CSD) of the mid right Internal Jugular Vein (IJV).
CSD measures 1.25cm. (normal)
(Permission granted by patient #26)

3.4.3.2 Colour Doppler of the internal jugular vein:

The proximal, mid and distal segments of the IJV are assessed with the colour box parallel to the vessel being sampled. Colour Doppler is used to identify any filling defects as well as abnormal flow within the vessel. Whilst Colour Doppler is activated, the patient is asked to do

the valsalva manoeuvre to determine if there is reflux/reversed flow in the segment of the IJV being sampled. The same technique is applied to the vertebral vein; however the patient is in the semi-erect position.

3.5.3.3 Pulsed wave (PW) Doppler of the internal jugular vein:

Evaluation by PW Doppler includes sampling the IJV segments with the patient in the supine position using a Doppler angle of 60° and a sample volume of size of 2mm. The Doppler angle should be parallel to the vessel wall and the centre of the vessel lumen should be sampled as seen in Figure 3.11. The same technique is used for assessment of the vertebral vein (Figure 3.12); however the patient is in the semi-erect position.

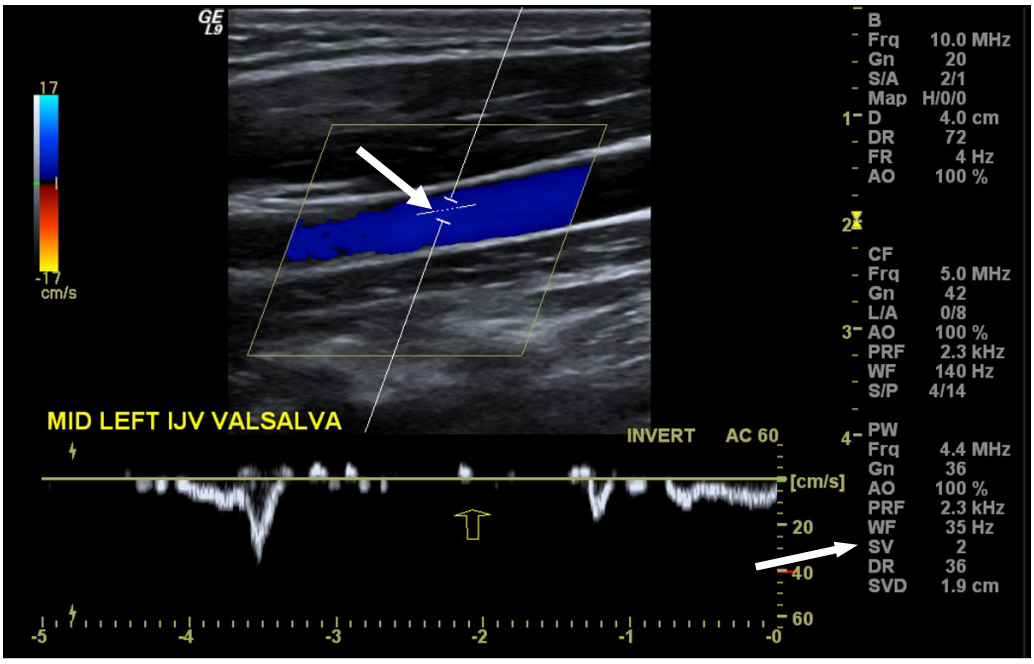

Figure 3.11: Colour and PW Doppler of the Left Internal Jugular Vein (IJV) during valsalva.
The vein is patent with normal directional flow away from the brain (blue). The sample volume (SV) is at 2mm (*solid arrows).* During valsalva there is cessation of flow within the mid segment of the IJV (*block arrow).* (Permission granted by patient #18)

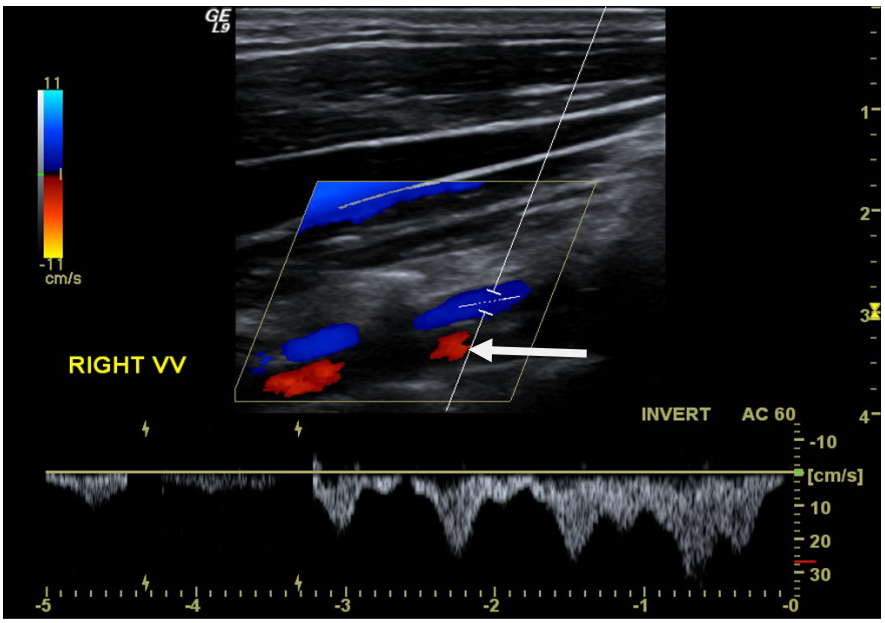

Figure 3.12: Right Vertebral Vein (VV).
Colour and Pulsed-wave Doppler demonstrates a patent VV (blue) with normal retrograde flow (away from the brain). Adjacent to the VV is the vertebral artery (VA) *(solid arrow*) which on Colour Doppler appears patent with antegrade flow (towards the brain). (Permission granted by patient #18)

Each ultrasound examination (Carotid and Extracranial Venous Ultrasound) took approximately 40 minutes. All ultrasound examinations were done by the researcher, who is an experienced vascular sonographer. All cases were reported by a Radiologist and participants with abnormal findings would be referred to their respective Neurologist for further management.

3.6. PATIENT DATA COLLECTION

The participants' age, gender and ultrasound findings were captured from the ultrasound data sheets. Biochemical data and lifestyle factors such as physical activity and smoking were accessed from the main study, and were entered into a Microsoft Excel database. The number of MS patients on and not on the nutritional programme and number of years with MS were also entered into the database.

3.7 BIOCHEMICAL ANALYSIS

Blood was drawn for biochemistry testing in the morning between 9h00 and 10h30 to standardise for diurnal variation. Plasma homocysteine was measured in 60 patients and 87 controls using a Siemens Centaur XP auto-analyser. Fibrinogen was determined by the Hemos IL-Fibrinogen C method using an ACL TOP (Beckman Coulter SA).

3.8 DATA ANALYSIS

Mixed model repeated measures ANOVA was used to compare left and right vessel measurements. One way ANOVA was used for comparison of measurements between groups (on nutritional programme and not on programme, smoking and non-smoking MS patients). ANOVA F-test and the Mann-Whitney U test were used to test the same hypothesis. Spearman correlations were used for testing relationships between biochemical variables and ultrasound measurements.

3.9 ETHICS

The present study was granted approval by the Faculty Research Committee (FRC) of the Cape Peninsula University of Technology (CPUT). Ethical approval was granted by the Faculty Ethics Research Committee (Registration Number NHREC: REC-230408-014) (Appendix F). Informed consent was obtained from each participant, stating that withdrawal from the study at anytime is their prerogative. Patients were asked to read and sign an informed consent form which was available in 2 languages (English and Afrikaans) (see Appendix B). The signed consent forms were kept as part of the study documentation. The participants were given an information leaflet explaining the study and giving contact details if they wished to contact the relevant persons.

A radiology service was provided to all participants and each ultrasound examination was reported by a radiologist. Because of the system used in the private radiology practice, each participant's name had to be entered onto the Picture Archives and Communication System (PACS) which enabled the radiologist to identify the patient and dictate a report. This prevents the patient from receiving the incorrect report which will result in an incorrect ultrasound diagnosis. However the MS status and data information of the participants were not captured onto the PACS system as it was only available to the principal researcher, supervisors and statistician involved in the study.

Patients who presented with an abnormal finding of Internal Jugular Vein stenosis, deep vein thrombosis and/or significant carotid artery disease were referred by the reporting Radiologist to their respective Neurologist for management thereof.

Patient confidentiality was maintained at all times and the research participants' rights with regard to respect for human dignity, the safeguarding of confidentiality or anonymity, and the right to information was upheld.

Only the researcher involved in the study had access to data and participant information. The principal researcher was responsible for inventory and organisation of the data collection forms. Participants were assigned a study number which was used on all study forms. Data with participant code/number was only accessible to the supervisors and statistician.

The As Low as Reasonable Achievable (ALARA) Doppler ultrasound thermal index of <100mW/cm^2 was maintained at all times. Biological effects of Doppler ultrasound may include heating and cavitations of tissues. However, evidence has shown that there are no known biological effects when diagnostic imaging ultrasound machines are used appropriately by trained sonographers (Fowlkes & Holland, 1998:53).

Ethical considerations according to CPUT research ethics guidelines have been strictly adhered to throughout the study.

Permission, in writing, to scan the MS patients at Drs Bergman, Ross and Partners Radiologists (previously known as Drs Symington and partners Radiologists) has been granted (Appendix G).

CHAPTER FOUR

RESULTS

Twenty-nine (n=29) MS patients with mean age of 47.72 were assessed. The patients suffered from multiple sclerosis for 0.83-27years. The clinical characteristics of the patients are illustrated in Table 4.1.

Table 4.1: Clinical Characteristics

Number of patients	29
Age	28-64yrs (47.72 mean ± 5.5)
Sex	26 females, 3 males
Race	22 Caucasian, 7 Mixed ancestry

4.1 The prevalence of thrombus, reflux and stenosis of the proximal, mid and distal segments of the right and left Internal Jugular Veins (IJVs)

4.1.1 Thrombus

B-mode, pulsed-wave (PW) and Colour Doppler ultrasound demonstrated no thrombus within the proximal, mid and distal segments of the right and left internal jugular veins (IJVs).

4.1.2 Reflux (reversed flow)

Colour and PW Doppler ultrasound imaging demonstrated reversed flow of >0.88s in IJV in (four) 13.79% of MS patients. No vertebral vein reflux was identified.

4.1.3 Stenosis

No stenosis of the internal jugular veins (IJVs) was demonstrated on B-mode, Colour or PW Doppler ultrasound imaging. However, a larger proximal and mid cross-sectional diameter (CSD) of the right IJV compared to the left (difference significant, P= 0.026 and P=0.023) was demonstrated, which is similar to data found in the literature (Khatri et al, 2001:286).

The mean cross-sectional diameter of the right IJV was 8.79mm +/-1.67 (SD) and the left IJV was 7.96mm +/-1.26 (SD) in the 26 female subjects. In the 3 male subjects (10.34%) the mean cross-sectional diameter of the right IJV was 11.05mm +/-2.27 (SD) and of the left IJV was 7.36mm +/-1.78 (SD) as seen in Figure 4.1.

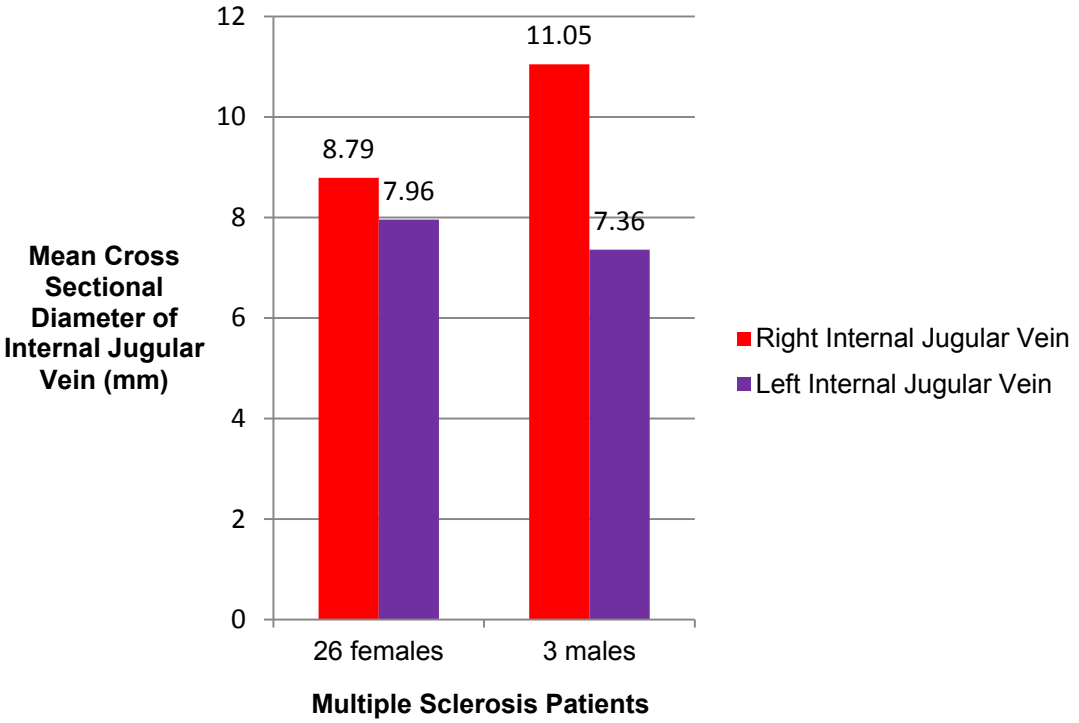

Figure 4.1: Cross-sectional Diameters of the Internal Jugular Veins.

4.2 The prevalence of stenosis, occlusion, plaque formation, and increased intima media thickness (IMT) of the right and left common carotid artery (CCA).

4.2.1 Stenosis

The right and left common carotid artery (CCA) displayed no stenosis on B-mode, Colour and PW Doppler ultrasound imaging. No blood low disturbances were identified.

4.2.2 Occlusion

The right and left CCAs were patent throughout with normal antegrade flow. No evidence of absent CCA blood flow was identified with B-mode, Colour and PW Doppler ultrasound imaging.

4.2.3 Plaque formation

No calcified, echogenic, smooth/soft or ulcerated plaque was noted in the right or left CCA in the 29 MS patients.

4.2.4 Intima media thickness (IMT) of the right and left common carotid artery (CCA)

Four (13.79%) MS patients displayed an increased CCA IMT of >0.8mm on B-mode ultrasound imaging. The increased IMT ranged between 0.9 -1.0mm.

4.3 The prevalence of stenosis, occlusion and plaque formation of the right and left internal carotid artery (ICA) and external carotid artery (ECA)

4.3.1 Stenosis of ICA and ECA

No evidence of vessel narrowing or increased PSV and EDV of the ICA (PSV>125cm/s, EDV>100cm/s) and ECA (PSV>200cm/s) was identified on B-mode, Colour and PW Doppler imaging.

4.3.2 Occlusion of ICA and ECA

All 29 MS patients displayed patent ICAs and ECAs on B-mode, Colour and PW Doppler ultrasound imaging. No vessel filling defect was identified.

4.3.3 Plaque formation of the ICA and ECA

Echogenic plaque in the ICA was identified in two (6.89%) of the 29 MS patients on B-mode ultrasound imaging. This small amount of plaque did not cause any disturbances to blood flow. No plaque formation was noted in the ECAs.

4.4 The prevalence of stenosis, occlusion, plaque formation and reversed flow of the right and left vertebral artery.

The right and left vertebral arteries in 29 MS patients were patent throughout with normal antegrade flow. The average PSV of the right vertebral artery was 49.14cm/s and the left 54.99cm/s. No evidence of stenosis, occlusion, plaque formation and reversed flow was identified on B-mode, Colour and PW Doppler ultrasound imaging.

4.5 Difference in the ultrasound findings of MS patients on the nutritional programme versus those MS patients not on the nutritional programme

Only 8 MS patients were on the nutritional programme, whilst the remaining 21 patients were not.

4.5.1 Cross-sectional Diameter (CSD) of the Internal Jugular Vein (IJV)

No significant differences ($p>0.05$) were illustrated in the tabulated CSD of the IJV in patients on and off the nutritional programme (Table 4.2)

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Table 4.2: Cross-sectional diameter of the IJV

CSD of IJV	p-value
Proximal right IJV CSD	0.32
Mid right IJV CSD	0.39
Distal right IJV CSD	0.34
Proximal Left IJV CSD	0.79
Mid left IJV CSD	0.71
Distal left IJV CSD	0.31

4.5.2 Ultrasound findings of Carotid Arteries

No significant difference ($p>0.05$) was illustrated in the tabulated Carotid ultrasound findings of patients on and off the nutritional programme (Table 4.3).

Table 4.3: Carotid Ultrasound variables

Ultrasound variable	p-value
Right CCA IMT	0.24
Right ICA PSV	0.23
Right ICA EDV	0.59
Right ECA PSV	0.98
Right VA PSV	0.64
Right ICA/CCA	0.12
Left CCA IMT	0.32
Left CCA PSV	0.64
Left CCA EDV	0.53
Left ICA PSV	0.75
Left ICA EDV	0.42
Left ECA PSV	0.34
Left VA PSV	0.88
Left (PSV) ICA/CCA	0.98
Left (EDV) ICA/CCA	0.57

4.5.3 Right common carotid artery (CCA) peak systolic velocity (PSV)

Figure 4.2 illustrates a significant difference (Mann-Whitney U test, $p=0.03$) in the PSVs of the right CCA in MS patients not on the nutritional programme versus those on the nutritional programme. The speed of blood flow within the CCA in systole was thus significantly higher in MS patients not on the nutritional programme.

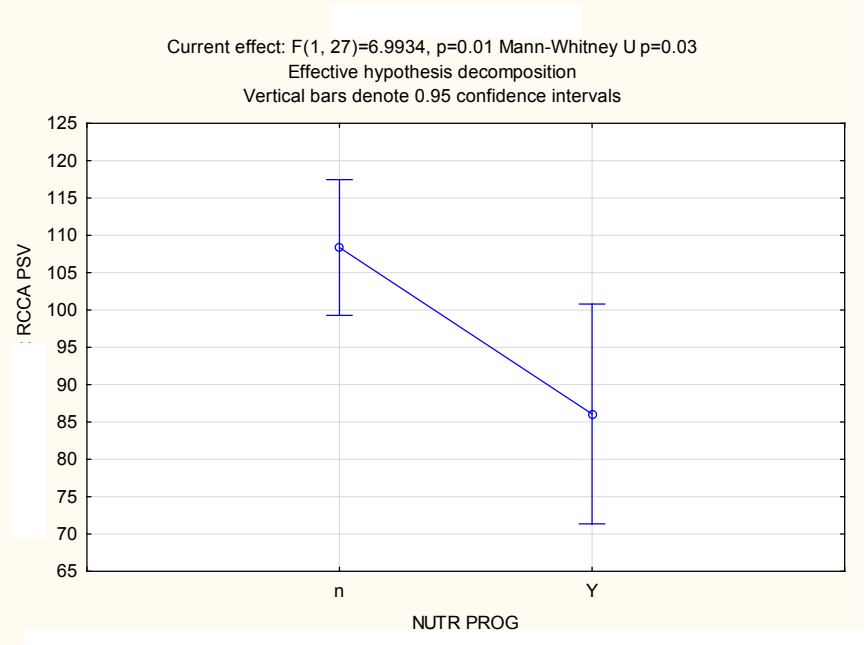

Figure 4.2: Higher PSV in the right CCA in MS patients not on the nutritional programme versus those on the programme.

n=MS patients not on nutritional programme, Y=MS patients on the nutritional programme, NUTR PROG=nutritional programme, CCA=common carotid artery, R=right, PSV=peak systolic velocity. There was a significant difference between the 2 groups (p=0.03). Maximum PSV of those not on the programme ~118cm/s, Maximum PSV of those on the programme~105cm/s

4.5.4 Right common carotid artery (CCA) end diastolic velocity (EDV)

Figure 4.3 illustrates a significant difference (Mann-Whitney U test, $p<0.01$) in the EDVs of MS patients on the nutritional programme versus those not on the programme. The speed of blood flow within the right common carotid artery in diastole was thus significantly lower in the group of MS patients on the nutritional programme.

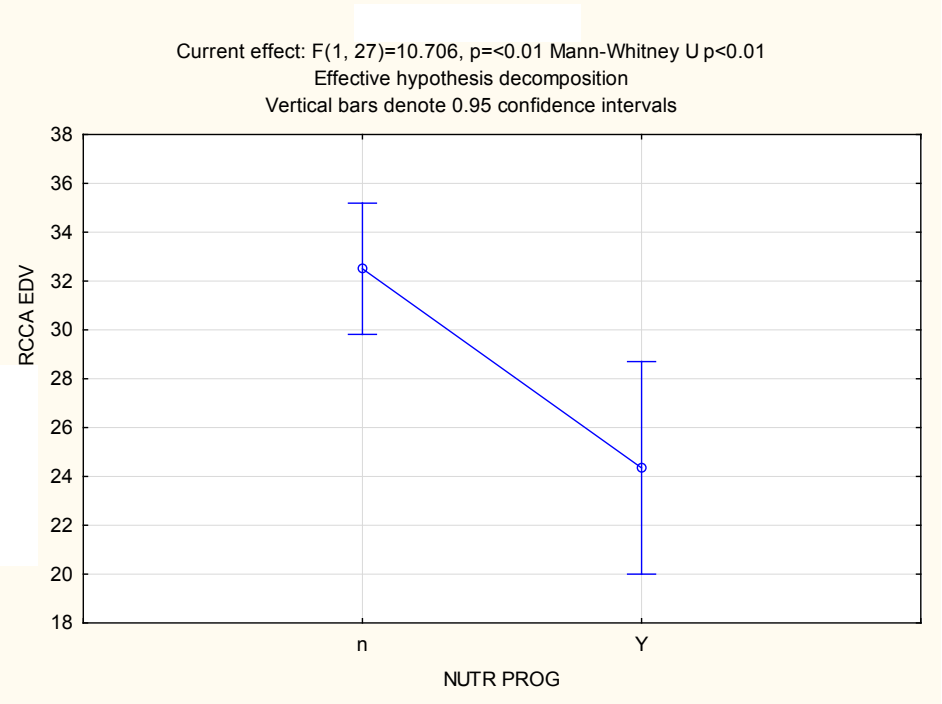

Figure 4.3: Higher EDV in the right CCA in MS patients not on the nutritional programme versus those on the programme.
n=MS patients not on nutritional programme, Y=MS patients on the nutritional programme, NUTR PROG=nutritional programme, CCA=common carotid artery, EDV=end-diastolic velocity, R=right. There was a significant difference between the 2 groups (p<0.01). Maximum EDV of those not on the programme~35cm/s. Maximum EDV of those on the programme~28.5cm/s

4.6 Correlation between biochemical markers (homocysteine and fibrinogen) and ultrasound findings

4.6.1 Homocysteine and Ultrasound findings

Homocysteine assessments were only available for 20 MS patients. Table 4.4 illustrates that there was a significant correlation between homocysteine and the right ECA PSV (Spearman p-value <0.01).

4.6.2 Fibrinogen and Ultrasound findings

Fibrinogen assessments were available for 16 MS patients. Table 4.5 illustrates that there was a significant correlation between fibrinogen and the left ECA PSV (Spearman p-value 0.03).

Table 4.4: Correlation between Homocysteine and Ultrasound findings

	Biochemical variable	Ultrasound variable	Spearman	Spearman p-value	# cases
1	Homocysteine	PROX IJV CSD(R)	-0.20	0.40	20
2	Homocysteine	MID IJV CSD (R)	-0.10	0.69	20
3	Homocysteine	DIST IJV CSD (R)	-0.05	0.83	20
4	Homocysteine	PROX IJV CSD(L)	-0.45	0.05	20
5	Homocysteine	MID IJV CSD (L)	-0.31	0.18	20
6	Homocysteine	DIST IJV CSD (L)	-0.23	0.32	20
7	Homocysteine	CCA IMT (R)	0.22	0.36	20
8	Homocysteine	CCA PSV (R)	-0.31	0.18	20
9	Homocysteine	CCA EDV (R)	-0.05	0.83	20
10	Homocysteine	ICA PSV (R)	0.23	0.33	20
11	Homocysteine	ICA EDV (R)	0.18	0.44	20
12	Homocysteine	ECA PSV (R)	-0.58	<0.01	20
13	Homocysteine	VA PSV (R)	0.02	0.94	20
14	Homocysteine	PSV ICA/CCA (R)	0.39	0.09	20
15	Homocysteine	EDV ICA/CCA (R)	0.35	0.13	20
16	Homocysteine	CCA IMT (L)	0.07	0.79	20
17	Homocysteine	CCA PSV (L)	-0.41	0.07	20
18	Homocysteine	CCA EDV (L)	0.03	0.91	20
19	Homocysteine	ICA PSV (L)	-0.16	0.51	20
20	Homocysteine	ICA EDV (L)	-0.06	0.81	20
21	Homocysteine	ECA PSV (L)	-0.30	0.20	20
22	Homocysteine	VA PSV (L)	0.11	0.65	20
23	Homocysteine	PSV ICA/CCA (L)	0.27	0.25	20
24	Homocysteine	EDV ICA/CCA (L)	-0.21	0.37	20

(R) = right, (L) = left, PROX= proximal, MID = mid, DIST= distal, IJV=internal jugular vein, CSD=cross-sectional diameter, CCA= common carotid artery, ICA=internal carotid artery, IMT=intima media thickness, PSV=peak systolic velocity, EDV=end diastolic velocity, VA=vertebral artery

Table 4.5: Correlation between Fibrinogen and Ultrasound findings

	Biochemical variable	Ultrasound variable	Spearman	Spearman p-value	# cases
1	Fibrinogen	PROX IJV CSD(R)	-0.38	0.14	16
2	Fibrinogen	MID IJV CSD(R)	-0.36	0.17	16
3	Fibrinogen	DIST IJV CSD(R)	-0.40	0.12	16
4	Fibrinogen	PROX IJV CSD(L)	-0.05	0.86	16
5	Fibrinogen	MID IJV CSD(L)	0.02	0.94	16
6	Fibrinogen	DIST IJV CSD(L)	-0.19	0.49	16
7	Fibrinogen	CCA IMT(R)	-0.25	0.35	16
8	Fibrinogen	CCA PSV(R)	0.49	0.05	16
9	Fibrinogen	CCA EDV(R)	0.45	0.08	16
10	Fibrinogen	ICA PSV(R)	0.25	0.35	16
11	Fibrinogen	ICA EDV(R)	0.22	0.41	16
12	Fibrinogen	ECA PSV(R)	0.11	0.69	16
13	Fibrinogen	VA PSV(R)	-0.32	0.23	16
14	Fibrinogen	PSV ICA/CCA(R)	-0.42	0.11	16
15	Fibrinogen	EDV ICA/CCA(R)	-0.14	0.59	16
16	Fibrinogen	CCA IMT(L)	-0.46	0.07	16
17	Fibrinogen	CCA PSV(L)	0.28	0.30	16
18	Fibrinogen	CCA EDV(L)	0.46	0.07	16
19	Fibrinogen	ICA PSV(L)	0.23	0.39	16
20	Fibrinogen	ICA EDV(L)	0.39	0.13	16
21	Fibrinogen	ECA PSV(L)	0.54	0.03	16
22	Fibrinogen	VA PSV(L)	0.15	0.58	16
23	Fibrinogen	PSV ICA/CCA(L)	-0.03	0.93	16
24	Fibrinogen	EDV ICA/CCA(L)	0.01	0.98	16

(R) = right, (L) = left, PROX= proximal, MID = mid, DIST= distal, IJV=internal jugular vein, CSD=cross-sectional diameter, CCA= common carotid artery, ICA=internal carotid artery, IMT=intima media thickness, PSV=peak systolic velocity, EDV=end diastolic velocity, VA=vertebral artery

4.7 Effects of lifestyle factors (smoking and physical activity) on the Internal Jugular Veins and Carotid arteries in MS patients.

4.7.1 Smoking: CSD of IJV and Carotid arteries

No significant differences ($p>0.05$) were demonstrated in the tabulated CSD of the IJV and Carotid arteries in smoking versus non-smoking MS patients (Table 4.6).

Table 4.6: Smoking versus Non-smoking MS patients

ULTRASOUND VARIABLE	p-value
PROX IJV CSD(R)	0.48
MID IJV CSD(R)	0.19
DIST IJV CSD(R)	0.34
DIST IJV CSD(L)	0.14
CCA IMT(R)	0.26
CCA PSV(R)	0.83
CCA EDV(R)	0.93
ICA PSV(R)	0.76
ICA EDV(R)	0.93
ECA PSV(R)	0.34
VA PSV(R)	0.57
PSV ICA/CCA(R)	0.79
EDV ICA/CCA(R)	0.60
CCA IMT(L)	0.24
CCA PSV(L)	0.66
CCA EDV(L)	1.00
ICA PSV(L)	0.54
ICA EDV(L)	0.14
ECA PSV(L)	0.66
VA PSV(L)	0.43
PSV ICA/CCA(L)	0.90
EDV ICA/CCA(L)	0.29

R=right, L=left, PROX=proximal, DIST=distal, IJV=internal jugular vein, CSD=cross-sectional diameter, CCA=common carotid artery, ECA=external carotid artery, ICA=internal carotid artery, VA=vertebral artery, IMT=intima media thickness, PSV=peak systolic velocity, EDV=end-diastolic velocity

However, a significant difference (Mann-Whitney U test; p=0.03, p=0.02) was demonstrated in the CSD of the proximal and mid left IJV in smokers versus non-smokers as seen in Figure 4.4 and 4.5.

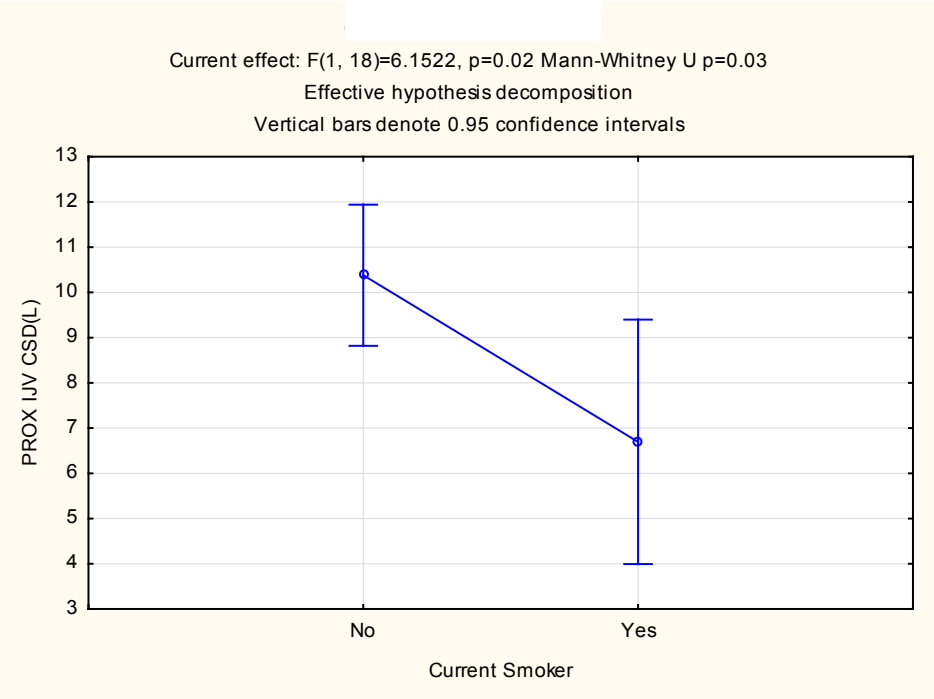

Figure 4.4: CSD of the proximal left IJV of smoking MS patients versus non-smoking MS patients.
PROX=proximal, IJV=internal Jugular Vein, L=left, CSD=cross-sectional diameter, no=non-smoking MS patients, yes=smoking MS patients. There is a significant difference between the 2 groups (Mann-Whitney U test, p=0.03). The maximum CSD of the left proximal IJV in non-smoking MS patients~12mm (1.20cm). The maximum CSD of the left proximal IJV in smoking MS patients~9.5mm (0.95cm).

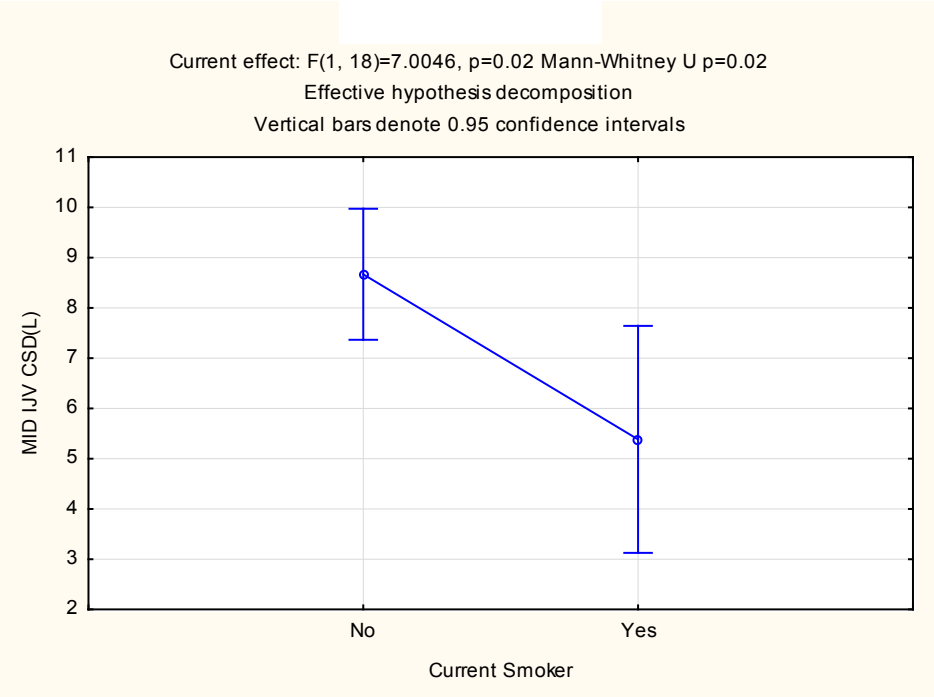

Figure 4.5: CSD of the mid left IJV in smoking MS patients versus non-smoking MS patients.
IJV=internal Jugular Vein, L=left, CSD=cross-sectional diameter, no=non-smoking MS patients, yes=smoking MS patients. There is a significant difference between the 2 groups (Mann-Whitney U test, p=0.02). The maximum CSD of the mid proximal IJV in non-smoking MS patients~10mm (1.00cm). The maximum CSD of the left mid IJV in smoking MS patients <8mm (0.80cm).

4.7.2 Smoking: IMT of CCA

The IMT of the right CCA was thicker in smokers compared to non-smokers. However the difference demonstrated was not significant (p=0.26).

4.7.3 Physical Activity: Intima Media Thickness of CCA

Figure 4.6 demonstrates a significant inverse association (Spearman p-value=0.03) between physical activity and the intima media thickness (IMT) of the left CCA.

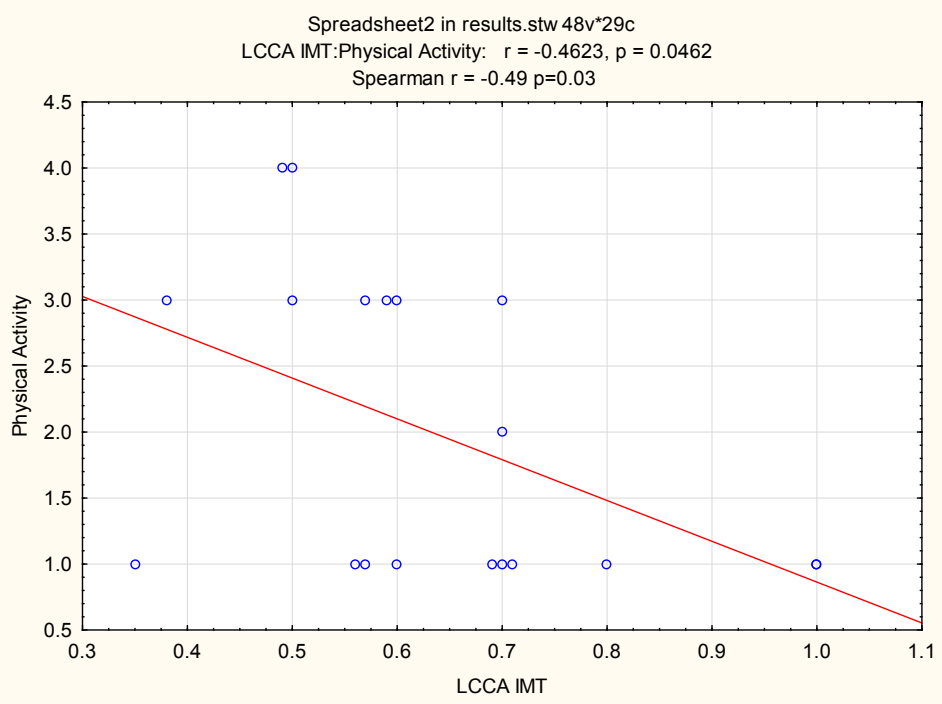

Figure 4.6: A significant association between Physical Activity and the IMT of the CCA.
L=left, CCA=common carotid artery, IMT=intima media thickness. (Spearman p-value=0.03)

4.7.4 Physical Activity: CSD of IJV

No significant association was demonstrated between physical activity and cross-sectional diameters of the Internal Jugular Veins.

CHAPTER FIVE

DISCUSSION

The internal jugular veins (IJV) are considered to be the main outflow pathway for intracranial venous blood (Sundström et al, 2010). This study demonstrated patency of the IJVs in 29 MS patients sampled with no evidence of thrombus on B-mode and Colour Doppler ultrasound imaging. This can be regarded as a new finding as no literature is available on the prevalence of internal jugular vein thrombus in MS patients. Previous research studies, however have demonstrated IJV intraluminal defects such as webs, septa and/or malformed valves which affects the venous outflow patterns (Zamboni et al, 2011). No IJV intraluminal defects were demonstrated in this study.

According to Zamboni et al (2009), reflux of flow directed towards the brain for a duration of >0.88s is regarded as significant. They defined this abnormal cerebrospinal venous drainage in MS patients as chronic cerebrospinal venous insufficiency (CCSVI) and hypothesised that CCSVI is strongly associated with MS and could play a causative role in the development of the disease. In this study reversed flow in the internal jugular veins during valsalva was demonstrated in 13.79% of MS patients; no vertebral vein reflux was identified.

Zamboni et al (2011) describes the ultrasound technique and criteria used to demonstrate CCSVI in the MS population, where reflux of the IJV should be present with the patient in any position and without valsalva. Zivadinov et al (2011) also describes the ultrasound technique and criteria used to demonstrate CCSVI in MS patients. The technique used for this study was not based on Zamboni's or Zivadinov's ultrasound technique and criteria as their techniques and criteria were only published after this research study commenced in January 2011. No literature describing the ultrasound technique and criteria for diagnosing CCSVI was published before 2011. The difference in the ultrasound technique in this study compared to that of Zamboni's and Zivadinov is that: 1) the valsalva manoeuvre was used to illustrate reflux within the IJV. Use of the valsalva manoeuvre was based on Zamboni's incidental finding of reflux in the IJV whilst an MS patient coughed during a Carotid Ultrasound examination (Zamboni, 2006); 2) the cross-sectional diameter of the IJV was used to illustrate stenosis of the vein instead of the cross-sectional area. A narrowing of the IJV would cause a reduction in the cross-sectional area (CSA) as well as the cross-sectional diameter (CSD).

Reflux in the IJV could be due to valve incompetence or stenosis. Stenosis is described as a severe reduction of the CSA of the IJV with the CSA <0.3cm^2. (Zamboni et al, 2011; Zivadinov et al, 2011). The antero-posterior CSD of the IJVs was used in this study as a measure of stenosis. A reduction of ≥50% of the CSD of the IJV was considered stenotic. No

stenosis of the IJV was identified in this study. The reflux identified in the 13.79% of MS patients was due to valve incompetence and not stenosis. Thus, this study does not support Zamboni's CCSVI theory

Other published studies could also not reproduce the findings published by Zamboni and his colleagues. Mayer et al (2011) found no supportive evidence for the presence of CCSVI in patients with MS. Awad et al (2011) also states that there is no conclusive evidence to support CCSVI and its association with MS. John Gever (2012) wrote a report on the "Largest CCSVI study fails to support theory" where approximately 2000 persons with blinded central imaging analysis found CCSVI in approximately 3% of MS patients. Based on this finding, the Italian group AISM (Associazone Italiana Sclerosi Multipla) declared that CCSVI is not a disease connected to MS. Baracchini et al (2013) state that CCSVI appears to be an alien condition and its existence should be questioned.

This study however demonstrated several features of vascular abnormalities in the MS patients, and associations of ultrasound results were found with lifestyle factors such as physical activity, smoking and a nutritional intervention programme. These effects have not been investigated in relation to ultrasound before.

Thompson et al (2003) defines physical activity as movement induced by skeletal muscles which results in the use of energy beyond that used whilst resting. Physical activity prevents development of coronary artery diseases and helps treat atherosclerotic risk factors such as high blood pressure, insulin resistance and obesity. An inverse association between physical activity and the intima media thickness of the CCA was demonstrated in this study. Literature states that a thickened intima media is associated with early atherosclerotic disease (Carroll, 2005). Bath and Lees (2000) state that vessel occlusion arises from atherosclerosis, particularly in the internal carotid artery. Thus the increase in physical activity helps prevent thickening of the intima media of the CCA which in turn will reduce the risk of developing early atherosclerotic disease.

Smoking is associated with an increased risk of atherothrombotic stroke and cerebral aneurysm (Powell, 1998: 22). Epidemiological studies demonstrate a close association of smoking with sudden death and occlusive peripheral arterial disease. Smoking causes an increase in the stiffness of the arterial wall which could lead to an increase in the risk for plaque rupture resulting in an acute cardiovascular event (Kool et al, 1993). This study demonstrated an increase in the IMT of the CCA in smoking MS patients compared to non-smoking MS patients, however the difference was not significant. Gourgou et al (2002) suggest that smoking is significantly associated with lower limb venous insufficiency, conforming to biological data and pathophysiological mechanisms. Smokers have higher fibrinogen levels and these elevated levels are related to the risk of developing venous

thrombosis (Pomp et al, 2008:97). Not much literature is available on smoking and its association with extracranial and upper limb venous abnormalities. Dolic et al (2012) reported that being overweight and smoking are common risk factors responsible for intraluminal structural venous abnormalities of the IJV.

A larger CSD of the right IJV compared to the left was demonstrated in this study, which supports previous studies (Khatri et al, 2001). This study also demonstrated a significant reduction in the CSD of the IJV in smoking MS patients compared to non-smoking MS patients. This new finding suggests that smoking is not only a risk factor for atherosclerotic disease but could be related to narrowing of the major neck veins.

To our knowledge, no literature has been published on carotid artery disease in MS patients. In this study, the carotid arteries in all MS patients (n=29) appeared patent with no evidence of stenosis or occlusions. The intima media of the common carotid artery (CCA) was thickened (0.9 and 1.0mm) in 13.79% of MS patients. An IMT >0.8mm is regarded as a marker for early atherosclerotic disease. A recent evidence-based review found that MS was associated with an increased risk of cerebrovascular diseases compared with the general population (Christiansen, 2012).

No plaque was identified in the external carotid arteries (ECAs). Echogenic plaque in the internal carotid artery (ICA) was identified in two of the MS patients (n=29). This small amount of plaque did not cause any disturbance to blood flow nor significant intraluminal reduction. Echogenic plaque is considered to be more benign than heterogeneous plaques (Thrush and Hartshorne, 2005:94).

The vertebral arteries appeared patent with normal antegrade flow. The average peak systolic velocity (PSV) of the right vertebral artery (VA) was 49.14cm/s and left VA 54.99cm/s. The normal vertebral artery peak systolic velocities range between 40 and 60cm/s (Myers and Clough, 2004:104).

When comparing the blood flow patterns of those MS patients on the nutritional programme to those who were not on the programme, no significant difference ($p>0.05$) was demonstrated in the cross-sectional diameter (CSD) of the right and left IJVs, IMTs of the right and left CCA's, PSV's of right and left ICA's, EDV's of right and left ICA's, PSV's of right and left ECA's, PSV's of the right and left vertebral arteries, PSV's of the right and left CCA's, left PSV ICA/CCA ratio and left EDV ICA/CCA ratio. However, a significant difference in the PSV ($p<0.05$) and EDV ($p<0.01$) of the right CCA in the MS patients not on the programme versus those on the nutritional programme was demonstrated in this study. The blood flow rate within the CCA in systole and diastole was significantly higher in MS patients not on the nutritional programme. This increased blood flow rate was present in the absence of plaque

formation. Thus the elevation is not due to a stenotic lesion within the vessel. However, according to Alexandrov et al. (1997), measuring PSV is the most important component of the carotid Doppler examination since it gives information about the diameter of the vessels. As a function of the area of the residual lumen, PSV increases with the narrowing of an artery. This indicates that the patients not following the nutritional program had narrower arteries, i.e. more vascular abnormalities than the patients following the nutritional programme. To our knowledge, no literature on carotid artery blood flow patterns in MS patients has been published to date.

There was no significant difference ($p>0.05$) in the right PSV ICA/CCA ratio in MS patients on the nutritional programme versus those not on the programme. However there appears to be a trend, according to the F-test there is a significance at 10% ($p<0.1$).

No significant difference ($p>0.05$) was illustrated in the right EDV ICA/CCA ratio in MS patients on the nutritional programme versus those not on the programme. There however appeared to be a trend with the F-test p-value just below 0.05.

A significant inverse correlation between homocysteine and the PSV of the right ECA (Spearman p-value <0.01) and direct correlation between fibrinogen and the PSV of the left ECA (Spearman p-value 0.03) was demonstrated in this study. The inverse correlation found with homocysteine and PSV is contradictory, since increased homocysteine levels in MS patients are associated with vascular damage and with an increased risk for carotid artery disease (Van Rensburg et al, 2006 and Selhub et al, 1995). Increased homocysteine levels causes proliferation of smooth muscle cells which can cause narrowing of a blood vessel. However no ECA stenosis was demonstrated in this study. The PSV of the ECAs was <200cm/s. According to Carroll, B (2005:959), there are no criteria for grading ECA stenosis, but if the ECA velocity does not exceed 200cm/s then there is not a significant stenosis. Elevated fibrinogen levels are associated with the severity of peripheral arterial disease and also accumulate in atherosclerotic plaques (Kerlin et al, 2004:1728 & Reinhardt, 2003:212). No plaque lesions were identified in the ECAs in this study. Further research in this field needs to be undertaken to ascertain the clinical significance of the association between homocysteine, fibrinogen and increased blood flow rates, which could produce shear stress within the vessels.

This study also showed for the first time that patients who regularly took a nutritional regimen designed to promote myelin maintenance had lower PSV measurements, and by implication healthier vasculature and greater luminal area than patients not on the regimen (Appendix A).

Limitations of the study

A limitation of this study is the absence of a control group and a relatively small sample size. A few trends were demonstrated with certain results such as the intima media being thicker in smoking MS patients compared to non-smoking MS patients, but not significantly. If a larger study is performed then these trends could reach significant values.

Conclusion

This cross-sectional observational study, supports findings of other studies viz that there's no significant correlation between extracranial venous abnormalities and MS. Further research needs to be undertaken to establish the existence of CCSVI and its relation to MS. This study demonstrated that lifestyle factors and nutrition have various effects on the vascular system. A new finding of the effect of smoking on the internal jugular veins was demonstrated. Smoking caused a significant reduction in the cross-sectional diameter on the internal jugular veins which over time, could lead to significant stenosis of the major neck veins. An inverse association was demonstrated between physical activity and the intima media thickness of the common carotid artery. Literature states that an increased intima media is associated with early atherosclerosis. It can thus be concluded that physical activity is a preventative mechanism for the development of early atherosclerotic disease.

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APPENDICES

Appendix A: Nutritional Programme - The Raphah Regimen

		Vitamins and Minerals		% RDA
Iron*	15 mg/day	Beta Carotene	3 mg	—
		Vitamin C	350 mg	777
Essential amino acids (e.g. diet milk shake)		Vitamin E	40 mg	266
		Vitamin B1	3 mg	200
Leucine	1235 mg	Vitamin B2	4 mg	222
Isoleucine	890 mg	Nicotinamide	20 mg	100
Lysine	995 mg	Folic Acid	5 mg	1250
Tryptophan	175 mg	Vitamin B12**	24 μg	800
Methionine	315 mg	Vitamin B6	10 mg	500
Phenylalanine	610 mg	Pantothenate	10 mg	130
Threonine	570 mg	Calcium	28.5 mg	2
Valine	840 mg	Magnesium	150 mg	37
Histidine	340 mg	Copper	1 mg	50
		Zinc	15 mg	100
Lipids		Manganese	2.5 mg	—
Evening Primrose Oil	500 mg	Chromium	100 μg	—
Salmon Oil	500 mg	Molybdenum	100 μg	—
Lecithin	300 mg	Selenium	60 μg	—

Note. *If patients do not present with excessively high iron parameters, 15 mg/day of an iron supplement (chosen for its ability not to promote constipation) is prescribed. **For the first three months the methylation metabolic pathway may be enhanced by additional weekly vitamin B12 injections, *or* a 1 mg/day vitamin B12 supplement that is dissolved under the tongue, *or* by taking S-adenosyl methionine (SAM), 200 mg/day.

(Depicted from Van Rensburg, S.J., Kotze, M.J., Hon, D., Haug, P., Kuyler, J., Hendricks, M., Botha, J., Potocnik, F.C.V., Matsha, T., Erasmus, R.T.2006: 125). [Permission. as per license agreement, granted by Springer (license number 3311730794101)].

Appendix B: Informed Consent (English and Afrikaans)

CEREBROSPINAL VENOUS AND CAROTID ARTERY ULTRASOUND STUDY

INFORMED CONSENT

INVESTIGATORS STATEMENT:

We are asking you to be part of this research study. The purpose of this consent form is to give you the information you will need to help you decide whether or not to join this study. Please read it carefully. You may ask questions about the purpose of the research, what you need to do, the possible risks and benefits, your rights as a volunteer and anything else about the research. Please ask if you do not understand any part of this form. When your questions have been answered you can decide if you want to join this study. This process is called 'informed consent'.

VOLUNTARY PARTICIPATION:

You have the right to withdraw from this study at any time. There will be no penalty or loss of benefits to which you are entitled.

BACKGROUND:

Previous research has shown that some of MS patients have narrowing of the neck veins (demonstrated with ultrasound) which cause reflux (backflow) of blood to the brain. Scientists' suggest that the narrowed veins in the neck are strongly associated with MS and could possibly be the cause of MS.

An ultrasound machine uses sound waves to image parts of the body including blood vessels. This study will use such a machine to look at the veins and arteries in your neck for narrowing, backflow and clot formation as well as plaque formation in the arteries of the neck.

THE PURPOSE OF THE STUDY:

The purpose of this study is to use Ultrasound to investigate the presence abnormal blood flow in the veins and arteries of the neck. Furthermore, if any abnormal flow is detected then you will be referred to the appropriate medical practitioner for further management.

ULTRASOUND EXAMINATION:

This study will require the patients to undergo one ultrasound examination of the arteries and veins of the neck. This test takes about 30-40 minutes to carry out. It is a painless examination and requires the patients to lie on their back and sitting up while a small instrument called a probe is moved across the skin. A water based gel is first applied to the skin so the probe has good contact and can move smoothly. Some patients find this test relaxing.

RISK AND BENEFITS:

There are no known risks to an ultrasound examination. The most important benefit for this group of patients is that abnormal blood flow (if present) will be identified on ultrasound. If abnormal blood flow is identified then appropriate steps can be taken to manage the patient as necessary.

OTHER INFORMATION:

Joining this study is voluntary. You can stop at any time. If you choose to join the study or not it will not affect your health care. Information about you is confidential. We will code the study records. The link between your name and code will be kept separate in a secure location. Only the study investigators will have access to that information. The link between your name and the code will be kept for 10 years then destroyed.

SUBJECT'S STATEMENT:

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have further questions about the research I can ask the principal researcher. If I have questions about my rights as a research subject I can contact the ethics committee at CPUT (021 442 6162) or the principal researcher (Merlisa Nelson) at 0767244185. I will receive a leaflet explaining this study to take away with me.

Signature of subject	Date	Printed name of subject

Signature of witness	Date	Printed name of witness

Appendix B – Afrikaans

INGELIGTE TOESTEMMING VIR SEREBROSPINAAL VENEUSE EN KAROTIS ARTERIE ULTRAKLANK STUDIE

Verklarende ondersoek:
Ons vra dat U deel sal wees van hierdie navorsingstudie. Die doel van hierdie toestemmingsvorm is om inligting deur te gee wat help met die besluit om deel van die studie te wees of nie. Lees asseblief deeglik. Voel gerus on vrae to vra oor die doel van die navorsing, wat U as 'n pasient moet doen, die risiko's en voordele, U regte as 'n vrywilliger en enige iets met betrekking tot die navorsing.

Vra asseblief vrae as daar dele van die vorm is wat U nie verstaan nie. Na beantwoording van vrae kan u besluit of U wel aan die studie wil deelneem. Die proses staan bekend as ingeligte toestemming.

Vrywillige deelname:
U het die reg om enige tyd van die studie te onttrek. Daar sal geen verlies of penalisering van voordele wees nie.

Agtergrond:
Vorige navorsing het bewys dat pasiente met veelvuldige skleroise vernouing van die nek venes het en terugvloei van die bloed na die brein veroorsaak. Wetenskaplike vermoed dat die vernoude venes in die nek kan geassosieer word met VS.
'n Ultraklankmasjien gebruik klankgolwe om dele van die liggaam te visualiseer insluitend bloedvate.
Hierdie studie gaan gebruik maak van so 'n masjien om te kyk na die vene en arteries in die nek vir vernouing, terugvloei, klont- en plaakformasie.

Die doel van die studie:
Die doel van hierdie studie is om ultraklank te gebruik om die teenwoordigheid van abnormal bloedvloei in die vene en arteries in die nek te ondersoek. Indien daar enige abnormale vloei opgespoor word sal U verwys word na die mees gepasde medies praktisyn vir verder behandeling.

Ultraklank ondersoek:
Hierdie studie vereis dat pasiente 'n verdure ultraklank ondersoek van die arteries en venes van die nek sal ondergaan, hierdie ondersoek duur ongeveer 30-40 minute. Dit is 'n pynlose ondersoek en daar sal van U verwag word om op die rug te le of regop te sit terwyl 'n klein instrument oor die vel sal aangewend word, sodat die instrument in goeie kontak met die vel sal wees en maklik sal beweeg. Sommige pasiente ervaar hierdie studie as ontspannend.

Risiko's en voordele:
Daar is geen gevare aan 'n ultraklank ondersoek verbande nie. Die mees belangrike voordeel vir hierdie groep pasiente is dat indien teenwoordig, abnormale bloedvloei geindentifiseer word, sal die nodige stappe in die behandeling van die pasient toegepas word.

Ander inligting:
Deelname aan hierdie studie is vrywillig. U kan enige tyd onttrek. Indien U kies om aan hierdie studie deel te neem sal dit nie U gesondheid affekteer nie. U informasie is vertroulik. Ons sal U studie rekords kodeer. Die skakel tussen U naam en kode sal op 'n aparte en veilige bestemming gehou word. Slegs die persone wat vol mag het, sal toegang he tot die informasie. Die skakel tussen U naam en kode sal vir 10 jaar gehou word en dan vernietig word.

Onderwerp verklaring:
Die studie was aan my verduidelik. Ek bied aan om deel te neem aan hierdie navorsing. Ek het ‘n kans gehad om vrae to vra. Indien ek enige ander vrae oor die studie het, kan ek die hoor navorser kontak. In dien ek enige vrae as ‘n navorsende onderwerp het, kan ek die etiese komitee by Kaaapse Universiteit van Tegnologie (021 442 6162) of die hoof narvorser (Merlisa) by 076 7244 185 skakel. Ek sal ‘n pamflet ontvang wat die studie verduidelik.

Handtekening van Navorsings Onderwerp Onderwerp	Datum	Naam van Navorsings in Drukskrif

Handtekening van Getuie	Datum	Naam Van Getuie in Drukskrif

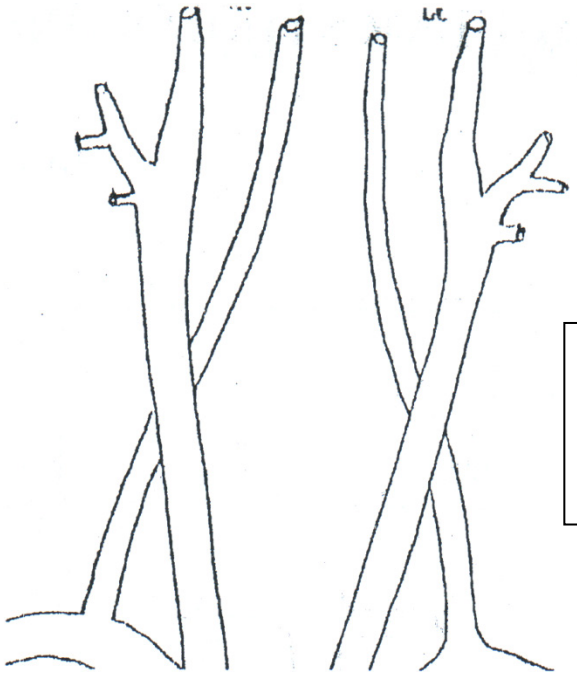
Appendix C: Carotid Duplex Report

CAROTID DUPLEX REPORT

PATIENT NAME: **DATE:**

AGE: **GENDER:** **PROGRAMME(Y/N):**

PLAQUE:
Calcified
Echogenic
Smooth,Soft
Ulcerated



PLAQUE:
Calcified
Echogenic
Smooth,Soft
Ulcerated

RIGHT **LEFT**

	COMMON CAROTID intima media thickness (mm)	
	peak systolic velocity (cm/s)	
	peak diastolic velocity (cm/s)	
	INTERNAL CAROTID peak systolic velocity	
	Peak diastolic velocity	
	EXTERNAL CAROTID peak systolic velocity	
	VERTEBRAL ARTERY peak systolic velocity	
	Peak systolic ICA/CCA ratio	
	Peak diastolic ICA/CCA ratio	
	INTERNAL CAROTID %age stenosis	
	EXTERNAL CAROTID %age stenosis	

(Adapted from Groote Schuur Hospital Carotid Artery Duplex template.)

Appendix D: Doppler Spectral Analysis

Diameter stenosis	Peak-Systolic velocity	ICA/CCA Systolic ratio	ICA EDV
0-49%	25cm/s, <125 cm/sec	<2	<40 cm/sec
50-69%	125-210 cm/sec	2-3	40-70 cm/sec
70-79%	>210 cm/sec	>3	70-100 cm/sec
80-99%*	>280 cm/sec	>3.7	>100 cm/sec
Occlusion	No flow detectable		

*Near occlusion velocities may be low

(Adapted from the North American Symptomatic Carotid Endarterectomy Trial (NASCET) Collaborators: Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis, 1991, ICA=internal carotid artery, ECA=external carotid artery, EDV=end-diastolic velocity).

Appendix E: Cerebrospinal Venous Duplex Report

PATIENT NAME: **AGE:** **GENDER:** **PROGRAMME(Y/N)** **DATE**

	PATENT	NON-OCCLUSIVE THROMBUS	OCCLUSIVE THROMBUS	STENOSIS	RFLUX TIME	CSD (mm)
RIGHT						
PROX IJV						
MID IJV						
DISTAL IJV						
VV						
LEFT						
PROX IJV						
MID IJV						
DISTAL IJV						
VV						
COMMENT:						

Appendix F: Ethical Approval

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)

Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
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2 October 2012
CPUT/HW-REC 2012/H09

Faculty of Health and Wellness Sciences
Nursing & Radiography Department

Dear Ms M Nelson

YOUR APPLICATION TO THE HW-REC FOR EXTENSION

At the meeting of the Health and Wellness Sciences-REC on 20 September 2012 approval was granted to Merlisa Nelson for your application. This approval is for research activities related to an MTech: Radiography at this institution.

TITLE: Ultrasound Evaluation of the extracranial cerebrospinal venous system and carotid arteries in patients with Multiple Sclerosis (MS).

INTERNAL SUPERVISOR: Ms F Isaacs

EXTERNAL CO-SUPERVISOR: Prof S Janse van Rensburg

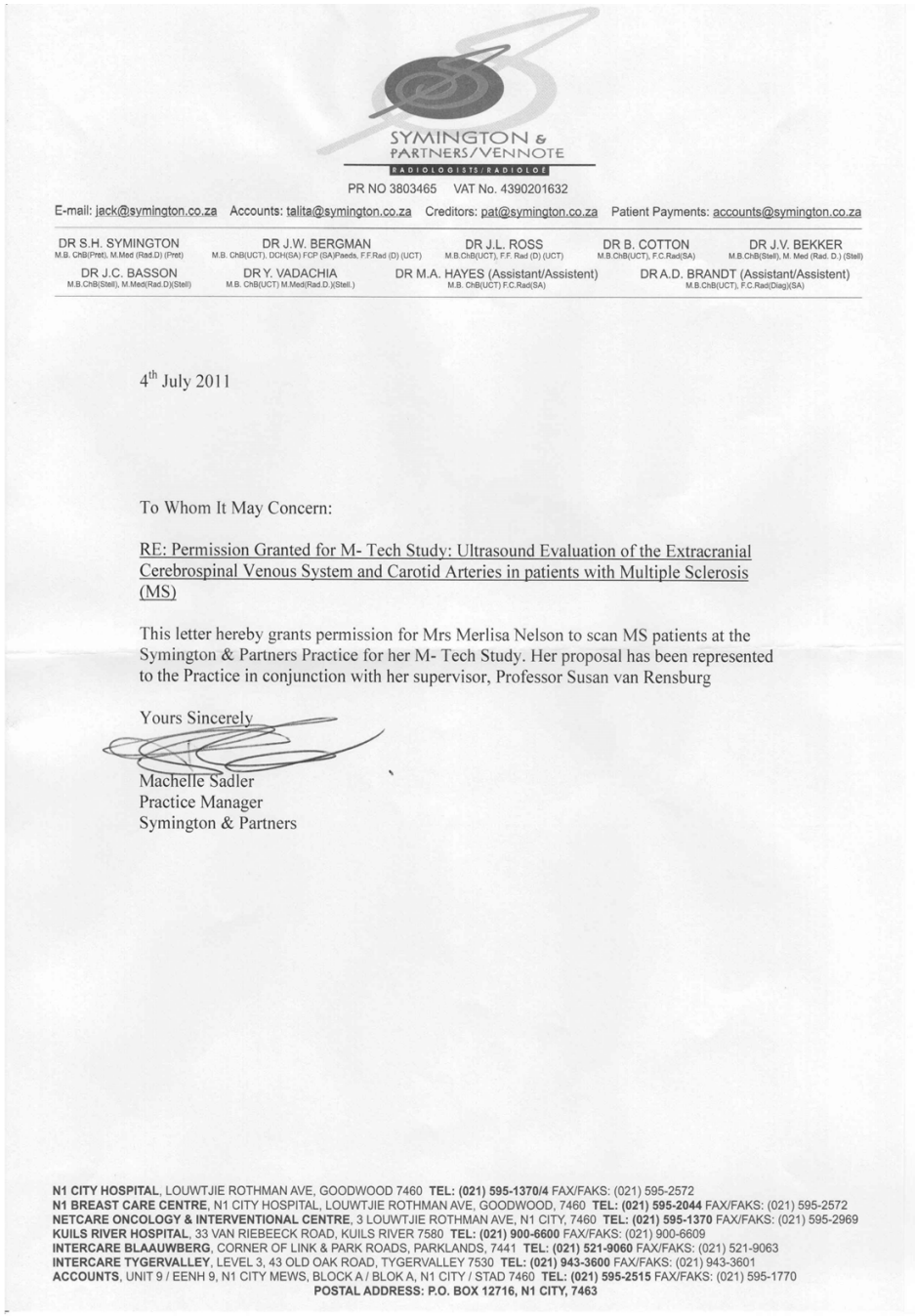
Comment:
Approval will not extend beyond 2 October 2013. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

Note:
The investigator(s) should understand the conditions under which they are authorized to carry out this study and they should be compliant to these conditions. **It is required that the investigator(s) complete an annual progress report that should be submitted to the HW-REC in December of that particular year, for the HW-REC to be kept informed of the progress and of any problems you may encounter.**

Kind Regards

Prof JL Marnewick
CHAIRPERSON: HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE

Appendix G: Written permission from Dr Bergman, Ross and partners Radiologists



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4th July 2011

To Whom It May Concern:

RE: Permission Granted for M- Tech Study: Ultrasound Evaluation of the Extracranial Cerebrospinal Venous System and Carotid Arteries in patients with Multiple Sclerosis (MS)

This letter hereby grants permission for Mrs Merlisa Nelson to scan MS patients at the Symington & Partners Practice for her M- Tech Study. Her proposal has been represented to the Practice in conjunction with her supervisor, Professor Susan van Rensburg

Yours Sincerely

Machelle Sadler
Practice Manager
Symington & Partners

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