ULTRASOUND INVESTIGATION OF RISK FACTORS FOR EXTRACRANIAL VASCULAR PATHOLOGY IN PATIENTS WITH MULTIPLE SCLEROSIS

by

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Bellville
November 2019

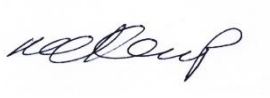
Gloria

in excelsis

Deo

DECLARATION

I, Merlisa Claudia Kemp, 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.



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ABSTRACT

Introduction: Cerebral hypoperfusion and impaired cerebral venous drainage are reported to be risk factors for multiple sclerosis (MS). Furthermore, lifestyle and biochemistry have significant effects on the brain and vascular system. This study investigates, with vascular ultrasound, the risk factors for extracranial vascular disease in patients with MS.

Methods: Grey-scale imaging, Colour and Spectral Doppler analysis of the carotid arteries and internal jugular veins were performed on a cohort of 50 female participants (25 MS patients and 25 age-matched controls). The major neck vessels were sonographically interrogated to determine patency, carotid intima media thickness (cIMT), carotid artery cross-sectional diameters (CSD), internal jugular vein (IJV) cross-sectional area (CSA), stenosis, occlusions and abnormal blood flow patterns. Both cohorts underwent blood tests, genetic tests and a lifestyle assessment. The MS participants had a neurological assessment to determine MS disability status (Expanded Disability Status Scale, EDSS).

Results: Statistically significant associations (p-value <0.05) were found between the extracranial vascular ultrasound variables and biochemical markers (s-iron, s-transferrin, %Tf saturation, ferritin, haemoglobin, vitamin B12, s-folate, homocysteine, CRP, 25-OH vitamin D, total cholesterol, HDL and triglycerides), lifestyle factors, genetic factors (HLA DRB1*1501 allele) and MS disability in both cohorts. The carotid artery blood flow parameters were negatively associated with MS disability, whereas the cIMT was positively associated with MS disability. Physical activity was positively associated with carotid artery blood flow velocities and passive smoking was found to have a negative association in the MS cohort of participants. Passive smokers also demonstrated a larger IJV CSA in comparison to non-smokers in both cohorts. In addition, drinking 1-13 units of alcohol/week was positively associated with carotid blood flow velocities in MS participants.

Conclusion: Several significant positive and negative associations between extracranial vascular ultrasound variables and genetic, lifestyle, biochemical and vascular factors as well as MS disability were demonstrated in this study. The new MS findings that unfolded in this study include significant associations between: 1) the carotid vessel diameters and biochemical and lifestyle parameters as well as the presence of the HLA DRB1*1501 allele; 2) IJV CSA in MS and biochemical and lifestyle parameters, specifically passive smoking; 3) MS disability and carotid artery blood flow velocities; and 4) carotid artery blood flow parameters and biochemical markers. Further studies are therefore needed to establish the clinical relevance of these new findings

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DEDICATION

To,

My husband, Deon

My son, Joshua

And my daughter, Jemma

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GLOSSARY

ADEM	Acute Disseminated Encephalomyelitis
AGE	Advanced Glycation End (products)
ALARA	As Low as Reasonably Achievable
ASCVD	Atherosclerotic Cardiovascular Disease
BMI	Body Mass Index
BP	Blood Pressure
BRP	Bergman, Ross & Partners
CA	Carotid Artery
CAD	Coronary Artery Disease
CAs	Carotid Arteries
CBF	Cerebral Blood Flow
CCA	Common carotid Artery
CCAs	Common carotid Arteries
CCSVI	Chronic Cerebrospinal Venous Insufficiency
CD	Compact Disk
CHD	Coronary Heart Disease
CIMT	Carotid Intima Media Thickness
CNS	Central Nervous System
CO	Carbon Monoxide
CPUT	Cape Peninsula University of Technology
CRP	C-reactive protein
CSA	Cross Sectional Area
CSD	Cross Sectional Diameter
CSF	Cerebrospinal Fluid
CVD	Cardiovascular Disease
CVR	Cardiovascular Risk
DIS	Dissemination in space
Dist	Distal
DIT	Dissemination in time
DMT	Disease Modifying Treatments
DNA	Deoxyribonucleic Acid
EC	Endothelial Cell
ECA	External carotid Artery
ECAs	External Carotid Arteries
EDSS	Expanded Disability Status Scale
EDV	End Diastolic Velocity

EP	Evoked potential
FS	Functional System
FRC	Faculty Research Committee
FREC	Faculty of Health and Wellness Sciences Research Ethics Committee
GA	Glatiramer Acetate
GE	General Electric
GM	Grey Matter
HB	Haemoglobin
Hcy	Homocysteine
H-Cyst	Homocysteine
HDL	High-density Lipoprotein
HDL-c	High-density Lipoprotein Cholesterol
Het	Heterogeneous
HLA	Human Leukocyte Antigen
Hom	Homogeneous
HRQOL	Health Related Quality of Life
HRT	Hormone Replacement Therapy
HW-REC	Health & Wellness Sciences Research Ethics Committee
IBD	Irritable Bowel Disease
ICA	Internal Carotid Artery
ICAs	Internal Carotid Arteries
IgG	Immunoglobulin
IJV	Internal Jugular Vein
IJVs	Internal Jugular Veins
IL6	Inflammatory Factor
IM	Intima Media
IMT	Intima Media Thickness
ISNVD	International Society for Neurovascular Disease
LDL	Low- Density Lipoprotein
LDL-c	Low-Density Lipoprotein cholesterol
LT	Left
MBP	Myelin Basic Protein
ME	Myalgic Encephalomyelitis (Yuppie flu)
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
MRV	Magnetic Resonance Venography
MS	Multiple Sclerosis
NASCET	North American Symptomatic Carotid Endarterectomy Trials

NO	Nitric Oxide
OCB	Oligoclonal Bands
OL	Oligodendrocyte
OCP	Oral Contraceptive Pills
Ox-LDL	Oxidized Low-density Lipoprotein
PACS	Picture Archives & Communications System
PI	Principal Investigator
PPMS	Primary- Progressive Multiple Sclerosis
PRF	Pulse Repetition frequency
Prox	Proximal
PSV	Peak Systolic Velocity
PwMS	Persons with Multiple Sclerosis
RAGE	Receptor for AGE
REC	Research Ethics Committee
RRMS	Relapsing Remitting Multiple Sclerosis
Rt	Right
SD	Standard Deviation
SFA	Saturated Fatty Acids
S-fol	S-Folate
S-Iron	Serum Iron (concentration)
SLE	Systemic Lupus Erythematous
SNP	Single Nucleotide Polymorphism
SPMS	Secondary-Progressive Multiple Sclerosis
S-transf	S-Transferrin
SV	Sample Volume
Tf	Transferrin
Tregs	Regulatory T Lymphocytes
Trigs	Triglycerides
VA	Vertebral Artery
VAs	Vertebral Arteries
Veg	Vegetables
VEP	Visual Evoked Potential
Vit B12	Vitamin B12
Vit D	Vitamin D
VS	Vaskulere Siekte
VV	Vertebral Vein
WM	White Matter
WML	White Matter Lesions

WT	Wild Type
25(OH)D	25-hydroxyvitamin D3
25(OH) Vit D	Serum 25 hydroxyvitamin D
1,25(OH)2VD	1,25-dihydroxy vitamin D (calcitriol)
% Sat Transf	Percentage Transferrin saturation
% Tf sat	Percentage Transferrin saturation

CHAPTER ONE

INTRODUCTION

In this chapter, an overview of the study is described. The overview includes description of multiple sclerosis (MS), pathogenesis of MS and disability progression, background and rationale of the study, research methodology, hypothesis, research objectives, risks and outcome.

Multiple sclerosis (MS) is described as a chronic inflammatory demyelinating disease affecting the central nervous system (CNS). The disease, which frequently starts in young adulthood, affects both the white and grey matter of the CNS. (Stadelmann et al., 2011; Van Horssen et al., 2011) According to the Multiple Sclerosis International Federation (2013), the number of persons with MS worldwide has increased from 2.1 million in 2008 to 2.3 million in 2013. The prevalence of MS is heterogeneous worldwide, however this demyelinating disease is commonly seen in countries of high-income (Leray et al., 2016).

Symptoms associated with MS include physical and mental disturbances caused by the neural damage, resulting in distortion of nerves impulses travelling to and from the brain (National MS Society, 2016).

1.1 PATHOGENESIS OF MS

Inflammation associated with MS may be due to a phenomenon caused by cytodegeneration of neurons and/or oligodendrocytes without involvement of immune cells or a physiological process secondary to the immune system activation (Bogie et al., 2014; Haider, 2015 and Palumbo, 2017).

Several factors contribute to the development, exacerbation and disability progression of MS, however the exact aetiology and pathogenesis of the disease remains unclear (Mike et al., 2011). The several factors associated with the MS pathophysiological processes are environmental, immunological, genetic, lifestyle, biochemical and vascular in origin. According to Loy et al. (2017), fatigue is perceived to be the most common and disabling symptom in persons with MS.

1.2 BACKGROUND AND RATIONALE OF THE STUDY

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).

The aetiology of MS is still not yet well understood, however immune dysregulation caused by a complex interplay of genetic and environmental factors appears to be associated with the disease process (Hadgkiss et al., 2014). 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).

Recent studies have linked impaired cerebral venous outflow with MS and surgical correction thereof results in rapid improvement of MS symptoms (Zamboni, 2006). Currently, no information is available in the literature on the prevalence of carotid artery disease in MS and its association with disability progression.

Ultrasound technology has become an important tool for healthcare professionals as it offers many advantages compared to other imaging modalities. It is cost effective, easily available, and does not use ionizing radiation. The use of diagnostic ultrasound as an imaging tool to interrogate the extracranial vascular ultrasound systems, allows for immediate identification of vascular pathology (if present) which can then be treated appropriately, thus preventing progression of vascular disease, reduce disease burden and extend the person's life expectancy

This case-control study will determine, by ultrasound and laboratory tests, the prevalence of extracranial cerebrospinal venous and carotid artery disease, abnormal biochemical parameters, genetic and lifestyle risk factors in the selected sample of MS patients within the region of the Western Cape.

1.2 OVERVIEW OF METHODOLOGY

A case-control study was performed on a cohort of MS participants and age-matched controls.

The research participants had the following examinations, tests and assessments in the Western Cape, South Africa:

- Carotid artery and internal jugular vein ultrasound examinations which were performed at a private Radiology practice by the (principal investigator) PI who is an experienced vascular sonographer
- Blood tests performed by a private pathology laboratory
- Genetic tests conducted at a pathology research facility affiliated with the University of Stellenbosch
- The neurological assessments were conducted by a clinician at a tertiary hospital to determine the disability status of the MS participants.

An ultrasound system with Grey-scale imaging and Doppler facilities were used to interrogate the carotid arteries (CAs) and the internal jugular veins (IJVs).
The following carotid artery (CA) variables were captured:

- blood flow velocities,
- blood flow ratios,
- common carotid artery (CCA) intima media thickness,
- cross-sectional diameters of the CAs,
- plaque formation,
- and percentage vessel stenosis (if present)

The following internal jugular vein (IJV) ultrasound variables were captured:

- blood flow velocities
- cross-sectional area (CSA) of the proximal IJV

The blood tests included assessment of multiple biochemical markers which influences vascular function and disability progression in MS. Genotyping was performed using single nucleotide polymorphism (SNP) genotyping assays. The HLA DRB1*1501 was investigated by use of a haplotype tagging SNP. This human leukocyte antigen is strongly associated with MS in the majority of populations tested worldwide (Alcina et al., 2012).

A neurological assessment included the testing of the cranial nerves, sensation, reflexes, coordination, balance and walking in the cohort of MS participants.

1.3 HYPOTHESIS

Multiple sclerosis is a complex disease influenced by numerous contributing factors. Therefore, it is hypothesised that genetic, vascular, biochemical and lifestyle factors are significantly associated with the development of extracranial vascular pathology in multiple sclerosis.

1.4 RESEARCH OBJECTIVES

The main objectives of the study were to:

- Compare the Ultrasound findings of the extracranial vascular systems in the two cohorts (MS and free from MS)
- Establish an association between the Human Leukocyte Antigen (HLA*) DRB1*1501* gene and the Grey-scale, Colour and Spectral Doppler analysis of the carotid arteries (CAs) and internal jugular veins (IJVs) in the two cohorts.
- Establish an association between biochemical markers (homocysteine, Vitamin D, iron, Vitamin B12, C-reactive protein (CRP), blood lipids) and the grey-scale, colour and spectral Doppler analysis of the CAs and IJVs in the two participant groups.

- Establish an association between lifestyle factors and the grey-scale, colour and spectral Doppler analysis of the CAs and IJVs in the two cohorts.
- Establish an association between disability and the grey-scale, colour and spectral Doppler analysis of the CAs and IJVs in the active MS cohort.

1.5 RISKS

There are no known risks associated with diagnostic ultrasound imaging, however the as low reasonably achievable (ALARA) principle should be adhered to when interrogating soft tissue and vascular structures within the human body. Fowlkes and Hollard (1998:52) postulates that there are no known biological effects when diagnostic ultrasound imaging machines are used appropriately. Diagnostic ultrasound is an operator-dependent imaging modality, thus should only be used by trained sonographers.

CONCLUSION

Multiple statistically significant associations and comparisons between the ultrasound variables, genetic, biochemical and lifestyle parameters were found. Some of which is novel and that which corroborates with previous MS studies of similar context.

CHAPTER TWO

LITERATURE REVIEW

INTRODUCTION

MS is described as the most frequently seen inflammatory demyelinating disease affecting the central nervous system (CNS) resulting from the interaction of genetic and environmental factors which are currently still not well understood (Kidd, 2001; Didonna &.Oksenberg, 2015; Sand, 2015; Leray et al., 2016). According to the Multiple Sclerosis International Federation (2013), the number of persons with MS worldwide has increased from 2.1 million in 2008 to 2.3 million in 2013. The prevalence of the disease varies from high levels in North America and Europe to low levels in Eastern Asia and sub-Saharan Africa (2/100 000 population) (Leray et al, 2016). Epidemiological studies also indicate a higher prevalence of MS in countries of affluence further from the equator where high caloric, high fat diets are common (Hadgkiss et al., 2014: 125). Multiple sclerosis is a relatively common disease in Europe, the United States, Canada, New Zealand, and parts of Australia. In high-risk populations, the lifetime risk for MS is about 1 in 200 for women and fewer for men (Ascherio & Munger, 2007) with the mean age of onset at approximately 30 years (Giampaolo et al., 2013). The female-to-male ratio for MS ranges between 1.5 and 2.5 in most populations (Ascherio & Munger, 2007).

In 1967, Geoffrey Dean reported results of a survey on MS in the White South African population. The incidence, prevalence and mortality of the disease were high amongst the White South Africans with it being higher in the English-speaking and lower in Afrikaans-speaking populations. The disease has been reported to be less common amongst the Coloured and Asian South Africans, and rare amongst Black Africans (Dean, 1967). Further studies on MS in South Africa by Modi et al (2008) state that there are approximately 5 000 persons suffering from MS in South Africa with Gauteng and the Western Cape having the most persons with MS. The study concludes that MS is widely diagnosed in the White South African population and affects females more than males (F:M=3:1).

Multiple sclerosis is associated with many symptoms which include physical (muscle weakness, weak reflexes, muscle spasm), mental and at times psychiatric disturbances due to the neural damage which occurs and blocks the communication among different parts of the nervous system (National MS Society, 2016).

2.1 DESCRIPTION: MS

Multiple sclerosis is stated to be the most common chronic disabling neurological disease of young adults that attacks the central nervous system (brain, spinal cord and optic nerves) (Paz Soldan & Rodriguez, 2002 ; National MS Society, 2016). This chronic autoimmune inflammatory and demyelinating disease of the central nervous system causes destruction of

the fatty substance (myelin) that surrounds and insulates the nerve fibres as well as the nerve fibres themselves (Taylor et al., 2014; National MS Society, 2016).

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 the variety of symptoms found in MS patients. The damaged myelin forms scar tissue, termed sclerosis, giving the disease its name (National MS Society, 2016).

There are 3 common disease courses of MS: relapsing remitting, primary progressive, and secondary progressive (National MS Society, 2017a; MS Association of America, 2019).

2.2 CLASSIFICATION

2.2.1 Relapsing-remitting Multiple Sclerosis

Relapsing remitting MS (RRMS) is the most common disease course which is characterised by acute attacks of new or increasing neurologic symptoms. Approximately 85 per cent of persons with MS are diagnosed with RRMS. In RRMS, the nerve fibres and myelin are affected by the inflammatory attacks. The patients will experience periods when symptoms flare up aggressively (relapse/an exacerbation) followed by periods of good recovery (remission) (National MS Society, 2017a; MS Association of America, 2019). Some of the symptoms experienced by patients include visual impairment, numbness, intestinal and urinary disorders as well as learning and memory impairment. Each stage may set off new symptoms or exacerbate the existing ones. (Millefiorini et al*.,* 1997; Ghasemi et al., 2017).

2.2.2 Primary-progressive Multiple Sclerosis

Primary-progressive MS (PPMS) affects approximately 10-15% of MS patients who experience a slow progressive downhill course without any distinct clinical episodes and who tend to have fewer MRI lesions (Hafler, 1999; Ghasemi et al., 2017). In this disease course, the patient's neurologic function and disability worsens from the onset of symptoms without periodic relapses or remissions (National MS Society, 2017a; MS Association of America, 2019). It has been suggested by Rovaris et al (2006) that the median time between onset of PPMS and progression to the secondary progressive stage is 19 years. One third of relapsing- remitting disease patients go on to have progressive disease (Hafler, 1999).

2.2.3 Secondary-progressive Multiple Sclerosis

Secondary-progressive MS (SPMS) disease course is characterised as a progression from the initial relapsing-remitting course. Most MS persons diagnosed with RRMS will eventually transition to SPMS where there is a progressive worsening of neurologic function and disability over time (National MS Society, 2017a; MS Association of America, 2019). There are no occasional relapses, plateaus or remissions (Lublin & Reingold, 1996). Many MS patients

experience increased weakness, intestinal and urinary system disorders, fatigue, stiffness, mental orders and psychological impairment (Ghasemi et al., 2017).

2.3 DIAGNOSTIC CRITERIA

The criteria for diagnosis of MS, as seen in Table 2.1, which is based on the integration of clinical, imaging and laboratory assessments is known as the McDonald criteria. The criteria were updated in 2010 and revised again in 2017 by the International Panel on the Diagnosis of Multiple Sclerosis. The panel met to examine the requirements for demonstrating dissemination of lesions in space (DIS) and time (DIT) and focus on the application of the McDonald criteria (National MS Society, 2010; Polman et al., 2011; Thompson et al., 2017; Thompson et al., 2018).

Table 2.1: The 2017 McDonald Criteria for Diagnosis of MS in patients with an attack at onset

Number of clinical attacks	Number of lesions with objective clinical evidence	Additional Data Needed for the diagnosis of MS
≥2 clinical attacks	≥ 2	None*
≥2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location†)	None*
≥2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡
1 clinical attack	≥ 2	Dissemination in time demonstrated by an additional clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡ AND Dissemination in time demonstrated by an additional clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶

**No additional tests are required to demonstrate dissemination in space and time. ‡The MRI criteria for dissemination in space are described in Table 2.2. ¶The presence of CSF-specific oligoclonal bands does not demonstrate dissemination in time per se but can substitute for the requirement for demonstration of this measure*. (Adapted from McDonald et al., 2001; Polman et al., 2011; Giampaolo et al., 2013; Thompson et al., 2017)

Table 2.2: 2017 McDonald criteria for demonstration of dissemination in space and time by MRI in a patient with a clinically isolated syndrome

Dissemination in space can be demonstrated by one or more T2-hyperintense lesions* that are characteristic of multiple sclerosis in two or more of four areas of the CNS: periventricular, cortical or juxtacortical, and infratentorial brain regions, and the spinal cord
Dissemination in time can be demonstrated by the simultaneous presence of gadolinium-enhancing and non-enhancing lesions* at any time or by a new T2-hyperintense or gadolinium-enhancing lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI

**Unlike the 2010 McDonald criteria, no distinction between symptomatic and asymptomatic MRI lesions is required.* (Adapted from Polman et al., 2011; Thompson et al., 2017)

2.3.1 TOOLS FOR MAKING A DIAGNOSIS

2.3.1.1 Medical History and Neurological exam

Early detection of MS is key as it provides an opportunity to seek treatment and plan for the future. Obtaining a history about the onset of the first symptoms, neurological disorders and other illnesses such as diabetes, thyroid diseases, food habits, geographic locations and history of medications taken as well as substance abuse is important (National MS Society, 2010; Ghasemi et al., 2017). A full neurological exam is performed which includes testing the cranial nerves (vision, hearing, facial sensation, strength, swallowing), sensation, reflexes, coordination, balance and walking (National MS Society, 2017b).

2.3.1.2 Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) is the imaging modality of choice, with 95-99% specificity, and allows for an earlier and more definitive diagnosis of MS. Presence of white matter lesions (WML) in different parts of the central nervous system can be detected with a high sensitivity and specificity. MRI can also differentiate between old, new and active lesions (Barkhof et al., 1997; Tintoré et al., 2003; Dähnert, 2007; National MS Society, 2010). This imaging modality offers 3 main applications in MS namely: 1) in conjunction with the characteristic symptoms, it can provide an earlier diagnosis than symptoms alone; 2) enhance the understanding of the pathophysiology of MS and the pathophysiological changes which are related to the clinical manifestation of the disease; 3) it can monitor the effects of therapies and identify the response to therapy in MS patients (Lövblad et al., 2010). In addition, contrast agents, such as Gadolinium, are used as part of the MRI imaging protocol to enhance the WML in the brain (Gulani et al., 2017). Bakshi and colleagues (2014) also postulate that the application of MRI techniques such as dynamic contrast-enhanced imaging and double-inversion recovery imaging has improved the visualisation and characterisation of damage to white and grey matter. These techniques reveal new insights into the morphology and evolution of MS lesions.

2.3.1.3 Visual Evoked Potential (VEP)

Visual Evoked Potential (VEP) tests are recordings of the nervous system's electrical responses to the stimulation of specific sensory pathways. VEP tests are useful in identifying a clinically silent lesion which sometimes provide evidence of scarring along the nerve pathways that do not show up during the neurological examination (National MS Society, 2010; Giampaolo et al., 2013).

2.3.1.4 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 (OCBs). These bands are found in the spinal fluid of approximately 90-95% of persons with MS and indicate immune response within the CSF. The

presence of OCBs or an increased immunoglobulin (IgG) index indicates the presence of intrathecal immunoglobulin synthesis which is considered the most important abnormality in the CSF (National MS Society, 2010; Giampaolo et al., 2013). However OCBs cannot be used as positive proof of MS because they are present in other diseases as well, therefore results must be interpreted in the context of the clinical diagnosis (National MS Society, 2010; (Giampaolo et al., 2013).

2.3.1.5 Blood tests

Blood sample analysis for detection of abnormal biochemical parameters, specifically vitamin deficiencies, may be diagnostically helpful (National MS Society, 2010; Ghasemi et al., 2017). In addition, several abnormal levels of homocysteine, total cholesterol, fibrinogen, high-density lipoprotein, low-density lipoprotein and c-reactive protein have been associated with peripheral arterial and cardiovascular disease. If these abnormal levels are demonstrated in MS, then it can be deduced that vascular risk factors play a role in the development and progression of the disease.

2.4 PATHOGENESIS OF MULTIPLE SCLEROSIS AND DISABILITY PROGRESSION

There are several factors that contribute to the development, exacerbation and progression of MS, however the pathogenesis of MS still remains unclear. Scientists believe that a combination of several factors (immunological, environmental, infectious and genetic) may be involved (Kidd, 2001; Zamboni et al., 2009a:392; National MS Society, 2010; Ghasemi et al., 2017). Additional factors such as smoking, stress, diet, lifestyle and vascular disease may also play a causative role in the development of MS (D'haeseleer et al., 2011; Hedström et al., 2014a; Koriem, 2016).

Disability progression is a major challenge in MS, and there is no consensus as to the causes of disability progression (Thompson et al., 2018). Other studies (Davis et al., 2014) point to lifestyle factors as the main promoters or ameliorators of disability, suggesting that the cells that synthesise and repair myelin, oligodendrocytes (OLs), are the main effectors of disease outcome. Myelination of axons in the CNS by OLs is described as an extremely complex interaction, specific to vertebrates. Myelination allows the solitary conduction between nodes of Ranvier and increases both speed and efficiency of nerve conduction (Dulamea, 2017). In addition, to myelination, there is evidence that OLs also provide trophic support and serve as a source of energy (Funfschilling et al., 2013). According to Bartzokis (2011), myelin contributes to congitive capacity and physical functions. Therefore, it can be deduced that an association exists between myelin destruction and status of disability.

Disability status in MS can be accurately determined by clinicians using a specially designed instrument called the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). It is

considered to be the gold standard and is used in all clinical trials. The EDSS ranges from 0 to 10, with higher values denoting greater disability (Table 2.3).

Table 2.3 Kurtzke Expanded Disability Status Scale (EDSS)

Score	Description
0	Normal neurological examination
1	No disability, minimal signs in FS*
1.5	No disability, minimal signs in >1 FS
2	Minimal disability in 1 FS
2.5	Mild disability in 1 FS or minimal disability in 2 FSs
3	Moderate disability in 1FS or mild disability in 3 or 4 FSs. Fully ambulatory
3.5	Fully ambulatory but with moderate disability in 1 FS and more than minimal disability in several others
4	Fully ambulatory without aid, self-sufficient, up and about ~12hrs/day despite relatively severe disability; able to walk without aid/rest for ~500m
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance, characterised by relatively severe disability, able to walk without the aid of rest for ~300m.
5	Ambulatory without aid or rest ~200m; disability severe enough to impair full daily activities (works a full day without special provision)
5.5	Ambulatory without aid or rest for ~100m; disability severe enough to preclude full daily activities
6	Intermittent or unilateral constant assistance (stick, crutch, brace) required to walk for ~100m with or without resting
6.5	Constant bilateral assistance (stick, crutches, braces) required to walk for ~20m without resting
7	Unable to walk beyond ~5m, even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair for ~12hrs/day
7.5	Unable to take more than a few steps, restricted to wheelchair; may need aid in transfer; wheels him-/herself, but cannot carry on in standard wheelchair for a full day, may require motorised wheelchair.
8	Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed much of the day; retains many self-care functions; generally has effective use of arms
8.5	Essentially restricted to bed much of the day; has some effective use of arms, retains some self-care functions
9	Confined to bed; can still communicate and eat
9.5	Totally helpless bed-bound patient; unable to communicate effectively or eat/swallow
10	Death due to MS

FS = functional system, FSs = functional systems, MS = multiple sclerosis

*Each system (visual, pyramidal, etc.) has a separate FS scale; scores are compiled to assist designation of the overall score.

(Adapted from Kurtzke, 1983; Giampaolo et al., 2013)

2.4.1 ENVIRONMENTAL FACTORS

Environmental factors including vitamin deficiency, diet, physical activity and obesity as well as smoking are associated with the onset of MS (Ghasemi et al., 2017).

2.4.1.1 Vitamin D deficiency

Vitamin D is a lipid-soluble vitamin, which acts like a hormone. Compared to other vitamins which have an essential organic compound that cannot be synthesized by the body and needs to be ingested, vitamin D can be synthesized (Ross et al., 2011). The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25[OH]2VD), also known as calcitriol, has chemical similarities to hormones such as testosterone, oestrogen, and cortisol. The active form of vitamin D plays a crucial role in lymphocyte activation and proliferation, T-helper cell differentiation, tissue-specific lymphocyte homing, the production of specific antibody isotopes, and regulation of the immune response (Mora et al., 2008). According to Sintzel et al (2018) the main sources of vitamin D are sunlight, diet, and supplementation.

MS is more prevalent in populations in colder climates which is related to lack of vitamin D due to lack of sunlight (Koriem, 2016). Mokry and colleagues (2015) conducted a randomized study to evaluate whether genetically lowered vitamin D level influences the risk of MS. The findings of the study were consistent with evidence from observational studies which demonstrated that low vitamin D levels influence the risk of MS and also reflect findings from functional studies that have implicated vitamin D as an important regulator in the expression of major histocompatibility complex (MHC) class II genes. This evidence provides reason to further investigate whether vitamin D supplementation may reduce MS susceptibility in those most at risk. Berezowska and colleageus (2019) postulate that other epidemiologic and experimental studies (Runia et al., 2012, Wacker & Holick, 2013, Duan et al., 2014, Harandi et al., 2014, Fitzgerald et al., 2015) investigating the effectiveness of vitamin D supplementation in MS have shown that low serum vitamin D levels may exacerbate MS symptoms and therefore are associated with higher relapse rates, occurrence of new lesions, and greater degree of disability. Therefore it can be concluded that higher serum 25(OH)D is significantly associated with lower MS incidence (Leray et al., 2016).

The risk of MS is 40% lower among women reporting regular intake of at least 400 IU/day of vitamin D supplements. High circulating levels of vitamin D have been associated with lower risk of MS, and higher serum 25-hydroxy-vitamin D (25 (OH)D) levels (>40 ng/ml) correlated with fewer MRI lesions and relapses (Correale & Gaitán, 2015). Although the correction of vitamin D insufficiency plays an important role in MS risk and disease activity (Thompson et al., 2018), the impact of vitamin D supplementation on MS disability remains inadequately investigated.(Sintzel et al., 2018).

2.4.1.2 Diet

Several studies have found associations between diet and disease outcome in MS. Davis et al. (2014) showed significant associations between disability as measured by the EDSS, and a low intake of fruits and vegetables. Van Rensburg et al. (2006) state that some MS patients, who present with low blood iron parameters, benefit from iron supplementation. Iron and a functional folate-vitamin B12-methylation pathway are needed for regeneration of myelin. In a pilot study these MS patients were taking a regimen of nutritional supplements designed to promote the regeneration of myelin. Patients participating in the study also had blood tests done to determine the level of haemoglobin, serum iron, transferrin, Tf saturation, Ferritin, CRP (C-reactive protein), cholesterol, homocysteine, serum folate, vitamin B12, 25-OH Vitamin D, Fibrinogen and D-dimer. The results of the study indicated that the patients on the regimen displayed significant neurological improvement.

A study conducted by Hadgkiss et al (2014) to explore the association between dietary factors (including fat, fruit and vegetable intake, dairy and meat consumption), and health-related quality of life (HRQOL), the disability and relapse rate in a large international sample of people with multiple sclerosis (MS) demonstrated a strong and significant association of healthy dietary habits with better physical and mental HRQOL and a lower level of disability in people with MS.

Modern diets, which are predominantly heat-processed, contain high levels of advanced glycation end products (AGEs) which are naturally present in uncooked animal-derived foods. Grilling, broiling, roasting, searing and frying of these foods result in acceleration of new AGE formation (Chen et al., 2013). Maillard-Lefebvre et al (2009) postulate that these products result from a non-enzymatic reaction between sugars and free amino acids of proteins. Furthermore, AGEs and their specific receptor for AGEs (RAGEs), were reported to be involved in microvascular and macrovascular complications. AGEs accumulate in the vessel wall where the cell structure and function is perturbed (Goldin et al., 2006). This pathological effect promotes oxidative stress and inflammation by binding with the cell surface (Chen et al., 2013).

The increased formation and accumulation of AGEs is associated with diabetes, renal failure, aging, inflammation and Alzheimer's disease (Maillard-Lefebvre et al., 2009). According to Goldin et al (2006), AGEs also contribute to the development of atherosclerosis which is a major vascular complication of diabetes. In a study, "Advanced glycation end products: from precursor to RAGE: round and round we go" by Ramasamy et al (2012), a positive correlation was discovered between coronary artery disease and RAGE. Burke and colleagues (2004) examined atherosclerotic plaques in diabetic and non-diabetic subjects and observed that the extent of RAGE expression was significantly higher in the diabetic versus the non-diabetic population.

Jelinek and colleagues (2013) conducted a study on the role of fish consumption and omega 3 supplementation in an international cohort of people with MS. The findings indicated that those consuming fish most frequently and those taking the largest doses of omega 3s were more likely to have normal mobility or only some disability. On the contrary, those not taking omega 3s were more likely to require major mobility support, and those taking omega 3s were more likely to be in the normal/some disability group. The study thus proves that frequent consumption of fish and omega 3 fatty acid supplementation are strongly associated with improved quality of life, reduced disease activity and disability in this large international cohort of people with MS.

Fragoso (2014) states that a diet rich in omega-3 unsaturated fatty acids, polyphenols and probiotics has been described to influence the development and character of regulatory T lymphocytes (Tregs). These cell types are emerging as key targets for the dietary prevention of chronic inflammatory diseases. In addition, a diet high in salt generates Th17 cells which appear to be highly pathogenic and related to proinflammatory cytokines (Issazadeh-Navikas et al., 2012; Kim & Lee, 2013). Cytokines are described by Dinarello (2000) as regulators of the host's responses to infection, immune responses, inflammation, and trauma. Proinflammatory cytokines act to make the disease worse whereas others serve to reduce inflammation and promote healing (anti-inflammatory).

Diet is not only strongly associated with reduced risk of relapse but also associated with depression risk, measures of fatigue, BMI and metabolic markers in MS (Taylor et al., 2014; Yadav et al., 2016).

2.4.1.3 Obesity and Physical Activity

Overweight or obese individuals have twice the risk of developing MS in adulthood. Exposure to a diet high in fat and cholesterol, high protein, high sugar and excess salt intake promotes obesity, cardiovascular disease and autoimmune diseases (Fragoso, 2014). Higher BMI correlates with increased intake of saturated fats and low physical activity (Davis et al., 2014). Increased BMI is also a risk factor for mortality from cardiovascular disease (CVD) – including coronary heart disease (CHD), ischaemic and haemorrhage stroke. According to Kavak (2015), women who had higher weight in adolescence and BMI in early adulthood were younger at MS onset. In addition to this, Hedström and colleagues (2014b) assessed the interaction between adolescent obesity and HLA risk genes in the aetiology of MS and observed a significant interaction between the HLA-DRB1*15 allele and obesity with regard to MS risk. It can thus be hypothesized that obesity-related inflammatory/immunological mechanisms are a contributory factor in explaining the association between adolescent obesity and increased risk of MS.

Exercise in MS is a form of treatment that does not lead to adverse effects and is beneficial in relation to various aspects of the disease, including fatigue, depression and disability (Fragoso,

2014: 891). According to Hägg et al (2005), physical activity improves cardiac and vascular function by lowering the carotid artery intima media thickness (IMT) and arterial wall stiffness. Arterial stiffness and increased IMT are the two main predictors of CVD. Increased IMT of the common carotid artery also serves as a surrogate marker for early atherosclerosis.

Marck et al (2016) conducted a study on the prevalence of comorbidities, overweight and obesity and associations with modifiable factors in an international sample of Persons with Multiple Sclerosis (PwMS) recruited online through social media, MS societies and websites. The study data indicated that overweight and obese PwMS reported lower mental and physical health HRQOL compared to those with normal weight. BMI was significantly associated with levels of disability, with obese participants 1.4 times more likely to have moderate to severe disability. Obesity and comorbidities including back pain, depression and anxiety may lead directly to decreased quality of life, and may indirectly interfere with health and wellbeing through lack of physical activity and quality of sleep. Prevention or treatment of comorbidities and obesity in PwMS should be an imperative goal in MS management (Merlino et al., 2009, Motl et al., 2011, Culpepper & Wallin, 2015).

2.4.1.4 Smoking

Smoking increases MS disability and is considered one of the most established environmental factors influencing MS risk (Davis et al., 2014; Hedström et al., 2014a). Cigarette smoking increases the risk of developing autoimmune diseases; and the risk of developing MS among smokers is two-fold that of non-smokers. Smoking MS patients have a more severe disease course and a faster disability progression rate (Fragoso, 2014). Smoking does not only increase MS risk, it also shortens the time to the secondary progressive course of MS (SPMS). The cessation of smoking will therefore slowly decrease the negative effects of MS, independent of the cumulative dose of smoking (van der Vuurst de Vries et al., 2018). Current evidence (Ghasemi et al., 2017) suggests that the nitric oxide (NO) and carbon monoxide (CO) produced by smoking plays a causative role in the destruction of nerve fibres. NO is a toxic soluble gas which, in pathological concentrations, can damage neurons and oligodendrocytes (Dawson et al., 1993, Merrill et al., 1993). Lipid peroxidation and mitochondrial damage that result from NO can lead to oligodendrocytes apoptosis (cell death), axonal degeneration, and demyelination (Mitrovic et al., 1995) . Ghasemi et al (2017) also state that a previous study by Somogyi et al (1981) has shown that CO exposure leads to blockage of tissue oxygenation, degradation of myelin basic protein (MBP), axonal injury and a subsequent inflammatory response including activated microglia and CD4+ lymphocyte invasion of the CNS, which results in demyelination (Thom et al., 2004). Smoking also increases the level of C-reactive protein, fibrinogen and inflammatory factors (IL-6) and may lead to dysregulation of B-cell and T-cell homeostasis (Abbasi, 2016). Smoking, which also plays an important role in the development of early atherosclerosis, is also found to cause

reduction in the cross-sectional diameter of the internal jugular veins (Nelson et al., 2014; Qu & Qu, 2015).

2.4.2 GENETIC FACTORS

MS is not a monogenetic disease although there are numerous genetic changes which are responsible for MS symptoms. In the 1970s, the human leukocyte antigen (HLA) locus was the first genetic factor related to the disease. This locus, which plays a role in MS diagnosis, is located in the short arm of chromosome 6, in a region called major histocompatibility complex (MHC) (Muñoz-Culla et al., 2013, Yaldizli et al., 2016, Goodin, 2015). Within this region, the genes encode highly polymorphic cell-surface glycoproteins that are key components of the immune system (Muñoz-Culla et al., 2013). The HLA locus contains many genes that have important functions in the immune system (Küçükali et al., 2015). Within this region HLA-DR2+, HLA-DQ6 , DQA 0102 and DQB1 0602 , HLA-DRB1, DR15 , DRB1*1501, and DRB1*1503 are genes susceptible to the onset of MS (Ghasemi et al., 2017). Furthermore, according to Ghasemi et al (2017), there are studies which show that the risk of MS in family members of a patient depends on the amount of genetic information they share (Ebers et al., 1995, Sadovnick et al., 1996, Sadovnick et al., 1999).

HLA genes are also related to other autoimmune disease such as Diabetes Type I. Specifically, the HLA-DRB*1501 haplotype is the genetic factor most strongly associated with the development of MS (Yaldizli et al., 2016). Okuda et al (2009) conducted a study on the "Genotype–Phenotype correlations in multiple sclerosis: genes influence disease severity inferred by 1HMR spectroscopy and MRI measures" and observed a clear association between DRB1 * 1501* status and four domains of disease severity. In a sample of 505 MS patients, DRB1*1501+ patients had significantly more women (74% versus 63%; P=0.009) and a younger mean age at disease onset (32.4 years versus 34.3 years; P=0.025). Their findings suggest that DRB1*1501+ increases disease severity in MS. However, recent hereditary studies showed that at least twelve genes outside HLA in chromosome 6 are also related to MS (Koriem, 2016). It is therefore clear that HLA-DRB1*15:01 has the strongest association with MS risk (Didonna & Oksenberg, 2015).

2.4.3 VASCULAR AND BIOCHEMICAL FACTORS

There are indications that vascular factors may play a role in demyelination since myelin production and maintenance in the brain is dependent on the delivery of nutrients and removal of toxic waste products by the blood (Davis et al., 2014). According to D'haeseleer et al (2011) there are three forms of vascular abnormalities associated with MS: first, an increased risk for ischaemic disease; secondly, global cerebral hypoperfusion and thirdly, a chronic state of impaired venous drainage.

2.4.3.1 Vessel Wall

The arterial and venous vessel wall is comprised of 3 layers; the intima (innermost luminal layer), media (middle) and adventitia (outermost layer). The intimal layer is lined by the endothelium which is a continuous layer of flat polygonal endothelial cells (ECs) that come into direct contact with blood flow (Sumpio & Chin, 2014:34). The endothelium, which participates in physiologic and pathologic processes, also reacts to physical forces, chemical signalling, and immunologic mediators (Sumpio & Chin, 2014:34). The endothelium provides a continuous lining throughout the arteries, capillaries, veins, heart valves, and endocardial surfaces. The ECs are covered by a glycoprotein coat (glycocalix) that is accountable for the antithrombogenic properties of the endothelial surface (Levy & Tedgui, 1999). The glycocalix also reduces friction from blood flow and serves as a barrier for fluid loss through the vessel wall. The thickness of the glycoprotein coat varies across the vascular tree (van den Berg et al, 2003). During inflammation, the glycocalix is sheared off, permitting the attachment of leukocytes and the transport of water from microvessels, resulting in the possible initiation of atherosclerotic lesion development (Sumpio & Chin, 2014). A positive association exists between lumen diameter and acute myocardial infarction (Kappus et al., 2014). Carotid artery lumen diameter is influenced by carotid intima media thickness (cIMT), where an increase in cIMT causes reduction of the lumen diameter. According to Kamenskiy et al. (2015), the carotid artery bulb diameter increases with increasing age. This could be related to degradation of intramural elastin in the vessel walls.

Tettey et al (2014) postulate that vascular risk factors and vascular comorbidities such as obesity, dyslipidaemia, type-2 diabetes and cardiovascular disease have been associated with MS onset and disease progression.

2.4.3.2 Homocysteine and vascular disease

Increased homocysteine (Hcy) levels in MS patients are associated with vascular damage and iron deficiency as described by Van Rensburg et al (2006). The risk for the development of atherosclerotic vascular disease is increased with mild to moderate elevation of homocysteinemia (Guo et al, 2009). There are two types of hyperhomocysteinemia: firstly, the rare but severe form due to major genetic mutations of the enzymes implicated in homocysteine metabolism; secondly the more common form causes moderately elevated homocysteine levels related to a pathogenesis such as genetic and environmental factors (Hankey & Eikenboom, 1999). Increased levels of homocysteinemia are also associated with endothelial dysfunction in blood vessels (Woo et al, 2002). Hyperhomocysteinemia can cause injury to the endodermis of the blood vessels, activate the platelets and enhance the production of fibrinogen, which is associated with vascular damage (Reinhart, 2003). This results in the proliferation of smooth muscle cells, which can cause narrowing (stenosis) of the lumen of

blood vessels. Long term use of folic acid improves arterial endothelial function and has potential of preventing atherosclerosis in persons with hyperhomocysteinemia (Guo et al, 2009).

According to Ďurfinová et al (2018), an elevated homocysteine serum level is also an independent cardiovascular disease risk factor modifiable by nutrition. Hyperhomocysteinemia (> 15 μmol/l) may lead to an enhancement of the adverse effects of risk factors like hypertension, lipid and lipoprotein metabolism, as well as promotion of the inflammation development (Ganguly & Alam, 2015).

2.4.3.3 High-density lipoprotein (HDL) and vascular disease

High-density lipoprotein (HDL), a biomarker, is a heterogeneous complex of differing size, density, surface charge, and lipoprotein content. Serum levels of this biomarker is thought to be inversely related with atherosclerotic vascular disease risk (Chang et al., 2015) . Risk factors for coronary artery disease (CAD) are the same as for cerebrovascular disease. Measurement of the IMT of the carotid artery (CIMT) by B-mode ultrasound is regarded as the technique used to quantify atherosclerosis. Prediction of patients at risk of atherosclerosis allows preventative measures to be implemented to avoid progress of the disease (Stein, 2004). Increased CIMT is widely accepted to be a surrogate marker for early atherosclerosis, and risk factors include increased hypertension, body mass index (BMI), LDL cholesterol, homocysteine and fibrinogen. IMT also increases with age (Grebe et al., 2010).

2.4.3.4 Cholesterol, Low-density lipoprotein (LDL) and vascular disease

Cholesterol plays an important role in neuronal physiology. Most of the cholesterol in the adult brain is in the myelin sheath formed by oligodendrocytes to insulate axons. The important source of cholesterol for peripheral tissues are low density lipoprotein particles (LDL) (Ďurfinová et al., 2018). Low density lipoprotein cholesterol (LDL-C) plays a causative role in the development of atherosclerotic cardiovascular disease (ASCVD) (Penson et al., 2018). The LDLs which are driven by arterial blood pressure are carried through the arterial wall by fluid flux. In the arterial intima, LDLs could become prone to oxidation or other modification. (Ďurfinová et al., 2018). As described by Penson et al (2018), the radical chain oxidation of LDL fatty acids causes modification of ApoB100 on LDL surface. Scavenger receptors on macrophages recognise the modified ApoB100 and the immune cells digest the ox-LDL (oxidised LDL) and transform into foam cells. The foam cells grow until they rupture, which attracts more immune cells which finally results in the inflammation of the arterial wall and vascular dysfunction.

2.4.3.5 Carotid intima-media thickness (CIMT) and cardiovascular disease (CVD)

The arterial wall is comprised of 3 layers (intima, media and adventitia) where intima-media (IM) thickening (>0.8mm) of the carotid arteries serves as a surrogate marker for early atherosclerosis. Carotid artery atherosclerosis is known to be associated with symptoms of cerebral ischaemia. The carotid intima-media thickness (cIMT), measured by carotid ultrasound, is also used to predict the occurrence and identify persons at risk of cardiovascular disease (Lee & Park, 2014; Qu & Qu, 2015; Müller-Scholden et al., 2019).

Atherosclerosis, which may begin in childhood as arterial damage in the intima, is described as an inflammatory disease of the vascular wall (usually arterial), where components of the immune system are involved in the steps of the atherosclerotic process (Ross,1999; Blake & Ridker, 2001; Glass & Witztum, 2001; Hallenbeck et al., 2005; Hansson, 2005; Calder, 2012; Owens, 2014; Akbari-Sedigh et al., 2019). The endothelium lines all blood vessels and in normal physiological conditions, contributes to vascular homoeostasis, by actively regulating vascular tone, controlling permeability between the bloodstream and the underlying vascular wall, regulating medial smooth muscle cell growth, and controlling platelet function, coagulation and fibrinolysis (Baker et al., 2018). The mean and maximum CIMT are independently influenced by age, blood creatinine levels and non-high density lipoprotein (HDL) cholesterol levels (Bosevski, 2011). Circulating low-density lipoprotein concentrations are associated with CIMT (Ross et al., 2012; Dessein et al., 2013), and high levels of oxidized LDL (OxLDL) are significantly associated with increased levels of CIMT (Lopes-Virella et al., 2011, Hunt et al., 2013, Paim et al., 2013). Several studies have also shown that low serum 25-hydroxyvitamin D3 (25(OH)D) levels in childhood were associated with increased CIMT in adulthood (Robinson et al., 2014) Therefore, early application of 25(OH)D or coupling with oral lipid-lowering drugs such as statins may be helpful for preventing subclinical atherosclerosis (Qu & Qu, 2015).

Multiple sclerosis patients could have an increased CVR (cardiovascular risk), based on elevation of some aggressive and atherogenic molecules in serum such as Ox-LDL and small HDL-cholesterol (HDL-c) (Palavra et al., 2013). Lipoproteins are involved in the regulation of neural functions in the CNS through local mechanisms that are linked to systemic lipid metabolism (Gotthardt et al., 2000, Stockinger et al., 2000).

Palavra and colleagues (2013) conducted a study aimed to characterize a population of multiple sclerosis (MS) patients in terms of traditional and new cardiovascular risk factors and assess their putative correlation with clinical disease activity (evaluated by the Expanded Disability Status Scale [EDSS]). Their findings indicated that worsening MS disability was associated with higher levels of LDL, total cholesterol and triglycerides. MS patients appear to present a profile indicative of an increased CVR, when compared with healthy subjects of well-matched age and sex. The clinical activity of the disease (given by the EDSS score) correlated significantly and directly with some markers of CVR, specifically Ox-LDL. It is therefore

recommended that early pharmacotherapeutic intervention to prevent premature development of cardiovascular disease in these patients should be considered.

2.4.3.6 Extracranial arterial and venous pathology

Current literature does not demonstrate any studies or study data on the prevalence of carotid artery disease in persons with MS. However, there has been renewed interest in studying extracranial venous changes associated with the development and MS. This is a result of a controversial finding that abnormalities in the extracranial venous system is associated with the disease (Beggs, 2013). This condition, known as chronic cerebrospinal venous insufficiency (CCSVI), is a venous pathology that is believed to play a causative role in the development of the disease (Zamboni et al. 2009a; Zamboni et al. 2009a; Simka et al. 2010; Monti et al., 2011; Nicolaides et al., 2011; Zivadinov et al., 2011; Barreto et al., 2013; Comi et al., 2013; Farina et al., 2013; Krsmanovic et al. 2013; Leone et al., 2013, Zavoreo et al., 2013; Zivadinov et al., 2013; Ciciarello et al., 2014, Rasman, 2015). CCSVI is characterized by multiple intraluminal stenotic malformations of the internal jugular veins (IJVs) and the azygos vein, and has been shown to be associated with impaired blood flow from the brain to the heart in patients with MS (Zamboni et al., 2012). CCSVI also appears to be associated with changes in the intracranial vasculature, with a strong correlation shown between CCSVI severity and decreased cerebral blood flow (CBF) in both the white matter (WM) and grey matter (GM) of patients with MS (Beggs, 2013).

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). A cross-sectional study on the prevalence of abnormal blood flow patterns and effects of biochemistry and lifestyle factors on the major neck vessels in patients with Multiple Sclerosis, demonstrated a significant difference in cross-sectional diameters of IJVs. Smoking MS patients displayed a smaller IJV diameter in comparison to non-smoking MS patients on Doppler Ultrasound (Nelson, et al., 2014). Haemodynamic of the IJVs may reflect cerebral venous drainage conditions and appear to influence intracranial hemodynamics, perfusion of the brain as well as the dynamics of CSF system (Mancini et al., 2014).

Doppler Ultrasound is useful for identifying outflow disturbances and morphological abnormalities associated with the IJVs and vertebral veins (VVs). Krsmanović et al (2013) conducted a study to assess the prevalance of CCSVI and small IJVs among MS patients and controls using Doppler ultrasound. Their findings illustrated a higher EDSS among small IJV-positive patients in comparison to those presenting with normal veins. IJV stenosis, is defined either as a cross-sectional area (CSA) ≤ 0.3 cm^2 or as a local CSA reduction of ≥50% (Zamboni

et al., 2009a; Zamboni et al., 2009b; Doepp et al., 2010) and is one of the most frequently observed CCSVI criteria in patients with MS (Krsmanović et al., 2013).

On the contrary, Wattjes and colleagues (2011) conducted a study aimed to investigate the intracranial and extracranial venous anatomy and the venous flow profile in patients with MS and healthy controls using magnetic resonance venography (MRV). Their findings of anomalies of the cranial venous outflow anatomy were frequently observed in both MS patients and healthy controls. It is likely that these findings reveal anatomical variations of venous drainage rather than clinical relevant venous outflow obstructions. These findings were corroborated by a recent prospective 5-year follow-up study (Gandhi et al., 2019), assessing the relationship of variations in extracranial venous anatomy, indicative of chronic cerebrospinal venous insufficiency (CCSVI) on Doppler sonography, according to the International Society for Neurovascular Disease (ISNVD) proposed consensus criteria, with clinical outcomes and disease progression in MS patients. The study data confirmed that the presence of variations in extracranial venous anatomy is not exclusive to the MS patients, as there was no significant difference in the prevalence of those in MS patients versus His (healthy individuals), at the follow-up. Thus, a causal association between CCSVI and MS cannot be implied.

2.5 TREATMENT

The treatment of MS is challenging and involves the use of immunomodulator drugs and symptom management to treat the specific symptoms such as fatigue, spasticity, and pain. The indication ultimately depends on the clinical course of the disease (Garg & Smith, 2015; Huang et al., 2017).

Treatments for MS are divided into 3 categories namely:1) acute relapse management, 2) disease-modifying treatments (DMTs) and 3) symptomatic treatments.

1) Acute relapse management - For a relapse of moderate functional severity or worse, Doshi & Chataway (2017) report that a high-dose of 500–1 000 mg per day of methylprednisolone therapy for 3–5 days should be considered. Although corticosteroids are not disease modifying, they can be used to shorten the duration of the relapse.

2) Disease-modifying treatments (DMTs) - There are over 10 approved MS DMTs which vary in degrees of efficacy for reducing relapse risk and preserving neurological function. However, their long-term benefits remain unclear. The approved DMTs include 4 interferon beta preparations and glatiramer acetate (GA), Mitoxantrone, Natalizumab, 3 oral DMTs (fingolimod, teriflunomide, and dimethyl fumarate/BG-12), Alemtuzumab, Ocrelizumab, Laquinimod and Daclizumab. (Wingerchuk & Carter, 2014; Garg & Smith, 2015; Huang et al., 2017).

3) Symptomatic treatments - Patients with MS experience a large range of significant and disabling symptoms, including fatigue, cognitive impairment, bladder dysfunction, faecal incontinence, pain and spasticity as illustrated in Table 2.4 (Garg & Smith, 2015, Doshi & Chataway, 2017).

Table 2.4 Suggested symptomatic treatment in MS

Symptom	Drug	Interventional	Multidisciplinary input
Fatigue	Amantadine		Occupational therapy and physiotherapy: fatigue management assessment and exercise programme
Cognition/low mood	Depression: e.g. citalopram duloxetine		Neuropsychology service, cognitive behavioural therapy, occupational therapy
Spasticity	Baclofen, abapentin, tizanidine,clonazepam, dantrolene	Intrathecalclofen, botulinum toxin	Physiotherapy
Bladder	Frequency/urgency: oxybutynin, solifenacin, tolterodine, mirabegron Nocturia: desmopressin/DDAVP spray	Residual bladder volume >100 ml: intermittent self-catheterisation or permanent catheter; intravesicular botulinum toxin,	Uro-neurology
Sexual dysfunction	Sildenafil, tadalafil, alprostadil, yohimbine		Uro-neurology
Constipation	Fibre/fluid, bulking agents, osmotic stimulant laxatives, suppositories, transanal irrigation		
Faecal incontinence	Codeine, loperamide		Biofeedback, neuro-gastroenterology
Pain	Amitriptyline, pregabalin, gabapentin, lamotrigine		
Ataxia/tremor	Propranolol, clonazepam, levetiracetam, isoniazid (with pyridoxine), carbamazepine, ondansetron	Botulinum toxin, thalamotomy	Physiotherapy, occupational therapy, audiovestibular therapy
Oscillopsia	Gabapentin, memantine, levetiracetam, clonazepam, baclofen		Neuro-ophthalmology

(Doshi & Chataway, 2017).

According to Feinstein et al (2015) physiotherapy has a small, but a significant beneficial effect on balance in MS patients with mild to moderate disability. However, the evidence for the effects in severely disabled persons are scarce. In addition, exercise may delay disease progression by reducing inflammation and encouraging neuronal repair (Feinstein et al., 2015). Huang and colleagues (2017) state that vitamin D provides beneficial therapeutic effects in small studies and administration of simvastatin may be beneficial for MS.

CONCLUSION

MS is a complex disease attributed to multiple genetic, biochemical, lifestyle, environmental and vascular factors which influences the onset and progression of the disease. Various studies have been performed to establish a significant association between the various factors and MS, however some of the findings are equivocal and inconclusive This means that there is still a demanding need for further research studies to be undertaken to identify the causes for the development and disability progression of the disease, which will inform treatment methods for improved quality of life in MS patients.

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

INTRODUCTION

A case-control study was performed on a cohort of 50 participants which included 25 controls and 25 participants clinically diagnosed with multiple sclerosis (MS). The purpose of the study was to use ultrasound imaging, in conjunction with lifestyle factors, biochemical and genetic testing, to identify risk factors associated with extracranial vascular pathology in MS. All participants received a carotid artery (CA) and internal jugular vein (IJV) ultrasound that included colour and spectral Doppler analysis.

This ultrasound study was a component of another ethically approved study titled: *"The implementation of a comprehensive gene-based, pathology supported intervention program in patients with disorders that include a component of demyelination such as multiple sclerosis (MS)"* NO7/09/203 (Division of Chemical Pathology, Tygerberg Hospital and University of Stellenbosch). MS patients were age-matched with MS free controls which were recruited by the principal investigator of the ultrasound study (PI). Ultrasound examinations of all the participants were performed by the PI at a Radiology practice, Cape Town, Western Cape, South Africa.

3.1 HYPOTHESIS

Genetic, vascular, biochemical and lifestyle factors are significantly associated with the development of extracranial vascular pathology in Multiple Sclerosis.

3.2 RESEARCH OBJECTIVES

The main objectives of the study were to:

- Compare the Ultrasound findings of the extracranial vascular systems in the two cohorts (MS and free from MS)
- Establish an association between the Human Leukocyte Antigen (HLA*) DRB1*1501* gene and the Grey-scale, Colour and Spectral Doppler analysis of the carotid arteries (CAs) and internal jugular veins (IJVs) in the two cohorts.
- Establish an association between biochemical markers (homocysteine, Vitamin D, iron, Vitamin B12, C-reactive protein (CRP), blood lipids) and the grey-scale, colour and spectral Doppler analysis of the CAs and IJVs in the two participant groups.
- Establish an association between lifestyle factors such as physical activity, body mass index (BMI) and diet; and the grey-scale, colour and spectral Doppler analysis of the CAs and IJVs in the two cohorts.
- Establish an association between disability and the grey-scale, colour and spectral Doppler analysis of the CAs and IJVs in the active MS cohort.

3.3 SAMPLE

3.3.1 Sample size

A minimum of 25 participants with MS were required to demonstrate statistically significant results. The formula used for calculation of the sample size 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%)

A sample of 50 participants were recruited with one group of 25 as the active MS cohort and the other of 25 as the control group.

3.3.2 Inclusion and Exclusion criteria

Inclusion criteria were as follows:

Active cohort,

- participants clinically diagnosed with MS according to McDonald's criteria (McDonald et al., 2001).

Control group,

- age-matched participants free of MS

Both cohorts included:

- only female patients from all race groups and ages (>18 years). According to Modi et al (2008), more females than males are diagnosed with MS in South Africa, therefore only females were included in the study.
- persons who were normotensive (including persons on medical management for hypertension)
- non-smokers exposed to passive smoking

Exclusion criteria were as follows:

- Persons who have been diagnosed with other neurological diseases including neuromyelitis and acute disseminated encephalomyelitis (ADEM).
- Persons previously diagnosed with carotid artery and extracranial venous disease.
- Persons who have a previous medical history of diabetes mellitus and cardiovascular disease.
- Male patients

3.4 DATA COLLECTION

The participants' age, gender and ultrasound findings were captured from the ultrasound reporting templates onto the research datasheet. Biochemical data (full blood count, haematinics, inflammatory markers, cardiac markers/risk factors, lipids and vitamins) were retrieved from the participants' blood results. Medical and lifestyle data were retrieved from the questionnaire that participants completed on entry to the study and the EDSS from the disability status score sheet. Genetic data was extracted from the password-protected website Gknowmix.com where only registered users have access. All retrieved data was captured onto the Microsoft Excel research database.

3.5 DIAGNOSTIC ULTRASOUND

The as low as reasonably achievable (ALARA) Doppler ultrasound thermal index of <100 mW/cm^2 was maintained at all times during the ultrasound examinations to prevent the possible adverse biological effects of Doppler ultrasound which may include heating and cavitation of tissues (Kremkau, 2006: 352).

The GE (General Electric) Logiq S8 and Logiq E9 ultrasound systems with Grey-scale imaging and Doppler facilities (colour and Spectral Doppler) and a 9-12 MHz multifrequency linear transducer with coupling gel were used to assess the carotid arteries and extracranial venous system. A high frequency linear transducer was used to optimally visualise the superficial vasculature, namely the common carotid arteries (CCAs), internal carotid arteries (ICAs), external carotid arteries (ECAs), vertebral arteries (VAs) and internal jugular veins (IJVs).

Grey-scale imaging was used to:

- interrogate the major neck vessels for tortuosity, anatomical variation, plaque formation in the carotid arteries,
- measure the intima media thickness (IMT) of the CCA,
- measure the cross-sectional diameter (CSD) of the proximal, mid and distal CCA,
- measure the CSD of the proximal ICA, ECA and proximal VA
- measure the cross-sectional area (CSA) of the proximal IJV at the level of the supraclavicular notch.

Colour Doppler was used to assess the vessels for patency, direction of blood flow, any colour-filling defects and presence of an occlusion.

Spectral Doppler analysis was used to detect carotid artery stenosis by measuring the peak systolic and end diastolic velocities within the carotid vessel. The pulse repetition frequency (PRF) for this study was adjusted to 150 cm/s for assessment of the carotid arteries, 100 cm/s for the VAs and 60 cm/s for the IJVs. These frequencies were adjusted to detect blood flow at the specified velocities in each vessel and prevent the artefact, aliasing which is a limitation associated with spectral Doppler analysis. The resistance of the distal vascular bed the vessels feed influences the blood flow velocity and blood flow profile exhibited within the vessel. The waveforms of the carotid vessels, VAs and IJVs were illustrated using spectral Doppler analysis.

The captured ultrasound images were stored onto the ultrasound system's hard drive and picture archive communications system (PACS). The ultrasound images were also copied onto a compact disc (CD) using the Epson PP-100 II CD writer.

3.5.1 Carotid Ultrasound protocol

The carotid and vertebral arteries were imaged using grey-scale imaging, colour and spectral Doppler analysis. The vessels were assessed for plaque formation, patency and stenosis.

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

Table 3.1 illustrates the recommended normal and abnormal values of ultrasound findings.

Table 3.1 Carotid Ultrasound findings

DOCUMENTED ULTRASOUND FINDING	NORMAL and ABNORMAL VALUES
Intima media thickness (IMT) of CCA	IMT >0.8 mm is regarded as abnormal (Stone & Hass, 2014)
ICA (internal carotid artery)	Peak systolic velocity (PSV) should be <125 cm/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 200 cm/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 B).

3.5.1.1 Grey-scale imaging of the carotid artery

Using grey-scale imaging the CCA was assessed in the transverse (Figure 3.1) and longitudinal planes (Figure 3.2) from the supraclavicular notch up to its bifurcation and along the ICA and ECA to its distal extent. This assessment was performed to identify any anatomical variation, tortuosity and plaque formation.

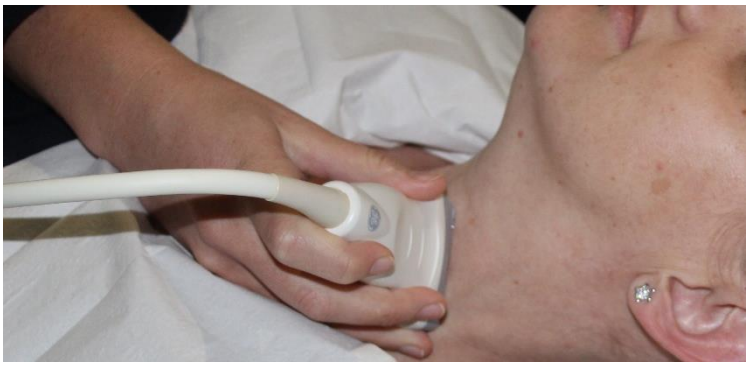

Figure 3.1.Ultrasound model in supine position with transducer in transverse plane.

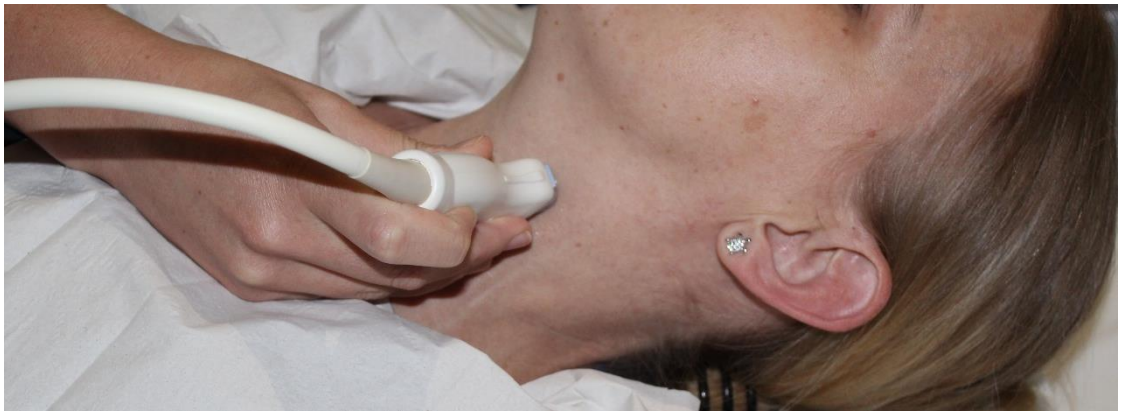

Figure 3.2 Ultrasound model in supine position with the transducer in the longitudinal plane assessing the CCA.

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

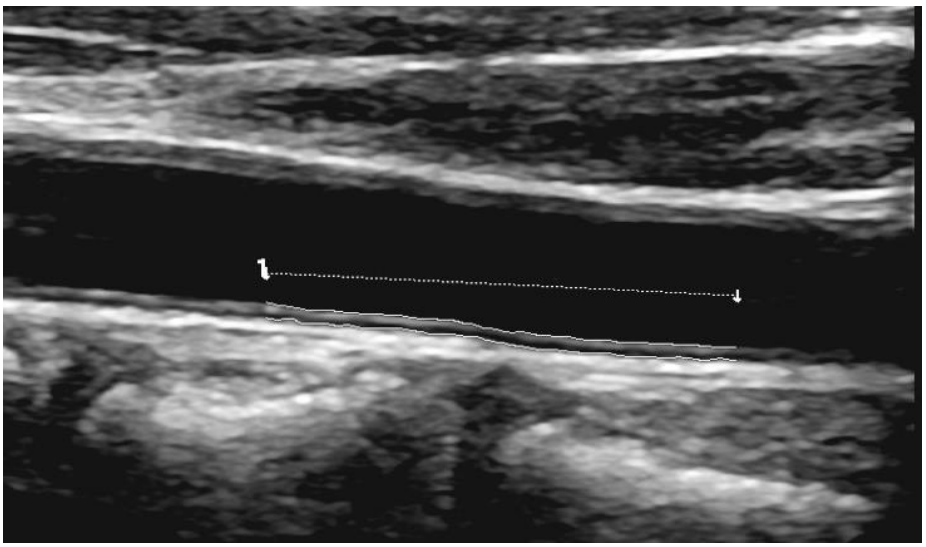

Figure 3.3 Grey-scale image of a normal left CCA in the longitudinal plane. Average IMT = 0.57mm (upper limit of normal = 0.8mm) (Permission granted by patient #C15).

The cross-sectional diameter (CSD) of the proximal, mid and distal CCA and proximal ICA and ECA were measured wall to wall and recorded (See Figure 3.4).

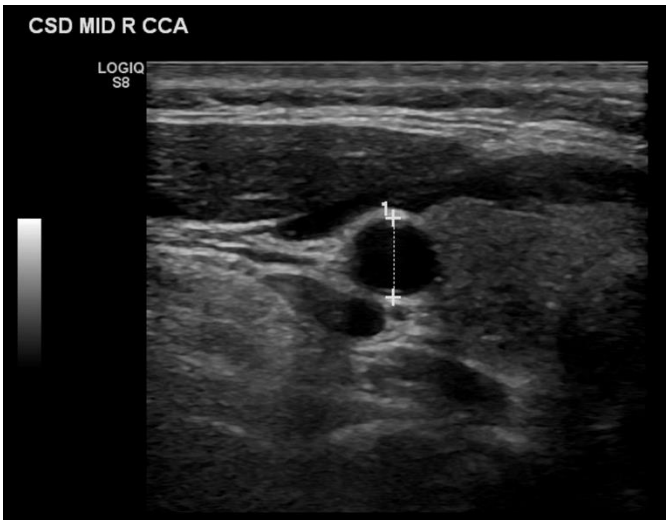

Figure 3.4. Cross-sectional diameter (CSD) of the mid right CCA. CSD = 7 mm (Permission granted by patient #CO7)

3.5.1.2 Colour Doppler of the carotid artery

Colour Doppler is useful in assessing patency of the vessel, direction of blood flow as well as detecting colour filling defects which are indicative of abnormal blood flow within the vessel.

The CCA, ICA, ECA and vertebral artery were scanned in the longitudinal plane and assessed using colour Doppler. The gain was adjusted so that only the lumen of the vessel displayed colour flow, thus preventing colour aliasing. The colour box was placed in a position parallel to the vessel being sampled.

3.5.1.3 Spectral Doppler analysis of the carotid artery

Once the vessels have been assessed with grey-sale and colour Doppler imaging, spectral Doppler was 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 2 mm was used to produce an accurate velocity measurement. The angle cursor should be parallel to the wall of the segment of the vessel being sampled as illustrated in Figure 3.5. At least 3 waveforms of each vessel should be captured and recorded.

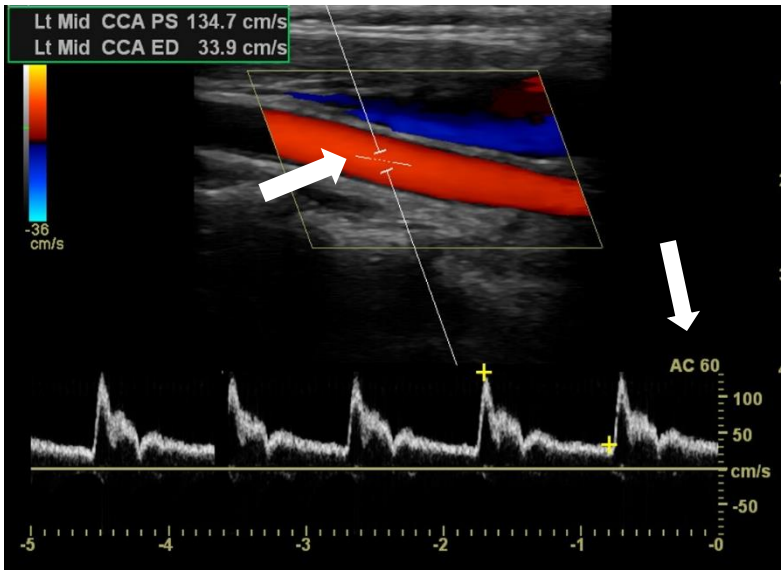

Figure 3.5. Colour and spectral Doppler analysis of a normal left common carotid artery (CCA) at a Doppler angle of 60 degrees (solid arrows). The peak systolic velocity = 134.7 cm/s, end diastolic velocity = 33.9 cm/s.
(Permission granted by patient #MS03)

A spectral waveform of the mid CCA (Figure 3.5) approximately 3 cm distal to the carotid bulb was recorded and the proximal and mid segments of the ICA (Figure 3.6) and ECA (Figure 3.7). If the carotid bifurcation is at the level of the angle of mandible then only the proximal segments of the ICA and ECA can be optimally visualized and assessed. The PSV and EDV of the mid VA (Figure 3.8) was also recorded.

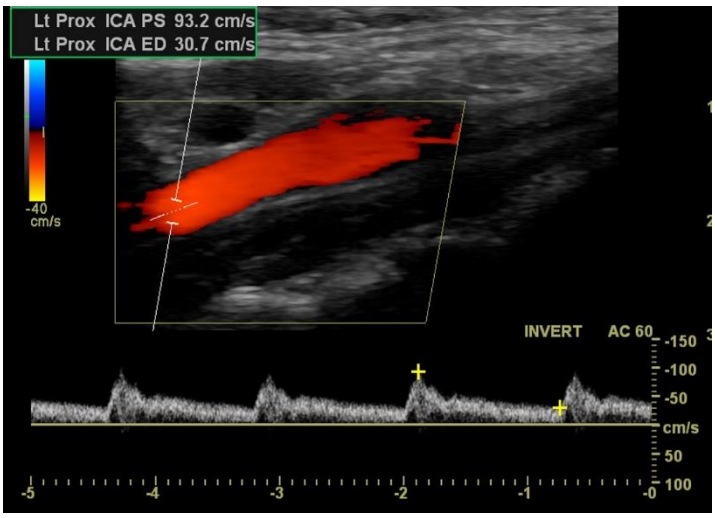

Figure 3.6. Colour and spectral Doppler analysis of a normal left internal carotid artery (ICA).
peak systolic velocity = 93.2cm/s, end diastolic velocity= 30.7 cm/s. (Permission granted by patient #CO2)

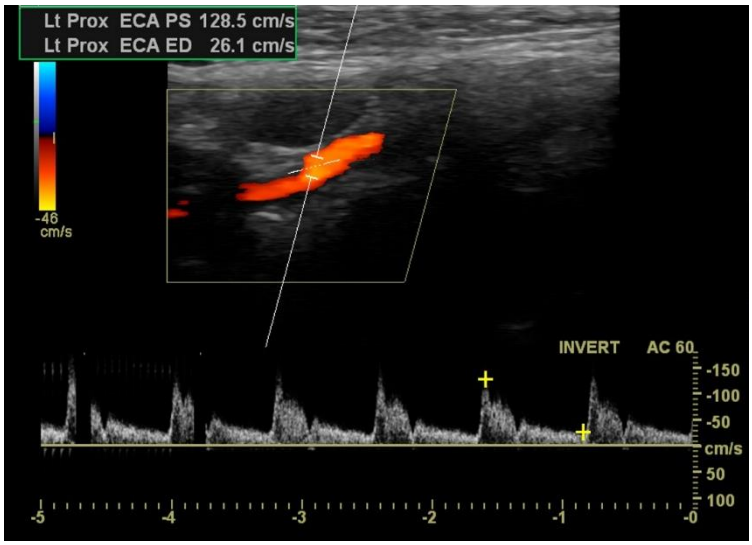

Figure 3.7. Spectral Doppler analysis of a normal left proximal external carotid artery (ECA). PSV=128.5 cm/s, EDV=26.1 cm/s (Permission granted by patient #CO12)

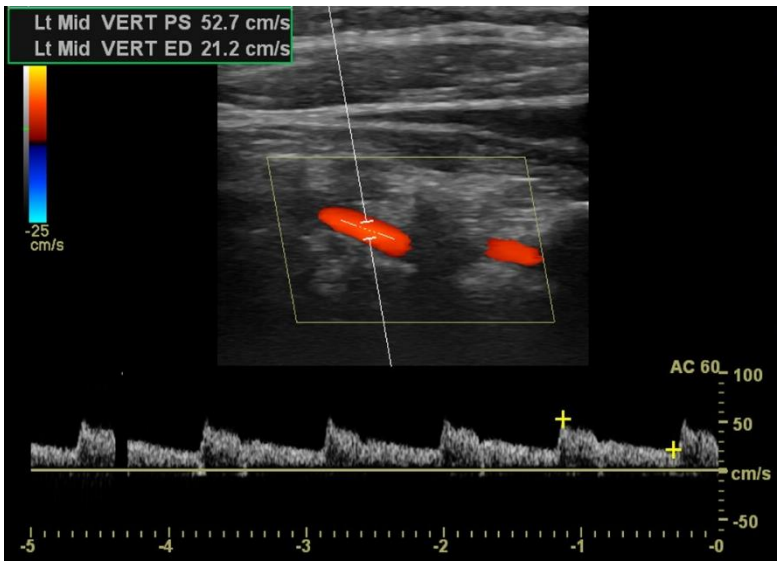

Figure 3.8. Colour and spectral Doppler analysis of a normal left mid vertebral artery. PSV=52.7 cm/s, EDV=21.2 cm/s. (Permission granted by patient #CO1)

3.5.2 Extracranial cerebrospinal venous ultrasound protocol

Grey-scale imaging, colour Doppler and spectral Doppler were employed to assess the internal jugular vein in longitudinal and transverse planes.

The IJV was assessed with the patient in the supine position as the IJV is the dominant outflow pathway with the patient in this position. As a result, the IJV can be easily visualised on ultrasound imaging. The IJV was assessed for patency and stenosis. The IJV findings of each participant were captured onto a template (See Appendix C):

- Patency and blood flow velocities of the proximal, mid and distal right and left IJVs.
- Cross-sectional area (CSA) of the proximal right and left IJV at the level of the supraclavicular notch.

3.5.2.1 Grey-scale imaging of the internal jugular vein

The IJV was 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 was interrogated in the longitudinal and transverse planes, keeping the probe pressure on the vessel to a minimum. The CSA of the proximal right and left IJV was measured at the level of supraclavicular notch as seen in Figure 3.9.

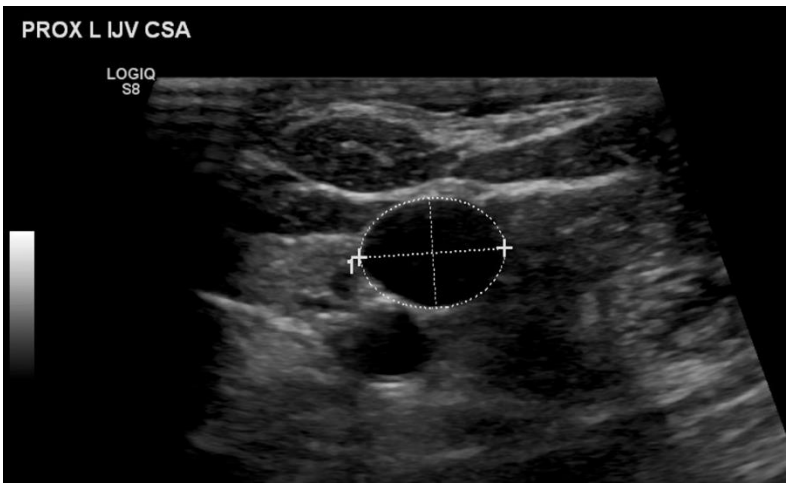

Figure 3.9. Cross-sectional area of the proximal left IJV. CSA=99 mm^2. (Permission granted by patient #MS11)

3.5.2.2 Colour Doppler of the internal jugular vein

Colour Doppler was used to assess patency of a vessel, identify any colour filling defects and abnormal flow within the vessel. The proximal, mid and distal segments of the IJV were assessed with the colour box parallel to the vessel being sampled.

3.5.2.3 Spectral Doppler analysis of the internal jugular vein

Spectral Doppler interrogation included sampling of the proximal, mid and distal segments of the IJV using a Doppler angle of 60° and a sample volume size of 2 mm. The Doppler angle was parallel to the vessel wall and the centre of the vessel lumen was sampled as seen in Figure 3.10.

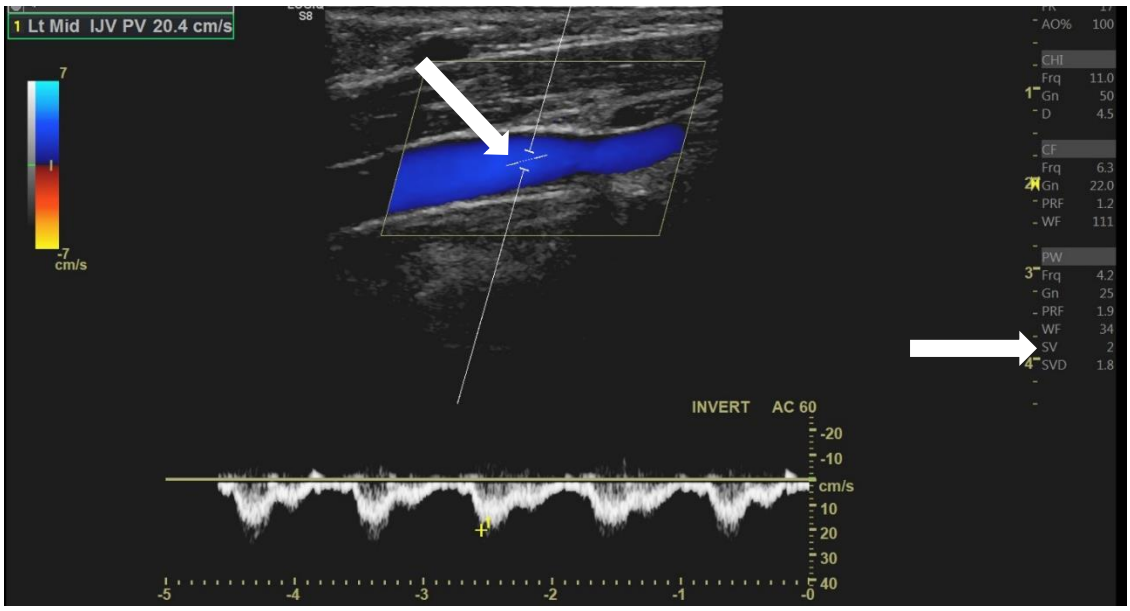

Figure 3.10. Colour and spectral Doppler analysis of the left mid IJV. The vein is patent with normal directional flow away from the brain (blue). The sample volume (SV) is at 2 mm (solid arrows). The peak blood flow velocity is at 20.4 cm/s (Permission granted by patient #MS20)

Each ultrasound examination (carotid and extracranial venous ultrasound) took approximately 40 minutes. All ultrasound examinations were done by the PI who is an experienced vascular sonographer. All cases were reported by a radiologist and participants with abnormal findings were referred to their respective neurologist or general practitioner for further management.

3.6 LIFESTYLE AND DISABILITY DATA

Participants completed a medical and lifestyle questionnaire on entry to the study (See Appendix D). The questionnaire recorded the number of days per week of eating certain foods retrospectively over a period of 3 months. Intake of 5 or more fruits and vegetables was also recorded. Information on family history, own medical history, clinical symptoms and medication was documented. Physical activity was self-reported and categorised into high (exercise 4 or more times a week), moderate (exercise 2-3 times a week) or low (exercise occasionally or complete lack of exercise).

The disability status of the MS participants was assessed by a clinician using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). According to Madras (2014), this scale quantifies disability in MS patients according to their signs and symptoms observed during a neurological assessment.

3.7 BIOCHEMICAL ANALYSIS

Blood was drawn for biochemistry and genetic testing in the morning between 09h00 and 10h30 to standardise for diurnal variation.

The biochemical parameters were determined using the following methods:

- Immunoturbidimetry
- Chemilluminescense
- Enzymatic method
- Glycerol phosphate oxidase
- Accelerator selective detergent method
- Measuring liquid selective detergent

3.8 GENETIC ANALYSIS

Genotyping was performed by real-time polymerase chain reaction (PCR) assays on the Roche Lightcycler LC480-II using hydrolysis probe TaqMan® single nucleotide polymorphism (SNP) genotyping assays. The HLA DRB1*1501 was investigated by use of a haplotype tagging SNP, rs9271366 (Field et al., 2010).

3.9 DATA ANALYSIS

3.9.1 Study data of the cohort of 50 participants (25 MS and 25 controls)

Mixed model repeated measures ANOVA were used to compare left and right vessel measurements. One way ANOVA was used for comparison of measurements between groups (patients with MS and patients free of MS). The ANOVA F-test and the Mann-Whitney U test were used to test the same hypothesis. Spearman and Pearson's' correlations were used for testing relationships between biochemical, genetic, lifestyle variables and ultrasound measurements.

3.9.2 Match-merged data

The data of the current case-control study (Ultrasound investigation of risk factors for extracranial vascular pathology in patients with multiple sclerosis) were matched and merged with data of a previous cross-sectional observational ethically approved study titled: Ultrasound evaluation of the extracranial cerebrospinal venous system and carotid arteries in patients with multiple sclerosis (Nelson et al., 2014). Ethical approval for the previous study was granted by the Cape Peninsula University of Technology (CPUT), study Registration Number NHREC: REC-230408-014. The previous (study A) and current (study B) studies were conducted by the same principal investigator.

The data of studies A and B were merged to demonstrate a stronger association between lifestyle, biochemistry, genetics, disability status and the ultrasound parameters in MS. The matching genetic, lifestyle, biochemical and ultrasound variables including disability status of the cohort of 23 MS participants for study A (excluding males) and 25 MS participants on study B, were merged and analysed.

The same ultrasound technique for study B, as described in 3.5, was employed in study A. The ultrasound variables assessed in study A were the:

- Intima media thickness (IMT) of the right and left common carotid artery (CCA)
- peak systolic and end-diastolic velocities of the right and left CCA and internal carotid artery (ICA),
- peak systolic velocities of the right and left vertebral artery (VA) and external carotid artery (ECA)
- right and left peak systolic velocity ratios: ICA/CCA
- right and left end-diastolic velocity ratios: ICA/CCA
- patency and cross-sectional diameters (CSDs) of the right and left proximal, mid and distal internal jugular veins (IJVs)

Mixed model repeated measures ANOVA were used to compare left and right vessel measurements in the combined cohort of MS participants on study A and B. Spearman correlations were used in addition for testing relationships between biochemical, genetic, lifestyle variables and ultrasound measurements in the combined cohort of MS participants (studies A and B).

3.10 ETHICAL DIMENSIONS

The primary purpose of this study involving human participants was to determine the possible risks for the development of extracranial vascular disease in persons diagnosed with multiple sclerosis. If the possible risks can be demonstrated, then persons with MS can be advised of preventative measures that can be implemented for improved quality of life.

Various factors are taken into consideration when undertaking health research. In this study, the following was considered:

3.10.1 Permission to perform ultrasound examinations

Permission, in writing, to scan the research participants at a private radiology practice has been granted (Appendix E).

3.10.2 Research ethics committee

The study was granted ethical approval by the Faculty of Health and Wellness Sciences Research Ethics Committee (FREC) of the Cape Peninsula University of Technology (CPUT) [Approval reference no: CPUT/HW-REC 2017/H4 (renewal)] (See Appendix F). The study was conducted according to the code of ethics of the World Medical Association (Declaration of Helsinki).

3.10.3 Informed consent

All participants were asked to voluntarily read and sign a written informed consent. Participants were informed that they have a right to withdraw from the study at any time. The informed consent was available in 2 languages (English and Afrikaans) (Appendix G). Consent in isiXhosa, the third regional language, was not available as MS is a very rare disease amongst Black Africans. The incidence is reported to be highest amongst English-speaking white South Africans and lower in Afrikaans-speaking white South Africans (Dean, 1967).

3.10.4 Risks, burdens and benefits

No interventional procedures were performed on the participants; thus no significant risks or burdens were expected. The benefits included a free Ultrasound examination of the carotid arteries and internal jugular veins with a radiological report. If any pathology was identified,

then the participants were referred to the appropriate physician for further management and treatment.

There are no known risks associated with ultrasound imaging. Fowlkes and Hollard (1998:52) postulates that there are no known biological effects when diagnostic ultrasound imagining machines are used appropriately by trained sonographers.

3.10.5 Privacy, confidentiality and respect

Every precaution was taken to protect the privacy of the participants and the confidentiality of the personal information. Respect was maintained at all times. Only the PI involved in the study had access to data and participant information. The principal investigator was responsible for inventory and organisation of the data collection forms. Participants were assigned a code which was used on all study paperwork. All data was kept in a locked room and the electronic database was locked with coded access. Data with the patient codes was made accessible to the supervisor and statistician.

3.10.6 Publication of results

Results of the study will be made publicly available by publication in peer-reviewed journals where persons in the medical and scientific community can evaluate the results. Publication of the results also allows other researchers to repeat the study to confirm the study findings. The applied research methods and design can also be used in clinical and academic teaching. The knowledge gained through this study will also be presented at conferences, congresses and seminars.

CONCLUSION

This chapter presents a detailed description of the research design and methodology undertaken which ensures trustworthiness of the study. This detailed description also allows for easy transferability of the design and methods to other studies of similar context where the findings of this study can be replicated, improved or disproved.

CHAPTER FOUR
RESULTS

INTRODUCTION

This chapter provides the significant and non-significant associations between the extracranial vascular ultrasound variables and the biochemical markers genetic, lifestyle and disability parameters in the MS group of participants and healthy controls.

Significant and non-significant associations and differences between 2 variables are represented by the p-value (calculated probability) and the correlation coefficient, *r*.

A *p* value of >0.05 is considered non-significant, whilst significant correlations are associated with a *p* value <0.05. A *p* value is described as the product of hypothesis testing using multiple statistical tests. The *r* value ranges from +1.0 where there's a positive relationship to -1.0, where there is a negative relationship (inverse association) (Marshall & Jonker, 2011).

The results of this study are presented in a graphic and tabular format.

4.1 ANTHROPOMETRIC AND DEMOGRAPHIC DATA

From October 2017 through to December 2018, 50 extracranial vascular ultrasound examinations were completed. Of these, 25 were MS participants and 25 age-matched healthy controls.

The demographic and anthropometric data of the MS participants and controls are summarised in Table 4.1.

Table 4.1: Characteristics of MS participants and controls

Parameter	MS participants (n=25)	Controls (n=25)
Gender	female	female
Mean age (years ± SD)	49.2 ± 11.53	49.6 ± 11.74
BMI (kg/m^2 ± SD)	25.29 ± 6.48 (n=24)	27.73 ± 5.82
Left Systolic BP (mmHg ± SD)	123.4 ± 9.13	120.56 ± 9.88
Left Diastolic BP (mmHg ± SD)	79.36 ± 9.25	77.96 ± 10.63
Right Systolic BP (mmHg ± SD)	125.72 ± 9.48	123.36 ± 11.43
Right Diastolic BP (mmHg ± SD)	81.4 ± 6.94	78.92 ± 9.65
Mean age at onset (years ± SD)	36 ± 9.85	–
Disease duration (years ± SD)	13.2 ± 7.27	–
EDSS (mean ± SD), (n=23)	2.8 ± 2.02	–

BMI, body mass index; BP, blood pressure; EDSS, Expanded Disability Status Scale; SD, standard deviation.

4.2 COMPARISON BETWEEN THE ULTRASOUND FINDINGS OF THE EXTRACRANIAL VASCULAR SYSTEMS IN THE TWO COHORTS (ACTIVE MS AND CONTROL GROUP)

4.2.1 Comparison between the ultrasound findings of carotid arteries: CCA, ICA, ECA and VA

No significant difference (p>0.05) was demonstrated between the right and left carotid artery ultrasound variables in the MS group and control group (Table 4.2 and 4.3).

Table 4.*2*: Comparison between the right carotid artery ultrasound variables in MS participants and controls

Ultrasound variable	MS participants (n=25)	Controls (n=25)	p-value*
Right CCA IMT (mm)	0.53 (0.49-0.57)	0.55 (0.51-0.59)	0.48
Right CCA PSV (cm/s)	96.64 (88.80-104.47)	98.49 (90.11-106.87)	0.75
Right CCA EDV (cm/s)	29.54 (27.04-32.05)	27.30 (24.62-29.98)	0.22
Right ICA PSV (cm/s)	88.47 (81.41-95.62)	89.68 (82.25-97.25)	0.82
Right ICA EDV (cm/s)	35.98 (32.08-39.89)	36.19 (32.05-40.42)	0.94
Right ECA PSV (cm/s)	85.17 (76.96-93.39)	83.69 (74.91-92.47)	0.81
Right VA PSV (cm/s)	53.13 (47.52-58.74)	54.99 (48.99-60.98)	0.65
Right VA EDV (cm/s)	18.01 (15.74-20.29)	18.69 (16.26-21.12)	0.69
Right PSV ICA/CCA ratio	0.93 (0.85-1.01)	0.94 (0.85-1.03)	0.82
Right EDV ICA/CCA ratio	1.22 (1.08-1.37)	1.34 (1.19-1.50)	0.25
CSD PROX right CCA (mm)	6.65 (6.39-6.91)	6.83 (6.55-7.11)	0.35
CSD mid right CCA (mm)	6.46 (6.18-6.74)	6.56 (6.26-6.86)	0.62
CSD distal right CCA (mm)	6.85 (6.54-7.15)	6.99 (6.66-7.31)	0.53
CSD PROX right ICA (mm)	6.54 (6.13-6.91)	6.33 (5.91-6.75)	0.49
CSD PROX right ECA (mm)	4.38 (4.10-4.66)	4.59 (4.29-4.89)	0.3
CSD PROX right VA (mm)	3.40 (3.19-3.61)	3.48 (3.26-3.70)	0.6

Results are presented as mean (range), *F-test; CCA, common carotid artery; ECA, external carotid artery; EDV, end-diastolic velocity; ICA, internal carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; PROX, proximal; VA, vertebral artery.

Table 4.3: Comparison between the left carotid artery ultrasound variables in the MS participants and controls

Ultrasound variable	MS participants (n=25)	Controls (n=25)	p-value*
Left CCA IMT (mm)	0.57 (0.52-0.62)	0.58 (0.52-0.64)	0.81
Left CCA PSV (cm/s)	100.22 (92.24-108.20)	102.75 (94.21-111.28)	0.67
Left CCA EDV (cm/s)	29.91 (27.15-32.66)	27.88 (24.94-30.82)	0.32
Left ICA PSV (cm/s)	92.05 (82.41-101.69)	91.89 (81.58-102.20)	0.98
Left ICA EDV (cm/s)	39.22 (35.03-43.41)	35.36 (30.88-39.84)	0.21
Left ECA PSV (cm/s)	82.44 (74.68-90.19)	82.35 (74.06-90.64)	0.99
Left VA PSV (cm/s)	59.14 (52.90-65.37)	57.69 (51.02-64.35)	0.75
Left VA EDV (cm/s)	21.94 (19.14-24.73)	20.25 (17.26-23.24)	0.41
Left PSV ICA/CCA ratio	0.96 (0.85-1.08)	0.95 (0.83-1.08)	0.88
Left EDV ICA/CCA ratio	1.33 (1.18-1.47)	1.30 (1.15-1.46)	0.81
CSD PROX left CCA (mm)	6.46 (6.23-6.68)	6.61 (6.37-6.84)	0.35
CSD mid left CCA (mm)	6.42 (6.25-6.60)	6.42 (6.23-6.60)	0.96
CSD distal left CCA (mm)	6.75 (6.52-6.97)	6.81 (6.56-7.05)	0.72
CSD PROX left ICA (mm)	6.30 (5.91-6.68)	6.79 (6.38-7.20)	0.09
CSD PROX left ECA (mm)	4.17 (3.94-4.40)	4.44 (4.20-4.69)	0.11
CSD PROX left VA (mm)	3.44 (3.20-3.69)	3.52 (3.26-3.78)	0.67

Results are presented as mean (range), *F-test; CCA, common carotid artery; EDV, end-diastolic velocity; ECA, external carotid artery; ICA, internal carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; PROX, proximal; VA, vertebral artery.

4.2.2 Comparison between the ultrasound findings of the internal jugular veins

No significant difference (p>0.05) was demonstrated between the right and left IJV (proximal and distal segments) ultrasound variables (Table 4.4) in the MS group and control group. However, a significant difference (F-test p-value 0.02) was demonstrated between the PV of the mid right IJV in the MS group (55.76, 48.24-63.28) and control group (41.94, 33.90-49.98) (Figure 4.1, Table 4.4).

Table 4.4: Comparison between the right and left IJV ultrasound variables in MS participants and controls

Ultrasound variable	MS participants (n=25)	Controls (n=25)	p-value*
PV PROX right IJV (cm/s)	49.34 (41.86-56.81)	40.04 (32.05-48.03)	0.09
PV mid right IJV	55.76 (48324-63.28)	41.94 (33.90-49.98	0.02
PV distal right IJV (cm/s)	56.22 (47.02-65.42)	49.58 (39.75-59.42)	0.33
PV PROX left IJV (cm/s)	43.68 (33.61-53.75)	39.42 (28.65-50.19)	0.56
PV mid left IJV (cm/s)	43.52 (37.12-49.92)	36.08 (29.24-42.93)	0.12
PV distal left IJV (cm/s)	49.77 (41.34-58.19)	40.66 (31.66-49.67)	0.14
CSA PROX right IJV (mm^2)	142.49 (110.60-174.37)	151.62 (117.53-185.70)	0.7
CSA PROX left IJV (mm^2)	102.71 (67.92-137-51)	134.02 (96.83-171.22)	0.22

Results are presented as mean (range), *F-test; PV, peak velocity; PROX, proximal; IJV, internal jugular vein; CSA-cross-sectional area.

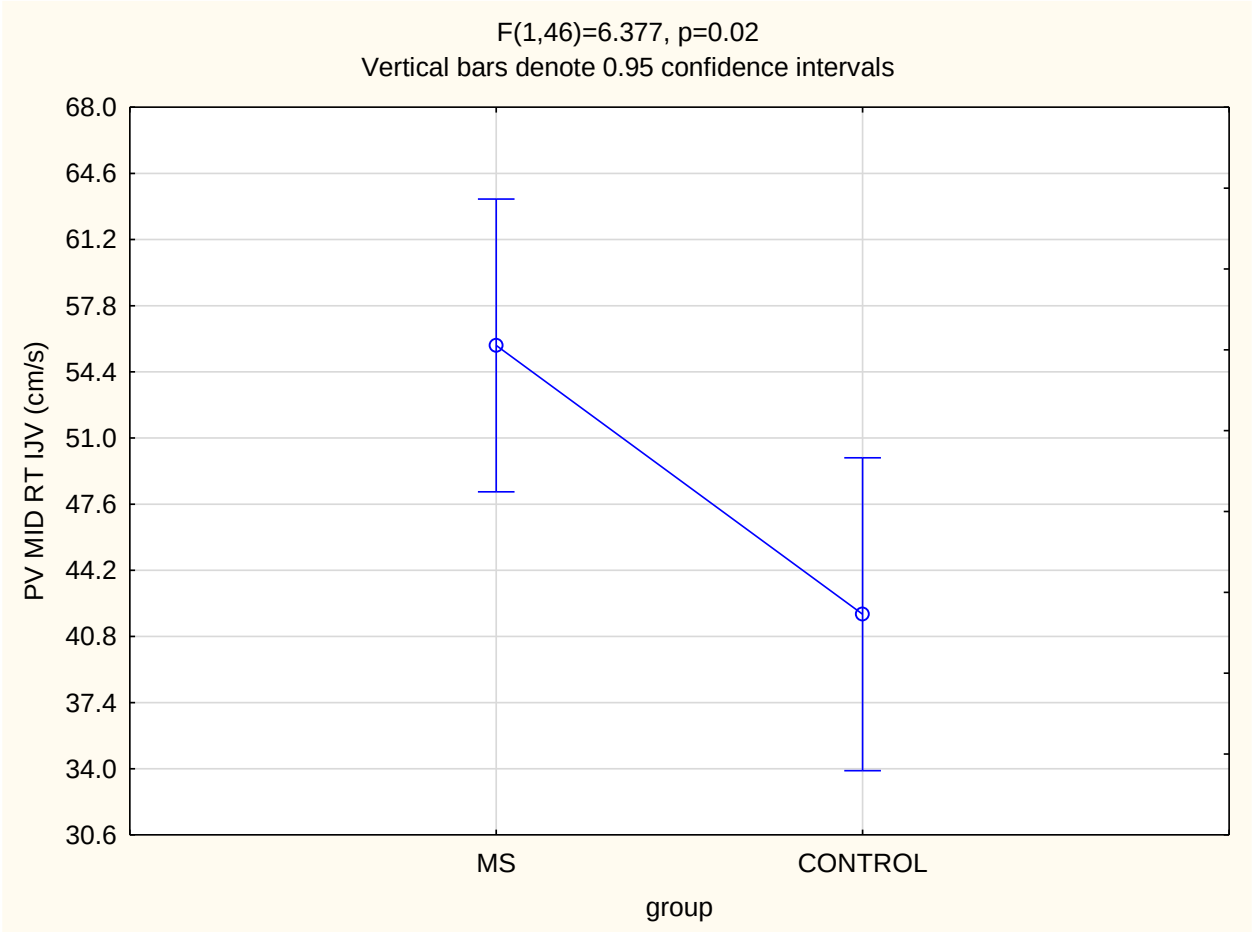

Figure 4.1: Significant difference between the PV of the mid right IJV in the MS group and control group

PV, peak velocity; RT, right; IJV, internal jugular vein; MS group: mean PSV MID RT IJV=55.76cm/s, CONTROL group: mean PV MID RT IJV=41.94cm/s

4.3 ASSOCIATION BETWEEN THE HUMAN LEUKOCYTE ANTIGEN (HLA*) DRB1*1501* ALLELE AND THE GREY-SCALE, COLOUR AND SPECTRAL DOPPLER ANALYSIS OF THE CAROTID ARTERIES (CAs) AND INTERNAL JUGULAR VEINS (IJVs) IN THE TWO COHORTS.

4.3.1 Association between the Human Leukocyte Antigen (HLA) DRB1*1501 allele and the carotid arteries: CCA, ICA, ECA and VA.

In a correlation analysis, no significant association ($p>0.05$) was demonstrated between the right and left CCA, ICA and VA ultrasound variables and the HLA DRB1*1501 allele in the active MS and control groups (Table 4.5 and 4.6). However, a significant association (p-value 0.03) between the HLA) DRB1*1501 allele and the CSD of the proximal right ECA was determined in the two cohorts (Figure 4.2, Table 4.5) as follows:

- CSD PROX RT ECA (mean ± SD), 4.56 ± 0.79 mm (WT, MS group, n=9)
- CSD PROX RT ECA (mean ± SD), 4.21 ± 0.64 mm (HET/HOM, MS group, n=16)
- CSD PROX RT ECA (mean ± SD), 4.31 ± 0.61 mm (WT, control group, n=18)
- CSD PROX RT ECA (mean ± SD), 4.87 ± 0.67 mm (HET/HOM, control group, n=7)

Table 4.5: Association between HLA DRB1*1501 allele and the right carotid artery ultrasound variables

Ultrasound variable	MS participants (n=25)		Controls (n=25)		p-value*
	HLA DRB1*1501 allele		HLA DRB1*1501 allele		
	WT (n=9)	HET/HOM (n=16)	WT (n=18)	HET/HOM (n=7)	
RT CCA IMT (mm)	0.52 ± 0.11	0.54 ± 0.11	0.52 ± 0.06	0.58 ± 0.11	0.53
RT CCA PSV (cm/s)	99.17 ± 14.66	94.11 ± 15.33	97.07 ± 20.85	99.91 ± 23.87	0.49
RT CCA EDV (cm/s)	31.24 ± 5.21	27.84 ± 7.25	25.76 ± 4.14	28.84 ± 7.56	0.08
RT ICA PSV (cm/s)	91.22 ± 23.74	85.72 ± 15.89	88.52 ± 14.62	90.84 ± 14.28	0.45
RT ICA EDV (cm/s)	37.24 ± 10.91	34.73 ± 9.45	34.64 ± 8.00	37.74 ± 10.40	0.33
RT ECA PSV (cm/s)	83.79 ± 21.82	86.56 ± 16.58	83.02 ± 17.98	84.37 ± 26.50	0.91
RT VA PSV (cm/s)	49.90 ± 15.01	56.37 ± 13.34	58.98 ± 12.88	51.00 ± 12.45	0.08
RT VA EDV (cm/s)	17.98 ± 7.78	18.05 ± 5.67	18.65 ± 3.64	18.73 ± 5.19	1
RT PSV ICA/CCA ratio	0.93 ± 0.22	0.93 ± 0.18	0.93 ± 0.20	0.95 ± 0.19	0.89
RT EDV ICA/CCA ratio	1.19 ± 0.27	1.26 ± 0.35	1.37 ± 0.40	1.32 ± 0.17	0.6
CSD PROX RT CCA (mm)	6.68 ± 0.48	6.63 ± 0.85	6.71 ± 0.38	6.96 ± 0.63	0.43
CSD mid RT CCA (mm)	6.33 ± 0.48	6.59 ± 0.84	6.43 ± 0.37	6.69 ± 0.92	1
CSD dist RT CCA (mm)	6.72 ± 0.55	6.97 ± 0.89	6.89 ± 0.60	7.09 ± 0.82	0.91
CSD PROX RT ICA (mm)	6.61 ± 0.96	6.44 ± 0.86	6.26 ± 0.99	6.40 ± 0.88	0.58
CSD PROX RT ECA (mm)	4.56 ± 0.79	4.21 ± 0.64	4.31 ± 0.61	4.87 ± 0.67	0.03
CSD PROX RT VA (mm)	3.22 ± 0.39	3.49 ± 0.42	3.49 ± 0.40	3.47 ± 0.91	0.23

Results are presented as mean ± SD (standard deviation); *F-test; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery, CSD, cross-sectional diameter; PROX, proximal; dist, distal

Table 4.6: Association between HLA DRB1*1501 allele and the left carotid artery ultrasound variables

Ultrasound variable	MS participants (n=25)		Controls (n=25)		p-value*
	HLA DRB1*1501 allele		HLA DRB1*1501 allele		
	WT (n=9)	HET/HOM (n=16)	WT (n=18)	HET/HOM (n=7)	
LT CCA IMT (mm)	0.57 ± 0.08	0.57 ± 0.12	0.59 ± 0.15	0.57± 0.13	0.84
Left CCA PSV (cm/s)	100.61 ± 24.54	99.83 ± 20.77	101.25 ± 13.22	104.24 ± 20.03	0.75
Left CCA EDV (cm/s)	29.71 ± 4.03	30.1 ± 9.08	27.26 ± 3.97	28.5 ± 7.59	0.83
Left ICA PSV (cm/s)	94.78 ± 28.58	89.33 ± 29.52	91.84 ± 15.7	91.94 ± 9.37	0.69
Left ICA EDV (cm/s)	41 ± 13.54	37.44 ± 10.47	34.88 ± 8	35.84 ± 8.16	0.46
Left ECA PSV (cm/s)	83.13 ± 1.99	81.74 ± 16.6	82.56 ± 19.57	82.14 ± 19.16	0.93
Left VA PSV (cm/s)	60.6 ± 12.07)	57.68 ± 13.31	55.54 ± 16.48	59.83 ± 17	0.43
Left VA EDV (cm/s)	23.41 ± 7.86	20.46 ± 7.3	19.49 ± 5.52	21.01 ± 6.23	0.28
Left PSV ICA/CCA ratio	0.97 ± 0.23	0.96 ± 0.25	1.01 ± 0.36	0.9 ± 0.16	0.53
Left EDV ICA/CCA ratio	1.38 ± 0.38	1.28 ± 0.33	1.29 ± 0.36	1.31 ± 0.32	0.54
CSD PROX left CCA (mm)	6.39 ± 0.3	6.53 ± 0.58	6.59 ± 0.53	6.63 ± 0.65	0.77
CSD mid left CCA (mm)	6.23 ± 0.33	6.61 ± 0.47	6.36 ± 0.39	6.47 ± 0.49	0.3
CSD distal left CCA (mm)	6.66 ± 0.55	6.84 ± 0.62	6.78 ± 0.45	6.83 ± 0.56	0.68
CSD PROX left ICA (mm)	5.98 ± 1.01	6.62 ± 0.98	6.67 ± 0.87	6.91 ± 0.73	0.49
CSD PROX left ECA mm)	3.9 ± 0.67	4.44 ± 0.59	4.37 ± 0.38	4.51 ± 0.71	0.25
CSD PROX left VA (mm)	3.41 ± 0.61	3.48 ± 0.46	3.43 ± 0.65	3.6 ± 0.61	0.78

Results are presented as mean ± SD (standard deviation); CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSD, cross-sectional diameter; PROX, proximal

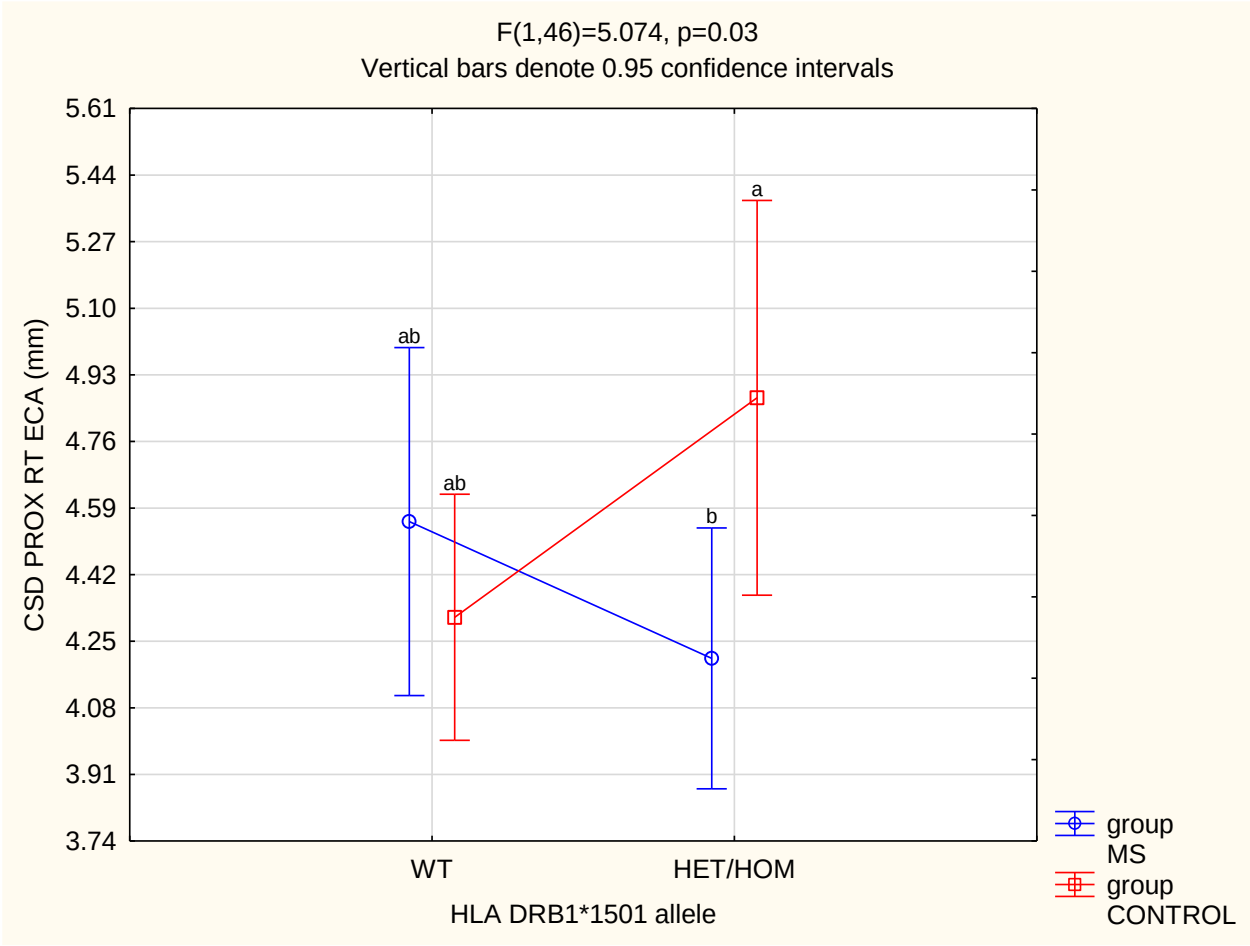

Figure 4.*2*: Significant association between the HLA DRB1*1501 allele and the CSD of the proximal right ECA in the MS group and control group.

CSD, cross-sectional diameter; PROX, proximal; RT, right; ECA, external carotid artery; WT, wild type; HET, heterogeneous; HOM, homogenous
MS group: Mean CSD PROX RT ECA 4.56mm (WT), 4.21mm (HET/HOM)
CONTROL group: Mean CSD PROX RT ECA 4.31 mm (WT), 4.87 mm (HET/HOM)

4.3.2 Association between the Human Leukocyte Antigen (HLA*) DRB1*1501* allele and the internal jugular veins.

No significant association (p>0.05) between the right and left IJV ultrasound variables and the HLA DRB1*1501 allele in the active MS and control groups was demonstrated (Table 4.7).

Table 4.7: Association between the HLA DRB1*1501 allele and the internal jugular vein ultrasound variables

Ultrasound variable	MS participants (n=25)		Controls (n=25)		p-value*
	HLA DRB1*1501 allele		HLA DRB1*1501 allele		
	WT (n=9)	HET/HOM (n=16)	WT (n=18)	HET/HOM (n=7)	
PV prox right IJV (cm/s)	52.92 ± 21.45	45.76 ± 15.01	39.15 ± 20.36	40.93 ± 9.07	0.41
PV mid right IJV (cm/s)	54.33 ± 15.20	57.18 ± 24.95	34.94 ± 12.38	48.94 ± 12.97	0.31
PV distal right IJV (cm/s)	59.5 ± 17.88	52.93 ± 27.22	40.96 ± 10.96	58.2 ± 33.49	0.08
PV prox left IJV (cm/s)	46.78 ± 28.74	40.58 ± 17.83	41.04 ± 29.50	37.8 ± 7.79	0.84
PV mid left IJV (cm/s)	44.19 ± 16.80	42.85 ± 18.58	32.16 ± 12.40	40.01 ± 10.49	0.33
PV distal left IJV (cm/s)	49.24 ± 14.31	50.29 ±25.84	42.06 ± 19.29	39.27 ± 9.80	0.76
CSA PROX right IJV (mm^2)	156.22 ± 47.11	128.75 ± 67.01	155.94 ± 78.26	147.29 ±113.05	0.69
CSA PROX left IJV (mm^2)	116.11 ± 81.54	89.31 ± 40.2	136.33 ± 114.81	131.71 ± 50.24	0.66

Results are presented as mean ± SD (standard deviation); *F-test; PS, peak velocity, PROX, proximal; IJV, internal jugular vein; CSA, cross-sectional area

4.4 ASSOCIATION BETWEEN BIOCHEMICAL MARKERS AND THE GREY-SCALE, COLOUR AND SPECTRAL DOPPLER ANALYSIS OF THE CAs AND IJVs IN PARTICIPANTS WITH MS (MS GROUP) AND PARTICIPANTS FREE FROM MS (CONTROL GROUP).

4.4.1 Association between serum iron (s-iron) concentrations and the extracranial vascular ultrasound variables

MS group: A statistically significant positive correlation was demonstrated between serum iron (s-iron) concentrations and the PSV of the right ICA (Pearson p-value 0.02, Spearman p-value 0.03), the right ICA/CCA PSV ratio (Pearson p-value 0.04, Spearman p-value 0.01) and the left CCA IMT (Spearman 0.03). A significant negative correlation was found between s-iron concentrations and the CSD of the proximal left vertebral artery (Pearson p-value 0.02, Spearman p-value 0.02) in the MS group (Table 4.8).

***Control group*:** In the control group, a statistically significant negative correlation was demonstrated between s-iron concentrations and the PSV of the proximal right ECA (Spearman p-value 0.01) and the PV of the distal left IJV (Spearman p-value 0.04) (Table 4.9).

Table 4.8: Correlation between serum iron concentration and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
s-iron	RT CCA IMT (mm)	0.15	0.46	0.31	0.13	25	MS
s-iron	RT CCA PSV (cm/s)	0.04	0.85	-0.05	0.82	25	MS
s-iron	RT CCA EDV (cm/s)	0	1	-0.11	0.6	25	MS
s-iron	CSD PROX RT CCA (mm)	-0.3	0.14	-0.35	0.09	25	MS
s-iron	CSD MID RT CCA (mm)	-0.12	0.57	-0.07	0.74	25	MS
s-iron	CSD DIST RT CCA (mm)	-0.08	0.72	-0.05	0.8	25	MS
s-iron	RT ICA PSV (cm/s)	0.47	0.02	0.44	0.03	25	MS
s-iron	RT ICA EDV (cm/s)	0.42	0.04	0.29	0.15	25	MS
s-iron	CSD PROX RT ICA (mm)	-0.08	0.7	-0.04	0.83	25	MS
s-iron	PROX ECA RT PSV cm/s)	0.06	0.78	-0.04	0.83	25	MS
s-iron	CSD PROX RT ECA (mm)	-0.1	0.64	-0.04	0.86	25	MS
s-iron	RT VA PSV (cm/s)	0.07	0.74	0.03	0.88	25	MS
s-iron	RT VA EDV (cm/s)	0.15	0.47	0.11	0.59	25	MS
s-iron	CSD PROX RT VA (mm)	0.05	0.79	0.09	0.66	25	MS
s-iron	RT PSV ICA/CCA ratio	0.42	0.04	0.48	0.01	25	MS
s-iron	RT EDV ICA/ECA ratio	0.48	0.01	0.35	0.08	25	MS
s-iron	LT CCA IMT (mm)	0.23	0.26	0.44	0.03	25	MS
s-iron	LT CCA PSV (cm/s)	-0.02	0.91	-0.14	0.5	25	MS
s-iron	LT CCA EDV (cm/s)	-0.02	0.92	-0.08	0.69	25	MS
s-iron	CSD PROX LT CCA (mm)	-0.22	0.29	-0.16	0.45	25	MS
s-iron	CSD MID LT CCA (mm)	-0.12	0.57	-0.06	0.76	25	MS
s-iron	CSD DIST LT CCA (mm)	-0.15	0.47	-0.11	0.61	25	MS
s-iron	LT ICA PSV (cm/s)	0.08	0.72	0.05	0.8	25	MS
s-iron	LT ICA EDV (cm/s)	0.31	0.14	0.18	0.4	25	MS
s-iron	CSD PROX LT ICA (mm)	-0.15	0.48	-0.07	0.74	25	MS
s-iron	PROX LT ECA PSV (cm/s)	-0.04	0.83	-0.12	0.55	25	MS
s-iron	CSD PROX LT ECA (mm)	-0.09	0.68	-0.14	0.5	25	MS
s-iron	LT VA PSV (cm/s)	-0.3	0.15	-0.24	0.25	25	MS
s-iron	LT VA EDV (cm/s)	-0.22	0.3	-0.15	0.48	25	MS
s-iron	CSD PROX LT VA (mm)	-0.45	0.02	-0.45	0.02	25	MS
s-iron	LT PSV ICA/CCA ratio	0.28	0.18	0.35	0.08	25	MS
s-iron	LT EDV ICA/ECA ratio	0.31	0.13	0.33	0.11	25	MS
s-iron	PV PROX RT IJV (cm/s)	0.19	0.36	0.13	0.54	25	MS
s-iron	PV MID RT IJV (cm/s)	-0.08	0.7	-0.06	0.76	25	MS
s-iron	PV DIST RT IJV (cm/s)	0.03	0.88	0.17	0.41	25	MS
s-iron	CSA PROX RT IJV (mm^2)	-0.29	0.15	-0.34	0.09	25	MS
s-iron	PV PROX LT IJV (cm/s)	0.19	0.36	0.06	0.77	25	MS
s-iron	PV MID LT IJV (cm/s)	0.17	0.42	0.17	0.43	25	MS
s-iron	PV DIST LT IJV (cm/s)	-0.03	0.88	0.04	0.84	25	MS
s-iron	CSA PROX LT IJV (mm^2)	0.01	0.95	-0.14	0.51	25	MS

s-iron, serum iron concentration; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

Table 4.9: Correlation between serum iron (s-iron) concentration and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
s-iron	RT CCA IMT (mm)	0.14	0.52	0.12	0.57	25	CONTROL
s-iron	RT CCA PSV (cm/s)	-0.12	0.57	-0.09	0.68	25	CONTROL
s-iron	RT CCA EDV (cm/s)	-0.13	0.54	-0.15	0.49	25	CONTROL
s-iron	CSD PROX RT CCA (mm)	0.18	0.39	0.28	0.18	25	CONTROL
s-iron	CSD MID RT CCA (mm)	0.19	0.36	0.28	0.18	25	CONTROL
s-iron	CSD DIST RT CCA (mm)	-0.03	0.88	0.01	0.96	25	CONTROL
s-iron	RT ICA PSV (cm/s)	-0.04	0.84	0.06	0.78	25	CONTROL
s-iron	RT ICA EDV (cm/s)	0.25	0.23	0.23	0.26	25	CONTROL
s-iron	CSD PROX RT ICA (mm)	0.03	0.89	-0.02	0.93	25	CONTROL
s-iron	PROX ECA RT PSV (cm/s)	-0.36	0.07	-0.49	0.01	25	CONTROL
s-iron	CSD PROX RT ECA (mm)	0.04	0.84	0.05	0.8	25	CONTROL
s-iron	RT VERT A PSV (cm/s)	-0.26	0.21	-0.24	0.26	25	CONTROL
s-iron	RT VERT A EDV (cm/s)	-0.25	0.22	-0.16	0.46	25	CONTROL
s-iron	CSD PROX RT VERT A (mm)	0.23	0.27	0.15	0.47	25	CONTROL
s-iron	RT PSV ICA/CCA ratio	0.07	0.73	0.11	0.61	25	CONTROL
s-iron	RT EDV ICA/ECA ratio	0.34	0.09	0.31	0.13	25	CONTROL
s-iron	LT CCA IMT (mm)	-0.04	0.86	0.01	0.97	25	CONTROL
s-iron	LT CCA PSV (cm/s)	-0.24	0.24	-0.25	0.23	25	CONTROL
s-iron	LT CCA EDV (cm/s)	-0.15	0.48	-0.02	0.94	25	CONTROL
s-iron	CSD PROX LT CCA (mm)	0.01	0.95	-0.06	0.77	25	CONTROL
s-iron	CSD MID LT CCA (mm)	0.13	0.54	0.08	0.69	25	CONTROL
s-iron	CSD DIST LT CCA (mm)	0.11	0.61	0.03	0.88	25	CONTROL
s-iron	LT ICA PSV (cm/s)	-0.05	0.82	0.08	0.72	25	CONTROL
s-iron	LT ICA EDV (cm/s)	-0.05	0.82	-0.04	0.85	25	CONTROL
s-iron	CSD PROX LT ICA (mm)	-0.11	0.62	-0.02	0.94	25	CONTROL
s-iron	PROX LT ECA PSV (cm/s)	-0.11	0.6	-0.09	0.66	25	CONTROL
s-iron	CSD PROX LT ECA (mm)	-0.02	0.94	-0.08	0.71	25	CONTROL
s-iron	LT VERT A PSV (cm/s)	-0.2	0.34	-0.13	0.53	25	CONTROL
s-iron	LT VERT A EDV (cm/s)	-0.2	0.33	-0.07	0.76	25	CONTROL
s-iron	CSD PROX LT VERT A (mm)	0.17	0.43	0.16	0.44	25	CONTROL
s-iron	LT PSV ICA/CCA ratio	0.27	0.19	0.39	0.06	25	CONTROL
s-iron	LT EDV ICA/ECA ratio	0.08	0.7	0.12	0.56	25	CONTROL
s-iron	PV PROX RT IJV (cm/s)	-0.08	0.7	0.03	0.87	25	CONTROL
s-iron	PV MID RT IJV (cm/s)	0.22	0.3	0.32	0.12	25	CONTROL
s-iron	PV DIST RT IJV (cm/s)	0.21	0.3	0.28	0.17	25	CONTROL
s-iron	CSA PROX RT IJV (mm^2)	0.05	0.82	0.09	0.66	25	CONTROL
s-iron	PV PROX LT IJV (cm/s)	-0.24	0.25	-0.01	0.95	25	CONTROL
s-iron	PV MID LT IJV (cm/s)	-0.28	0.18	-0.17	0.41	25	CONTROL
s-iron	PV DIST LT IJV (cm/s)	-0.42	0.04	-0.34	0.09	25	CONTROL
s-iron	CSA PROX LT IJV (mm^2)	-0.14	0.51	0	1	25	CONTROL

RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

4.4.2 Association between serum transferrin (s-transferrin) and the extracranial vascular ultrasound variables

MS group*:* In the MS group, a statistically significant negative correlation was found between s-transferrin and the CSD of the proximal left ECA (Pearson p-value 0.04) and a positive correlation between the PV of the proximal left IJV (Pearson p-value <0.01). No significant association was demonstrated between the remaining variables in this group (Table 4.10).

Control group: In the control group, as illustrated in Table 4.11, a statistically significant negative association was found between s-transferrin and the EDV of the right ICA (Pearson p-value 0.03), right EDV ICA/ECA ratio (Pearson p-value 0.02) and the PSV of the proximal left ECA (Spearman p-value 0.03). In addition, a statistically significant positive association was found between s-transferrin and the CSD of the proximal left ECA (Pearson p-value 0.04) and the PV of the proximal right and left IJV (Spearman p-value <0.01, Pearson p-value <0.01). No significant association was found between the remaining variables.

Table 4.10: Correlation between serum transferrin and the extracranial ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
S-transf	RT CCA IMT (mm)	0.15	0.47	0.25	0.24	25	MS
S-transf	RT CCA PSV (cm/s)	0.27	0.19	0.36	0.08	25	MS
S-transf	RT CCA EDV (cm/s)	-0.03	0.89	0.07	0.75	25	MS
S-transf	CSD PROX RT CCA (mm)	0.19	0.36	0.33	0.11	25	MS
S-transf	CSD MID RT CCA (mm)	-0.01	0.97	0.19	0.35	25	MS
S-transf	CSD DIST RT CCA (mm)	-0.11	0.61	0.11	0.61	25	MS
S-transf	RT ICA PSV (cm/s)	0.36	0.08	0.08	0.71	25	MS
S-transf	RT ICA EDV (cm/s)	0.17	0.42	-0.03	0.88	25	MS
S-transf	CSD PROX RT ICA (mm)	-0.32	0.12	-0.07	0.75	25	MS
S-transf	PROX ECA RT PSV (cm/s)	0.08	0.72	-0.02	0.93	25	MS
S-transf	CSD PROX RT ECA (mm)	-0.19	0.36	0.01	0.98	25	MS
S-transf	RT VA PSV (cm/s)	0.21	0.32	0.15	0.46	25	MS
S-transf	RT VA EDV (cm/s)	-0.18	0.4	-0.16	0.45	25	MS
S-transf	CSD PROX RT VA (mm)	-0.33	0.11	-0.28	0.17	25	MS
S-transf	RT PSV ICA/CCA ratio	0.09	0.65	-0.24	0.26	25	MS
S-transf	RT EDV ICA/ECA ratio	0.07	0.75	-0.16	0.43	25	MS
S-transf	LT CCA IMT (mm)	0.16	0.45	0.25	0.22	25	MS
S-transf	LT CCA PSV (cm/s)	0.36	0.07	0.14	0.52	25	MS
S-transf	LT CCA EDV (cm/s)	0.05	0.82	-0.14	0.49	25	MS
S-transf	CSD PROX LT CCA (mm)	0.11	0.61	0.39	0.05	25	MS
S-transf	CSD MID LT CCA (mm)	0.02	0.94	0.05	0.8	25	MS
S-transf	CSD DIST LT CCA (mm)	0.07	0.74	0.08	0.72	25	MS
S-transf	LT ICA PSV (cm/s)	0.29	0.16	0.18	0.38	25	MS
S-transf	LT ICA EDV (cm/s)	0.14	0.49	-0.22	0.3	25	MS
S-transf	CSD PROX LT ICA (mm)	-0.14	0.51	-0.16	0.45	25	MS
S-transf	PROX LT ECA PSV (cm/s)	-0.02	0.92	0.14	0.51	25	MS
S-transf	CSD PROX LT ECA (mm)	-0.41	0.04	-0.16	0.44	25	MS
S-transf	LT VA PSV (cm/s)	0.19	0.37	0.22	0.3	25	MS
S-transf	LT VA EDV (cm/s)	0.1	0.63	0.06	0.76	25	MS
S-transf	CSD PROX LT VA (mm)	0.09	0.68	0.12	0.56	25	MS
S-transf	LT PSV ICA/CCA ratio	0.02	0.92	-0.07	0.74	25	MS
S-transf	LT EDV ICA/ECA ratio	0.16	0.46	-0.02	0.92	25	MS
S-transf	PV PROX RT IJV (cm/s)	-0.1	0.65	-0.09	0.67	25	MS
S-transf	PV MID RT IJV (cm/s)	0.14	0.51	0.17	0.43	25	MS
S-transf	PV DIST RT IJV (cm/s)	-0.08	0.69	-0.02	0.92	25	MS
S-transf	CSA PROX RT IJV (mm^2)	-0.37	0.07	-0.09	0.67	25	MS
S-transf	PV PROX LT IJV (cm/s)	0.7	<0.01	0.02	0.92	25	MS
S-transf	PV MID LT IJV (cm/s)	0.38	0.06	0.06	0.79	25	MS
S-transf	PV DIST LT IJV (cm/s)	0.21	0.31	0.09	0.66	25	MS
S-transf	CSA PROX LT IJV (mm^2)	-0.21	0.32	0.04	0.85	25	MS

S-transf, serum trasnferrin; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

Table 4.11: Correlation between serum transferrin and the extracranial ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
S-transf	RT CCA IMT (mm)	-0.12	0.56	-0.16	0.45	25	CONTROL
S-transf	RT CCA PSV (cm/s)	-0.14	0.51	0.03	0.87	25	CONTROL
S-transf	RT CCA EDV (cm/s)	0.08	0.72	0.15	0.47	25	CONTROL
S-transf	CSD PROX RT CCA (mm)	-0.16	0.45	-0.15	0.47	25	CONTROL
S-transf	CSD MID RT CCA (mm)	-0.1	0.62	-0.01	0.96	25	CONTROL
S-transf	CSD DIST RT CCA (mm)	0.02	0.94	-0.02	0.94	25	CONTROL
S-transf	RT ICA PSV (cm/s)	-0.09	0.66	-0.25	0.23	25	CONTROL
S-transf	RT ICA EDV (cm/s)	-0.43	0.03	-0.22	0.29	25	CONTROL
S-transf	CSD PROX RT ICA (mm)	0.14	0.51	0.16	0.45	25	CONTROL
S-transf	PROX ECA RT PSV (cm/s)	0.02	0.93	-0.24	0.25	25	CONTROL
S-transf	CSD PROX RT ECA (mm)	0.07	0.75	0.4	0.05	25	CONTROL
S-transf	RT VA PSV (cm/s)	-0.15	0.48	-0.08	0.69	25	CONTROL
S-transf	RT VA EDV (cm/s)	-0.22	0.29	-0.11	0.59	25	CONTROL
S-transf	CSD PROX RT VA (mm)	-0.17	0.41	-0.18	0.4	25	CONTROL
S-transf	RT PSV ICA/CCA ratio	0.05	0.8	-0.19	0.36	25	CONTROL
S-transf	RT EDV ICA/CCA ratio	-0.46	0.02	-0.21	0.31	25	CONTROL
S-transf	LT CCA IMT (mm)	-0.18	0.4	-0.32	0.12	25	CONTROL
S-transf	LT CCA PSV (cm/s)	0.11	0.62	-0.04	0.87	25	CONTROL
S-transf	LT CCA EDV (cm/s)	0.24	0.25	-0.15	0.48	25	CONTROL
S-transf	CSD PROX LT CCA (mm)	0.22	0.28	0.14	0.51	25	CONTROL
S-transf	CSD MID LT CCA (mm)	0.15	0.48	0.01	0.97	25	CONTROL
S-transf	CSD DIST LT CCA (mm)	-0.01	0.96	-0.17	0.42	25	CONTROL
S-transf	LT ICA PSV (cm/s)	-0.02	0.92	-0.15	0.46	25	CONTROL
S-transf	LT ICA EDV (cm/s)	-0.17	0.41	-0.06	0.78	25	CONTROL
S-transf	CSD PROX LT ICA (mm)	0.33	0.11	-0.05	0.82	25	CONTROL
S-transf	PROX LT ECA PSV (cm/s)	-0.13	0.54	-0.43	0.03	25	CONTROL
S-transf	CSD PROX LT ECA (mm)	0.28	0.17	0.41	0.04	25	CONTROL
S-transf	LT VA PSV (cm/s)	-0.05	0.79	-0.02	0.91	25	CONTROL
S-transf	LT VA EDV (cm/s)	0.05	0.82	0.01	0.97	25	CONTROL
S-transf	CSD PROX LT VA (mm)	-0.18	0.38	-0.09	0.67	25	CONTROL
S-transf	LT PSV ICA/CCA ratio	-0.12	0.57	-0.07	0.75	25	CONTROL
S-transf	LT EDV ICA/ECA ratio	-0.29	0.16	0.09	0.68	25	CONTROL
S-transf	PV PROX RT IJV (cm/s)	0.25	0.23	0.54	<0.01	25	CONTROL
S-transf	PV MID RT IJV (cm/s)	-0.23	0.27	0.11	0.58	25	CONTROL
S-transf	PV DIST RT IJV (cm/s)	-0.15	0.47	0.16	0.44	25	CONTROL
S-transf	CSA PROX RT IJV (mm^2)	0.05	0.8	-0.03	0.89	25	CONTROL
S-transf	PV PROX LT IJV (cm/s)	0.66	<0.01	0.36	0.08	25	CONTROL
S-transf	PV MID LT IJV (cm/s)	0.22	0.29	0.3	0.15	25	CONTROL
S-transf	PV DIST LT IJV (cm/s)	0.11	0.6	0.32	0.12	25	CONTROL
S-transf	CSA PROX LT IJV (mm^2)	0.03	0.89	0.17	0.42	25	CONTROL

S-transf, serum transferrin; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

4.4.3 Association between percentage (%) transferrin saturation and the extracranial vascular ultrasound variables

***MS group*:** A significant positive association was demonstrated between percentage transferrin saturation and the following extracranial vascular ultrasound variables in the MS group as tabulated in Table 4.12:

- Right ICA PSV; Pearson p-value 0.03, Spearman p-value 0.03
- Right PSV ICA/CCA ratio; Pearson p-value 0.01, Spearman p-value <0.01
- Right EDV ICA/CCA ratio; Pearson p-value 0.03, Spearman p-value 0.04

Similarly, a significant negative association was found between percentage transferrin saturation and the CSD of the proximal right CCA (Spearman p-value 0.04), CSD proximal left VA (Spearman p-value 0.04) and the CSA proximal right IJV (Spearman p-value 0.04). No significant association was found between the remaining extracranial vascular ultrasound variables and percentage transferrin saturation (Table 4.12).

***Control group**:* In the control group, a significant negative correlation was found between the PV of the distal left IJV (Pearson p-value 0.01). No significant association (p>0.05) was demonstrated between percentage transferrin saturation and the remaining 39 extracranial vascular ultrasound variables (Table 4.13).

Table 4.12: Correlation between percentage transferrin saturation and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
% Tf sat	RT CCA IMT (mm)	0.13	0.55	0.26	0.21	25	MS
% Tf sat	RT CCA PSV (cm/s)	-0.11	0.6	-0.17	0.4	25	MS
% Tf sat	RT CCA EDV (cm/s)	-0.06	0.76	-0.21	0.32	25	MS
% Tf sat	CSD PROX RT CCA (mm)	-0.33	0.1	-0.42	0.04	25	MS
% Tf sat	CSD MID RT CCA (mm)	-0.15	0.48	-0.05	0.83	25	MS
% Tf sat	CSD DIST RT CCA (mm)	-0.12	0.57	-0.06	0.76	25	MS
% Tf sat	RT ICA PSV (cm/s)	0.43	0.03	0.43	0.03	25	MS
% Tf sat	RT ICA EDV (cm/s)	0.37	0.07	0.32	0.12	25	MS
% Tf sat	CSD PROX RT ICA (mm)	-0.12	0.56	-0.12	0.57	25	MS
% Tf sat	PROX ECA RT PSV (cm/s)	0.11	0.59	0.06	0.76	25	MS
% Tf sat	CSD PROX RT ECA (mm)	-0.16	0.45	-0.16	0.43	25	MS
% Tf sat	RT VA PSV (cm/s)	0.04	0.83	0.08	0.7	25	MS
% Tf sat	RT VA EDV (cm/s)	0.09	0.66	0.09	0.67	25	MS
% Tf sat	CSD PROX RT VA (mm)	0.06	0.76	0.05	0.8	25	MS
% Tf sat	RT PSV ICA/CCA ratio	0.49	0.01	0.54	<0.01	25	MS
% Tf sat	RT EDV ICA/CCA ratio	0.44	0.03	0.41	0.04	25	MS
% Tf sat	LT CCA IMT (mm)	0.22	0.3	0.34	0.1	25	MS
% Tf sat	LT CCA PSV (cm/s)	0.02	0.93	-0.04	0.85	25	MS
% Tf sat	LT CCA EDV (cm/s)	0.06	0.79	0.09	0.66	25	MS
% Tf sat	CSD PROX LT CCA (mm)	-0.3	0.14	-0.23	0.26	25	MS
% Tf sat	CSD MID LT CCA (mm)	-0.13	0.54	-0.06	0.78	25	MS
% Tf sat	CSD DIST LT CCA (mm)	-0.23	0.26	-0.23	0.27	25	MS
% Tf sat	LT ICA PSV (cm/s)	0.1	0.65	0.11	0.62	25	MS
% Tf sat	LT ICA EDV (cm/s)	0.35	0.09	0.26	0.21	25	MS
% Tf sat	CSD PROX LT ICA (mm)	-0.07	0.74	-0.07	0.74	25	MS
% Tf sat	PROX LT ECA PSV (cm/s)	-0.1	0.64	-0.03	0.88	25	MS
% Tf sat	CSD PROX LT ECA (mm)	0.01	0.95	-0.14	0.5	25	MS
% Tf sat	LT VA PSV (cm/s)	-0.31	0.13	-0.25	0.23	25	MS
% Tf sat	LT VA EDV (cm/s)	-0.23	0.26	-0.12	0.58	25	MS
% Tf sat	CSD PROX LT VA (mm)	-0.4	0.05	-0.42	0.04	25	MS
% Tf sat	LT PSV ICA/CCA ratio	0.25	0.22	0.32	0.12	25	MS
% Tf sat	LT EDV ICA/ECA ratio	0.3	0.14	0.23	0.27	25	MS
% Tf sat	PV PROX RT IJV (cm/s)	0.17	0.41	0.14	0.52	25	MS
% Tf sat	PV MID RT IJV (cm/s)	-0.07	0.74	-0.01	0.96	25	MS
% Tf sat	PV DIST RT IJV (cm/s)	0.01	0.96	0.17	0.41	25	MS
% Tf sat	CSA PROX RT IJV (mm^2)	-0.31	0.13	-0.41	0.04	25	MS
% Tf sat	PV PROX LT IJV (cm/s)	0.19	0.36	0.12	0.58	25	MS
% Tf sat	PV MID LT IJV (cm/s)	0.22	0.28	0.22	0.29	25	MS
% Tf sat	PV DIST LT IJV (cm/s)	0	0.99	0.11	0.6	25	MS
% Tf sat	CSA PROX LT IJV (mm^2)	0	1	-0.19	0.37	25	MS

% Tf sat, percentage transferrin saturation; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal

Table 4.13: Correlation between percentage transferrin saturation and the extracranial ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
% Tf sat	RT CCA IMT (mm)	0.25	0.22	0.13	0.54	25	CONTROL
% Tf sat	RT CCA PSV (cm/s)	-0.1	0.65	-0.03	0.89	25	CONTROL
% Tf sat	RT CCA EDV (cm/s)	-0.1	0.63	-0.13	0.52	25	CONTROL
% Tf sat	CSD PROX RT CCA (mm)	0.06	0.77	0.19	0.37	25	CONTROL
% Tf sat	CSD MID RT CCA (mm)	0.11	0.59	0.14	0.52	25	CONTROL
% Tf sat	CSD DIST RT CCA (mm)	-0.04	0.84	-0.06	0.79	25	CONTROL
% Tf sat	RT ICA PSV (cm/s)	0.05	0.82	0.15	0.46	25	CONTROL
% Tf sat	RT ICA EDV (cm/s)	0.26	0.21	0.22	0.29	25	CONTROL
% Tf sat	CSD PROX RT ICA (mm)	-0.02	0.91	-0.09	0.66	25	CONTROL
% Tf sat	PROX ECA RT PSV (cm/s)	-0.19	0.36	-0.28	0.17	25	CONTROL
% Tf sat	CSD PROX RT ECA (mm)	-0.1	0.64	-0.04	0.84	25	CONTROL
% Tf sat	RT VA PSV (cm/s)	-0.15	0.48	-0.11	0.61	25	CONTROL
% Tf sat	RT VA EDV (cm/s)	-0.14	0.51	-0.08	0.71	25	CONTROL
% Tf sat	CSD PROX RT VA (mm)	0.31	0.14	0.23	0.27	25	CONTROL
% Tf sat	RT PSV ICA/CCA ratio	0.11	0.62	0.1	0.63	25	CONTROL
% Tf sat	RT EDV ICA/ECA ratio	0.31	0.13	0.25	0.23	25	CONTROL
% Tf sat	LT CCA IMT (mm)	0.13	0.53	0.07	0.75	25	CONTROL
% Tf sat	LT CCA PSV (cm/s)	-0.15	0.47	-0.1	0.64	25	CONTROL
% Tf sat	LT CCA EDV (cm/s)	-0.05	0.83	0.07	0.73	25	CONTROL
% Tf sat	CSD PROX LT CCA (mm)	-0.05	0.81	-0.18	0.39	25	CONTROL
% Tf sat	CSD MID LT CCA (mm)	0.12	0.56	0.05	0.83	25	CONTROL
% Tf sat	CSD DIST LT CCA (mm)	0.11	0.59	0.03	0.88	25	CONTROL
% Tf sat	LT ICA PSV (cm/s)	0.04	0.85	0.12	0.56	25	CONTROL
% Tf sat	LT ICA EDV (cm/s)	0	0.98	-0.04	0.85	25	CONTROL
% Tf sat	CSD PROX LT ICA (mm)	-0.01	0.95	0	1	25	CONTROL
% Tf sat	PROX LT ECA PSV (cm/s)	0.12	0.56	0.12	0.56	25	CONTROL
% Tf sat	CSD PROX LT ECA (mm)	-0.2	0.33	-0.2	0.33	25	CONTROL
% Tf sat	LT VA PSV (cm/s)	-0.11	0.6	-0.03	0.88	25	CONTROL
% Tf sat	LT VA EDV (cm/s)	-0.18	0.39	-0.01	0.95	25	CONTROL
% Tf sat	CSD PROX LT VA (mm)	0.21	0.32	0.18	0.4	25	CONTROL
% Tf sat	LT PSV ICA/CCA ratio	0.25	0.23	0.24	0.25	25	CONTROL
% Tf sat	LT EDV ICA/ECA ratio	0.02	0.91	0.01	0.95	25	CONTROL
% Tf sat	PV PROX RT IJV (cm/s)	-0.26	0.21	-0.11	0.61	25	CONTROL
% Tf sat	PV MID RT IJV (cm/s)	0.17	0.41	0.25	0.23	25	CONTROL
% Tf sat	PV DIST RT IJV (cm/s)	0.13	0.52	0.14	0.51	25	CONTROL
% Tf sat	CSA PROX RT IJV (mm^2)	0.05	0.82	0.08	0.71	25	CONTROL
% Tf sat	PV PROX LT IJV (cm/s)	-0.31	0.13	-0.09	0.67	25	CONTROL
% Tf sat	PV MID LT IJV (cm/s)	-0.35	0.08	-0.27	0.2	25	CONTROL
% Tf sat	PV DIST LT IJV (cm/s)	-0.5	0.01	-0.38	0.06	25	CONTROL
% Tf sat	CSA PROX LT IJV (mm^2)	-0.16	0.44	0	0.99	25	CONTROL

% Tf sat, percentage transferrin saturation; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

4.4.4 Association between serum ferritin and the extracranial vascular ultrasound variables

MS group: As seen in Table 4.14, a statistically significant negative association was found between ferritin and the PV of the proximal left IJV (Pearson p-value 0.02). No statistically significant association was found between ferritin and the remaining 39 extracranial vascular ultrasound variables in the MS group.

Control group: In the control group, a significant positive correlation was found between ferritin and the IMT of the left CCA (Pearson p-value 0.01). No significant associations were demonstrated between ferritin and the remaining 39 extracranial vascular ultrasound variables (Table 4.15).

Table 4.14: Correlation between ferritin and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Ferritin	RT CCA IMT (mm)	0.02	0.94	0.04	0.87	25	MS
Ferritin	RT CCA PSV (cm/s)	-0.18	0.39	0.02	0.91	25	MS
Ferritin	RT CCA EDV (cm/s)	0.03	0.88	0.25	0.23	25	MS
Ferritin	CSD PROX RT CCA (mm)	-0.15	0.48	-0.17	0.42	25	MS
Ferritin	CSD MID RT CCA (mm)	0.05	0.81	-0.06	0.78	25	MS
Ferritin	CSD DIST RT CCA (mm)	0.1	0.64	-0.04	0.84	25	MS
Ferritin	RT ICA PSV (cm/s)	0.09	0.68	0.23	0.28	25	MS
Ferritin	RT ICA EDV (cm/s)	0.18	0.4	0.32	0.12	25	MS
Ferritin	CSD PROX RT ICA (mm)	0.11	0.62	-0.03	0.89	25	MS
Ferritin	PROX ECA RT PSV (cm/s)	-0.21	0.32	-0.02	0.93	25	MS
Ferritin	CSD PROX RT ECA (mm)	0.11	0.6	-0.02	0.92	25	MS
Ferritin	RT VA PSV (cm/s)	0.18	0.4	0.22	0.28	25	MS
Ferritin	RT VA EDV (cm/s)	0.14	0.51	0.12	0.56	25	MS
Ferritin	CSD PROX RT VA (mm)	-0.32	0.12	-0.18	0.4	25	MS
Ferritin	RT PSV ICA/CCA ratio	0.27	0.18	0.29	0.16	25	MS
Ferritin	RT EDV ICA/ECA ratio	0.13	0.53	0.15	0.47	25	MS
Ferritin	LT CCA IMT (mm)	0.09	0.65	0.12	0.56	25	MS
Ferritin	LT CCA PSV (cm/s)	0.04	0.84	0.08	0.72	25	MS
Ferritin	LT CCA EDV (cm/s)	0.24	0.25	0.4	0.05	25	MS
Ferritin	CSD PROX LT CCA (mm)	0.02	0.91	0.01	0.96	25	MS
Ferritin	CSD MID LT CCA (mm)	-0.18	0.4	-0.24	0.24	25	MS
Ferritin	CSD DIST LT CCA (mm)	-0.18	0.39	-0.33	0.11	25	MS
Ferritin	LT ICA PSV (cm/s)	0.12	0.57	0.29	0.16	25	MS
Ferritin	LT ICA EDV (cm/s)	0.18	0.4	0.17	0.42	25	MS
Ferritin	CSD PROX LT ICA (mm)	-0.21	0.32	-0.18	0.38	25	MS
Ferritin	PROX LT ECA PSV (cm/s)	0.14	0.51	0.2	0.34	25	MS
Ferritin	CSD PROX LT ECA (mm)	-0.12	0.56	-0.08	0.69	25	MS
Ferritin	LT VA PSV (cm/s)	0.03	0.9	0.11	0.6	25	MS
Ferritin	LT VA EDV (cm/s)	0.15	0.47	0.15	0.47	25	MS
Ferritin	CSD PROX LT VA (mm)	-0.25	0.23	-0.06	0.77	25	MS
Ferritin	LT PSV ICA/CCA ratio	0.16	0.45	0.11	0.61	25	MS
Ferritin	LT EDV ICA/ECA ratio	-0.04	0.86	-0.17	0.42	25	MS
Ferritin	PV PROX RT IJV (cm/s)	0.07	0.74	0.18	0.4	25	MS
Ferritin	PV MID RT IJV (cm/s)	-0.07	0.73	0.05	0.8	25	MS
Ferritin	PV DIST RT IJV (cm/s)	0	1	0.03	0.91	25	MS
Ferritin	CSA PROX RT IJV (mm^2)	0.28	0.18	0.2	0.33	25	MS
Ferritin	PV PROX LT IJV (cm/s)	-0.35	0.09	-0.46	0.02	25	MS
Ferritin	PV MID LT IJV (cm/s)	-0.24	0.24	-0.08	0.69	25	MS
Ferritin	PV DIST LT IJV (cm/s)	-0.12	0.58	-0.05	0.81	25	MS
Ferritin	CSA PROX LT IJV (mm^2)	-0.04	0.85	-0.01	0.94	25	MS

RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

Table 4.15: Correlation between ferritin and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Ferritin	RT CCA IMT (mm)	-0.04	0.85	0.02	0.93	25	CONTROL
Ferritin	RT CCA PSV (cm/s)	-0.11	0.61	-0.17	0.42	25	CONTROL
Ferritin	RT CCA EDV (cm/s)	-0.24	0.24	-0.33	0.11	25	CONTROL
Ferritin	CSD PROX RT CCA (mm)	0.02	0.91	0.21	0.31	25	CONTROL
Ferritin	CSD MID RT CCA (mm)	-0.07	0.75	0.01	0.95	25	CONTROL
Ferritin	CSD DIST RT CCA (mm)	0.25	0.23	0.23	0.28	25	CONTROL
Ferritin	RT ICA PSV (cm/s)	0.25	0.23	0.14	0.52	25	CONTROL
Ferritin	RT ICA EDV (cm/s)	0.15	0.48	0.06	0.79	25	CONTROL
Ferritin	CSD PROX RT ICA (mm)	0.02	0.91	0.01	0.96	25	CONTROL
Ferritin	PROX ECA RT PSV (cm/s)	0.36	0.07	0.16	0.43	25	CONTROL
Ferritin	CSD PROX RT ECA (mm)	-0.19	0.35	-0.14	0.51	25	CONTROL
Ferritin	RT VA PSV (cm/s)	-0.13	0.52	-0.28	0.17	25	CONTROL
Ferritin	RT VA EDV (cm/s)	0.02	0.92	-0.16	0.44	25	CONTROL
Ferritin	CSD PROX RT VA (mm)	0.2	0.33	0.39	0.06	25	CONTROL
Ferritin	RT PSV ICA/CCA ratio	0.3	0.14	0.23	0.28	25	CONTROL
Ferritin	RT EDV ICA/ECA ratio	0.36	0.08	0.26	0.21	25	CONTROL
Ferritin	LT CCA IMT (mm)	0.48	0.01	0.24	0.24	25	CONTROL
Ferritin	LT CCA PSV (cm/s)	0.03	0.88	-0.01	0.98	25	CONTROL
Ferritin	LT CCA EDV (cm/s)	-0.11	0.6	-0.06	0.77	25	CONTROL
Ferritin	CSD PROX LT CCA (mm)	-0.11	0.61	-0.19	0.36	25	CONTROL
Ferritin	CSD MID LT CCA (mm)	0.15	0.47	0.07	0.72	25	CONTROL
Ferritin	CSD DIST LT CCA (mm)	0.29	0.16	0.16	0.45	25	CONTROL
Ferritin	LT ICA PSV (cm/s)	0.13	0.53	0.03	0.88	25	CONTROL
Ferritin	LT ICA EDV (cm/s)	-0.12	0.56	-0.2	0.34	25	CONTROL
Ferritin	CSD PROX LT ICA (mm)	0.21	0.31	0.14	0.51	25	CONTROL
Ferritin	PROX LT ECA PSV (cm/s)	0.36	0.08	0.33	0.11	25	CONTROL
Ferritin	CSD PROX LT ECA (mm)	-0.36	0.08	-0.33	0.11	25	CONTROL
Ferritin	LT VA PSV (cm/s)	-0.19	0.36	-0.28	0.17	25	CONTROL
Ferritin	LT VA EDV (cm/s)	-0.2	0.33	-0.31	0.13	25	CONTROL
Ferritin	CSD PROX LT VA (mm)	0.4	0.05	0.22	0.28	25	CONTROL
Ferritin	LT PSV ICA/CCA ratio	0.17	0.41	0.07	0.76	25	CONTROL
Ferritin	LT EDV ICA/ECA ratio	-0.05	0.81	-0.18	0.4	25	CONTROL
Ferritin	PV PROX RT IJV (cm/s)	-0.16	0.44	-0.12	0.56	25	CONTROL
Ferritin	PV MID RT IJV (cm/s)	0	0.98	0.09	0.67	25	CONTROL
Ferritin	PV DIST RT IJV (cm/s)	0.07	0.73	0.18	0.38	25	CONTROL
Ferritin	CSA PROX RT IJV (mm^2)	-0.01	0.95	-0.15	0.47	25	CONTROL
Ferritin	PV PROX LT IJV (cm/s)	-0.3	0.14	-0.25	0.24	25	CONTROL
Ferritin	PV MID LT IJV (cm/s)	-0.14	0.5	-0.06	0.77	25	CONTROL
Ferritin	PV DIST LT IJV (cm/s)	-0.11	0.58	-0.15	0.48	25	CONTROL
Ferritin	CSA PROX LT IJV (mm^2)	-0.39	0.05	-0.37	0.07	25	CONTROL

RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

4.4.5 Association between haemoglobin and the extracranial vascular ultrasound variables

MS group: No significant association (p>0.05) was observed between haemoglobin and most of the extracranial vascular ultrasound variables in the MS group. However, a significant negative correlation was observed between haemoglobin and the following extracranial vascular ultrasound variables (Table 4.16):

- EDV of the right ICA; Pearson p-value 0.03
- EDV of the right VA; Pearson p-value 0.01, Spearman p-value <0.01
- PSV of the left VA; Pearson p-value 0.03, Spearman p-value 0.02
- EDV of the left VA; Pearson p-value 0.02, Spearman p-value 0.03
- PV of the proximal right IJV; Pearson p-value <0.01, Spearman p-value <0.01
- PV of the mid right IJV; Pearson p-value <0.01, Spearman p-value 0.01
- PV of the distal right IJV; Pearson p-value 0.02, Spearman p-value 0.04

***Control group*:** The association between haemoglobin and the extracranial vascular ultrasound variables in the control group is given in Table 4.17. A significant negative correlation was found between haemoglobin and the EDV of the right VA (Spearman p-value 0.04) and EDV of the left ICA (Spearman p-value 0.02, Pearson p-value 0.01). No significant association (p>0.05) was found between haemoglobin and the remaining extracranial vascular ultrasound variables in this specific group of participants.

Table 4.16: Correlation between haemoglobin and the extracranial ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
HB	RT CCA IMT (mm)	0.19	0.37	0.2	0.33	25	MS
HB	RT CCA PSV (cm/s)	-0.13	0.53	-0.04	0.87	25	MS
HB	RT CCA EDV (cm/s)	-0.31	0.13	-0.27	0.19	25	MS
HB	CSD PROX RT CCA (mm)	0.14	0.5	0.15	0.48	25	MS
HB	CSD MID RT CCA (mm)	0.31	0.13	0.27	0.2	25	MS
HB	CSD DIST RT CCA (mm)	0.4	0.05	0.36	0.07	25	MS
HB	RT ICA PSV (cm/s)	-0.19	0.36	-0.11	0.59	25	MS
HB	RT ICA EDV (cm/s)	-0.42	0.03	-0.39	0.05	25	MS
HB	CSD PROX RT ICA (mm)	0.27	0.19	0.23	0.26	25	MS
HB	PROX ECA RT PSV (cm/s)	0.15	0.46	0.21	0.31	25	MS
HB	CSD PROX RT ECA (mm)	0.03	0.91	0.02	0.92	25	MS
HB	RT VA PSV (cm/s)	-0.29	0.15	-0.33	0.11	25	MS
HB	RT VA EDV (cm/s)	-0.49	0.01	-0.53	<0.01	25	MS
HB	CSD PROX RT VERT A (mm)	-0.24	0.24	-0.23	0.27	25	MS
HB	RT PSV ICA/CCA ratio	-0.12	0.56	-0.11	0.59	25	MS
HB	RT EDV ICA/ECA ratio	-0.07	0.74	-0.18	0.38	25	MS
HB	LT CCA IMT (mm)	0.08	0.7	0.12	0.57	25	MS
HB	LT CCA PSV (cm/s)	-0.02	0.91	0.13	0.54	25	MS
HB	LT CCA EDV (cm/s)	-0.19	0.36	-0.16	0.45	25	MS
HB	CSD PROX LT CCA (mm)	-0.08	0.72	-0.03	0.88	25	MS
HB	CSD MID LT CCA (mm)	0.09	0.66	0.2	0.33	25	MS
HB	CSD DIST LT CCA (mm)	0.08	0.7	0.11	0.6	25	MS
HB	LT ICA PSV (cm/s)	-0.26	0.21	-0.24	0.25	25	MS
HB	LT ICA EDV (cm/s)	-0.19	0.36	-0.09	0.66	25	MS
HB	CSD PROX LT ICA (mm)	-0.02	0.92	0.05	0.81	25	MS
HB	PROX LT ECA PSV (cm/s)	0.06	0.76	0.11	0.59	25	MS
HB	CSD PROX LT ECA (mm)	-0.06	0.77	-0.04	0.85	25	MS
HB	LT VA PSV (cm/s)	-0.43	0.03	-0.47	0.02	25	MS
HB	LT VA EDV (cm/s)	-0.47	0.02	-0.42	0.03	25	MS
HB	CSD PROX LT VERT A (mm)	0.12	0.56	0.08	0.7	25	MS
HB	LT PSV ICA/CCA ratio	-0.18	0.38	-0.17	0.42	25	MS
HB	LT EDV ICA/ECA ratio	-0.04	0.84	-0.16	0.44	25	MS
HB	PV PROX RT IJV (cm/s)	-0.62	<0.01	-0.6	<0.01	25	MS
HB	PV MID RT IJV (cm/s)	-0.58	<0.01	-0.47	0.02	25	MS
HB	PV DIST RT IJV (cm/s)	-0.45	0.02	-0.41	0.04	25	MS
HB	CSA PROX RT IJV (mm^2)	-0.22	0.3	-0.25	0.24	25	MS
HB	PV PROX LT IJV (cm/s)	0.04	0.86	0.11	0.6	25	MS
HB	PV MID LT IJV (cm/s)	-0.11	0.61	0.08	0.71	25	MS
HB	PV DIST LT IJV (cm/s)	-0.14	0.52	-0.04	0.86	25	MS
HB	CSA PROX LT IJV (mm^2)	0.06	0.77	-0.08	0.72	25	MS

HB, haemoglobin; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

Table 4.17: Correlation between haemoglobin and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
HB	RT CCA IMT (mm)	-0.15	0.48	-0.1	0.63	25	CONTROL
HB	RT CCA PSV (cm/s)	0.01	0.95	-0.03	0.9	25	CONTROL
HB	RT CCA EDV (cm/s)	-0.34	0.09	-0.36	0.08	25	CONTROL
HB	CSD PROX RT CCA (mm)	0.07	0.73	0.01	0.95	25	CONTROL
HB	CSD MID RT CCA (mm)	0.07	0.75	-0.09	0.69	25	CONTROL
HB	CSD DIST RT CCA (mm)	0.04	0.83	-0.08	0.71	25	CONTROL
HB	RT ICA PSV (cm/s)	-0.2	0.33	-0.23	0.28	25	CONTROL
HB	RT ICA EDV (cm/s)	-0.12	0.58	-0.18	0.39	25	CONTROL
HB	CSD PROX RT ICA (mm)	0.25	0.23	0.16	0.44	25	CONTROL
HB	PROX ECA RT PSV (cm/s)	0.14	0.49	-0.01	0.95	25	CONTROL
HB	CSD PROX RT ECA (mm)	-0.03	0.88	-0.09	0.67	25	CONTROL
HB	RT VA PSV (cm/s)	-0.23	0.27	-0.23	0.26	25	CONTROL
HB	RT VA EDV (cm/s)	-0.4	0.05	-0.42	0.04	25	CONTROL
HB	CSD PROX RT VA (mm)	0.17	0.42	0.29	0.15	25	CONTROL
HB	RT PSV ICA/CCA ratio	-0.03	0.88	-0.03	0.87	25	CONTROL
HB	RT EDV ICA/ECA ratio	0.2	0.34	0.25	0.23	25	CONTROL
HB	LT CCA IMT (mm)	0.05	0.8	-0.1	0.62	25	CONTROL
HB	LT CCA PSV (cm/s)	-0.07	0.76	-0.16	0.44	25	CONTROL
HB	LT CCA EDV (cm/s)	0.02	0.92	0.04	0.85	25	CONTROL
HB	CSD PROX LT CCA (mm)	0	0.99	-0.04	0.86	25	CONTROL
HB	CSD MID LT CCA (mm)	0.3	0.14	0.23	0.26	25	CONTROL
HB	CSD DIST LT CCA (mm)	0.35	0.09	0.27	0.19	25	CONTROL
HB	LT ICA PSV (cm/s)	-0.14	0.51	-0.16	0.45	25	CONTROL
HB	LT ICA EDV (cm/s)	-0.46	0.02	-0.49	0.01	25	CONTROL
HB	CSD PROX LT ICA (mm)	0.2	0.35	0.18	0.4	25	CONTROL
HB	PROX LT ECA PSV (cm/s)	0.35	0.09	0.28	0.17	25	CONTROL
HB	CSD PROX LT ECA (mm)	0.05	0.8	0.01	0.95	25	CONTROL
HB	LT VA PSV (cm/s)	-0.31	0.14	-0.26	0.21	25	CONTROL
HB	LT VA EDV (cm/s)	-0.39	0.05	-0.37	0.07	25	CONTROL
HB	CSD PROX LT VA (mm)	0.07	0.76	0	1	25	CONTROL
HB	LT PSV ICA/CCA ratio	0.17	0.41	0.12	0.58	25	CONTROL
HB	LT EDV ICA/ECA ratio	-0.35	0.09	-0.34	0.1	25	CONTROL
HB	PV PROX RT IJV (cm/s)	0.1	0.65	0.13	0.54	25	CONTROL
HB	PV MID RT IJV (cm/s)	0.27	0.2	0.28	0.18	25	CONTROL
HB	PV DIST RT IJV (cm/s)	-0.03	0.89	0.16	0.45	25	CONTROL
HB	CSA PROX RT IJV (mm^2)	0.06	0.77	-0.02	0.93	25	CONTROL
HB	PV PROX LT IJV (cm/s)	0.13	0.52	0.18	0.39	25	CONTROL
HB	PV MID LT IJV (cm/s)	0.12	0.56	0.09	0.67	25	CONTROL
HB	PV DIST LT IJV (cm/s)	-0.08	0.71	-0.02	0.93	25	CONTROL
HB	CSA PROX LT IJV (mm^2)	-0.26	0.2	-0.15	0.47	25	CONTROL

HB, haemoglobin; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

4.4.6 Association between serum vitamin B12 and the extracranial vascular ultrasound variables

MS group: In the MS group, a statistically significant positive correlation was found between vitamin B12 and PSV of the right CCA (Spearman p-value <0.01) as well as the PSV of the left CCA (Spearman p-value <0.01). Similarly, a significant negative correlation was found between vitamin B12 and the EDV of the left VA (Pearson p-value 0.03) and PV of the proximal right IJV (Spearman p-value 0.02). No other significant associations were found between vitamin B12 and the remainder of the extracranial ultrasound variables (Table 4.18).

Control group: The correlation between vitamin B12 and the extracranial vascular ultrasound variables in the control group are presented in Table 4.19. No significant associations were observed between vitamin B12 and ultrasound variables except for one significant positive association between vitamin B12 and the left PSV ICA/CCA ratio (Pearson p-value 0.02).

Table 4.18: Correlation between vitamin B12 and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Vit B12	RT CCA IMT (mm)	-0.14	0.51	-0.05	0.82	25	MS
Vit B12	RT CCA PSV (cm/s)	0.38	0.06	0.52	<0.01	25	MS
Vit B12	RT CCA EDV (cm/s)	-0.06	0.76	-0.02	0.93	25	MS
Vit B12	CSD PROX RT CCA (mm)	-0.08	0.69	0	0.99	25	MS
Vit B12	CSD MID RT CCA (mm)	-0.12	0.57	-0.06	0.76	25	MS
Vit B12	CSD DIST RT CCA (mm)	0.1	0.64	0.19	0.37	25	MS
Vit B12	RT ICA PSV (cm/s)	0.2	0.35	0.11	0.61	25	MS
Vit B12	RT ICA EDV (cm/s)	0.18	0.38	0.16	0.46	25	MS
Vit B12	CSD PROX RT ICA (mm)	-0.14	0.52	-0.14	0.51	25	MS
Vit B12	PROX ECA RT PSV (cm/s)	0.14	0.51	0.13	0.54	25	MS
Vit B12	CSD PROX RT ECA (mm)	0.04	0.85	0.05	0.82	25	MS
Vit B12	RT VA PSV (cm/s)	0.22	0.29	0.21	0.33	25	MS
Vit B12	RT VA EDV (cm/s)	-0.06	0.76	-0.1	0.64	25	MS
Vit B12	CSD PROX RT VA (mm)	-0.26	0.21	-0.25	0.24	25	MS
Vit B12	RT PSV ICA/CCA ratio	-0.12	0.57	-0.26	0.21	25	MS
Vit B12	RT EDV ICA/ECA ratio	0.3	0.15	0.12	0.58	25	MS
Vit B12	LT CCA IMT (mm)	0.13	0.54	0.19	0.37	25	MS
Vit B12	LT CCA PSV (cm/s)	0.39	0.05	0.52	<0.01	25	MS
Vit B12	LT CCA EDV (cm/s)	0.09	0.66	0.16	0.44	25	MS
Vit B12	CSD PROX LT CCA (mm)	0.16	0.46	0.28	0.18	25	MS
Vit B12	CSD MID LT CCA (mm)	0.08	0.72	0.17	0.43	25	MS
Vit B12	CSD DIST LT CCA (mm)	0.02	0.93	0.06	0.78	25	MS
Vit B12	LT ICA PSV (cm/s)	0.02	0.91	0.12	0.58	25	MS
Vit B12	LT ICA EDV (cm/s)	0.2	0.35	0.1	0.62	25	MS
Vit B12	CSD PROX LT ICA (mm)	-0.12	0.57	-0.14	0.51	25	MS
Vit B12	PROX LT ECA PSV (cm/s)	0.08	0.7	0.11	0.61	25	MS
Vit B12	CSD PROX LT ECA (mm)	0.02	0.92	0.06	0.78	25	MS
Vit B12	LT VA PSV (cm/s)	-0.25	0.24	-0.17	0.41	25	MS
Vit B12	LT VA EDV (cm/s)	-0.44	0.03	-0.36	0.08	25	MS
Vit B12	CSD PROX LT VA (mm)	-0.18	0.38	-0.08	0.72	25	MS
Vit B12	LT PSV ICA/CCA ratio	-0.14	0.5	-0.2	0.33	25	MS
Vit B12	LT EDV ICA/ECA ratio	0.13	0.53	-0.11	0.62	25	MS
Vit B12	PV PROX RT IJV (cm/s)	-0.38	0.06	-0.45	0.02	25	MS
Vit B12	PV MID RT IJV (cm/s)	-0.31	0.13	-0.24	0.24	25	MS
Vit B12	PV DIST RT IJV (cm/s)	-0.28	0.17	-0.28	0.17	25	MS
Vit B12	CSA PROX RT IJV (mm^2)	0.08	0.71	0.1	0.64	25	MS
Vit B12	PV PROX LT IJV (cm/s)	0.01	0.95	-0.08	0.7	25	MS
Vit B12	PV MID LT IJV (cm/s)	-0.05	0.82	0.03	0.9	25	MS
Vit B12	PV DIST LT IJV (cm/s)	-0.29	0.16	-0.25	0.22	25	MS
Vit B12	CSA PROX LT IJV (mm^2)	-0.01	0.97	-0.18	0.4	25	MS

Vit B12, serum vitamin B12; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

Table 4.19: Correlation between serum vitamin B12 and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Vit B12	RT CCA IMT (mm)	-0.12	0.58	-0.17	0.43	25	CONTROL
Vit B12	RT CCA PSV (cm/s)	0.04	0.85	0.11	0.61	25	CONTROL
Vit B12	RT CCA EDV (cm/s)	-0.19	0.37	-0.2	0.34	25	CONTROL
Vit B12	CSD PROX RT CCA (mm)	0.05	0.81	0	1	25	CONTROL
Vit B12	CSD MID RT CCA (mm)	0.02	0.92	0	0.99	25	CONTROL
Vit B12	CSD DIST RT CCA (mm)	-0.19	0.37	-0.24	0.26	25	CONTROL
Vit B12	RT ICA PSV (cm/s)	-0.2	0.34	-0.06	0.77	25	CONTROL
Vit B12	RT ICA EDV (cm/s)	-0.06	0.76	-0.07	0.76	25	CONTROL
Vit B12	CSD PROX RT ICA (mm)	-0.04	0.84	-0.06	0.76	25	CONTROL
Vit B12	PROX ECA RT PSV (cm/s)	-0.35	0.09	-0.37	0.07	25	CONTROL
Vit B12	CSD PROX RT ECA (mm)	-0.12	0.57	-0.15	0.48	25	CONTROL
Vit B12	RT VA PSV (cm/s)	-0.29	0.16	-0.19	0.37	25	CONTROL
Vit B12	RT VA EDV (cm/s)	-0.23	0.26	-0.23	0.27	25	CONTROL
Vit B12	CSD PROX RT VA (mm)	0.14	0.52	0.03	0.9	25	CONTROL
Vit B12	RT PSV ICA/CCA ratio	-0.21	0.31	-0.2	0.33	25	CONTROL
Vit B12	RT EDV ICA/ECA ratio	0.08	0.69	0.14	0.51	25	CONTROL
Vit B12	LT CCA IMT (mm)	-0.25	0.24	-0.19	0.35	25	CONTROL
Vit B12	LT CCA PSV (cm/s)	-0.03	0.9	-0.05	0.81	25	CONTROL
Vit B12	LT CCA EDV (cm/s)	-0.11	0.62	-0.16	0.46	25	CONTROL
Vit B12	CSD PROX LT CCA (mm)	-0.06	0.78	0	0.99	25	CONTROL
Vit B12	CSD MID LT CCA (mm)	-0.14	0.51	-0.01	0.97	25	CONTROL
Vit B12	CSD DIST LT CCA (mm)	0.01	0.95	0.05	0.82	25	CONTROL
Vit B12	LT ICA PSV (cm/s)	-0.35	0.08	-0.12	0.56	25	CONTROL
Vit B12	LT ICA EDV (cm/s)	-0.29	0.15	-0.19	0.37	25	CONTROL
Vit B12	CSD PROX LT ICA (mm)	0.11	0.6	0.09	0.69	25	CONTROL
Vit B12	PROX LT ECA PSV (cm/s)	0	0.99	0.06	0.79	25	CONTROL
Vit B12	CSD PROX LT ECA (mm)	0.01	0.98	0	0.99	25	CONTROL
Vit B12	LT VA PSV (cm/s)	-0.04	0.87	0.05	0.8	25	CONTROL
Vit B12	LT VA EDV (cm/s)	0.04	0.85	0.08	0.72	25	CONTROL
Vit B12	CSD PROX LT VA (mm)	0.2	0.34	0.18	0.38	25	CONTROL
Vit B12	LT PSV ICA/CCA ratio	0.45	0.02	0.13	0.53	25	CONTROL
Vit B12	LT EDV ICA/ECA ratio	-0.18	0.38	-0.13	0.53	25	CONTROL
Vit B12	PV PROX RT IJV (cm/s)	0.04	0.86	0.07	0.73	25	CONTROL
Vit B12	PV MID RT IJV (cm/s)	0.28	0.17	0.25	0.23	25	CONTROL
Vit B12	PV DIST RT IJV (cm/s)	0.07	0.74	0.15	0.46	25	CONTROL
Vit B12	CSA PROX RT IJV (mm^2)	-0.24	0.25	-0.22	0.29	25	CONTROL
Vit B12	PV PROX LT IJV (cm/s)	0.09	0.66	0.26	0.2	25	CONTROL
Vit B12	PV MID LT IJV (cm/s)	0.05	0.8	0	0.98	25	CONTROL
Vit B12	PV DIST LT IJV (cm/s)	0.21	0.32	0.17	0.41	25	CONTROL
Vit B12	CSA PROX LT IJV (mm^2)	0.03	0.89	-0.08	0.7	25	CONTROL

Vit B12, serum vitamin B12; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein.

4.4.7 Association between serum folate (s-folate) and the extracranial vascular ultrasound variables

MS group: As seen in the MS group in Table 4.20, a significant positive correlation was found between s-folate and the PSV of the right ICA (Pearson p-value 0.04), EDV of the right ICA (Pearson p-value 0.01, Spearman p-value 0.03) and the IMT of the left CCA (Spearman p-value 0.02). Similarly, a significant negative correlation was found between s-folate of the CSD of the proximal left VA (Spearman p-value 0.04). No significant correlation was found between s-folate and the remaining ultrasound variables.

Control group: In the control group (Table 4.21), a significant negative correlation was found between s-folate and the EDV of the right ICA (Spearman p-value 0.04) and the EDV of the right VA (Spearman p-value 0.03).

Table 4.20: Correlation between serum folate (s-folate) and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
S-fol	RT CCA IMT (mm)	0.15	0.48	0.15	0.48	25	MS
S-fol	RT CCA PSV (cm/s)	0.24	0.26	0.24	0.24	25	MS
S-fol	RT CCA EDV (cm/s)	0.13	0.52	0.09	0.65	25	MS
S-fol	CSD PROX RT CCA (mm)	-0.14	0.5	-0.16	0.43	25	MS
S-fol	CSD MID RT CCA (mm)	-0.21	0.32	-0.14	0.5	25	MS
S-fol	CSD DIST RT CCA (mm)	-0.08	0.7	0.1	0.64	25	MS
S-fol	RT ICA PSV (cm/s)	0.41	0.04	0.38	0.06	25	MS
S-fol	RT ICA EDV (cm/s)	0.49	0.01	0.44	0.03	25	MS
S-fol	CSD PROX RT ICA (mm)	-0.14	0.5	-0.1	0.64	25	MS
S-fol	PROX ECA RT PSV (cm/s)	-0.05	0.81	0.05	0.82	25	MS
S-fol	CSD PROX RT ECA (mm)	0.29	0.16	0.28	0.18	25	MS
S-fol	RT VA PSV (cm/s)	0.33	0.1	0.27	0.2	25	MS
S-fol	RT VA EDV (cm/s)	0.24	0.25	0.14	0.5	25	MS
S-fol	CSD PROX RT VA (mm)	0.01	0.98	0.03	0.88	25	MS
S-fol	RT PSV ICA/CCA ratio	0.2	0.34	0.15	0.47	25	MS
S-fol	RT EDV ICA/ECA ratio	0.32	0.11	0.3	0.15	25	MS
S-fol	LT CCA IMT (mm)	0.36	0.08	0.47	0.02	25	MS
S-fol	LT CCA PSV (cm/s)	0.06	0.78	0.01	0.97	25	MS
S-fol	LT CCA EDV (cm/s)	0.11	0.6	0.12	0.56	25	MS
S-fol	CSD PROX LT CCA (mm)	0.15	0.48	0.22	0.3	25	MS
S-fol	CSD MID LT CCA (mm)	-0.02	0.91	0.08	0.7	25	MS
S-fol	CSD DIST LT CCA (mm)	-0.17	0.41	-0.09	0.66	25	MS
S-fol	LT ICA PSV (cm/s)	0.09	0.66	0.21	0.31	25	MS
S-fol	LT ICA EDV (cm/s)	0.32	0.12	0.32	0.12	25	MS
S-fol	CSD PROX LT ICA (mm)	-0.11	0.62	-0.11	0.59	25	MS
S-fol	PROX LT ECA PSV (cm/s)	0.14	0.5	0.18	0.39	25	MS
S-fol	CSD PROX LT ECA (mm)	0.2	0.34	0.25	0.23	25	MS
S-fol	LT VA PSV (cm/s)	0.11	0.59	0.1	0.63	25	MS
S-fol	LT VA EDV (cm/s)	-0.06	0.78	-0.08	0.7	25	MS
S-fol	CSD PROX LT VA (mm)	-0.39	0.06	-0.4	0.04	25	MS
S-fol	LT PSV ICA/CCA ratio	0.23	0.26	0.2	0.34	25	MS
S-fol	LT EDV ICA/ECA ratio	0.31	0.14	0.21	0.32	25	MS
S-fol	PV PROX RT IJV (cm/s)	0.05	0.81	0.01	0.96	25	MS
S-fol	PV MID RT IJV (cm/s)	0.11	0.6	0.17	0.43	25	MS
S-fol	PV DIST RT IJV (cm/s)	-0.04	0.84	0.04	0.84	25	MS
S-fol	CSA PROX RT IJV (mm^2)	0.17	0.42	0.16	0.43	25	MS
S-fol	PV PROX LT IJV (cm/s)	-0.25	0.22	-0.39	0.05	25	MS
S-fol	PV MID LT IJV (cm/s)	-0.01	0.94	-0.02	0.93	25	MS
S-fol	PV DIST LT IJV (cm/s)	-0.11	0.6	0	1	25	MS
S-fol	CSA PROX LT IJV ($mm^{2)}$	0.01	0.97	-0.16	0.43	25	MS

S-fol, serum folate; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.21: Correlation between serum folate (s-folate) and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
S-fol	RT CCA IMT (mm)	-0.07	0.73	-0.04	0.84	24	CONTROL
S-fol	RT CCA PSV (cm/s)	0.09	0.67	0.01	0.95	24	CONTROL
S-fol	RT CCA EDV (cm/s)	-0.09	0.67	-0.21	0.32	24	CONTROL
S-fol	CSD PROX RT CCA (mm)	-0.05	0.83	-0.02	0.94	24	CONTROL
S-fol	CSD MID RT CCA (mm)	-0.05	0.81	-0.04	0.86	24	CONTROL
S-fol	CSD DIST RT CCA (mm)	0.19	0.38	0.09	0.66	24	CONTROL
S-fol	RT ICA PSV (cm/s)	-0.24	0.27	-0.27	0.21	24	CONTROL
S-fol	RT ICA EDV (cm/s)	-0.25	0.24	-0.42	0.04	24	CONTROL
S-fol	CSD PROX RT ICA (mm)	0.26	0.21	0.15	0.47	24	CONTROL
S-fol	PROX ECA RT PSV (cm/s)	-0.01	0.97	-0.12	0.59	24	CONTROL
S-fol	CSD PROX RT ECA (mm)	0.13	0.54	0.02	0.91	24	CONTROL
S-fol	RT VA PSV (cm/s)	-0.03	0.9	-0.17	0.43	24	CONTROL
S-fol	RT VA EDV (cm/s)	-0.25	0.24	-0.44	0.03	24	CONTROL
S-fol	CSD PROX RT VA (mm)	0.21	0.32	0.29	0.17	24	CONTROL
S-fol	RT PSV ICA/CCA ratio	-0.24	0.26	-0.18	0.39	24	CONTROL
S-fol	RT EDV ICA/ECA ratio	-0.2	0.35	-0.24	0.26	24	CONTROL
S-fol	LT CCA IMT (mm)	0.13	0.55	-0.09	0.68	24	CONTROL
S-fol	LT CCA PSV (cm/s)	0.06	0.79	0.07	0.73	24	CONTROL
S-fol	LT CCA EDV (cm/s)	-0.03	0.88	0.04	0.86	24	CONTROL
S-fol	CSD PROX LT CCA (mm)	0.05	0.83	-0.04	0.84	24	CONTROL
S-fol	CSD MID LT CCA (mm)	0.28	0.19	0.29	0.16	24	CONTROL
S-fol	CSD DIST LT CCA (mm)	0.03	0.88	0.08	0.69	24	CONTROL
S-fol	LT ICA PSV (cm/s)	0.1	0.64	-0.02	0.93	24	CONTROL
S-fol	LT ICA EDV (cm/s)	-0.16	0.45	-0.27	0.2	24	CONTROL
S-fol	CSD PROX LT ICA (mm)	0.09	0.66	0.12	0.57	24	CONTROL
S-fol	PROX LT ECA PSV (cm/s)	0.28	0.19	0.16	0.44	24	CONTROL
S-fol	CSD PROX LT ECA (mm)	-0.3	0.15	-0.15	0.48	24	CONTROL
S-fol	LT VA PSV (cm/s)	-0.09	0.69	-0.05	0.82	24	CONTROL
S-fol	LT VA EDV (cm/s)	-0.21	0.32	-0.1	0.64	24	CONTROL
S-fol	CSD PROX LT VA (mm)	0.26	0.22	0.27	0.19	24	CONTROL
S-fol	LT PSV ICA/CCA ratio	0.02	0.92	-0.09	0.69	24	CONTROL
S-fol	LT EDV ICA/ECA ratio	-0.16	0.44	-0.31	0.15	24	CONTROL
S-fol	PV PROX RT IJV (cm/s)	-0.15	0.48	-0.12	0.58	24	CONTROL
S-fol	PV MID RT IJV (cm/s)	0.12	0.59	0.09	0.69	24	CONTROL
S-fol	PV DIST RT IJV (cm/s)	-0.01	0.96	0.09	0.69	24	CONTROL
S-fol	CSA PROX RT IJV (mm^2)	0.05	0.83	-0.04	0.84	24	CONTROL
S-fol	PV PROX LT IJV (cm/s)	-0.13	0.54	-0.03	0.89	24	CONTROL
S-fol	PV MID LT IJV (cm/s)	-0.22	0.31	-0.2	0.34	24	CONTROL
S-fol	PV DIST LT IJV (cm/s)	0.01	0.95	0.06	0.77	24	CONTROL
S-fol	CSA PROX LT IJV (mm^2)	0.15	0.49	0.13	0.53	24	CONTROL

S-fol, serum folate; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.4.8 Association between homocysteine and the extracranial vascular ultrasound variables

MS group: Homocysteine results were available for 24 MS participants. Table 4.22 (MS group) indicates that a significant negative correlation was found between homocysteine and the PSV of the right CCA (Pearson p-value <0.01, Spearman p-value 0.03) as well as the PV of the mid left IJV (Spearman p-value 0.04). A significant positive association was found between homocysteine and CSD of the proximal right ECA. No other significant associations were found between homocysteine and the extracranial vascular ultrasound variables in the MS group.

Control group: The correlations between homocysteine and the extracranial vascular ultrasound variables in the control group is tabulated in Table 4.23. A significant positive correlation was found between homocysteine and the following ultrasound variables:

- Right EDV ICA/CCA ratio; Spearman p-value <0.01
- Left CCA IMT; Spearman p-value 0.02
- CSA proximal right IJV; Spearman p-value 0.03

Similarly, a negative correlation was found between homocysteine and the PSV of the left CCA (Spearman p-value 0.04).

Table 4.22: Correlation between homocysteine and the extracranial ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
H-cyst	RT CCA IMT (mm)	0.12	0.57	0.15	0.48	24	MS
H-cyst	RT CCA PSV (cm/s)	-0.53	<0.01	-0.45	0.03	24	MS
H-cyst	RT CCA EDV (cm/s)	-0.31	0.14	-0.30	0.15	24	MS
H-cyst	CSD PROX RT CCA (mm)	0.26	0.23	0.24	0.25	24	MS
H-cyst	CSD MID RT CCA (mm)	0.10	0.65	0.17	0.43	24	MS
H-cyst	CSD DIST RT CCA (mm)	0.16	0.46	0.18	0.40	24	MS
H-cyst	RT ICA PSV (cm/s)	-0.11	0.60	-0.03	0.89	24	MS
H-cyst	RT ICA EDV (cm/s)	-0.24	0.25	-0.22	0.31	24	MS
H-cyst	CSD PROX RT ICA (mm)	0.19	0.37	0.14	0.50	24	MS
H-cyst	PROX ECA RT PSV (cm/s)	-0.11	0.60	-0.16	0.45	24	MS
H-cyst	CSD PROX RT ECA (mm)	0.38	0.06	0.48	0.02	24	MS
H-cyst	RT VA PSV (cm/s)	-0.28	0.19	-0.25	0.23	24	MS
H-cyst	RT VA EDV (cm/s)	-0.23	0.29	-0.25	0.23	24	MS
H-cyst	CSD PROX RT VA (mm)	-0.04	0.85	-0.15	0.48	24	MS
H-cyst	RT PSV ICA/CCA ratio	0.34	0.10	0.34	0.11	24	MS
H-cyst	RT EDV ICA/ECA ratio	-0.11	0.62	0.07	0.75	24	MS
H-cyst	LT CCA IMT (mm)	0.24	0.26	0.27	0.21	24	MS
H-cyst	LT CCA PSV (cm/s)	-0.07	0.75	-0.13	0.55	24	MS
H-cyst	LT CCA EDV (cm/s)	-0.21	0.31	-0.16	0.45	24	MS
H-cyst	CSD PROX LT CCA (mm)	0.03	0.90	0.18	0.40	24	MS
H-cyst	CSD MID LT CCA (mm)	-0.01	0.97	-0.02	0.94	24	MS
H-cyst	CSD DIST LT CCA (mm)	-0.08	0.70	-0.11	0.61	24	MS
H-cyst	LT ICA PSV (cm/s)	-0.11	0.60	-0.19	0.39	24	MS
H-cyst	LT ICA EDV (cm/s)	-0.16	0.45	-0.19	0.38	24	MS
H-cyst	CSD PROX LT ICA (mm)	0.25	0.25	0.19	0.37	24	MS
H-cyst	PROX LT ECA PSV (cm/s)	-0.25	0.24	-0.23	0.29	24	MS
H-cyst	CSD PROX LT ECA (mm)	0.27	0.19	0.33	0.11	24	MS
H-cyst	LT VA PSV (cm/s)	-0.24	0.26	-0.18	0.40	24	MS
H-cyst	LT VA EDV (cm/s)	-0.22	0.30	-0.20	0.35	24	MS
H-cyst	CSD PROX LT VA (mm)	0.10	0.63	0.04	0.84	24	MS
H-cyst	LT PSV ICA/CCA ratio	-0.08	0.71	-0.11	0.61	24	MS
H-cyst	LT EDV ICA/ECA ratio	0.03	0.88	0.03	0.88	24	MS
H-cyst	PV PROX RT IJV (cm/s)	0.16	0.46	0.27	0.20	24	MS
H-cyst	PV MID RT IJV (cm/s)	-0.16	0.45	-0.11	0.62	24	MS
H-cyst	PV DIST RT IJV (cm/s)	-0.09	0.66	-0.07	0.74	24	MS
H-cyst	CSA PROX RT IJV (mm^2)	0.33	0.12	0.41	0.05	24	MS
H-cyst	PV PROX LT IJV (cm/s)	-0.19	0.37	-0.21	0.33	24	MS
H-cyst	PV MID LT IJV (cm/s)	-0.38	0.07	-0.42	0.04	24	MS
H-cyst	PV DIST LT IJV (cm/s)	-0.17	0.42	-0.12	0.58	24	MS
H-cyst	CSA PROX LT IJV (mm^2)	0.23	0.28	0.32	0.13	24	MS

H-cyst, homocysteine; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.23: Correlation between homocysteine and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
H-cyst	RT CCA IMT (mm)	0.29	0.16	0.34	0.10	25	CONTROL
H-cyst	RT CCA PSV (cm/s)	-0.17	0.42	-0.14	0.51	25	CONTROL
H-cyst	RT CCA EDV (cm/s)	-0.12	0.58	-0.20	0.34	25	CONTROL
H-cyst	CSD PROX RT CCA (mm)	-0.07	0.72	0.03	0.88	25	CONTROL
H-cyst	CSD MID RT CCA (mm)	0.25	0.23	0.27	0.20	25	CONTROL
H-cyst	CSD DIST RT CCA (mm)	0.22	0.29	0.27	0.18	25	CONTROL
H-cyst	RT ICA PSV (cm/s)	0.24	0.25	0.25	0.24	25	CONTROL
H-cyst	RT ICA EDV (cm/s)	0.30	0.14	0.29	0.16	25	CONTROL
H-cyst	CSD PROX RT ICA (mm)	-0.07	0.75	-0.10	0.64	25	CONTROL
H-cyst	PROX ECA RT PSV (cm/s)	0.27	0.19	0.31	0.13	25	CONTROL
H-cyst	CSD PROX RT ECA (mm)	0.05	0.81	0.09	0.67	25	CONTROL
H-cyst	RT VA PSV (cm/s)	0.15	0.48	0.04	0.84	25	CONTROL
H-cyst	RT VA EDV (cm/s)	0.07	0.75	0.08	0.69	25	CONTROL
H-cyst	CSD PROX RT VA (mm)	-0.17	0.42	-0.13	0.53	25	CONTROL
H-cyst	RT PSV ICA/CCA ratio	0.34	0.10	0.39	0.05	25	CONTROL
H-cyst	RT EDV ICA/CCA ratio	0.38	0.06	0.54	<0.01	25	CONTROL
H-cyst	LT CCA IMT (mm)	0.31	0.13	0.46	0.02	25	CONTROL
H-cyst	LT CCA PSV (cm/s)	-0.37	0.07	-0.41	0.04	25	CONTROL
H-cyst	LT CCA EDV (cm/s)	-0.11	0.60	-0.17	0.41	25	CONTROL
H-cyst	CSD PROX LT CCA (mm)	-0.08	0.72	0.01	0.95	25	CONTROL
H-cyst	CSD MID LT CCA (mm)	0.02	0.92	0.04	0.85	25	CONTROL
H-cyst	CSD DIST LT CCA (mm)	0.30	0.14	0.26	0.20	25	CONTROL
H-cyst	LT ICA PSV (cm/s)	0.02	0.91	0.00	1.00	25	CONTROL
H-cyst	LT ICA EDV (cm/s)	0.19	0.37	0.12	0.56	25	CONTROL
H-cyst	CSD PROX LT ICA (mm)	-0.14	0.52	-0.21	0.32	25	CONTROL
H-cyst	PROX LT ECA PSV (cm/s)	0.06	0.77	0.08	0.71	25	CONTROL
H-cyst	CSD PROX LT ECA (mm)	0.17	0.43	0.13	0.53	25	CONTROL
H-cyst	LT VA PSV (cm/s)	-0.19	0.37	-0.25	0.22	25	CONTROL
H-cyst	LT VA EDV (cm/s)	-0.17	0.41	-0.19	0.36	25	CONTROL
H-cyst	CSD PROX LT VA (mm)	-0.08	0.71	0.05	0.83	25	CONTROL
H-cyst	LT PSV ICA/CCA ratio	0.11	0.60	0.33	0.11	25	CONTROL
H-cyst	LT EDV ICA/ECA ratio	0.22	0.29	0.37	0.07	25	CONTROL
H-cyst	PV PROX RT IJV (cm/s)	-0.09	0.65	-0.03	0.91	25	CONTROL
H-cyst	PV MID RT IJV (cm/s)	-0.06	0.79	0.08	0.71	25	CONTROL
H-cyst	PV DIST RT IJV (cm/s)	0.10	0.64	0.18	0.39	25	CONTROL
H-cyst	CSA PROX RT IJV (mm^2)	0.37	0.07	0.44	0.03	25	CONTROL
H-cyst	PV PROX LT IJV (cm/s)	-0.29	0.16	-0.30	0.15	25	CONTROL
H-cyst	PV MID LT IJV (cm/s)	-0.07	0.72	-0.06	0.78	25	CONTROL
H-cyst	PV DIST LT IJV (cm/s)	-0.12	0.58	-0.08	0.72	25	CONTROL
H-cyst	CSA PROX LT IJV (mm^2)	-0.08	0.70	-0.10	0.62	25	CONTROL

H-cyst, homocysteine; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.4.9 Association between C-reactive protein (CRP) and the extracranial vascular ultrasound variables

MS group: Association between CRP and the extracranial vascular ultrasound variables in the MS group are shown in Table 4.24. A significant positive correlation was found between CRP and the CSD of the distal left CCA (Spearman p-value 0.03); and significant negative correlation between CRP and the PSV of the mid left IJV (Spearman p-value 0.03).

Control group: A significant positive correlation was found between CRP and the CSD of the distal left CCA (Spearman p-value 0.01) and negative correlation with the PV of the mid left IJV (Spearman p-value 0.03) (Table 4.25).

Table 4.24: Correlation between C-reactive protein and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
CRP	RT CCA IMT (mm)	-0.11	0.61	0	0.98	25	MS
CRP	RT CCA PSV (cm/s)	-0.17	0.42	-0.21	0.31	25	MS
CRP	RT CCA EDV (cm/s)	-0.14	0.5	-0.13	0.55	25	MS
CRP	CSD PROX RT CCA (mm)	0.03	0.9	-0.06	0.78	25	MS
CRP	CSD MID RT CCA (mm)	0.11	0.6	0.15	0.49	25	MS
CRP	CSD DIST RT CCA (mm)	0.24	0.24	0.32	0.12	25	MS
CRP	RT ICA PSV (cm/s)	-0.2	0.33	0.04	0.86	25	MS
CRP	RT ICA EDV (cm/s)	-0.2	0.35	-0.16	0.43	25	MS
CRP	CSD PROX RT ICA (mm)	0.04	0.86	-0.01	0.97	25	MS
CRP	PROX ECA RT PSV (cm/s)	-0.19	0.36	-0.27	0.19	25	MS
CRP	CSD PROX RT ECA (mm)	0.18	0.4	0.22	0.28	25	MS
CRP	RT VA PSV (cm/s)	0.1	0.65	-0.21	0.31	25	MS
CRP	RT VA EDV (cm/s)	0.09	0.67	0.05	0.8	25	MS
CRP	CSD PROX RT VA (mm)	0.17	0.41	0.31	0.13	25	MS
CRP	RT PSV ICA/CCA ratio	-0.03	0.87	0.03	0.88	25	MS
CRP	RT EDV ICA/ECA ratio	-0.01	0.95	0.21	0.31	25	MS
CRP	LT CCA IMT (mm)	0.04	0.85	0.06	0.79	25	MS
CRP	LT CCA PSV (cm/s)	-0.04	0.83	-0.15	0.47	25	MS
CRP	LT CCA EDV (cm/s)	-0.19	0.37	-0.32	0.12	25	MS
CRP	CSD PROX LT CCA (mm)	0.1	0.65	0.04	0.84	25	MS
CRP	CSD MID LT CCA (mm)	0.23	0.27	0.3	0.15	25	MS
CRP	CSD DIST LT CCA (mm)	0.3	0.15	0.43	0.03	25	MS
CRP	LT ICA PSV (cm/s)	-0.25	0.23	-0.3	0.15	25	MS
CRP	LT ICA EDV (cm/s)	-0.28	0.17	-0.25	0.23	25	MS
CRP	CSD PROX LT ICA (mm)	0.18	0.39	-0.01	0.97	25	MS
CRP	PROX LT ECA PSV (cm/s)	-0.31	0.13	-0.12	0.58	25	MS
CRP	CSD PROX LT ECA (mm)	0.28	0.18	0.22	0.28	25	MS
CRP	LT VA PSV (cm/s)	-0.12	0.56	-0.29	0.16	25	MS
CRP	LT VA EDV (cm/s)	-0.11	0.6	-0.2	0.33	25	MS
CRP	CSD PROX LT VA (mm)	0.19	0.37	-0.13	0.52	25	MS
CRP	LT PSV ICA/CCA ratio	-0.28	0.18	-0.1	0.65	25	MS
CRP	LT EDV ICA/ECA ratio	-0.21	0.31	-0.19	0.36	25	MS
CRP	PV PROX RT IJV (cm/s)	-0.03	0.9	0.08	0.71	25	MS
CRP	PV MID RT IJV (cm/s)	-0.3	0.15	-0.21	0.32	25	MS
CRP	PV DIST RT IJV (cm/s)	-0.22	0.28	-0.32	0.12	25	MS
CRP	CSA PROX RT IJV (mm^2)	0.12	0.56	-0.11	0.61	25	MS
CRP	PV PROX LT IJV (cm/s)	-0.23	0.26	-0.24	0.25	25	MS
CRP	PV MID LT IJV (cm/s)	-0.39	0.06	-0.45	0.03	25	MS
CRP	PV DIST LT IJV (cm/s)	-0.27	0.19	-0.37	0.07	25	MS
CRP	CSA PROX LT IJV (mm^2)	-0.13	0.54	-0.01	0.95	25	MS

CRP, c-reactive protein; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.25: Correlation between C-reactive protein and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
CRP	RT CCA IMT (mm)	-0.01	0.95	0.00	0.98	25	CONTROL
CRP	RT CCA PSV (cm/s)	-0.17	0.43	-0.24	0.24	25	CONTROL
CRP	RT CCA EDV (cm/s)	-0.21	0.31	-0.19	0.36	25	CONTROL
CRP	CSD PROX RT CCA (mm)	0.10	0.62	0.30	0.14	25	CONTROL
CRP	CSD MID RT CCA (mm)	0.07	0.74	0.23	0.27	25	CONTROL
CRP	CSD DIST RT CCA (mm)	0.10	0.62	0.32	0.12	25	CONTROL
CRP	RT ICA PSV (cm/s)	0.11	0.61	0.02	0.91	25	CONTROL
CRP	RT ICA EDV (cm/s)	-0.08	0.69	0.00	0.98	25	CONTROL
CRP	CSD PROX RT ICA (mm)	-0.12	0.55	-0.18	0.40	25	CONTROL
CRP	PROX ECA RT PSV (cm/s)	0.08	0.71	-0.10	0.65	25	CONTROL
CRP	CSD PROX RT ECA (mm)	-0.19	0.37	-0.26	0.22	25	CONTROL
CRP	RT VA PSV (cm/s)	-0.03	0.88	-0.06	0.77	25	CONTROL
CRP	RT VA EDV (cm/s)	-0.05	0.81	-0.01	0.95	25	CONTROL
CRP	CSD PROX RT VA (mm)	-0.04	0.84	-0.08	0.71	25	CONTROL
CRP	RT PSV ICA/CCA ratio	0.20	0.34	0.22	0.29	25	CONTROL
CRP	RT EDV ICA/ECA ratio	0.12	0.57	0.26	0.20	25	CONTROL
CRP	LT CCA IMT (mm)	0.10	0.64	0.04	0.86	25	CONTROL
CRP	LT CCA PSV (cm/s)	-0.01	0.98	-0.16	0.43	25	CONTROL
CRP	LT CCA EDV (cm/s)	-0.20	0.33	-0.23	0.28	25	CONTROL
CRP	CSD PROX LT CCA (mm)	0.24	0.24	0.48	0.01	25	CONTROL
CRP	CSD MID LT CCA (mm)	0.10	0.63	0.09	0.68	25	CONTROL
CRP	CSD DIST LT CCA (mm)	0.24	0.25	0.10	0.62	25	CONTROL
CRP	LT ICA PSV (cm/s)	-0.18	0.38	-0.09	0.68	25	CONTROL
CRP	LT ICA EDV (cm/s)	-0.35	0.09	-0.25	0.22	25	CONTROL
CRP	CSD PROX LT ICA (mm)	-0.08	0.69	-0.08	0.71	25	CONTROL
CRP	PROX LT ECA PSV (cm/s)	-0.13	0.54	-0.36	0.08	25	CONTROL
CRP	CSD PROX LT ECA (mm)	0.02	0.94	0.12	0.55	25	CONTROL
CRP	LT VA PSV (cm/s)	0.04	0.86	-0.20	0.35	25	CONTROL
CRP	LT VA EDV (cm/s)	0.11	0.60	0.03	0.88	25	CONTROL
CRP	CSD PROX LT VA (mm)	-0.10	0.64	0.00	0.99	25	CONTROL
CRP	LT PSV ICA/CCA ratio	-0.11	0.61	0.13	0.53	25	CONTROL
CRP	LT EDV ICA/ECA ratio	-0.17	0.42	-0.14	0.51	25	CONTROL
CRP	PV PROX RT IJV (cm/s)	-0.15	0.47	-0.04	0.83	25	CONTROL
CRP	PV MID RT IJV (cm/s)	-0.53	<0.01	-0.52	<0.01	25	CONTROL
CRP	PV DIST RT IJV (cm/s)	-0.17	0.42	-0.09	0.66	25	CONTROL
CRP	CSA PROX RT IJV (mm^2)	-0.11	0.59	0.11	0.59	25	CONTROL
CRP	PV PROX LT IJV (cm/s)	0.09	0.66	0.02	0.91	25	CONTROL
CRP	PV MID LT IJV (cm/s)	-0.01	0.96	-0.02	0.92	25	CONTROL
CRP	PV DIST LT IJV (cm/s)	0.00	1.00	0.01	0.97	25	CONTROL
CRP	CSA PROX LT IJV ($mm^{2)}$	-0.05	0.83	0.06	0.77	25	CONTROL

CRP, c-reactive protein; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.4.10. Association between serum 25-OH vitamin D and the extracranial vascular ultrasound variables

***MS group**:* As shown in Table 4.26, a significant positive correlation was found between serum vitamin D and the following ultrasound variables:

- PSV of the right CCA; Pearson p-value 0.03, Spearman p-value 0.03
- PSV of the left CCA; Spearman p-value 0.03
- PSV of the left VA; Spearman p-value <0.01

Similarly, a significant negative correlation was found between serum vitamin D and the CSA of the proximal left IJV (Spearman p-value 0.02).

***Control group**:* Table 4.27 shows that a significant negative association was found between serum vitamin D and the following ultrasound variables in the control group:

- EDV of the left ICA; Pearson p-value 0.04, Spearman p-value <0.01
- CSD of the proximal left ICA; Pearson p-value 0.02
- Left EDV ICA/CCA ratio; Spearman p-value 0.04

In addition, a significant positive association was found between serum vitamin D and the CSD of the proximal left VA (Pearson p-value 0.02).

Table 4.26 Correlation between serum 25-OH vitamin D and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
25 OH- Vit D	RT CCA IMT (mm)	0.08	0.71	0.15	0.47	24	MS
25 OH -Vit D	RT CCA PSV (cm/s)	0.45	0.03	0.46	0.02	24	MS
25 OH- Vit D	RT CCA EDV (cm/s)	0.3	0.15	0.23	0.28	24	MS
25 OH- Vit D	CSD PROX RT CCA (mm)	0.02	0.94	0.04	0.87	24	MS
25 OH- Vit D	CSD MID RT CCA (mm)	-0.23	0.27	-0.15	0.48	24	MS
25 OH- Vit D	CSD DIST RT CCA (mm)	-0.09	0.69	0.02	0.94	24	MS
25 OH- Vit D	RT ICA PSV (cm/s)	0.34	0.11	0.31	0.14	24	MS
25 OH- Vit D	RT ICA EDV (cm/s)	0.46	0.02	0.33	0.11	24	MS
25 OH- Vit D	CSD PROX RT ICA (mm)	-0.03	0.9	-0.24	0.26	24	MS
25 OH- Vit D	PROX ECA RT PSV (cm/s)	-0.27	0.21	-0.08	0.69	24	MS
25 OH- Vit D	CSD PROX RT ECA (mm)	0.31	0.14	0.14	0.51	24	MS
25 OH- Vit D	RT VA PSV (cm/s)	0.18	0.4	0.09	0.68	24	MS
25 OH- Vit D	RT VA EDV (cm/s)	0.36	0.08	0.19	0.38	24	MS
25 OH- Vit D	CSD PROX RT VA (mm)	0.05	0.83	-0.05	0.82	24	MS
25 OH- Vit D	RT PSV ICA/CCA ratio	0.03	0.89	-0.14	0.53	24	MS
25 OH- Vit D	RT EDV ICA/ECA ratio	0.21	0.32	0.13	0.54	24	MS
25 OH- Vit D	LT CCA IMT (mm)	-0.02	0.94	-0.06	0.79	24	MS
25 OH- Vit D	LT CCA PSV (cm/s)	0.24	0.26	0.45	0.03	24	MS
25 OH- Vit D	LT CCA EDV (cm/s)	0.23	0.27	0.35	0.09	24	MS
25 OH- Vit D	CSD PROX LT CCA (mm)	0.07	0.76	0.11	0.6	24	MS
25 OH- Vit D	CSD MID LT CCA (mm)	0.02	0.92	0.08	0.73	24	MS
25 OH- Vit D	CSD DIST LT CCA (mm)	0.19	0.38	0.18	0.4	24	MS
25 OH- Vit D	LT ICA PSV (cm/s)	-0.17	0.42	-0.05	0.8	24	MS
25 OH- Vit D	LT ICA EDV (cm/s)	0.22	0.3	0.12	0.57	24	MS
25 OH- Vit D	CSD PROX LT ICA (mm)	-0.14	0.51	-0.17	0.42	24	MS
25 OH- Vit D	PROX LT ECA PSV (cm/s)	-0.1	0.64	-0.08	0.73	24	MS
25 OH- Vit D	CSD PROX LT ECA (mm)	-0.06	0.78	-0.03	0.88	24	MS
25 OH- Vit D	LT VA PSV (cm/s)	0.39	0.06	0.52	<0.01	24	MS
25 OH- Vit D	LT VA EDV (cm/s)	0.36	0.09	0.37	0.08	24	MS
25 OH- Vit D	CSD PROX LT VA (mm)	-0.31	0.14	-0.27	0.21	24	MS
25 OH- Vit D	LT PSV ICA/CCA ratio	-0.17	0.43	-0.38	0.07	24	MS
25 OH- Vit D	LT EDV ICA/ECA ratio	0.03	0.9	0.07	0.75	24	MS
25 OH- Vit D	PV PROX RT IJV (cm/s)	0.17	0.42	-0.03	0.89	24	MS
25 OH-Vit D	PV MID RT IJV (cm/s)	0.18	0.4	0.26	0.22	24	MS
25 OH- Vit D	PV DIST RT IJV (cm/s)	0.29	0.18	0.22	0.31	24	MS
25 OH- Vit D	CSA PROX RT IJV (mm^2)	0.13	0.53	0.08	0.71	24	MS
25 OH- Vit D	PV PROX LT IJV (cm/s)	-0.04	0.84	0.14	0.52	24	MS
25 OH- Vit D	PV MID LT IJV (cm/s)	-0.18	0.41	-0.02	0.93	24	MS
25 OH- Vit D	PV DIST LT IJV (cm/s)	-0.25	0.23	-0.05	0.81	24	MS
25 OH- Vit D	CSA PROX LT IJV (mm^2)	-0.38	0.07	-0.48	0.02	24	MS

25 OH-vit D, serum 25 hydroxyvtiamin D; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.27: Correlation between vitamin D and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
25 OH-Vit D	RT CCA IMT (mm)	-0.08	0.72	0.13	0.53	25	CONTROL
25 OH-Vit D	RT CCA PSV (cm/s)	-0.03	0.88	-0.03	0.90	25	CONTROL
25 OH-Vit D	RT CCA EDV (cm/s)	-0.08	0.70	-0.17	0.41	25	CONTROL
25 OH-Vit D	CSD PROX RT CCA (mm)	0.10	0.62	-0.09	0.66	25	CONTROL
25 OH-Vit D	CSD MID RT CCA (mm)	0.01	0.95	-0.05	0.81	25	CONTROL
25 OH-Vit D	CSD DIST RT CCA (mm)	-0.04	0.86	0.02	0.94	25	CONTROL
25 OH-Vit D	RT ICA PSV (cm/s)	-0.12	0.56	-0.10	0.63	25	CONTROL
25 OH-Vit D	RT ICA EDV (cm/s)	-0.09	0.66	-0.17	0.42	25	CONTROL
25 OH-Vit D	CSD PROX RT ICA (mm)	-0.32	0.12	-0.17	0.41	25	CONTROL
25 OH-Vit D	PROX ECA RT PSV (cm/s)	-0.21	0.31	0.04	0.87	25	CONTROL
25 OH-Vit D	CSD PROX RT ECA (mm)	-0.12	0.55	-0.20	0.34	25	CONTROL
25 OH-Vit D	RT VA PSV (cm/s)	-0.13	0.53	-0.05	0.83	25	CONTROL
25 OH-Vit D	RT VA EDV (cm/s)	-0.33	0.11	-0.47	0.02	25	CONTROL
25 OH-Vit D	CSD PROX RT VA (mm)	0.22	0.28	0.33	0.11	25	CONTROL
25 OH-Vit D	RT PSV ICA/CCA ratio	-0.10	0.64	0.05	0.83	25	CONTROL
25 OH-Vit D	RT EDV ICA/ECA ratio	-0.05	0.79	0.09	0.67	25	CONTROL
25 OH-Vit D	LT CCA IMT (mm)	-0.10	0.62	-0.05	0.80	25	CONTROL
25 OH-Vit D	LT CCA PSV (cm/s)	0.05	0.81	0.00	0.99	25	CONTROL
25 OH-Vit D	LT CCA EDV (cm/s)	-0.05	0.83	-0.01	0.96	25	CONTROL
25 OH-Vit D	CSD PROX LT CCA (mm)	-0.18	0.39	-0.28	0.18	25	CONTROL
25 OH-Vit D	CSD MID LT CCA (mm)	-0.10	0.63	0.07	0.75	25	CONTROL
25 OH-Vit D	CSD DIST LT CCA (mm)	-0.11	0.59	0.08	0.69	25	CONTROL
25 OH-Vit D	LT ICA PSV (cm/s)	-0.29	0.16	-0.21	0.31	25	CONTROL
25 OH-Vit D	LT ICA EDV (cm/s)	-0.41	0.04	-0.52	<0.01	25	CONTROL
25 OH-Vit D	CSD PROX LT ICA (mm)	-0.46	0.02	-0.32	0.12	25	CONTROL
25 OH-Vit D	PROX LT ECA PSV (cm/s)	-0.17	0.42	0.16	0.44	25	CONTROL
25 OH-Vit D	CSD PROX LT ECA (mm)	-0.06	0.79	-0.14	0.50	25	CONTROL
25 OH-Vit D	LT VA PSV (cm/s)	-0.16	0.44	-0.14	0.50	25	CONTROL
25 OH-Vit D	LT VA EDV (cm/s)	-0.15	0.47	-0.24	0.25	25	CONTROL
25 OH-Vit D	CSD PROX LT VA (mm)	0.46	0.02	0.32	0.12	25	CONTROL
25 OH-Vit D	LT PSV ICA/CCA ratio	0.30	0.15	-0.04	0.86	25	CONTROL
25 OH-Vit D	LT EDV ICA/CCA ratio	-0.36	0.07	-0.41	0.04	25	CONTROL
25 OH-Vit D	PV PROX RT IJV (cm/s)	0.15	0.48	0.04	0.85	25	CONTROL
25 OH-Vit D	PV MID RT IJV (cm/s)	0.21	0.31	0.16	0.45	25	CONTROL
25 OH-Vit D	PV DIST RT IJV (cm/s)	-0.01	0.97	0.17	0.41	25	CONTROL
25 OH-Vit D	CSA PROX RT IJV (mm^2)	-0.20	0.35	-0.18	0.38	25	CONTROL
25 OH-Vit D	PV PROX LT IJV (cm/s)	-0.02	0.94	-0.03	0.88	25	CONTROL
25 OH-Vit D	PV MID LT IJV (cm/s)	0.04	0.84	-0.04	0.85	25	CONTROL
25 OH-Vit D	PV DIST LT IJV (cm/s)	0.08	0.70	0.02	0.91	25	CONTROL
25 OH-Vit D	CSA PROX LT IJV (mm^2)	-0.16	0.46	-0.21	0.32	25	CONTROL

25 OH-vit D, serum 25 hydroxyvtiamin D; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.4.11 Association between total cholesterol and the extracranial vascular ultrasound variables

***MS group**:* A significant negative association was found between total cholesterol and the EDV of the left CCA (Pearson p-value 0.03, Spearman p-value 0.01) as well as the PSV of the left VA (Pearson p-value 0.03) (Table 4.28).

Control group: A significant correlation was found between total cholesterol and the EDV of the left ICA (Pearson p-value 0.04) (Table 4.29).

Table 4.28. Correlation between total cholesterol and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Total cholesterol	RT CCA IMT (mm)	0.1	0.63	0.09	0.67	25	MS
Total cholesterol	RT CCA PSV (cm/s)	-0.08	0.71	-0.16	0.44	25	MS
Total cholesterol	RT CCA EDV (cm/s)	-0.33	0.11	-0.29	0.16	25	MS
Total cholesterol	CSD PROX RT CCA (mm)	0.07	0.75	-0.03	0.87	25	MS
Total cholesterol	CSD MID RT CCA (mm)	0.17	0.41	0.16	0.45	25	MS
Total cholesterol	CSD DIST RT CCA (mm)	0.3	0.15	0.23	0.27	25	MS
Total cholesterol	RT ICA PSV (cm/s)	-0.04	0.86	-0.18	0.38	25	MS
Total cholesterol	RT ICA EDV (cm/s)	-0.13	0.54	-0.22	0.28	25	MS
Total cholesterol	CSD PROX RT ICA (mm)	0	0.99	0.01	0.97	25	MS
Total cholesterol	PROX ECA RT PSV (cm/s)	0.33	0.1	0.37	0.07	25	MS
Total cholesterol	CSD PROX RT ECA (mm)	-0.02	0.91	-0.06	0.78	25	MS
Total cholesterol	RT VA PSV (cm/s)	-0.37	0.07	-0.36	0.08	25	MS
Total cholesterol	RT VA EDV (cm/s)	-0.3	0.15	-0.31	0.13	25	MS
Total cholesterol	CSD PROX RT VA (mm)	0.4	0.05	0.28	0.17	25	MS
Total cholesterol	RT PSV ICA/CCA ratio	0.05	0.82	-0.03	0.9	25	MS
Total cholesterol	RT EDV ICA/ECA ratio	0.3	0.15	0.05	0.8	25	MS
Total cholesterol	LT CCA IMT (mm)	0.22	0.28	0.33	0.1	25	MS
Total cholesterol	LT CCA PSV (cm/s)	-0.32	0.11	-0.38	0.06	25	MS
Total cholesterol	LT CCA EDV (cm/s)	-0.43	0.03	-0.5	0.01	25	MS
Total cholesterol	CSD PROX LT CCA (mm)	0.09	0.68	-0.12	0.58	25	MS
Total cholesterol	CSD MID LT CCA (mm)	0.17	0.41	0.04	0.85	25	MS
Total cholesterol	CSD DIST LT CCA (mm)	0.08	0.69	-0.01	0.95	25	MS
Total cholesterol	LT ICA PSV (cm/s)	-0.31	0.13	-0.22	0.29	25	MS
Total cholesterol	LT ICA EDV (cm/s)	-0.25	0.22	-0.3	0.15	25	MS
Total cholesterol	CSD PROX LT ICA (mm)	-0.18	0.38	-0.18	0.4	25	MS
Total cholesterol	PROX LT ECA PSV (cm/s)	0.21	0.32	0.26	0.21	25	MS
Total cholesterol	CSD PROX LT ECA (mm)	0.22	0.3	0.09	0.69	25	MS
Total cholesterol	LT VA PSV (cm/s)	-0.44	0.03	-0.39	0.05	25	MS
Total cholesterol	LT VA EDV (cm/s)	-0.37	0.07	-0.33	0.1	25	MS
Total cholesterol	CSD PROX LT VA (mm)	-0.03	0.89	0	0.99	25	MS
Total cholesterol	LT PSV ICA/CCA ratio	0.22	0.3	0.15	0.48	25	MS
Total cholesterol	LT EDV ICA/ECA ratio	0.16	0.44	-0.09	0.66	25	MS
Total cholesterol	PV PROX RT IJV (cm/s)	-0.02	0.94	0.12	0.56	25	MS
Total cholesterol	PV MID RT IJV (cm/s)	0.02	0.94	0.06	0.76	25	MS
Total cholesterol	PV DIST RT IJV (cm/s)	-0.16	0.46	-0.14	0.52	25	MS
Total cholesterol	CSA PROX RT IJV (mm^2)	-0.23	0.26	-0.35	0.08	25	MS
Total cholesterol	PV PROX LT IJV (cm/s)	-0.35	0.09	-0.2	0.33	25	MS
Total cholesterol	PV MID LT IJV (cm/s)	-0.01	0.97	0.11	0.6	25	MS
Total cholesterol	PV DIST LT IJV (cm/s)	0.05	0.83	0.08	0.69	25	MS
Total cholesterol	CSA PROX LT IJV (mm^2)	0.21	0.32	0.22	0.3	25	MS

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

Table 4.29: Correlation between total cholesterol and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
Total cholesterol	RT CCA IMT (mm)	0.37	0.07	0.24	0.24	25	CONTROL
Total cholesterol	RT CCA PSV (cm/s)	-0.02	0.94	-0.08	0.69	25	CONTROL
Total cholesterol	RT CCA EDV (cm/s)	-0.19	0.37	-0.32	0.12	25	CONTROL
Total cholesterol	CSD PROX RT CCA (mm)	0.15	0.47	0.14	0.51	25	CONTROL
Total cholesterol	CSD MID RT CCA (mm)	0.24	0.25	0.20	0.35	25	CONTROL
Total cholesterol	CSD DIST RT CCA (mm)	0.16	0.46	0.23	0.28	25	CONTROL
Total cholesterol	RT ICA PSV (cm/s)	-0.33	0.10	-0.20	0.35	25	CONTROL
Total cholesterol	RT ICA EDV (cm/s)	-0.35	0.08	-0.35	0.09	25	CONTROL
Total cholesterol	CSD PROX RT ICA (mm)	-0.14	0.50	-0.10	0.64	25	CONTROL
Total cholesterol	PROX ECA RT PSV (cm/s)	0.08	0.70	0.05	0.81	25	CONTROL
Total cholesterol	CSD PROX RT ECA (mm)	-0.07	0.75	-0.06	0.78	25	CONTROL
Total cholesterol	RT VA PSV (cm/s)	-0.04	0.86	-0.17	0.40	25	CONTROL
Total cholesterol	RT VA EDV (cm/s)	-0.12	0.56	-0.18	0.38	25	CONTROL
Total cholesterol	CSD PROX RT VA (mm)	0.17	0.41	0.07	0.76	25	CONTROL
Total cholesterol	RT PSV ICA/CCA ratio	-0.20	0.33	-0.04	0.87	25	CONTROL
Total cholesterol	RT EDV ICA/ECA ratio	-0.25	0.23	0.02	0.93	25	CONTROL
Total cholesterol	LT CCA IMT (mm)	0.23	0.26	0.08	0.72	25	CONTROL
Total cholesterol	LT CCA PSV (cm/s)	0.09	0.68	-0.11	0.60	25	CONTROL
Total cholesterol	LT CCA EDV (cm/s)	0.05	0.82	0.06	0.78	25	CONTROL
Total cholesterol	CSD PROX LT CCA (mm)	0.29	0.16	0.23	0.28	25	CONTROL
Total cholesterol	CSD MID LT CCA (mm)	0.28	0.18	0.27	0.18	25	CONTROL
Total cholesterol	CSD DIST LT CCA (mm)	0.02	0.91	0.14	0.50	25	CONTROL
Total cholesterol	LT ICA PSV (cm/s)	-0.09	0.69	0.05	0.80	25	CONTROL
Total cholesterol	LT ICA EDV (cm/s)	-0.42	0.04	-0.39	0.06	25	CONTROL
Total cholesterol	CSD PROX LT ICA (mm)	0.17	0.43	0.22	0.30	25	CONTROL
Total cholesterol	PROX LT ECA PSV (cm/s)	0.17	0.42	0.17	0.41	25	CONTROL
Total cholesterol	CSD PROX LT ECA (mm)	-0.23	0.27	-0.18	0.39	25	CONTROL
Total cholesterol	LT VA PSV (cm/s)	0.15	0.48	0.04	0.83	25	CONTROL
Total cholesterol	LT VA EDV (cm/s)	0.00	0.98	0.04	0.85	25	CONTROL
Total cholesterol	CSD PROX LT VA (mm)	0.08	0.71	-0.03	0.90	25	CONTROL
Total cholesterol	LT PSV ICA/CCA ratio	0.06	0.79	0.20	0.34	25	CONTROL
Total cholesterol	LT EDV ICA/ECA ratio	-0.39	0.06	-0.19	0.36	25	CONTROL
Total cholesterol	PV PROX RT IJV (cm/s)	-0.34	0.10	-0.16	0.44	25	CONTROL
Total cholesterol	PV MID RT IJV (cm/s)	0.24	0.25	0.19	0.36	25	CONTROL
Total cholesterol	PV DIST RT IJV (cm/s)	-0.01	0.97	-0.15	0.47	25	CONTROL
Total cholesterol	CSA PROX RT IJV (mm^2)	-0.11	0.59	-0.06	0.76	25	CONTROL
Total cholesterol	PV PROX LT IJV (cm/s)	0.04	0.84	0.09	0.68	25	CONTROL
Total cholesterol	PV MID LT IJV (cm/s)	0.04	0.85	-0.01	0.98	25	CONTROL
Total cholesterol	PV DIST LT IJV (cm/s)	-0.16	0.45	-0.08	0.72	25	CONTROL
Total cholesterol	CSA PROX LT IJV (mm^2)	0.03	0.87	0.14	0.51	25	CONTROL

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

4.4.12 Association between High-density lipoprotein (HDL) and the extracranial vascular ultrasound variables

The correlations between HDL and the extracranial vascular ultrasound variables are shown in Tables 4.30 and 4.31. HDL results were available for 24 MS participants and 24 controls.

MS group: The negative correlation between HDL and the CSD of the proximal right ECA (Pearson p-value 0.03, Spearman p-value <0.01) and the CSA of the proximal right IJV (Pearson p-value 0.04) was found to be statistically significant. A positive correlation was found between HDL and the PV of the proximal right IJV (Pearson p-value 0.02) (Table 4.30).

Control group: No significant correlations (p-value >0.05) were found between HDL and the extracranial vascular ultrasound variables in this group of participants (Table 4.31).

Table 4.30: Correlation between HDL and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
HDL	RT CCA IMT (mm)	-0.13	0.53	-0.14	0.51	24	MS
HDL	RT CCA PSV (cm/s)	0.25	0.23	0.19	0.38	24	MS
HDL	RT CCA EDV (cm/s)	-0.11	0.62	-0.11	0.61	24	MS
HDL	CSD PROX RT CCA (mm)	0.13	0.54	0.07	0.76	24	MS
HDL	CSD MID RT CCA (mm)	0.02	0.93	0.04	0.86	24	MS
HDL	CSD DIST RT CCA (mm)	-0.09	0.69	-0.14	0.52	24	MS
HDL	RT ICA PSV (cm/s)	0.02	0.92	-0.02	0.93	24	MS
HDL	RT ICA EDV (cm/s)	0.15	0.48	0.16	0.46	24	MS
HDL	CSD PROX RT ICA (mm)	-0.26	0.23	-0.25	0.24	24	MS
HDL	PROX ECA RT PSV (cm/s)	-0.06	0.79	0.02	0.93	24	MS
HDL	CSD PROX RT ECA (mm)	-0.43	0.03	-0.57	<0.01	24	MS
HDL	RT VA PSV (cm/s)	-0.03	0.88	-0.01	0.97	24	MS
HDL	RT VA EDV (cm/s)	-0.03	0.87	0.06	0.77	24	MS
HDL	CSD PROX RT VA (mm)	0.14	0.53	0.07	0.76	24	MS
HDL	RT PSV ICA/CCA ratio	-0.19	0.38	-0.17	0.43	24	MS
HDL	RT EDV ICA/ECA ratio	0.32	0.13	0.25	0.24	24	MS
HDL	LT CCA IMT (mm)	-0.31	0.14	-0.41	0.05	24	MS
HDL	LT CCA PSV (cm/s)	0.24	0.26	0.25	0.25	24	MS
HDL	LT CCA EDV (cm/s)	0.06	0.78	0.1	0.64	24	MS
HDL	CSD PROX LT CCA (mm)	-0.01	0.97	-0.11	0.61	24	MS
HDL	CSD MID LT CCA (mm)	0.05	0.8	0	1	24	MS
HDL	CSD DIST LT CCA (mm)	0.18	0.41	0.13	0.56	24	MS
HDL	LT ICA PSV (cm/s)	0.02	0.93	-0.05	0.83	24	MS
HDL	LT ICA EDV (cm/s)	-0.08	0.71	-0.12	0.57	24	MS
HDL	CSD PROX LT ICA (mm)	-0.02	0.94	-0.09	0.67	24	MS
HDL	PROX LT ECA PSV (cm/s)	-0.06	0.78	-0.04	0.84	24	MS
HDL	CSD PROX LT ECA (mm)	-0.33	0.12	-0.37	0.07	24	MS
HDL	LT VA PSV (cm/s)	0.11	0.59	0.16	0.46	24	MS
HDL	LT VA EDV (cm/s)	0.23	0.28	0.28	0.18	24	MS
HDL	CSD PROX LT VA (mm)	0.16	0.45	0.23	0.29	24	MS
HDL	LT PSV ICA/CCA ratio	-0.19	0.38	-0.15	0.48	24	MS
HDL	LT EDV ICA/ECA ratio	-0.18	0.39	-0.11	0.61	24	MS
HDL	PV PROX RT IJV (cm/s)	0.03	0.89	0.03	0.9	24	MS
HDL	PV MID RT IJV (cm/s)	0.19	0.39	0.19	0.37	24	MS
HDL	PV DIST RT IJV (cm/s)	0.27	0.19	0.22	0.29	24	MS
HDL	CSA PROX RT IJV (mm^2)	-0.42	0.04	-0.4	0.05	24	MS
HDL	PV PROX LT IJV (cm/s)	0.4	0.05	0.49	0.02	24	MS
HDL	PV MID LT IJV (cm/s)	0.22	0.29	0.32	0.12	24	MS
HDL	PV DIST LT IJV (cm/s)	-0.02	0.94	0.08	0.7	24	MS
HDL	CSA PROX LT IJV (mm^2)	-0.26	0.22	-0.3	0.15	24	MS

HDL, high-density lipoprotein; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.31: Correlation between HDL and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
HDL	RT CCA IMT (mm)	0.13	0.54	0.33	0.11	24	CONTROL
HDL	RT CCA PSV (cm/s)	0.24	0.26	0.31	0.14	24	CONTROL
HDL	RT CCA EDV (cm/s)	0.11	0.61	0.03	0.90	24	CONTROL
HDL	CSD PROX RT CCA (mm)	0.03	0.88	0.01	0.95	24	CONTROL
HDL	CSD MID RT CCA (mm)	0.00	0.99	0.12	0.59	24	CONTROL
HDL	CSD DIST RT CCA (mm)	-0.08	0.71	-0.01	0.98	24	CONTROL
HDL	RT ICA PSV (cm/s)	0.04	0.84	0.05	0.81	24	CONTROL
HDL	RT ICA EDV (cm/s)	0.11	0.61	0.13	0.56	24	CONTROL
HDL	CSD PROX RT ICA (mm)	-0.18	0.41	-0.11	0.62	24	CONTROL
HDL	PROX ECA RT PSV (cm/s)	-0.26	0.23	-0.16	0.44	24	CONTROL
HDL	CSD PROX RT ECA (mm)	0.18	0.40	0.29	0.16	24	CONTROL
HDL	RT VA PSV (cm/s)	-0.01	0.96	0.00	1.00	24	CONTROL
HDL	RT VA EDV (cm/s)	-0.12	0.56	-0.11	0.60	24	CONTROL
HDL	CSD PROX RT VA (mm)	0.23	0.28	0.19	0.37	24	CONTROL
HDL	RT PSV ICA/CCA ratio	-0.28	0.19	-0.26	0.22	24	CONTROL
HDL	RT EDV ICA/ECA ratio	-0.02	0.92	0.09	0.66	24	CONTROL
HDL	LT CCA IMT (mm)	-0.11	0.61	0.05	0.82	24	CONTROL
HDL	LT CCA PSV (cm/s)	0.08	0.71	0.03	0.87	24	CONTROL
HDL	LT CCA EDV (cm/s)	-0.12	0.57	-0.10	0.65	24	CONTROL
HDL	CSD PROX LT CCA (mm)	-0.11	0.60	-0.19	0.39	24	CONTROL
HDL	CSD MID LT CCA (mm)	0.01	0.96	0.08	0.70	24	CONTROL
HDL	CSD DIST LT CCA (mm)	-0.12	0.56	0.00	0.99	24	CONTROL
HDL	LT ICA PSV (cm/s)	0.05	0.80	0.20	0.34	24	CONTROL
HDL	LT ICA EDV (cm/s)	-0.11	0.62	-0.08	0.73	24	CONTROL
HDL	CSD PROX LT ICA (mm)	-0.37	0.07	-0.31	0.14	24	CONTROL
HDL	PROX LT ECA PSV (cm/s)	-0.10	0.66	0.13	0.55	24	CONTROL
HDL	CSD PROX LT ECA (mm)	-0.18	0.40	-0.27	0.21	24	CONTROL
HDL	LT VA PSV (cm/s)	0.11	0.62	0.12	0.57	24	CONTROL
HDL	LT VA EDV (cm/s)	0.05	0.83	0.02	0.92	24	CONTROL
HDL	CSD PROX LT VA (mm)	0.20	0.36	-0.03	0.89	24	CONTROL
HDL	LT PSV ICA/CCA ratio	-0.03	0.90	0.10	0.66	24	CONTROL
HDL	LT EDV ICA/ECA ratio	-0.02	0.94	0.13	0.55	24	CONTROL
HDL	PV PROX RT IJV (cm/s)	0.05	0.80	0.00	0.99	24	CONTROL
HDL	PV MID RT IJV (cm/s)	0.29	0.16	0.33	0.12	24	CONTROL
HDL	PV DIST RT IJV (cm/s)	0.26	0.21	0.21	0.33	24	CONTROL
HDL	CSA PROX RT IJV (mm^2)	-0.25	0.24	-0.24	0.25	24	CONTROL
HDL	PV PROX LT IJV (cm/s)	-0.12	0.58	-0.04	0.84	24	CONTROL
HDL	PV MID LT IJV (cm/s)	-0.11	0.60	-0.15	0.49	24	CONTROL
HDL	PV DIST LT IJV (cm/s)	-0.06	0.79	-0.11	0.61	24	CONTROL
HDL	CSA PROX LT IJV (mm^2)	0.01	0.98	0.13	0.55	24	CONTROL

HDL, high-density lipoprotein; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.4.13 Association between Low-density lipoprotein and the extracranial vascular ultrasound variables

Low-density lipoprotein (LDL) results were available for 23 MS participants and 23 controls.

***MS group**:* There were no significant correlations (p-value >0.05) between LDL and the extracranial vascular ultrasound variables in the MS group of participants (Table 4.32).

***Control group**:* In this group, only the CSD of the proximal left CCA had a significant negative correlation (Spearman p-value 0.04) with LDL (Table 4.33).

Table 4.32: Correlation between LDL and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
LDL	RT CCA IMT (mm)	-0.13	0.54	0	1	23	MS
LDL	RT CCA PSV (cm/s)	0.06	0.8	-0.01	0.96	23	MS
LDL	RT CCA EDV (cm/s)	0.12	0.6	0.12	0.57	23	MS
LDL	CSD PROX RT CCA (mm)	-0.27	0.21	-0.31	0.15	23	MS
LDL	CSD MID RT CCA (mm)	-0.37	0.08	-0.24	0.27	23	MS
LDL	CSD DIST RT CCA (mm)	-0.14	0.54	0.01	0.98	23	MS
LDL	RT ICA PSV (cm/s)	-0.02	0.91	-0.02	0.92	23	MS
LDL	RT ICA EDV (cm/s)	0.1	0.65	0.13	0.54	23	MS
LDL	CSD PROX RT ICA (mm)	0	0.99	0.03	0.89	23	MS
LDL	PROX ECA RT PSV (cm/s)	0.38	0.07	0.39	0.07	23	MS
LDL	CSD PROX RT ECA (mm)	0.17	0.43	0.28	0.2	23	MS
LDL	RT VA PSV (cm/s)	-0.24	0.27	-0.27	0.21	23	MS
LDL	RT VA EDV (cm/s)	-0.1	0.66	-0.08	0.73	23	MS
LDL	CSD PROX RT VA (mm)	0.23	0.28	0.23	0.3	23	MS
LDL	RT PSV ICA/CCA ratio	-0.09	0.69	0.01	0.97	23	MS
LDL	RT EDV ICA/ECA ratio	0	1	-0.13	0.54	23	MS
LDL	LT CCA IMT (mm)	0.01	0.97	0.36	0.09	23	MS
LDL	LT CCA PSV (cm/s)	-0.13	0.57	-0.18	0.41	23	MS
LDL	LT CCA EDV (cm/s)	0.01	0.98	-0.02	0.92	23	MS
LDL	CSD PROX LT CCA (mm)	-0.3	0.16	-0.36	0.09	23	MS
LDL	CSD MID LT CCA (mm)	-0.14	0.51	-0.16	0.46	23	MS
LDL	CSD DIST LT CCA (mm)	-0.15	0.49	-0.25	0.24	23	MS
LDL	LT ICA PSV (cm/s)	-0.34	0.12	-0.22	0.32	23	MS
LDL	LT ICA EDV (cm/s)	0.17	0.43	0.22	0.31	23	MS
LDL	CSD PROX LT ICA (mm)	-0.19	0.39	-0.14	0.53	23	MS
LDL	PROX LT ECA PSV (cm/s)	0.11	0.63	0.11	0.61	23	MS
LDL	CSD PROX LT ECA (mm)	0.23	0.29	0.27	0.22	23	MS
LDL	LT VA PSV (cm/s)	-0.2	0.37	-0.14	0.51	23	MS
LDL	LT V A EDV (cm/s)	-0.17	0.43	-0.1	0.65	23	MS
LDL	CSD PROX LT VA (mm)	-0.15	0.49	-0.19	0.39	23	MS
LDL	LT PSV ICA/CCA ratio	0	1	0.03	0.88	23	MS
LDL	LT EDV ICA/ECA ratio	0.3	0.17	0.21	0.33	23	MS
LDL	PV PROX RT IJV (cm/s)	-0.08	0.73	0.07	0.75	23	MS
LDL	PV MID RT IJV (cm/s)	-0.02	0.93	0	0.98	23	MS
LDL	PV DIST RT IJV (cm/s)	-0.19	0.39	-0.07	0.74	23	MS
LDL	CSA PROX RT IJV (mm^2)	0.07	0.74	0.05	0.81	23	MS
LDL	PV PROX LT IJV (cm/s)	-0.26	0.24	-0.18	0.41	23	MS
LDL	PV MID LT IJV (cm/s)	0.14	0.53	0.17	0.43	23	MS
LDL	PV DIST LT IJV (cm/s)	-0.25	0.25	-0.07	0.74	23	MS
LDL	CSA PROX LT IJV (mm^2)	0.22	0.32	0.01	0.98	23	MS

LDL, low-density lipoprotein; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.33: Correlation between LDL and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
LDL	RT CCA IMT (mm)	0.29	0.17	0.18	0.41	23	CONTROL
LDL	RT CCA PSV (cm/s)	-0.07	0.75	-0.07	0.76	23	CONTROL
LDL	RT CCA EDV (cm/s)	-0.23	0.29	-0.27	0.21	23	CONTROL
LDL	CSD PROX RT CCA (mm)	0.14	0.53	0.22	0.32	23	CONTROL
LDL	CSD MID RT CCA (mm)	0.26	0.23	0.30	0.17	23	CONTROL
LDL	CSD DIST RT CCA (mm)	0.17	0.43	0.23	0.29	23	CONTROL
LDL	RT ICA PSV (cm/s)	-0.21	0.33	-0.13	0.54	23	CONTROL
LDL	RT ICA EDV (cm/s)	-0.35	0.11	-0.37	0.09	23	CONTROL
LDL	CSD PROX RT ICA (mm)	0.01	0.95	0.00	0.98	23	CONTROL
LDL	PROX ECA RT PSV (cm/s)	0.20	0.35	0.17	0.44	23	CONTROL
LDL	CSD PROX RT ECA (mm)	-0.07	0.75	-0.10	0.64	23	CONTROL
LDL	RT VA PSV (cm/s)	-0.01	0.97	-0.11	0.63	23	CONTROL
LDL	RT VA EDV (cm/s)	-0.05	0.83	-0.14	0.51	23	CONTROL
LDL	CSD PROX RT VA (mm)	-0.06	0.80	-0.23	0.30	23	CONTROL
LDL	RT PSV ICA/CCA ratio	-0.01	0.95	0.03	0.90	23	CONTROL
LDL	RT EDV ICA/ECA ratio	-0.16	0.45	-0.05	0.81	23	CONTROL
LDL	LT CCA IMT (mm)	0.25	0.24	0.10	0.66	23	CONTROL
LDL	LT CCA PSV (cm/s)	0.07	0.74	0.02	0.92	23	CONTROL
LDL	LT CCA EDV (cm/s)	0.11	0.60	0.21	0.35	23	CONTROL
LDL	CSD PROX LT CCA (mm)	0.36	0.09	0.43	0.04	23	CONTROL
LDL	CSD MID LT CCA (mm)	0.35	0.11	0.39	0.07	23	CONTROL
LDL	CSD DIST LT CCA (mm)	0.14	0.51	0.26	0.23	23	CONTROL
LDL	LT ICA PSV (cm/s)	-0.01	0.98	0.01	0.95	23	CONTROL
LDL	LT ICA EDV (cm/s)	-0.30	0.16	-0.38	0.07	23	CONTROL
LDL	CSD PROX LT ICA (mm)	0.36	0.09	0.31	0.15	23	CONTROL
LDL	PROX LT ECA PSV (cm/s)	0.25	0.25	0.24	0.27	23	CONTROL
LDL	CSD PROX LT ECA (mm)	-0.07	0.75	-0.04	0.87	23	CONTROL
LDL	LT VA PSV (cm/s)	0.15	0.48	0.11	0.61	23	CONTROL
LDL	LT VA EDV (cm/s)	0.04	0.85	0.14	0.53	23	CONTROL
LDL	CSD PROX LT VA (mm)	-0.09	0.70	-0.05	0.83	23	CONTROL
LDL	LT PSV ICA/CCA ratio	-0.06	0.80	-0.02	0.93	23	CONTROL
LDL	LT EDV ICA/ECA ratio	-0.31	0.14	-0.31	0.14	23	CONTROL
LDL	PV PROX RT IJV (cm/s)	-0.32	0.13	-0.22	0.31	23	CONTROL
LDL	PV MID RT IJV (cm/s)	0.12	0.60	0.02	0.92	23	CONTROL
LDL	PV DIST RT IJV (cm/s)	-0.05	0.83	-0.19	0.39	23	CONTROL
LDL	CSA PROX RT IJV (mm^2)	0.08	0.71	0.14	0.53	23	CONTROL
LDL	PV PROX LT IJV (cm/s)	0.16	0.45	0.20	0.36	23	CONTROL
LDL	PV MID LT IJV (cm/s)	0.08	0.73	0.08	0.73	23	CONTROL
LDL	PV DIST LT IJV (cm/s)	-0.14	0.51	-0.05	0.82	23	CONTROL
LDL	CSA PROX LT IJV (mm^2)	0.09	0.69	0.21	0.34	23	CONTROL

LDL, low-density lipoprotein; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.4.14 Association between triglycerides and the extracranial vascular ultrasound variables

Triglycerides results were available for 24 MS participants and 23 participants free from MS (control group).

MS group: Triglycerides had a significant negative correlation with PV of the proximal left IJV (Spearman p-value 0.02) (Table 4.34).

Control group: A significant negative correlation was found between triglycerides and the following extracranial vascular ultrasound variables:

- PSV of the right ICA; Pearson p-value <0.01, Spearman p-value 0.02
- PSV of the left ICA; Pearson p-value 0.04, Spearman p-value 0.04

Similarly, a significant positive correlation was found between triglycerides and the CSD of the proximal right VA (Pearson p-value 0.03, Spearman p-value <0.01) (Table 4.35).

Table 4.34: Correlation between triglycerides and the extracranial vascular ultrasound variables in the MS group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Trigs	RT CCA IMT (mm)	-0.17	0.41	-0.07	0.75	24	MS
Trigs	RT CCA PSV (cm/s)	0.02	0.92	-0.17	0.44	24	MS
Trigs	RT CCA EDV (cm/s)	0.07	0.73	0.07	0.76	24	MS
Trigs	CSD PROX RT CCA (mm)	-0.08	0.70	-0.24	0.26	24	MS
Trigs	CSD MID RT CCA (mm)	0.00	0.99	-0.05	0.80	24	MS
Trigs	CSD DIST RT CCA (mm)	0.04	0.86	-0.06	0.77	24	MS
Trigs	RT ICA PSV (cm/s)	-0.09	0.66	-0.16	0.45	24	MS
Trigs	RT ICA EDV (cm/s)	-0.10	0.63	-0.10	0.65	24	MS
Trigs	CSD PROX RT ICA (mm)	-0.07	0.75	-0.02	0.92	24	MS
Trigs	PROX ECA RT PSV (cm/s)	0.09	0.69	-0.15	0.47	24	MS
Trigs	CSD PROX RT ECA (mm)	-0.01	0.96	-0.03	0.88	24	MS
Trigs	RT VA PSV (cm/s)	0.17	0.43	0.08	0.73	24	MS
Trigs	RT VA EDV (cm/s)	0.10	0.65	0.15	0.48	24	MS
Trigs	CSD PROX RT VA (mm)	0.20	0.35	0.29	0.18	24	MS
Trigs	RT PSV ICA/CCA ratio	-0.07	0.73	0.08	0.70	24	MS
Trigs	RT EDV ICA/ECA ratio	-0.14	0.53	-0.03	0.89	24	MS
Trigs	LT CCA IMT (mm)	0.12	0.58	0.11	0.61	24	MS
Trigs	LT CCA PSV (cm/s)	-0.20	0.36	-0.36	0.08	24	MS
Trigs	LT CCA EDV (cm/s)	-0.23	0.28	-0.29	0.17	24	MS
Trigs	CSD PROX LT CCA (mm)	0.19	0.36	-0.06	0.76	24	MS
Trigs	CSD MID LT CCA (mm)	0.06	0.78	-0.14	0.52	24	MS
Trigs	CSD DIST LT CCA (mm)	-0.09	0.66	-0.16	0.45	24	MS
Trigs	LT ICA PSV (cm/s)	0.15	0.49	0.16	0.44	24	MS
Trigs	LT ICA EDV (cm/s)	-0.05	0.82	-0.13	0.53	24	MS
Trigs	CSD PROX LT ICA (mm)	-0.15	0.50	-0.08	0.72	24	MS
Trigs	PROX LT ECA PSV (cm/s)	0.12	0.58	0.16	0.47	24	MS
Trigs	CSD PROX LT ECA (mm)	0.17	0.44	0.05	0.81	24	MS
Trigs	LT VA PSV (cm/s)	-0.20	0.34	-0.22	0.30	24	MS
Trigs	LT VA EDV (cm/s)	-0.22	0.31	-0.24	0.25	24	MS
Trigs	CSD PROX LT VA (mm)	0.10	0.64	-0.04	0.86	24	MS
Trigs	LT PSV ICA/CCA ratio	0.32	0.12	0.34	0.11	24	MS
Trigs	LT EDV ICA/ECA ratio	0.23	0.28	-0.10	0.65	24	MS
Trigs	PV PROX RT IJV (cm/s)	0.07	0.73	0.11	0.62	24	MS
Trigs	PV MID RT IJV (cm/s)	-0.02	0.91	-0.18	0.40	24	MS
Trigs	PV DIST RT IJV (cm/s)	-0.23	0.28	-0.28	0.19	24	MS
Trigs	CSA PROX RT IJV (mm^2)	0.20	0.34	0.03	0.88	24	MS
Trigs	PV PROX LT IJV (cm/s)	-0.35	0.09	-0.49	0.02	24	MS
Trigs	PV MID LT IJV (cm/s)	-0.09	0.68	-0.15	0.48	24	MS
Trigs	PV DIST LT IJV (cm/s)	-0.14	0.53	-0.23	0.28	24	MS
Trigs	CSA PROX LT IJV (mm^2)	0.15	0.47	0.35	0.09	24	MS

Trigs, triglycerides; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.35: Correlation between triglycerides and the extracranial vascular ultrasound variables in the control group

Biochemical marker	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Trigs	RT CCA IMT (mm)	0.09	0.69	-0.01	0.97	23	CONTROL
Trigs	RT CCA PSV (cm/s)	-0.36	0.09	-0.22	0.31	23	CONTROL
Trigs	RT CCA EDV (cm/s)	-0.21	0.34	-0.27	0.22	23	CONTROL
Trigs	CSD PROX RT CCA (mm)	0.23	0.28	0.07	0.76	23	CONTROL
Trigs	CSD MID RT CCA (mm)	0.06	0.78	-0.04	0.84	23	CONTROL
Trigs	CSD DIST RT CCA (mm)	0.21	0.34	0.18	0.42	23	CONTROL
Trigs	RT ICA PSV (cm/s)	-0.56	<0.01	-0.48	0.02	23	CONTROL
Trigs	RT ICA EDV (cm/s)	-0.38	0.07	-0.38	0.07	23	CONTROL
Trigs	CSD PROX RT ICA (mm)	-0.16	0.47	-0.10	0.66	23	CONTROL
Trigs	PROX ECA RT PSV (cm/s)	-0.06	0.80	0.16	0.47	23	CONTROL
Trigs	CSD PROX RT ECA (mm)	0.05	0.80	0.02	0.92	23	CONTROL
Trigs	RT VA PSV (cm/s)	-0.02	0.93	0.15	0.48	23	CONTROL
Trigs	RT VA EDV (cm/s)	0.02	0.91	0.09	0.68	23	CONTROL
Trigs	CSD PROX RT VA (mm)	0.45	0.03	0.55	<0.01	23	CONTROL
Trigs	RT PSV ICA/CCA ratio	-0.09	0.67	-0.12	0.58	23	CONTROL
Trigs	RT EDV ICA/ECA ratio	-0.22	0.31	-0.06	0.78	23	CONTROL
Trigs	LT CCA IMT (mm)	0.19	0.38	0.12	0.60	23	CONTROL
Trigs	LT CCA PSV (cm/s)	-0.20	0.35	-0.18	0.40	23	CONTROL
Trigs	LT CCA EDV (cm/s)	-0.32	0.14	-0.37	0.08	23	CONTROL
Trigs	CSD PROX LT CCA (mm)	0.20	0.35	0.06	0.80	23	CONTROL
Trigs	CSD MID LT CCA (mm)	0.01	0.98	0.02	0.94	23	CONTROL
Trigs	CSD DIST LT CCA (mm)	0.00	1.00	0.04	0.84	23	CONTROL
Trigs	LT ICA PSV (cm/s)	-0.44	0.04	-0.44	0.04	23	CONTROL
Trigs	LT ICA EDV (cm/s)	-0.39	0.07	-0.36	0.09	23	CONTROL
Trigs	CSD PROX LT ICA (mm)	0.15	0.50	0.20	0.36	23	CONTROL
Trigs	PROX LT ECA PSV (cm/s)	-0.36	0.09	-0.14	0.51	23	CONTROL
Trigs	CSD PROX LT ECA (mm)	0.03	0.88	-0.10	0.67	23	CONTROL
Trigs	LT VA PSV (cm/s)	-0.01	0.97	-0.02	0.93	23	CONTROL
Trigs	LT VA EDV (cm/s)	-0.09	0.69	-0.16	0.47	23	CONTROL
Trigs	CSD PROX LT VA (mm)	0.08	0.72	0.13	0.56	23	CONTROL
Trigs	LT PSV ICA/CCA ratio	-0.11	0.61	-0.10	0.65	23	CONTROL
Trigs	LT EDV ICA/ECA ratio	-0.15	0.48	-0.10	0.65	23	CONTROL
Trigs	PV PROX RT IJV (cm/s)	0.01	0.95	-0.15	0.51	23	CONTROL
Trigs	PV MID RT IJV (cm/s)	-0.19	0.40	-0.11	0.61	23	CONTROL
Trigs	PV DIST RT IJV (cm/s)	-0.41	0.05	-0.36	0.09	23	CONTROL
Trigs	CSA PROX RT IJV (mm^2)	0.08	0.73	-0.03	0.89	23	CONTROL
Trigs	PV PROX LT IJV (cm/s)	-0.12	0.60	-0.18	0.42	23	CONTROL
Trigs	PV MID LT IJV (cm/s)	0.02	0.94	0.03	0.89	23	CONTROL
Trigs	PV DIST LT IJV (cm/s)	0.09	0.70	0.13	0.55	23	CONTROL
Trigs	CSA PROX LT IJV (mm^2)	-0.04	0.85	-0.18	0.40	23	CONTROL

Trigs, triglycerides; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.5 ASSOCIATION BETWEEN LIFESTYLE FACTORS AND THE GREY-SCALE, COLOUR AND SPECTRAL DOPPLER ANALYSIS OF THE CAs AND IJVs IN PARTICIPANTS WITH MS AND PARTICIPANTS FREE FROM MS (CONTROL GROUP).

The lifestyle parameters of the participants were retrieved from the medical and lifestyle questionnaire which was completed on entry to the study (Appendix D). The saturated fat, folate and fruit/vegetable/fibre intake scores, retrieved from the questionnaire, were based on the types of food the participants ate and the frequency of intake per week.

4.5.1 Association between saturated fat and the extracranial vascular ultrasound variables

MS group – A significant positive association was found between saturated fat and the right CCA IMT (Pearson p-value 0.04, Spearman p-vale <0.01) and negative correlation with the CSD of the proximal left VA (Pearson p-value 0.01, Spearman p-value <0.01) (Table 4.36).

Control group – Table 4.37 shows the significant positive correlations found between saturated fat and the following ultrasound variables:

- RT CCA EDV ; Spearman p-value 0.03
- LT CCA EDV; Spearman p-value 0.03
- PV PROX LT IJV; Pearson p-value <0.01, Spearman p-value 0.01

Table 4.36: Correlation between saturated fat and the extracranial vascular ultrasound variables in the MS group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Saturated Fat	RT CCA IMT (mm)	0.43	0.04	0.53	<0.01	24	MS
Saturated Fat	RT CCA PSV (cm/s)	-0.34	0.11	-0.33	0.11	24	MS
Saturated Fat	RT CCA EDV (cm/s)	-0.19	0.36	-0.21	0.32	24	MS
Saturated Fat	CSD PROX RT CCA (mm)	-0.16	0.46	-0.21	0.34	24	MS
Saturated Fat	CSD MID RT CCA (mm)	0.25	0.23	0.14	0.5	24	MS
Saturated Fat	CSD DIST RT CCA (mm)	0.28	0.19	0.19	0.38	24	MS
Saturated Fat	RT ICA PSV (cm/s)	-0.02	0.94	0.13	0.56	24	MS
Saturated Fat	RT ICA EDV (cm/s)	-0.06	0.78	-0.14	0.52	24	MS
Saturated Fat	CSD PROX RT ICA (mm)	0.19	0.38	0.2	0.35	24	MS
Saturated Fat	PROX ECA RT PSV (cm/s)	-0.07	0.73	-0.23	0.28	24	MS
Saturated Fat	CSD PROX RT ECA (mm)	0.23	0.28	0.23	0.28	24	MS
Saturated Fat	RT VA PSV (cm/s)	-0.05	0.83	-0.06	0.78	24	MS
Saturated Fat	RT VA EDV (cm/s)	0.17	0.42	0.12	0.58	24	MS
Saturated Fat	CSD PROX RT VA (mm)	0.23	0.27	0.27	0.2	24	MS
Saturated Fat	RT PSV ICA/CCA ratio	0.27	0.2	0.34	0.11	24	MS
Saturated Fat	RT EDV ICA/ECA ratio	0.16	0.46	0.21	0.34	24	MS
Saturated Fat	LT CCA IMT (mm)	0.19	0.38	0.35	0.1	24	MS
Saturated Fat	LT CCA PSV (cm/s)	-0.18	0.41	-0.2	0.34	24	MS
Saturated Fat	LT CCA EDV (cm/s)	-0.16	0.47	-0.22	0.3	24	MS
Saturated Fat	CSD PROX LT CCA (mm)	-0.24	0.26	-0.18	0.39	24	MS
Saturated Fat	CSD MID LT CCA (mm)	-0.12	0.58	-0.1	0.64	24	MS
Saturated Fat	CSD DIST LT CCA (mm)	-0.03	0.91	0.04	0.86	24	MS
Saturated Fat	LT ICA PSV (cm/s)	-0.16	0.46	-0.09	0.67	24	MS
Saturated Fat	LT ICA EDV (cm/s)	-0.17	0.42	-0.14	0.52	24	MS
Saturated Fat	CSD PROX LT ICA (mm)	0.03	0.88	0.1	0.63	24	MS
Saturated Fat	PROX LT ECA PSV (cm/s)	0.2	0.34	0.05	0.8	24	MS
Saturated Fat	CSD PROX LT ECA (mm)	0.06	0.79	0.01	0.97	24	MS
Saturated Fat	LT VA PSV (cm/s)	-0.12	0.58	-0.15	0.48	24	MS
Saturated Fat	LT VA EDV (cm/s)	-0.14	0.51	-0.17	0.41	24	MS
Saturated Fat	CSD PROX LT VA (mm)	-0.5	0.01	-0.61	<0.01	24	MS
Saturated Fat	LT PSV ICA/CCA ratio	-0.05	0.81	0.15	0.48	24	MS
Saturated Fat	LT EDV ICA/ECA ratio	-0.19	0.38	0.07	0.73	24	MS
Saturated Fat	PV PROX RT IJV (cm/s)	0.11	0.62	-0.01	0.97	24	MS
Saturated Fat	PV MID RT IJV (cm/s)	-0.25	0.24	-0.28	0.18	24	MS
Saturated Fat	PV DIST RT IJV (cm/s)	-0.12	0.59	-0.04	0.86	24	MS
Saturated Fat	CSA PROX RT IJV (mm^2)	-0.03	0.9	-0.01	0.96	24	MS
Saturated Fat	PV PROX LT IJV (cm/s)	-0.26	0.21	-0.2	0.35	24	MS
Saturated Fat	PV MID LT IJV (cm/s)	-0.34	0.1	-0.33	0.11	24	MS
Saturated Fat	PV DIST LT IJV (cm/s)	0.11	0.62	0.09	0.69	24	MS
Saturated Fat	CSA PROX LT IJV (mm^2)	0.09	0.69	0.1	0.63	24	MS

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

Table 4.37: Correlation between saturated fat and the extracranial vascular ultrasound variables in the control group

Lifestyle parameter	Ultrasound variables	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
Saturated Fat	RT CCA IMT (mm)	-0.08	0.69	-0.05	0.82	25	CONTROL
Saturated Fat	RT CCA PSV (cm/s)	0.14	0.5	0.17	0.43	25	CONTROL
Saturated Fat	RT CCA EDV (cm/s)	0.39	0.05	0.44	0.03	25	CONTROL
Saturated Fat	CSD PROX RT CCA (mm)	-0.16	0.44	-0.2	0.35	25	CONTROL
Saturated Fat	CSD MID RT CCA (mm)	-0.16	0.44	-0.21	0.31	25	CONTROL
Saturated Fat	CSD DIST RT CCA (mm)	-0.31	0.13	-0.32	0.11	25	CONTROL
Saturated Fat	RT ICA PSV (cm/s)	0.22	0.29	0.12	0.56	25	CONTROL
Saturated Fat	RT ICA EDV (cm/s)	0.01	0.98	0.13	0.54	25	CONTROL
Saturated Fat	CSD PROX RT ICA (mm)	0.07	0.76	0.09	0.67	25	CONTROL
Saturated Fat	PROX ECA RT PSV (cm/s)	-0.04	0.85	-0.06	0.79	25	CONTROL
Saturated Fat	CSD PROX RT ECA (mm)	0.01	0.97	0.02	0.92	25	CONTROL
Saturated Fat	RT VA PSV (cm/s)	-0.18	0.39	-0.2	0.34	25	CONTROL
Saturated Fat	RT VA EDV (cm/s)	-0.22	0.29	-0.28	0.18	25	CONTROL
Saturated Fat	CSD PROX RT VA (mm)	-0.27	0.19	-0.45	0.02	25	CONTROL
Saturated Fat	RT PSV ICA/CCA ratio	0.07	0.73	0.02	0.93	25	CONTROL
Saturated Fat	RT EDV ICA/ECA ratio	-0.29	0.16	-0.19	0.36	25	CONTROL
Saturated Fat	LT CCA IMT (mm)	-0.28	0.18	-0.3	0.15	25	CONTROL
Saturated Fat	LT CCA PSV (cm/s)	0.15	0.47	0.17	0.43	25	CONTROL
Saturated Fat	LT CCA EDV (cm/s)	0.34	0.1	0.43	0.03	25	CONTROL
Saturated Fat	CSD PROX LT CCA (mm)	0.08	0.69	0.17	0.41	25	CONTROL
Saturated Fat	CSD MID LT CCA (mm)	-0.01	0.97	0.02	0.92	25	CONTROL
Saturated Fat	CSD DIST LT CCA (mm)	-0.1	0.63	0	0.99	25	CONTROL
Saturated Fat	LT ICA PSV (cm/s)	0.04	0.87	0.04	0.83	25	CONTROL
Saturated Fat	LT ICA EDV (cm/s)	-0.07	0.75	0.05	0.83	25	CONTROL
Saturated Fat	CSD PROX LT ICA (mm)	0.06	0.79	-0.02	0.91	25	CONTROL
Saturated Fat	PROX LT ECA PSV (cm/s)	0.14	0.51	0.13	0.52	25	CONTROL
Saturated Fat	CSD PROX LT ECA (mm)	0.28	0.17	0.29	0.15	25	CONTROL
Saturated Fat	LT VA PSV (cm/s)	0.01	0.96	0.01	0.94	25	CONTROL
Saturated Fat	LT VA EDV (cm/s)	0.03	0.89	0.06	0.77	25	CONTROL
Saturated Fat	CSD PROX LT VA (mm)	-0.15	0.48	-0.19	0.37	25	CONTROL
Saturated Fat	LT PSV ICA/CCA ratio	-0.18	0.39	-0.14	0.49	25	CONTROL
Saturated Fat	LT EDV ICA/ECA ratio	-0.24	0.24	-0.13	0.55	25	CONTROL
Saturated Fat	PV PROX RT IJV (cm/s)	0.33	0.11	0.31	0.14	25	CONTROL
Saturated Fat	PV MID RT IJV (cm/s)	0.14	0.5	0.16	0.44	25	CONTROL
Saturated Fat	PV DIST RT IJV (cm/s)	0.09	0.67	0.09	0.66	25	CONTROL
Saturated Fat	CSA PROX RT IJV (mm^2)	-0.06	0.79	-0.05	0.83	25	CONTROL
Saturated Fat	PV PROX LT IJV (cm/s)	0.62	<0.01	0.48	0.01	25	CONTROL
Saturated Fat	PV MID LT IJV (cm/s)	0.35	0.08	0.28	0.17	25	CONTROL
Saturated Fat	PV DIST LT IJV (cm/s)	0.1	0.63	0.05	0.83	25	CONTROL
Saturated Fat	CSA PROX LT IJV (mm^2)	0.18	0.39	0.31	0.13	25	CONTROL

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

4.5.2 Association between consumption of chicken with skin and the extracranial vascular ultrasound variables

MS group – As seen in Table 4.38, a significant negative correlation was found between consumption of chicken with skin and the CSD of the proximal right CCA (Pearson p-value 0.04).

Control group – A significant positive correlation as found between consumption of chicken with skin and the following ultrasound variables (Table 4.39):

- Left CCA EDV; Pearson p-value 0.04
- PV of the proximal right IJV; Pearson p-value 0.02, Spearman p-value 0.03
- PV of the proximal left IJV; Pearson p-value 0.03

Table 4.38: Correlation between chicken with skin consumption and the extracranial vascular ultrasound variables in the MS group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Chicken with skin	RT CCA IMT (mm)	-0.24	0.27	-0.12	0.57	23	MS
Chicken with skin	RT CCA PSV (cm/s)	0.08	0.72	0.02	0.93	23	MS
Chicken with skin	RT CCA EDV (cm/s)	0.39	0.06	0.24	0.27	23	MS
Chicken with skin	CSD PROX RT CCA (mm)	-0.42	0.04	-0.31	0.15	23	MS
Chicken with skin	CSD MID RT CCA (mm)	-0.34	0.11	-0.38	0.08	23	MS
Chicken with skin	CSD DIST RT CCA (mm)	-0.34	0.11	-0.25	0.25	23	MS
Chicken with skin	RT ICA PSV (cm/s)	-0.2	0.36	-0.19	0.38	23	MS
Chicken with skin	RT ICA EDV (cm/s)	0.12	0.58	0.07	0.74	23	MS
Chicken with skin	CSD PROX RT ICA (mm)	-0.01	0.97	-0.02	0.92	23	MS
Chicken with skin	PROX ECA RT PSV (cm/s)	0.04	0.84	-0.14	0.52	23	MS
Chicken with skin	CSD PROX RT ECA (mm)	-0.02	0.94	0.2	0.37	23	MS
Chicken with skin	RT VA PSV (cm/s)	-0.05	0.84	-0.12	0.57	23	MS
Chicken with skin	RT VA EDV (cm/s)	0.25	0.25	0.24	0.26	23	MS
Chicken with skin	CSD PROX RT VA (mm)	0.18	0.4	0.19	0.4	23	MS
Chicken with skin	RT PSV ICA/CCA ratio	-0.21	0.33	-0.17	0.45	23	MS
Chicken with skin	RT EDV ICA/ECA ratio	-0.2	0.35	-0.12	0.58	23	MS
Chicken with skin	LT CCA IMT (mm)	-0.33	0.12	-0.28	0.19	23	MS
Chicken with skin	LT CCA PSV (cm/s)	0.1	0.64	0.04	0.86	23	MS
Chicken with skin	LT CCA EDV (cm/s)	0.36	0.09	0.29	0.17	23	MS
Chicken with skin	CSD PROX LT CCA (mm)	-0.37	0.08	-0.3	0.16	23	MS
Chicken with skin	CSD MID LT CCA (mm)	-0.42	0.05	-0.4	0.06	23	MS
Chicken with skin	CSD DIST LT CCA (mm)	-0.21	0.33	-0.11	0.61	23	MS
Chicken with skin	LT ICA PSV (cm/s)	-0.05	0.82	-0.15	0.49	23	MS
Chicken with skin	LT ICA EDV (cm/s)	0.03	0.9	-0.12	0.59	23	MS
Chicken with skin	CSD PROX LT ICA (mm)	-0.09	0.68	-0.02	0.92	23	MS
Chicken with skin	PROX LT ECA PSV (cm/s)	0.19	0.39	0.01	0.95	23	MS
Chicken with skin	CSD PROX LT ECA (mm)	-0.17	0.43	-0.14	0.53	23	MS
Chicken with skin	LT VA PSV (cm/s)	0.19	0.4	0.15	0.5	23	MS
Chicken with skin	LT VA EDV (cm/s)	0.29	0.19	0.17	0.42	23	MS
Chicken with skin	CSD PROX LT VA (mm)	-0.15	0.51	-0.16	0.46	23	MS
Chicken with skin	LT PSV ICA/CCA ratio	-0.15	0.49	-0.25	0.25	23	MS
Chicken with skin	LT EDV ICA/ECA ratio	-0.28	0.19	-0.2	0.36	23	MS
Chicken with skin	PV PROX RT IJV (cm/s)	-0.02	0.94	-0.12	0.57	23	MS
Chicken with skin	PV MID RT IJV (cm/s)	0.06	0.8	-0.14	0.53	23	MS
Chicken with skin	PV DIST RT IJV (cm/s)	-0.09	0.69	-0.1	0.64	23	MS
Chicken with skin	CSA PROX RT IJV (mm^2)	0.32	0.14	0.37	0.08	23	MS
Chicken with skin	PV PROX LT IJV (cm/s)	-0.29	0.17	-0.27	0.21	23	MS
Chicken with skin	PV MID LT IJV (cm/s)	0.1	0.64	-0.12	0.6	23	MS
Chicken with skin	PV DIST LT IJV (cm/s)	0.12	0.58	-0.13	0.55	23	MS
Chicken with skin	CSA PROX LT IJV (mm^2)	0.18	0.41	0.18	0.41	23	MS

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

Table 4.39: Correlation between chicken with skin consumption and the extracranial vascular ultrasound variables in the control group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Chicken with skin	RT CCA IMT (mm)	-0.28	0.17	-0.27	0.2	25	CONTROL
Chicken with skin	RT CCA PSV (cm/s)	0.17	0.4	0.08	0.71	25	CONTROL
Chicken with skin	RT CCA EDV (cm/s)	0.17	0.43	0.2	0.34	25	CONTROL
Chicken with skin	CSD PROX RT CCA (mm)	-0.16	0.45	-0.15	0.47	25	CONTROL
Chicken with skin	CSD MID RT CCA (mm)	-0.2	0.34	-0.24	0.24	25	CONTROL
Chicken with skin	CSD DIST RT CCA (mm)	-0.25	0.24	-0.23	0.27	25	CONTROL
Chicken with skin	RT ICA PSV (cm/s)	0.39	0.05	0.32	0.12	25	CONTROL
Chicken with skin	RT ICA EDV (cm/s)	0.27	0.2	0.26	0.2	25	CONTROL
Chicken with skin	CSD PROX RT ICA (mm)	-0.17	0.41	-0.11	0.6	25	CONTROL
Chicken with skin	PROX ECA RT PSV (cm/s)	0.27	0.19	0.27	0.18	25	CONTROL
Chicken with skin	CSD PROX RT ECA (mm)	-0.06	0.77	-0.07	0.74	25	CONTROL
Chicken with skin	RT VA PSV (cm/s)	0.02	0.92	-0.05	0.83	25	CONTROL
Chicken with skin	RT VA EDV (cm/s)	0.06	0.77	0.06	0.78	25	CONTROL
Chicken with skin	CSD PROX RT VA (mm)	-0.25	0.23	-0.18	0.4	25	CONTROL
Chicken with skin	RT PSV ICA/CCA ratio	0.16	0.45	0.14	0.51	25	CONTROL
Chicken with skin	RT EDV ICA/ECA ratio	0.18	0.4	0.13	0.55	25	CONTROL
Chicken with skin	LT CCA IMT (mm)	-0.22	0.29	-0.2	0.34	25	CONTROL
Chicken with skin	LT CCA PSV (cm/s)	0.16	0.46	0.17	0.43	25	CONTROL
Chicken with skin	LT CCA EDV (cm/s)	0.42	0.04	0.35	0.08	25	CONTROL
Chicken with skin	CSD PROX LT CCA (mm)	-0.28	0.18	-0.19	0.36	25	CONTROL
Chicken with skin	CSD MID LT CCA (mm)	-0.27	0.19	-0.28	0.17	25	CONTROL
Chicken with skin	CSD DIST LT CCA (mm)	0.01	0.97	-0.02	0.92	25	CONTROL
Chicken with skin	LT ICA PSV (cm/s)	0.05	0.81	-0.09	0.67	25	CONTROL
Chicken with skin	LT ICA EDV (cm/s)	0.09	0.69	0.02	0.93	25	CONTROL
Chicken with skin	CSD PROX LT ICA (mm)	-0.12	0.58	-0.05	0.82	25	CONTROL
Chicken with skin	PROX LT ECA PSV (cm/s)	0.03	0.9	0.08	0.7	25	CONTROL
Chicken with skin	CSD PROX LT ECA (mm)	0.4	0.05	0.45	0.03	25	CONTROL
Chicken with skin	LT VA PSV (cm/s)	-0.14	0.5	-0.16	0.44	25	CONTROL
Chicken with skin	LT VA EDV (cm/s)	-0.07	0.75	-0.07	0.75	25	CONTROL
Chicken with skin	CSD PROX LT VA (mm)	0.01	0.97	0.05	0.8	25	CONTROL
Chicken with skin	LT PSV ICA/CCA ratio	-0.06	0.76	-0.13	0.54	25	CONTROL
Chicken with skin	LT EDV ICA/ECA ratio	-0.15	0.46	-0.26	0.2	25	CONTROL
Chicken with skin	PV PROX RT IJV (cm/s)	0.47	0.02	0.43	0.03	25	CONTROL
Chicken with skin	PV MID RT IJV (cm/s)	0.13	0.52	0.14	0.5	25	CONTROL
Chicken with skin	PV DIST RT IJV (cm/s)	-0.02	0.93	0.13	0.53	25	CONTROL
Chicken with skin	CSA PROX RT IJV (mm^2)	0.23	0.28	0.16	0.45	25	CONTROL
Chicken with skin	PV PROX LT IJV (cm/s)	0.44	0.03	0.37	0.07	25	CONTROL
Chicken with skin	PV MID LT IJV (cm/s)	0.23	0.28	0.33	0.11	25	CONTROL
Chicken with skin	PV DIST LT IJV (cm/s)	0.24	0.25	0.23	0.27	25	CONTROL
Chicken with skin	CSA PROX LT IJV (mm^2)	0.07	0.73	-0.01	0.96	25	CONTROL

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

4.5.3 Association between fruit/veg/fibre intake and the extracranial vascular ultrasound variables

MS group – No significant correlations were found in this group of participants (Table 4.40).

Control group – A significant negative correlation was found between fruit/veg/fibre intake and the following ultrasound variables (Table 4.14):

- PV of the proximal right IJV; Pearson p-value 0.02, Spearman p-value <0.01
- PV of the proximal left IJV; Pearson p-value 0.02, Spearman p-value 0.04
- PV of the mid left IJV; Pearson p-value <0.01, Spearman p-value 0.01
- PV of the distal left IJV; Pearson p-value 0.02, Spearman p-value 0.02

Table 4.40: Correlation between fruit/veg/fibre intake and the extracranial vascular ultrasound variables in the MS group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Fruit/Veg/Fibre	RT CCA IMT (mm)	-0.04	0.87	-0.07	0.75	24	MS
Fruit/Veg/Fibre	RT CCA PSV (cm/s)	-0.13	0.55	-0.04	0.87	24	MS
Fruit/Veg/Fibre	RT CCA EDV (cm/s)	-0.32	0.12	-0.33	0.12	24	MS
Fruit/Veg/Fibre	CSD PROX RT CCA (mm)	0.24	0.26	0.16	0.45	24	MS
Fruit/Veg/Fibre	CSD MID RT CCA (mm)	0.23	0.29	0.22	0.29	24	MS
Fruit/Veg/Fibre	CSD DIST RT CCA (mm)	0.23	0.28	0.25	0.25	24	MS
Fruit/Veg/Fibre	RT ICA PSV (cm/s)	-0.08	0.7	-0.11	0.62	24	MS
Fruit/Veg/Fibre	RT ICA EDV (cm/s)	-0.22	0.3	-0.18	0.41	24	MS
Fruit/Veg/Fibre	CSD PROX RT ICA (mm)	0.08	0.7	0.06	0.79	24	MS
Fruit/Veg/Fibre	PROX ECA RT PSV (cm/s)	0.25	0.24	0.23	0.28	24	MS
Fruit/Veg/Fibre	CSD PROX RT ECA (mm)	-0.19	0.38	-0.14	0.52	24	MS
Fruit/Veg/Fibre	RT VA PSV (cm/s)	-0.04	0.87	0.06	0.76	24	MS
Fruit/Veg/Fibre	RT VA EDV (cm/s)	-0.29	0.17	-0.29	0.17	24	MS
Fruit/Veg/Fibre	CSD PROX RT VA (mm)	0.1	0.63	0.02	0.93	24	MS
Fruit/Veg/Fibre	RT PSV ICA/CCA ratio	0.07	0.74	0.05	0.81	24	MS
Fruit/Veg/Fibre	RT EDV ICA/ECA ratio	0.03	0.87	0.1	0.64	24	MS
Fruit/Veg/Fibre	LT CCA IMT (mm)	0.28	0.19	0.24	0.25	24	MS
Fruit/Veg/Fibre	LT CCA PSV (cm/s)	-0.06	0.8	-0.05	0.82	24	MS
Fruit/Veg/Fibre	LT CCA EDV (cm/s)	-0.15	0.47	-0.11	0.61	24	MS
Fruit/Veg/Fibre	CSD PROX LT CCA (mm)	0.39	0.06	0.4	0.05	24	MS
Fruit/Veg/Fibre	CSD MID LT CCA (mm)	0.26	0.22	0.2	0.36	24	MS
Fruit/Veg/Fibre	CSD DIST LT CCA (mm)	-0.02	0.91	-0.07	0.74	24	MS
Fruit/Veg/Fibre	LT ICA PSV (cm/s)	0.11	0.62	0.19	0.38	24	MS
Fruit/Veg/Fibre	LT ICA EDV (cm/s)	-0.15	0.49	-0.05	0.8	24	MS
Fruit/Veg/Fibre	CSD PROX LT ICA (mm)	0.05	0.83	-0.1	0.64	24	MS
Fruit/Veg/Fibre	PROX LT ECA PSV (cm/s)	-0.03	0.91	0.1	0.63	24	MS
Fruit/Veg/Fibre	CSD PROX LT ECA (mm)	0.11	0.62	0.14	0.53	24	MS
Fruit/Veg/Fibre	LT VA PSV (cm/s)	-0.25	0.24	-0.18	0.39	24	MS
Fruit/Veg/Fibre	LT VA EDV (cm/s)	-0.11	0.59	-0.12	0.58	24	MS
Fruit/Veg/Fibre	CSD PROX LT VA (mm)	0.28	0.19	0.19	0.38	24	MS
Fruit/Veg/Fibre	LT PSV ICA/CCA ratio	0.21	0.31	0.2	0.34	24	MS
Fruit/Veg/Fibre	LT EDV ICA/ECA ratio	0.03	0.88	-0.07	0.76	24	MS
Fruit/Veg/Fibre	PV PROX RT IJV (cm/s)	0.03	0.89	0.02	0.91	24	MS
Fruit/Veg/Fibre	PV MID RT IJV (cm/s)	0.29	0.18	0.25	0.24	24	MS
Fruit/Veg/Fibre	PV DIST RT IJV (cm/s)	0.35	0.1	0.26	0.22	24	MS
Fruit/Veg/Fibre	CSA PROX RT IJV (mm^2)	-0.01	0.97	-0.07	0.74	24	MS
Fruit/Veg/Fibre	PV PROX LT IJV (cm/s)	-0.2	0.35	-0.29	0.17	24	MS
Fruit/Veg/Fibre	PV MID LT IJV (cm/s)	-0.01	0.96	-0.02	0.91	24	MS
Fruit/Veg/Fibre	PV DIST LT IJV (cm/s)	0.04	0.85	0.01	0.97	24	MS
Fruit/Veg/Fibre	CSA PROX LT IJV (mm^2)	0.12	0.58	0.16	0.46	24	MS

Veg, vegetables; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

Table 4.41: Correlation between weekly fruit/vegetable/fibre intake and the extracranial vascular ultrasound variables in the control group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Fruit/Veg/Fibre	RT CCA IMT (mm)	0.35	0.08	0.34	0.1	25	CONTROL
Fruit/Veg/Fibre	RT CCA PSV (cm/s)	-0.26	0.21	-0.15	0.46	25	CONTROL
Fruit/Veg/Fibre	RT CCA EDV (cm/s)	-0.21	0.31	-0.25	0.22	25	CONTROL
Fruit/Veg/Fibre	CSD PROX RT CCA (mm)	-0.12	0.56	-0.04	0.85	25	CONTROL
Fruit/Veg/Fibre	CSD MID RT CCA (mm)	-0.03	0.87	0.14	0.51	25	CONTROL
Fruit/Veg/Fibre	CSD DIST RT CCA (mm)	-0.03	0.87	0.09	0.65	25	CONTROL
Fruit/Veg/Fibre	RT ICA PSV (cm/s)	-0.2	0.34	-0.11	0.6	25	CONTROL
Fruit/Veg/Fibre	RT ICA EDV (cm/s)	-0.09	0.67	-0.02	0.91	25	CONTROL
Fruit/Veg/Fibre	CSD PROX RT ICA (mm)	0.21	0.31	0.26	0.21	25	CONTROL
Fruit/Veg/Fibre	PROX ECA RT PSV (cm/s)	0.08	0.7	0.14	0.51	25	CONTROL
Fruit/Veg/Fibre	CSD PROX RT ECA (mm)	-0.18	0.38	-0.2	0.33	25	CONTROL
Fruit/Veg/Fibre	RT VA PSV (cm/s)	-0.17	0.41	-0.18	0.4	25	CONTROL
Fruit/Veg/Fibre	RT VA EDV (cm/s)	-0.23	0.28	-0.18	0.39	25	CONTROL
Fruit/Veg/Fibre	CSD PROX RT VA (mm)	-0.05	0.8	-0.02	0.93	25	CONTROL
Fruit/Veg/Fibre	RT PSV ICA/CCA ratio	0.01	0.95	-0.02	0.94	25	CONTROL
Fruit/Veg/Fibre	RT EDV ICA/ECA ratio	0.09	0.68	0.08	0.72	25	CONTROL
Fruit/Veg/Fibre	LT CCA IMT (mm)	0.18	0.38	0.27	0.19	25	CONTROL
Fruit/Veg/Fibre	LT CCA PSV (cm/s)	0.05	0.8	0.04	0.86	25	CONTROL
Fruit/Veg/Fibre	LT CCA EDV (cm/s)	-0.08	0.72	-0.05	0.83	25	CONTROL
Fruit/Veg/Fibre	CSD PROX LT CCA (mm)	-0.08	0.71	-0.1	0.65	25	CONTROL
Fruit/Veg/Fibre	CSD MID LT CCA (mm)	0.15	0.49	0.15	0.49	25	CONTROL
Fruit/Veg/Fibre	CSD DIST LT CCA (mm)	0.24	0.24	0.21	0.32	25	CONTROL
Fruit/Veg/Fibre	LT ICA PSV (cm/s)	-0.3	0.15	-0.17	0.41	25	CONTROL
Fruit/Veg/Fibre	LT ICA EDV (cm/s)	-0.08	0.69	0.03	0.89	25	CONTROL
Fruit/Veg/Fibre	CSD PROX LT ICA (mm)	0.23	0.27	0.26	0.22	25	CONTROL
Fruit/Veg/Fibre	PROX LT ECA PSV (cm/s)	0.06	0.77	0.14	0.51	25	CONTROL
Fruit/Veg/Fibre	CSD PROX LT ECA (mm)	-0.12	0.58	-0.06	0.77	25	CONTROL
Fruit/Veg/Fibre	LT VA PSV (cm/s)	0.26	0.2	0.23	0.27	25	CONTROL
Fruit/Veg/Fibre	LT VA EDV (cm/s)	0.19	0.36	0.25	0.23	25	CONTROL
Fruit/Veg/Fibre	CSD PROX LT VA (mm)	0	0.99	0	0.99	25	CONTROL
Fruit/Veg/Fibre	LT PSV ICA/CCA ratio	-0.17	0.42	-0.19	0.36	25	CONTROL
Fruit/Veg/Fibre	LT EDV ICA/ECA ratio	-0.04	0.85	-0.15	0.47	25	CONTROL
Fruit/Veg/Fibre	PV PROX RT IJV (cm/s)	-0.45	0.02	-0.57	<0.01	25	CONTROL
Fruit/Veg/Fibre	PV MID RT IJV (cm/s)	-0.23	0.27	-0.2	0.33	25	CONTROL
Fruit/Veg/Fibre	PV DIST RT IJV (cm/s)	-0.03	0.89	-0.14	0.5	25	CONTROL
Fruit/Veg/Fibre	CSA PROX RT IJV (mm^2)	-0.18	0.4	-0.12	0.57	25	CONTROL
Fruit/Veg/Fibre	PV PROX LT IJV (cm/s)	-0.45	0.02	-0.42	0.04	25	CONTROL
Fruit/Veg/Fibre	PV MID LT IJV (cm/s)	-0.51	<0.01	-0.5	0.01	25	CONTROL
Fruit/Veg/Fibre	PV DIST LT IJV (cm/s)	-0.46	0.02	-0.47	0.02	25	CONTROL
Fruit/Veg/Fibre	CSA PROX LT IJV (mm^2)	-0.34	0.09	-0.21	0.32	25	CONTROL

Veg, vegetables; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.5.4 Association between folate and the extracranial vascular ultrasound variables

MS group – In this group of participants, a significant positive correlation was found between folate and the CSA of the proximal left IJV (Pearson p-value 0.01). Similarly, a significant negative correlation was found between folate and the EDV of the left VA (Spearman p-value 0.02) (Table 4.42).

Control group – A significant positive correlation was found between the PSV of the left VA (Pearson p-value 0.03, Spearman p-value 0.02). Folate was also negatively correlated with the PSV of the right ICA (Pearson p-value 0.03, Spearman p-value 0.02) (Table 4.43).

Table 4.42: Correlation between folate and the extracranial vascular ultrasound variables in the MS group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Folate	RT CCA IMT (mm)	-0.02	0.93	-0.01	0.96	24	MS
Folate	RT CCA PSV (cm/s)	-0.24	0.26	-0.11	0.61	24	MS
Folate	RT CCA EDV (cm/s)	-0.31	0.14	-0.43	0.04	24	MS
Folate	CSD PROX RT CCA (mm)	-0.04	0.84	-0.07	0.74	24	MS
Folate	CSD MID RT CCA (mm)	-0.07	0.75	-0.05	0.8	24	MS
Folate	CSD DIST RT CCA (mm)	0	0.99	0.08	0.71	24	MS
Folate	RT ICA PSV (cm/s)	-0.14	0.52	-0.2	0.36	24	MS
Folate	RT ICA EDV (cm/s)	-0.14	0.52	-0.16	0.44	24	MS
Folate	CSD PROX RT ICA (mm)	-0.01	0.95	-0.08	0.71	24	MS
Folate	PROX ECA RT PSV (cm/s)	0.17	0.42	0.26	0.22	24	MS
Folate	CSD PROX RT ECA (mm)	0.14	0.53	0.02	0.93	24	MS
Folate	RT VA PSV (cm/s)	-0.14	0.52	-0.11	0.6	24	MS
Folate	RT VA EDV (cm/s)	-0.24	0.25	-0.18	0.4	24	MS
Folate	CSD PROX RT VA (mm)	0.14	0.52	0.23	0.29	24	MS
Folate	RT PSV ICA/CCA ratio	-0.03	0.87	-0.07	0.75	24	MS
Folate	RT EDV ICA/ECA ratio	0.11	0.6	0.07	0.73	24	MS
Folate	LT CCA IMT (mm)	0.16	0.46	0.25	0.25	24	MS
Folate	LT CCA PSV (cm/s)	-0.07	0.75	0.03	0.89	24	MS
Folate	LT CCA EDV (cm/s)	-0.12	0.57	-0.25	0.25	24	MS
Folate	CSD PROX LT CCA (mm)	-0.11	0.62	-0.17	0.43	24	MS
Folate	CSD MID LT CCA (mm)	-0.14	0.5	-0.06	0.77	24	MS
Folate	CSD DIST LT CCA (mm)	-0.22	0.3	-0.2	0.35	24	MS
Folate	LT ICA PSV (cm/s)	-0.02	0.94	-0.09	0.68	24	MS
Folate	LT ICA EDV (cm/s)	-0.06	0.78	-0.06	0.78	24	MS
Folate	CSD PROX LT ICA (mm)	0.18	0.4	0.13	0.54	24	MS
Folate	PROX LT ECA PSV (cm/s)	0.03	0.89	0.12	0.57	24	MS
Folate	CSD PROX LT ECA (mm)	0.23	0.28	0.32	0.13	24	MS
Folate	LT VA PSV (cm/s)	-0.31	0.13	-0.33	0.12	24	MS
Folate	LT VA EDV (cm/s)	-0.39	0.06	-0.48	0.02	24	MS
Folate	CSD PROX LT VA (mm)	0.05	0.83	0	1	24	MS
Folate	LT PSV ICA/CCA ratio	-0.03	0.91	0.01	0.96	24	MS
Folate	LT EDV ICA/ECA ratio	0.02	0.91	-0.06	0.79	24	MS
Folate	PV PROX RT IJV (cm/s)	-0.31	0.14	-0.19	0.38	24	MS
Folate	PV MID RT IJV (cm/s)	-0.19	0.37	-0.17	0.44	24	MS
Folate	PV DIST RT IJV (cm/s)	-0.14	0.51	-0.28	0.19	24	MS
Folate	CSA PROX RT IJV (mm^2)	0.06	0.79	-0.08	0.7	24	MS
Folate	PV PROX LT IJV (cm/s)	-0.19	0.38	-0.08	0.72	24	MS
Folate	PV MID LT IJV (cm/s)	0.06	0.77	0.09	0.67	24	MS
Folate	PV DIST LT IJV (cm/s)	0.05	0.8	0.14	0.5	24	MS
Folate	CSA PROX LT IJV (mm^2)	0.5	0.01	0.12	0.57	24	MS

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

Table 4.43: Correlation between folate and the extracranial vascular ultrasound variables in the control group

Lifestyle parameter	Ultrasound variable	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
Folate	RT CCA IMT (mm)	0.29	0.17	0.23	0.27	25	CONTROL
Folate	RT CCA PSV (cm/s)	-0.03	0.89	0.01	0.98	25	CONTROL
Folate	RT CCA EDV (cm/s)	-0.05	0.81	-0.05	0.82	25	CONTROL
Folate	CSD PROX RT CCA (mm)	0.08	0.69	0.11	0.59	25	CONTROL
Folate	CSD MID RT CCA (mm)	0.11	0.59	0.19	0.37	25	CONTROL
Folate	CSD DIST RT CCA (mm)	-0.08	0.72	-0.06	0.76	25	CONTROL
Folate	RT ICA PSV (cm/s)	-0.45	0.02	-0.38	0.06	25	CONTROL
Folate	RT ICA EDV (cm/s)	-0.21	0.31	-0.23	0.28	25	CONTROL
Folate	CSD PROX RT ICA (mm)	0.11	0.6	0.13	0.55	25	CONTROL
Folate	PROX ECA RT PSV (cm/s)	-0.22	0.29	-0.25	0.22	25	CONTROL
Folate	CSD PROX RT ECA (mm)	0.13	0.52	0.17	0.41	25	CONTROL
Folate	RT VA PSV (cm/s)	-0.02	0.92	-0.12	0.56	25	CONTROL
Folate	RT VA EDV (cm/s)	0.02	0.93	-0.07	0.72	25	CONTROL
Folate	CSD PROX RT VA (mm)	0.13	0.54	0.11	0.59	25	CONTROL
Folate	RT PSV ICA/CCA ratio	-0.32	0.12	-0.35	0.09	25	CONTROL
Folate	RT EDV ICA/ECA ratio	-0.19	0.37	-0.28	0.17	25	CONTROL
Folate	LT CCA IMT (mm)	-0.11	0.6	0	1	25	CONTROL
Folate	LT CCA PSV (cm/s)	0.22	0.28	0.11	0.59	25	CONTROL
Folate	LT CCA EDV (cm/s)	-0.01	0.94	-0.07	0.74	25	CONTROL
Folate	CSD PROX LT CCA (mm)	-0.05	0.8	-0.11	0.59	25	CONTROL
Folate	CSD MID LT CCA (mm)	0.05	0.81	0.04	0.86	25	CONTROL
Folate	CSD DIST LT CCA (mm)	-0.21	0.32	-0.13	0.53	25	CONTROL
Folate	LT ICA PSV (cm/s)	-0.24	0.24	-0.27	0.19	25	CONTROL
Folate	LT ICA EDV (cm/s)	-0.14	0.5	0	1	25	CONTROL
Folate	CSD PROX LT ICA (mm)	0.2	0.33	0.14	0.49	25	CONTROL
Folate	PROX LT ECA PSV (cm/s)	-0.1	0.62	-0.14	0.52	25	CONTROL
Folate	CSD PROX LT ECA (mm)	-0.12	0.58	0.02	0.92	25	CONTROL
Folate	LT VA PSV (cm/s)	0.43	0.03	0.45	0.02	25	CONTROL
Folate	LT VA EDV (cm/s)	0.27	0.2	0.3	0.15	25	CONTROL
Folate	CSD PROX LT VA (mm)	0.1	0.64	0.18	0.4	25	CONTROL
Folate	LT PSV ICA/CCA ratio	-0.2	0.34	-0.29	0.17	25	CONTROL
Folate	LT EDV ICA/ECA ratio	-0.09	0.66	-0.02	0.92	25	CONTROL
Folate	PV PROX RT IJV (cm/s)	-0.18	0.39	-0.24	0.26	25	CONTROL
Folate	PV MID RT IJV (cm/s)	0.33	0.1	0.28	0.18	25	CONTROL
Folate	PV DIST RT IJV (cm/s)	0.08	0.7	-0.07	0.75	25	CONTROL
Folate	CSA PROX RT IJV (mm^2)	-0.17	0.41	-0.19	0.35	25	CONTROL
Folate	PV PROX LT IJV (cm/s)	-0.22	0.3	-0.06	0.76	25	CONTROL
Folate	PV MID LT IJV (cm/s)	-0.18	0.38	-0.21	0.31	25	CONTROL
Folate	PV DIST LT IJV (cm/s)	-0.24	0.25	-0.28	0.18	25	CONTROL
Folate	CSA PROX LT IJV (mm^2)	-0.03	0.87	0.04	0.84	25	CONTROL

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

4.5.5 Association between physical activity and the extracranial vascular ultrasound variables

MS group – A significant positive correlation was found between physical activity and the following ultrasound variables (Table 4.44):

- PSV of the right CCA; Pearson p-value 0.03, Spearman p-value 0.02
- EDV of the right CCA; Pearson p-value 0.01, Spearman p-value <0.01
- EDV of the right VA; Pearson p-value 0.03
- PSV of the left ICA; Pearson p-value 0.02, Spearman p-value 0.03
- EDV of the left ICA; Pearson p-value, Spearman p-value 0.04

Control group – No significant correlations were found (Table 4.45).

Table 4.44: Correlation between physical activity and the extracranial vascular ultrasound variables in the MS group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	group
		r	p	r	p		
Physical activity	RT CCA IMT (mm)	-0.23	0.28	-0.18	0.41	24	MS
Physical activity	RT CCA PSV (cm/s)	0.45	0.03	0.46	0.02	24	MS
Physical activity	RT CCA EDV (cm/s)	0.51	0.01	0.54	<0.01	24	MS
Physical activity	CSD PROX RT CCA (mm)	-0.14	0.51	-0.08	0.71	24	MS
Physical activity	CSD MID RT CCA (mm)	-0.29	0.17	-0.29	0.17	24	MS
Physical activity	CSD DIST RT CCA (mm)	-0.39	0.06	-0.33	0.11	24	MS
Physical activity	RT ICA PSV (cm/s)	0.16	0.46	0.03	0.89	24	MS
Physical activity	RT ICA EDV (cm/s)	0.38	0.07	0.35	0.1	24	MS
Physical activity	CSD PROX RT ICA (mm)	-0.04	0.85	-0.07	0.73	24	MS
Physical activity	PROX ECA RT PSV (cm/s)	0.11	0.6	0.18	0.41	24	MS
Physical activity	CSD PROX RT ECA (mm)	-0.27	0.2	-0.31	0.15	24	MS
Physical activity	RT VA PSV (cm/s)	0.37	0.08	0.38	0.07	24	MS
Physical activity	RT VA EDV (cm/s)	0.44	0.03	0.41	0.05	24	MS
Physical activity	CSD PROX RT VA (mm)	0.24	0.26	0.18	0.39	24	MS
Physical activity	RT PSV ICA/CCA ratio	-0.19	0.37	-0.16	0.45	24	MS
Physical activity	RT EDV ICA/ECA ratio	-0.03	0.91	0.02	0.94	24	MS
Physical activity	LT CCA IMT (mm)	-0.31	0.14	-0.28	0.18	24	MS
Physical activity	LT CCA PSV (cm/s)	0.1	0.66	0.06	0.78	24	MS
Physical activity	LT CCA EDV (cm/s)	0.35	0.09	0.37	0.08	24	MS
Physical activity	CSD PROX LT CCA (mm)	-0.04	0.85	-0.09	0.69	24	MS
Physical activity	CSD MID LT CCA (mm)	-0.2	0.34	-0.21	0.32	24	MS
Physical activity	CSD DIST LT CCA (mm)	-0.2	0.35	-0.18	0.4	24	MS
Physical activity	LT ICA PSV (cm/s)	0.46	0.02	0.44	0.03	24	MS
Physical activity	LT ICA EDV (cm/s)	0.5	0.01	0.43	0.04	24	MS
Physical activity	CSD PROX LT ICA (mm)	-0.19	0.37	-0.32	0.13	24	MS
Physical activity	PROX LT ECA PSV (cm/s)	0.18	0.41	0.25	0.24	24	MS
Physical activity	CSD PROX LT ECA (mm)	-0.1	0.66	-0.19	0.37	24	MS
Physical activity	LT VA PSV (cm/s)	0.33	0.11	0.34	0.1	24	MS
Physical activity	LT VA EDV (cm/s)	0.33	0.12	0.28	0.19	24	MS
Physical activity	CSD PROX LT VA (mm)	-0.24	0.25	-0.19	0.37	24	MS
Physical activity	LT PSV ICA/CCA ratio	0.34	0.1	0.34	0.1	24	MS
Physical activity	LT EDV ICA/ECA ratio	0.31	0.14	0.32	0.13	24	MS
Physical activity	PV PROX RT IJV (cm/s)	0.21	0.32	0.16	0.46	24	MS
Physical activity	PV MID RT IJV (cm/s)	0.33	0.12	0.3	0.16	24	MS
Physical activity	PV DIST RT IJV (cm/s)	0.39	0.06	0.38	0.07	24	MS
Physical activity	CSA PROX RT IJV (mm^2)	0.25	0.23	0.16	0.45	24	MS
Physical activity	PV PROX LT IJV (cm/s)	0.04	0.85	0.11	0.62	24	MS
Physical activity	PV MID LT IJV (cm/s)	0.24	0.27	0.21	0.32	24	MS
Physical activity	PV DIST LT IJV (cm/s)	0.07	0.75	0.07	0.76	24	MS
Physical activity	CSA PROX LT IJV (mm^2)	-0.2	0.34	-0.05	0.82	24	MS

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

Table 4.45: Correlation between physical activity and the extracranial vascular ultrasound variables in the control group

Lifestyle parameter	Ultrasound variable	Pearson r	Pearson p	Spearman r	Spearman p	# cases	group
Physical activity	RT CCA IMT (mm)	-0.19	0.37	-0.08	0.69	25	CONTROL
Physical activity	RT CCA PSV (cm/s)	0.21	0.31	0.23	0.27	25	CONTROL
Physical activity	RT CCA EDV (cm/s)	0.28	0.18	0.26	0.21	25	CONTROL
Physical activity	CSD PROX RT CCA (mm)	-0.22	0.3	-0.23	0.27	25	CONTROL
Physical activity	CSD MID RT CCA (mm)	-0.14	0.49	-0.12	0.57	25	CONTROL
Physical activity	CSD DIST RT CCA (mm)	0.01	0.96	-0.04	0.84	25	CONTROL
Physical activity	RT ICA PSV (cm/s)	-0.09	0.66	-0.05	0.8	25	CONTROL
Physical activity	RT ICA EDV (cm/s)	0.13	0.53	0.08	0.72	25	CONTROL
Physical activity	CSD PROX RT ICA (mm)	-0.09	0.67	-0.11	0.59	25	CONTROL
Physical activity	PROX ECA RT PSV (cm/s)	0.13	0.53	0.17	0.43	25	CONTROL
Physical activity	CSD PROX RT ECA (mm)	0.2	0.35	0.2	0.35	25	CONTROL
Physical activity	RT VA PSV (cm/s)	0.18	0.38	0.21	0.3	25	CONTROL
Physical activity	RT VA EDV (cm/s)	0.28	0.18	0.29	0.16	25	CONTROL
Physical activity	CSD PROX RT VA (mm)	0.07	0.74	0.06	0.77	25	CONTROL
Physical activity	RT PSV ICA/CCA ratio	-0.3	0.14	-0.29	0.17	25	CONTROL
Physical activity	RT EDV ICA/ECA ratio	-0.16	0.46	-0.12	0.58	25	CONTROL
Physical activity	LT CCA IMT (mm)	-0.12	0.56	-0.08	0.7	25	CONTROL
Physical activity	LT CCA PSV (cm/s)	0.04	0.84	0.02	0.94	25	CONTROL
Physical activity	LT CCA EDV (cm/s)	0.02	0.91	0.04	0.85	25	CONTROL
Physical activity	CSD PROX LT CCA (mm)	-0.27	0.19	-0.26	0.22	25	CONTROL
Physical activity	CSD MID LT CCA (mm)	-0.19	0.36	-0.25	0.24	25	CONTROL
Physical activity	CSD DIST LT CCA (mm)	-0.03	0.9	-0.03	0.9	25	CONTROL
Physical activity	LT ICA PSV (cm/s)	-0.07	0.75	0.02	0.92	25	CONTROL
Physical activity	LT ICA EDV (cm/s)	-0.01	0.96	0.12	0.56	25	CONTROL
Physical activity	CSD PROX LT ICA (mm)	0	0.98	0.01	0.97	25	CONTROL
Physical activity	PROX LT ECA PSV (cm/s)	0.01	0.96	0.07	0.73	25	CONTROL
Physical activity	CSD PROX LT ECA (mm)	-0.29	0.16	-0.31	0.13	25	CONTROL
Physical activity	LT VA PSV (cm/s)	0.12	0.58	0.12	0.56	25	CONTROL
Physical activity	LT VA EDV (cm/s)	0.09	0.67	0.06	0.77	25	CONTROL
Physical activity	CSD PROX LT VA (mm)	0.19	0.36	0.26	0.21	25	CONTROL
Physical activity	LT PSV ICA/CCA ratio	-0.09	0.68	-0.07	0.74	25	CONTROL
Physical activity	LT EDV ICA/ECA ratio	-0.1	0.63	-0.02	0.92	25	CONTROL
Physical activity	PV PROX RT IJV (cm/s)	0.09	0.66	0.08	0.72	25	CONTROL
Physical activity	PV MID RT IJV (cm/s)	0.25	0.23	0.2	0.33	25	CONTROL
Physical activity	PV DIST RT IJV (cm/s)	0.16	0.43	0.08	0.7	25	CONTROL
Physical activity	CSA PROX RT IJV (mm^2)	-0.3	0.14	-0.29	0.16	25	CONTROL
Physical activity	PV PROX LT IJV (cm/s)	-0.28	0.18	-0.11	0.61	25	CONTROL
Physical activity	PV MID LT IJV (cm/s)	0.22	0.28	0.19	0.36	25	CONTROL
Physical activity	PV DIST LT IJV (cm/s)	0.26	0.2	0.24	0.25	25	CONTROL
Physical activity	CSA PROX LT IJV ($mm^{2)}$	-0.13	0.54	-0.05	0.83	25	CONTROL

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

4.5.6 Association between passive smoking and the extracranial vascular ultrasound variables

MS group:The smoking status results were available for 24 MS participants.

In total, 10 out of 24 (42%) MS participants were passive smokers and 14 (58%) non-smokers. In this analysis, a significant difference (Mann-Whitney U test p-value 0.03) in the EDV of the rigth ICA was found between passive smokers (31.20 ± 9.21) and non-smokers (36.35 ± 9.26) (Figure 4.3). Additionally,passive smokers displayed a significnatly larger CSA of the proximal left IJV (138 ± 74.17) in comparison to non-smokers (72.14 ± 22.4) (Mann-Whitney U test p-value <0.01) (Figure 4.4).

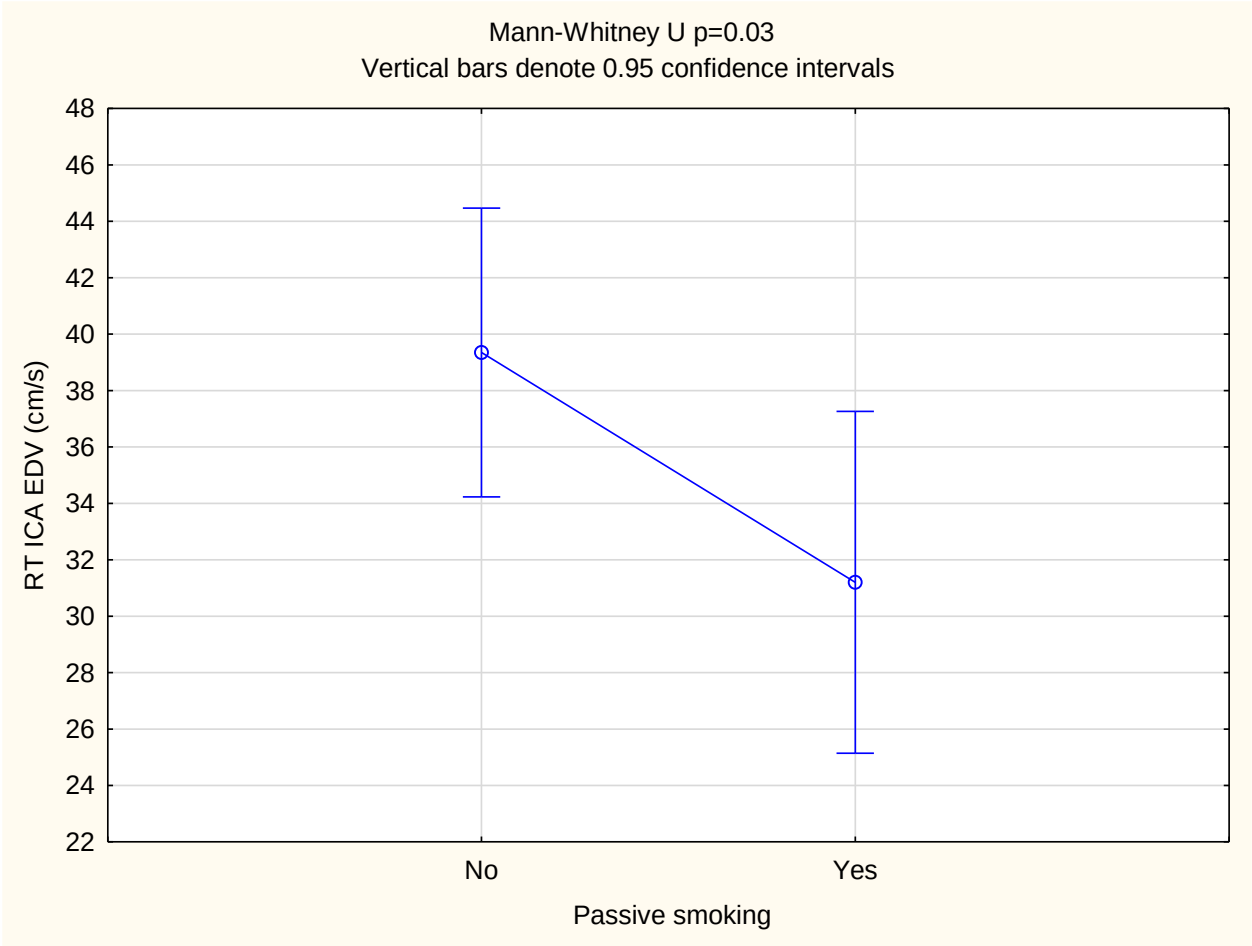

Figure 4.11: Significant difference in the EDV of the RT ICA in passive smokers and non-smokers in the MS group

RT, right, ICA, internal carotid artery; EDV, end-diastolic velocity; Passive smoking (no): mean EDV of the RT ICA = 36.35 ± 9.26; Passive smoking (yes): mean EDV of the RT ICA= 31.20 ± 9.21

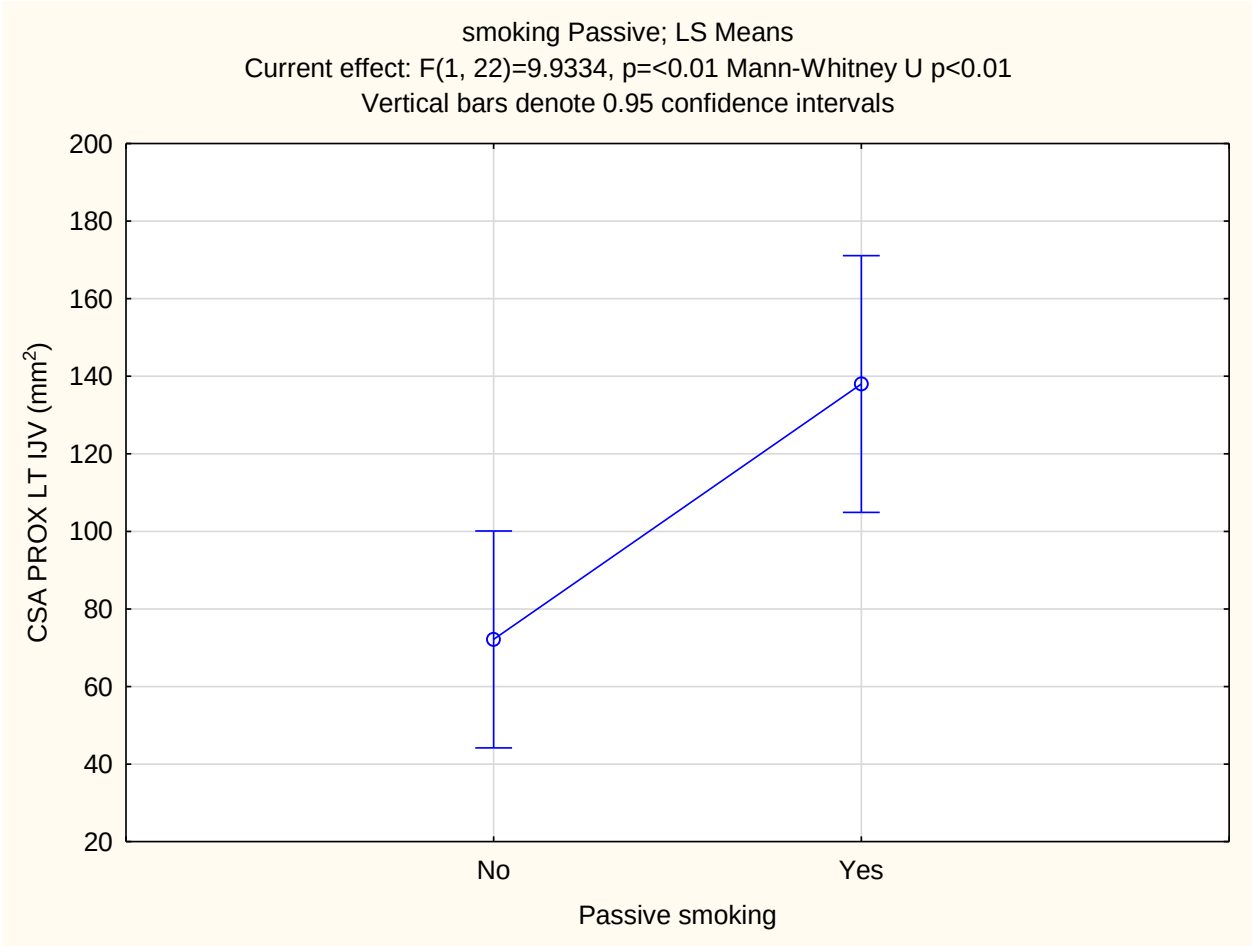

Figure 4.4: Significant difference in the CSA of the proximal left IJV in passive smokers and non-smokers in the MS group

CSA, cross-sectional area; PROX, proximal; LT, left; IJV, internal jugular vein; Passive smoking (no), mean CSA of the PROX LT IJV =72.14 ± 22.4; Passive smoking (yes), mean CSA of the PROX LT IJV=138 ± 74.17.

Control group: The smoking status results were available for 25 controls.

Ten (10) out of the 25 (40%) controls were passive smokers and 15 (60%) non-smokers.

A significant difference (Mann-Whitney U test p-value 0.02) was found between the PSV of the left ICA in passive smokers and non-smokers. The peak systolic blood flow velocity in the left ICA was lower in passive smokers (85.49, 76.82-94.16) in comparison to non-smokers (96.12, 89.04-103.20) (Figure 4.5).

Similarly, the cross-sectional area of the proximal left IJV in passive smokers (185, 124.34-245.66) were significantly larger (Pearson p-value 0.04) in comparison to non-smokers (101.73, 52.21-151.26) (Figure 4.6).

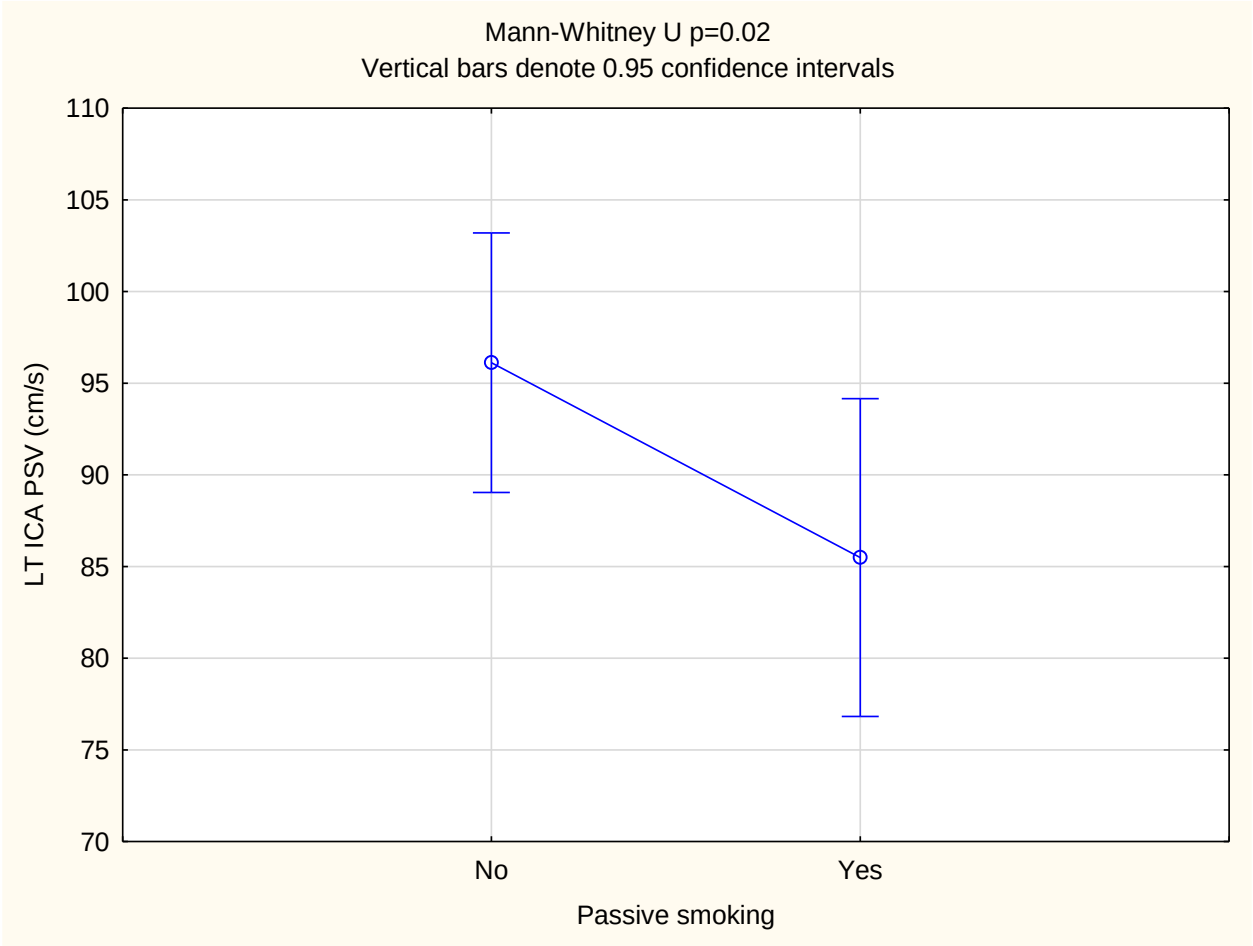

Figure 4.5: Significant difference between the PSV of left ICA in passive smokers and non-smokers in the control group

LT, left; ICA, internal carotid artery; PSV, peak systolic velocity; Passive smoking (no), mean CSA of the PROX LT ICA = 101.73 (52.21-151.26); Passive smoking (yes), mean CSA of the PROX LT ICA = 185 (124.34-245.66)

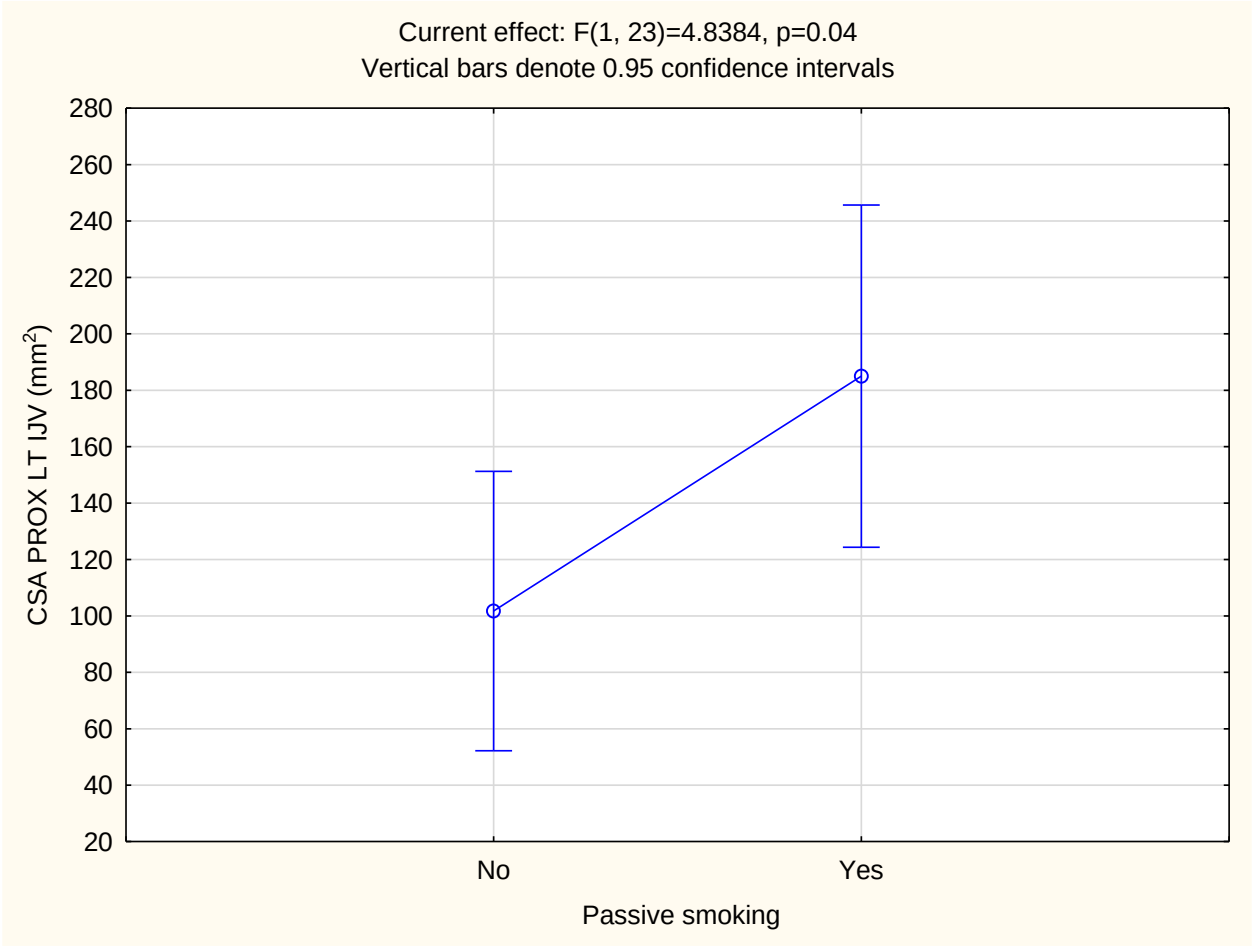

Figure 4.6: Significant difference in the CSA of the proximal left IJV in passive smokers and non-smokers in the control group

CSA, cross-sectional area; PROX, proximal; LT, left; IJV, internal jugular vein; Passive smoking (no): mean CSA PROX LT IJV = 101.73 (2.21-151.26); Passive smoking (yes): mean CSA PROX LT IJV = 185. (124.34-245.66)

4.5.7 Association between alcohol intake and the extracranial vascular ultrasound variables

Alcohol intake was categorised into scores 0, 1, 2 and 3 (0=abstain, 1= 1-2 units /week, 2= 3-13 units/week and 3= 14-21 units/week).

***MS group*:** The alcohol intake status was available for 24 MS participants. A significant difference (Kruskal-Wallis test p-value 0.02) in the PSV of the RT CCA was found between MS person consuming alcohol and those abstaining. The peak systolic blood flow velocity was higher in MS persons with an alcohol intake score of 1 (n=12; 97.41 ± 15.19) and 2 (n=6; 104.30 ± 13.80) in comparison to those with a score of 0 (n=6; 83.38 ± 9.55) (Figure 4.7).

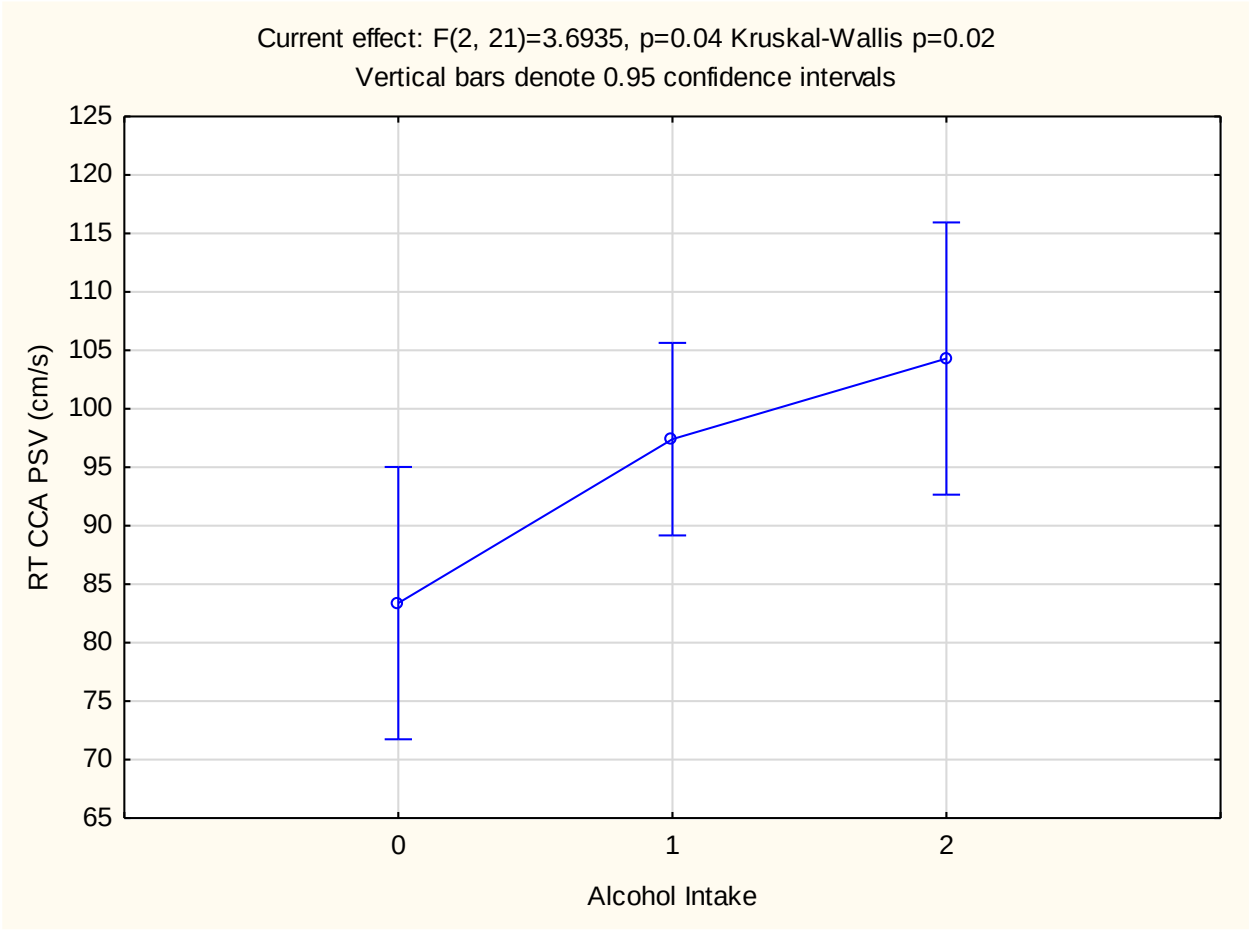

Figure 4.7: Significant difference in the PSV of the RT CCA in MS persons consuming alcohol in comparison to MS persons abstaining from alcohol

RT, right; CCA, common carotid artery; PSV, peak systolic velocity; 0=abstain; 1= 1-2 units /week; 2= 3-13 units/week; 3= 14-21 units/week; score 0=83.38 ± 9.55; score 1=97.41 ± 15.19; score 2=104.30 ± 13.80

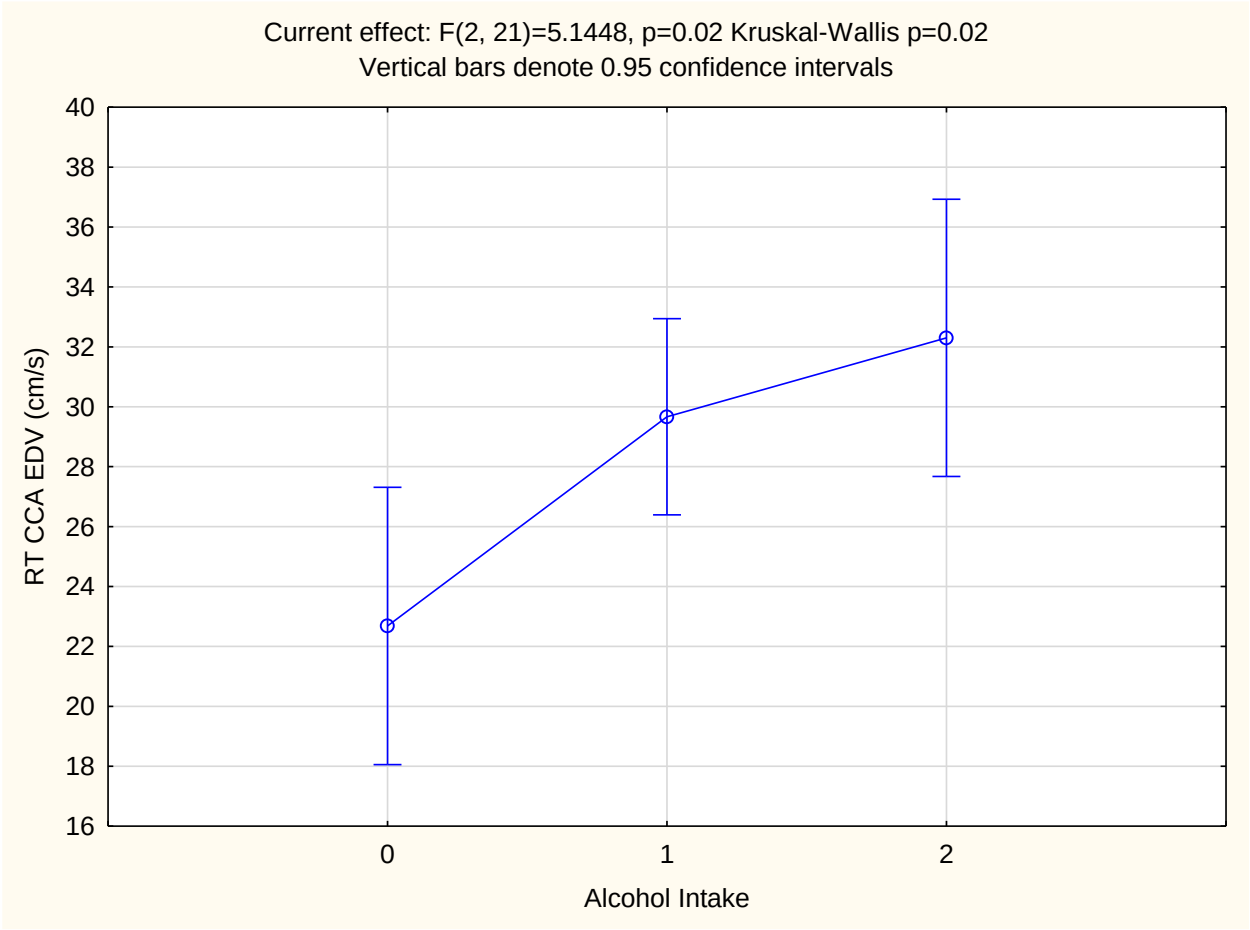

Figure 4.8: Significant difference in the RT CCA EDV in MS persons who consumed alcohol in comparison to MS persons abstaining

RT, right; CCA, common carotid artery EDV, end-diastolic velocity; sore 0=22.68 ± 5.20; score 1=29.67 ± 4.63; score 2=32.30 ± 7.11

The end-diastolic blood flow velocity in the right CCA and right VA was found to be significantly higher (Kruskal-Wallis test p-value 0.02) in MS persons who consumed alcohol in comparison to those who abstained.

The mean end-diastolic blood flow velocity in the right CCA in MS persons with score 1 = 29.67 ± 4.63, score 2 = 32.30 ± 7.11 and score 0= 22.68 ± 5.20 (Figure 4.8).

Figure 4.9 shows that the end-diastolic blood flow velocity was significantly higher (Kruskal-Wallis p-value 0.02) in MS persons consuming alcohol than MS person abstaining from alcohol. The mean end-diastolic velocity of the RT VA in persons with score 0 = 11.7± 1.99; score 1= 20.36 ± 6.44 and score 2= 18.53 ± 5.24.

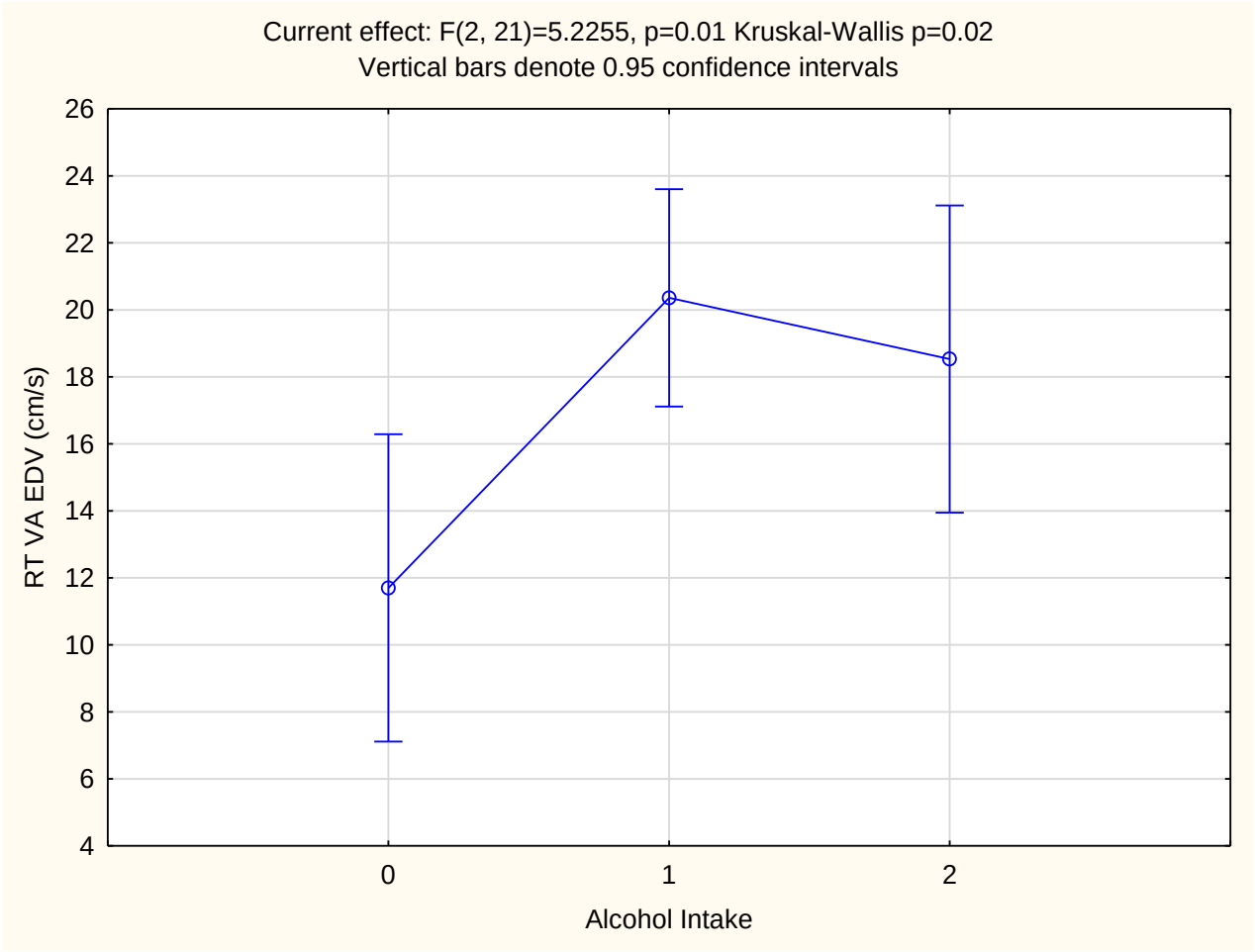

Figure 4.9: Significant difference in the EDV of the RT VERT artery in MS persons consuming alcohol compared to MS persons abstaining

score 0 = 11.7± 1.99; score 1= 20.36 ± 6.44 and score 2= 18.53 ± 5.24.

Control group: The alcohol intake results were available for 23 controls where 12 controls had a score of 0 and 11 controls had a score of 1. The cross-sectional diameter of the proximal right VA in controls was significantly higher (Mann-Whitney U test p-value 0.02) in controls with a score of 1 (3.81 ± 0.53) compared to controls with a score of 0 (3.30 ±0.41) (Figure 4.10). Additionally, the end-diastolic velocity of LT ICA was significantly higher (Mann-Whitney U test p-value 0.01) in controls with a score of 0 (38.76 ± 7.59) in comparison to controls with a score of 1 (30.69 ± 6.82) (Figure 4.11).

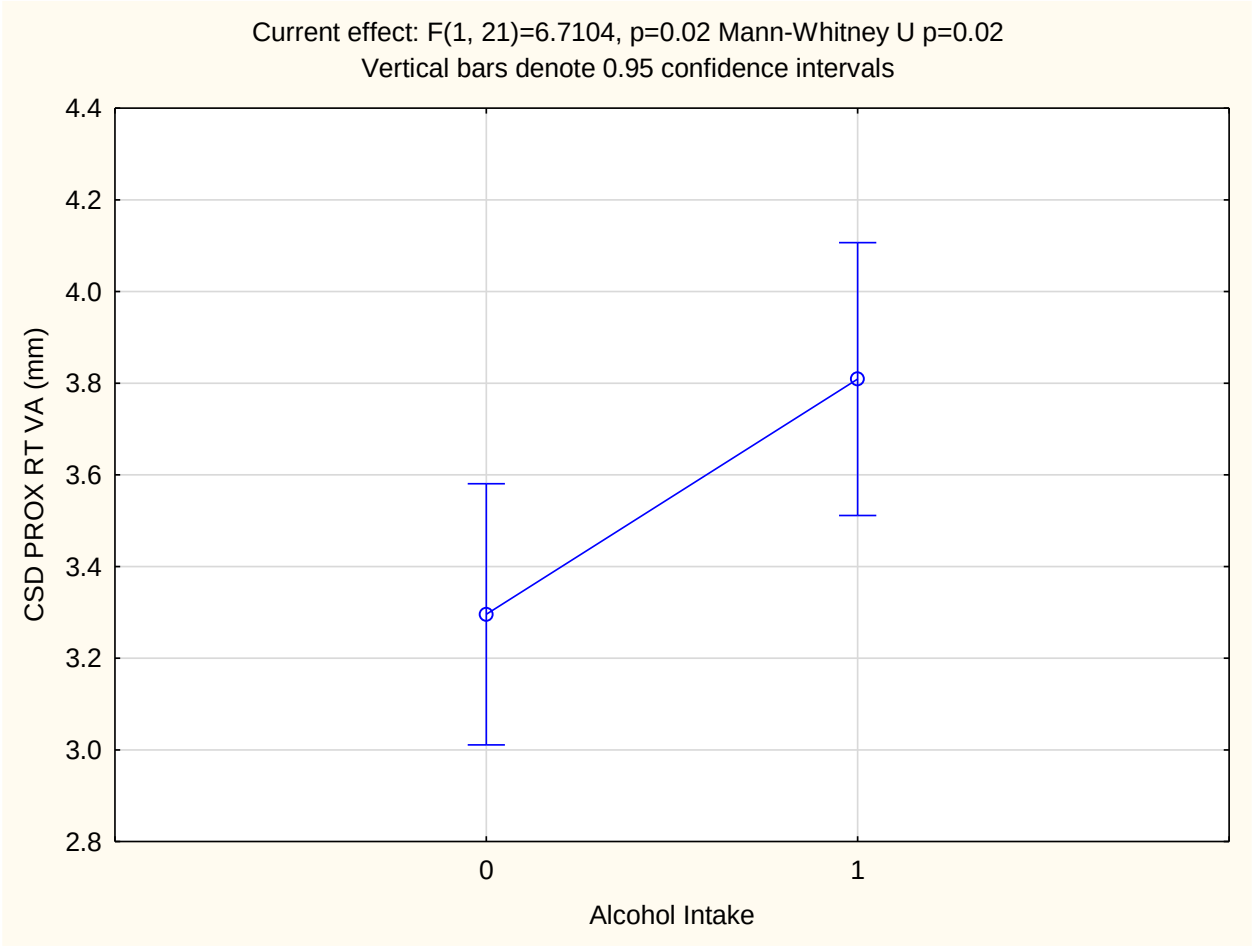

Figure 4.10: Significant difference in the CSD of the proximal RT VA in controls consuming alcohol compared to controls abstaining

CSD, cross-sectional diameter; PROX, proximal; RT, right; VA, vertebral artery; score 0=3.30 ±0.41; score 1=3.81 ± 0.53

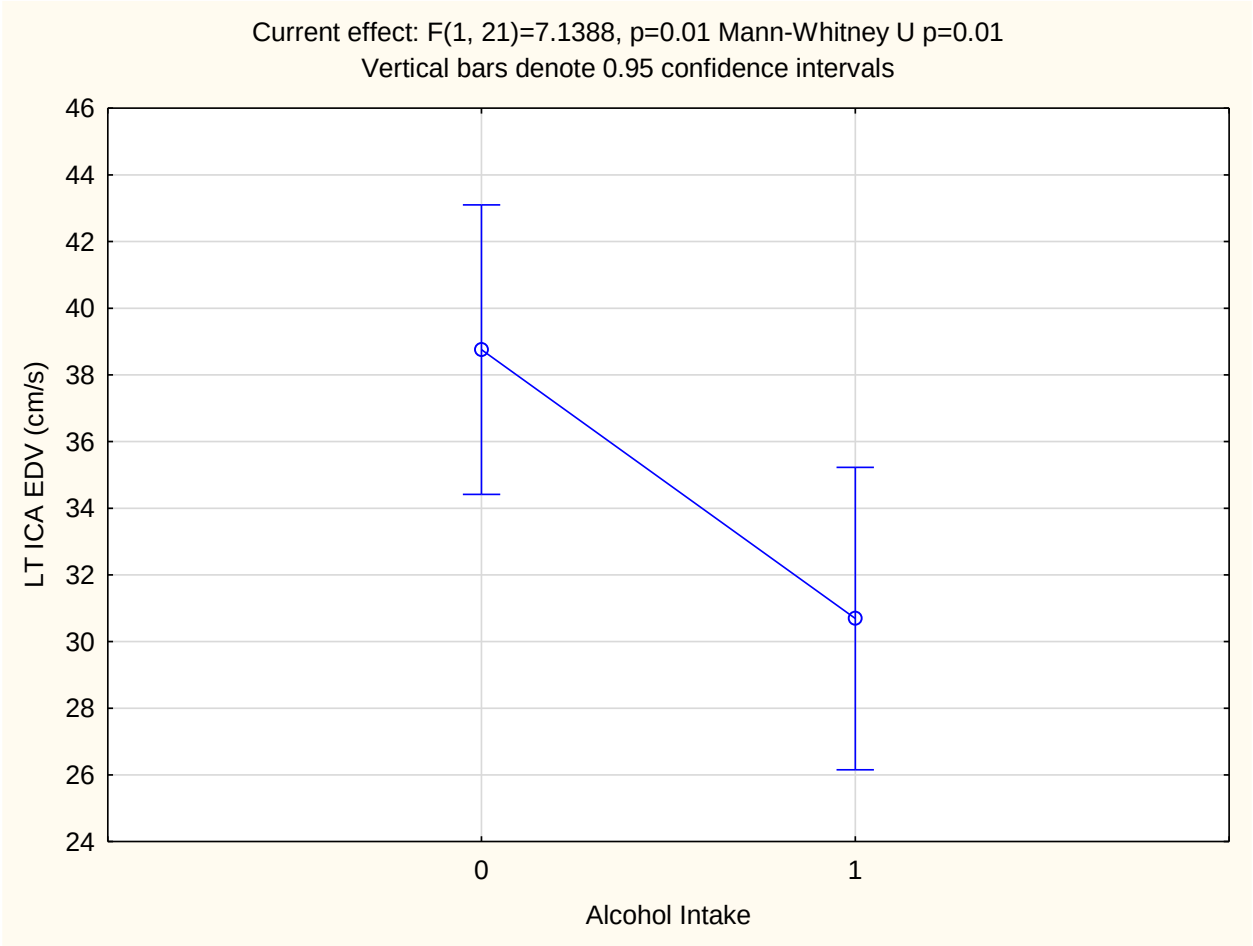

Figure 4.11: Significant difference in the EDV of the LT ICA in controls consuming alcohol and controls abstaining

LT, left; ICA, internal carotid artery; EDV, end-diastolic velocity; score 0=38.76 ± 7.59; score 1=30.69 ± 6.82.

4.6 ASSOCIATION BETWEEN DISABILITY AND THE GREY-SCALE, COLOUR AND SPECTRAL DOPPLER ANALYSIS OF THE CAs AND IJVs IN THE ACTIVE MS COHORT

Expanded disability status scale (EDSS) scores were available for 23 MS participants. Table 4.46 summarises the correlations between EDSS and the extracranial vascular ultrasound variables.

Significant positive correlations were found between EDSS and the following ultrasound variables:

- RT CCA IMT (mm); Pearson p-value <0.01 (Figure 4.12)

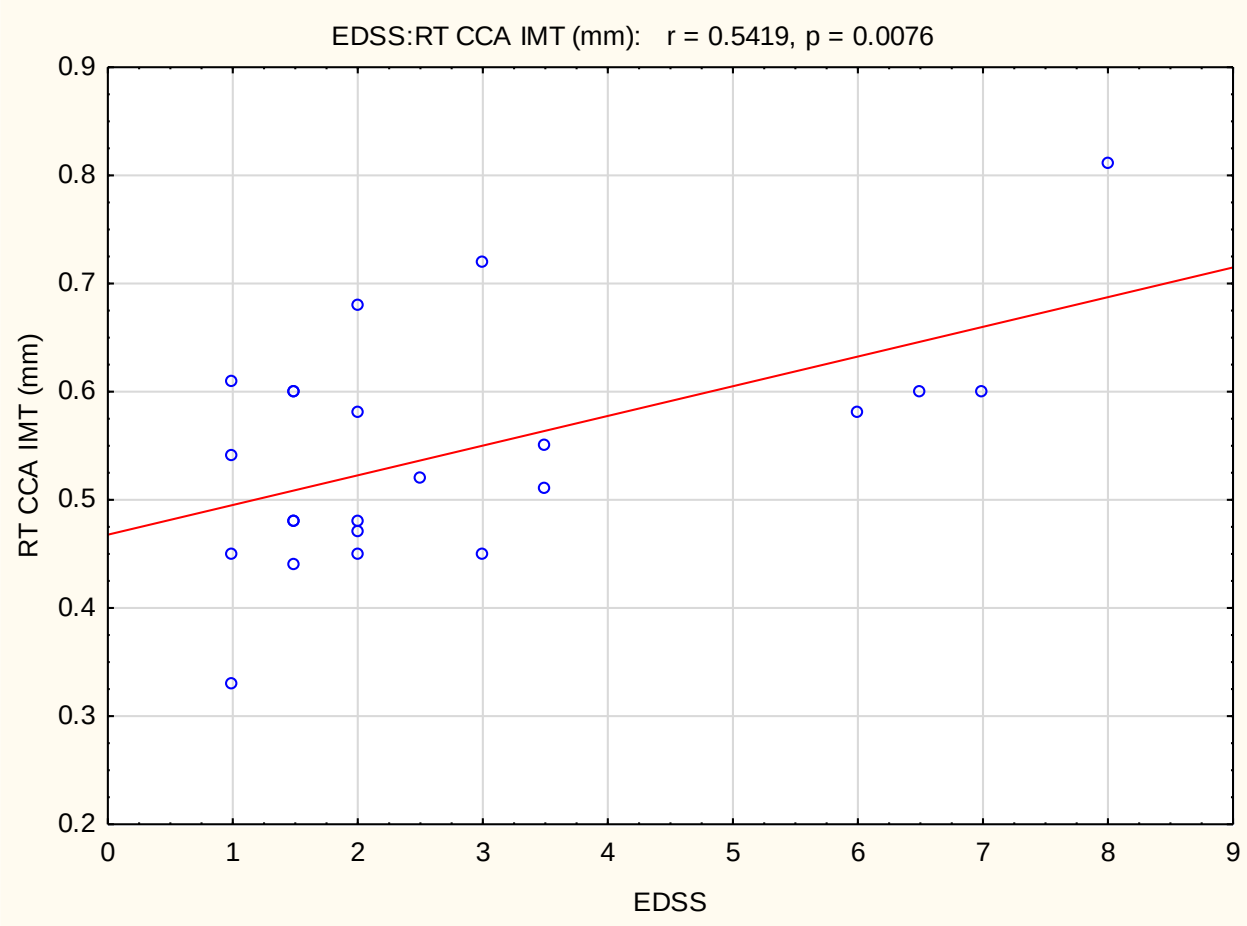

Figure 4.12: Significant positive association between disability and the RT CCA IMT

RT, right; CCA, common carotid artery; IMT, intima media thickness; EDSS, expanded disability status scale

- CSD MID RT CCA (mm); Pearson p-value <0.01, Spearman p-value 0.01 (Figure 4.13)

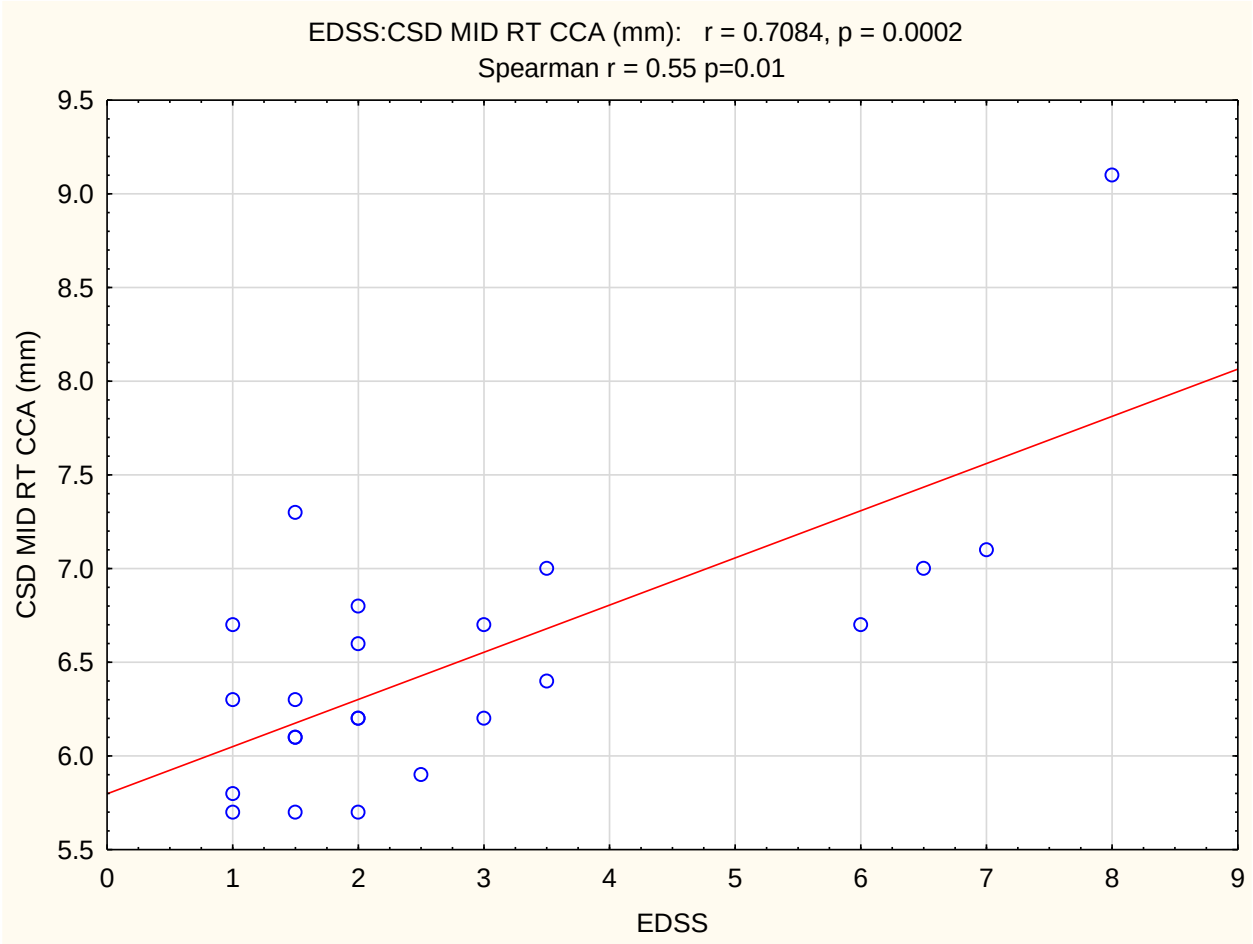

Figure 4.13: Significant positive association between disability and the CSD of the mid RT CCA

CSD, cross-sectional diameter; CCA; RT, right; CCA, common carotid artery

- CSD DIST RT CCA (mm); Pearson p-value <0.01, Spearman p-value 0.01 (Figure 4.14)

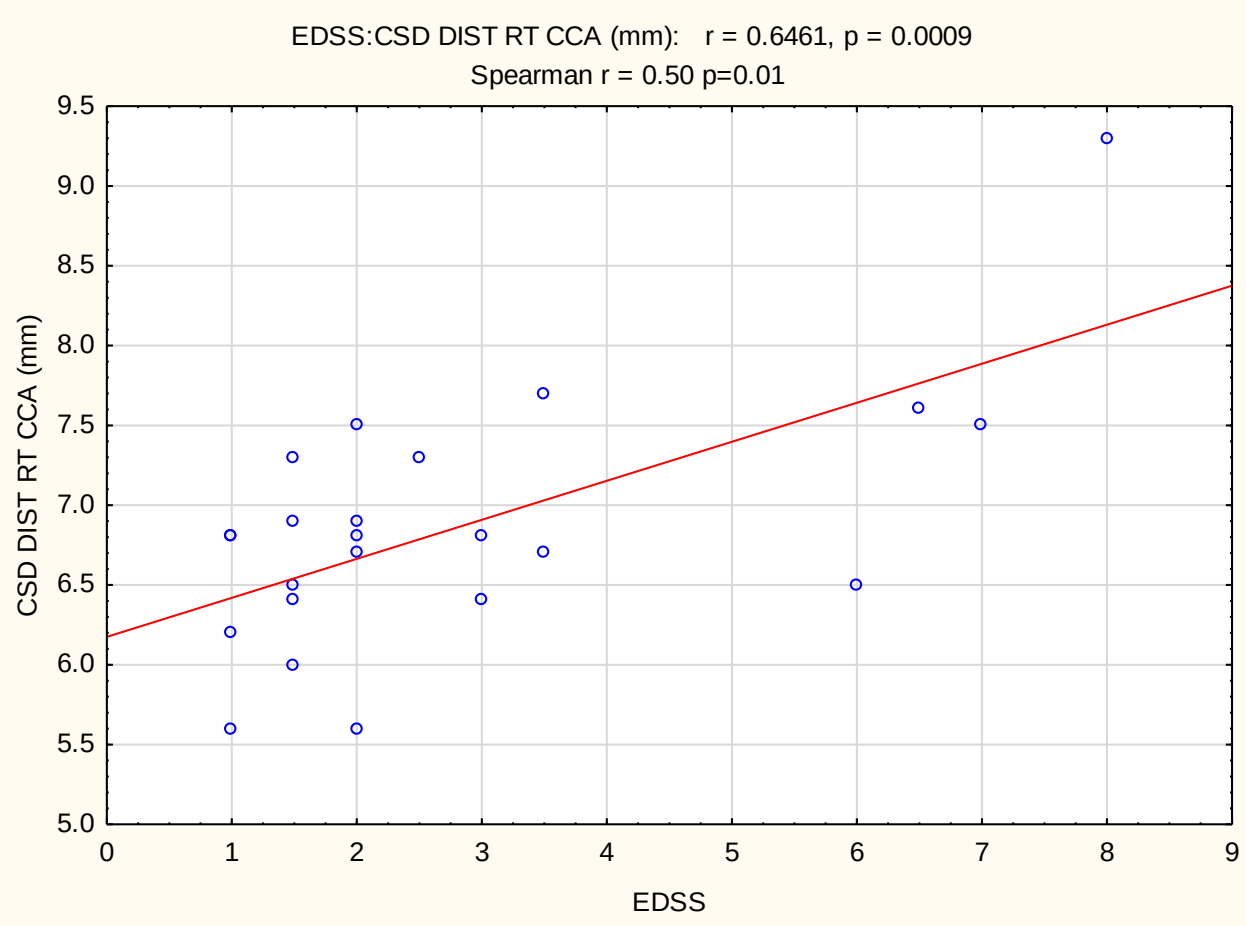

Figure 4.14: Significant positive association between the CSD of the distal RT CCA and disability

CSD, cross-sectional diameter; DIST, distal; RT, right; CCA, common carotid artery

- LT CCA IMT (mm); Pearson p-value 0.04 (Figure 4.15).

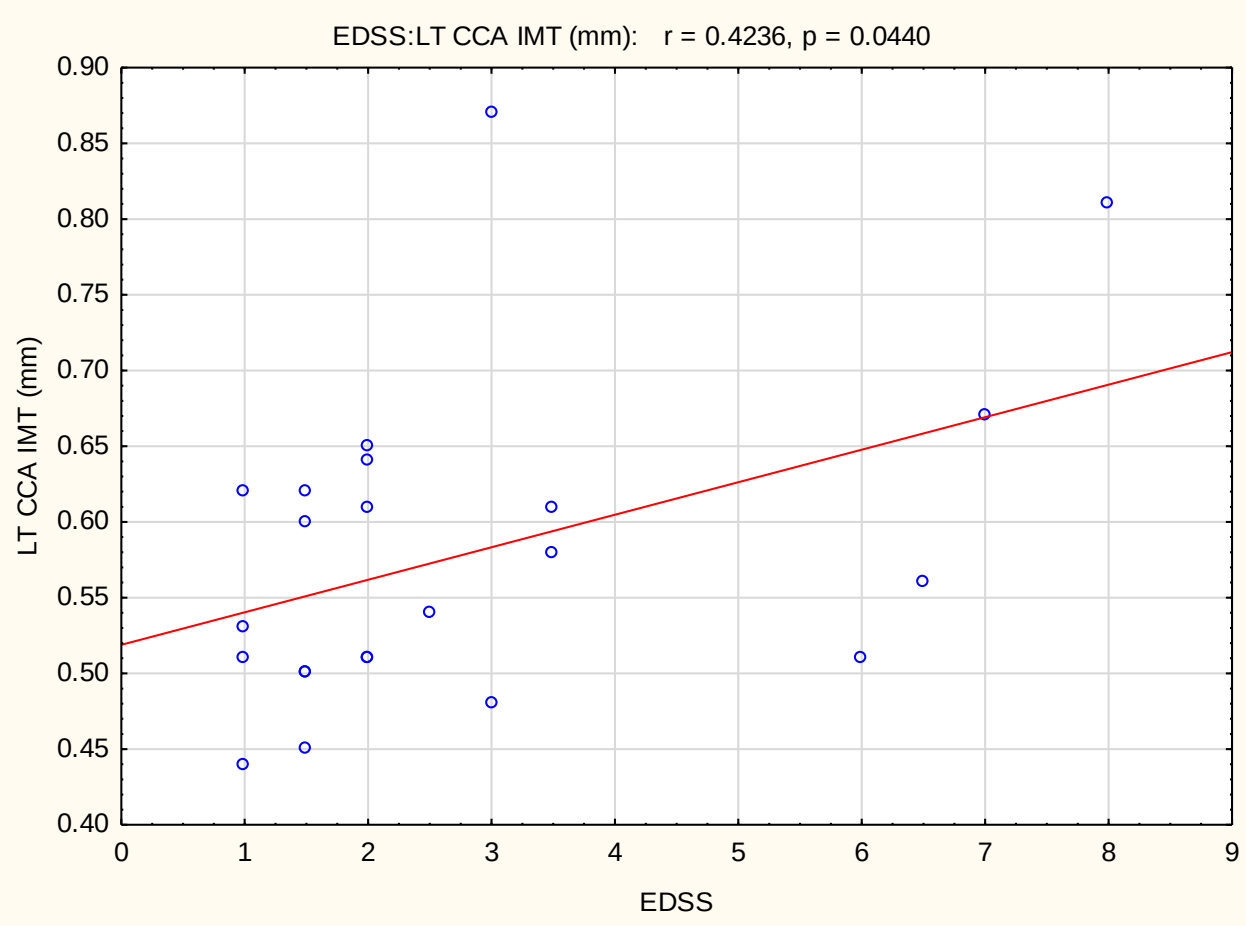

Figure 4.15: Significant positive association between disability and the left CCA IMT

LT, left; CCA, common carotid artery; IMT, intima media thickness; EDSS, expanded disability status scale

- CSD MID LT CCA (mm); Pearson p-value <0.01, Spearman p-value 0.01 (Figure 4.16).

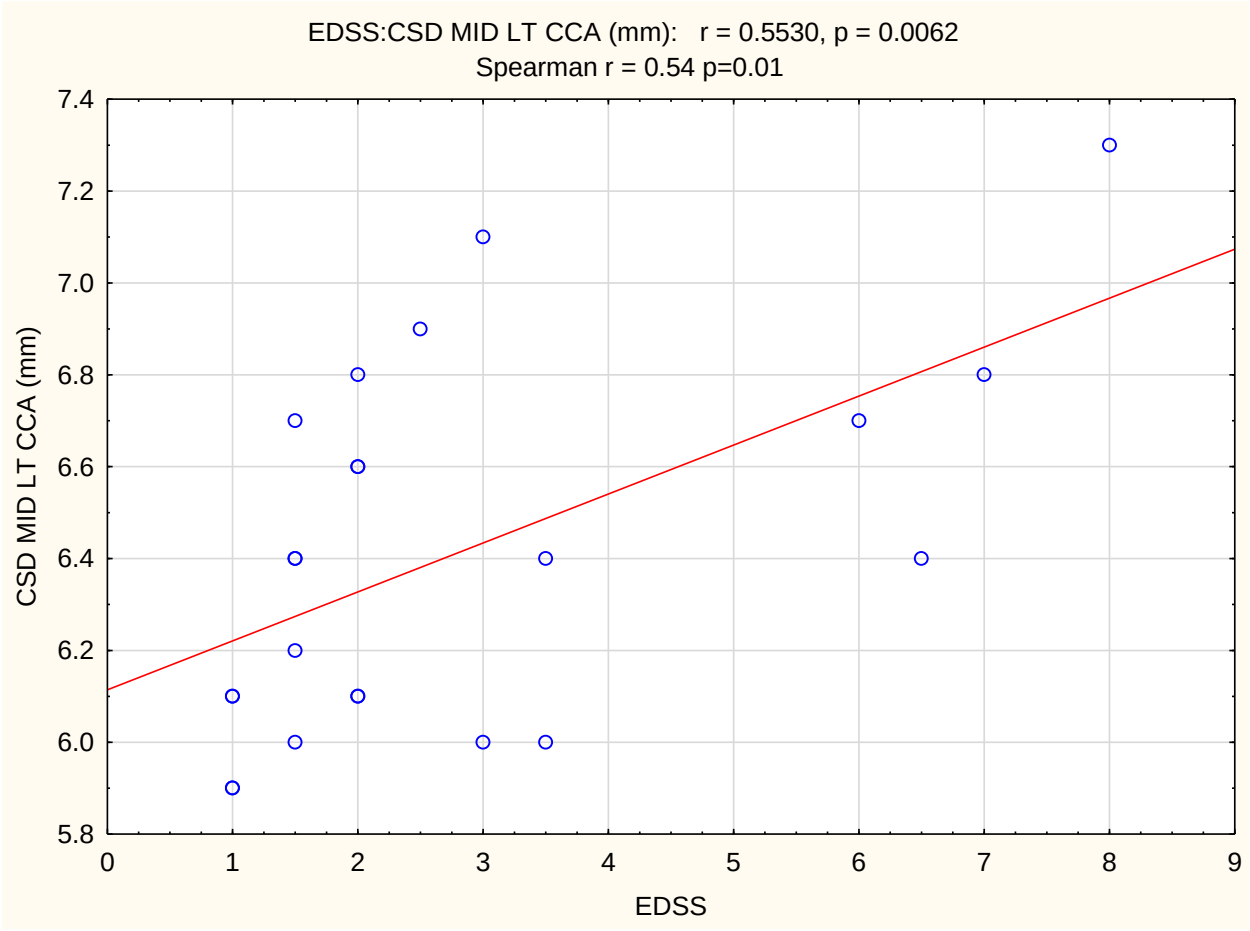

Figure 4.16: Significant positive association between disability and the CSD of the MID LT CCA

CSD, cross-sectional diameter; LT, left; CCA, common carotid artery; EDSS, expanded disability status scale

Similarly, significant negative associations were found between EDSS and the following ultrasound variables:

- RT CCA PSV (cm/s); Pearson p-value 0.02, Spearman p-value 0.01 (Figure 4.17)
- RT CCA EDV (cm/s); Pearson p-value 0.02 (Figure 4.18)
- RT ICA EDV (cm/s); Pearson p-value 0.04 (Figure 4.19)
- RT VERT A PSV (cm/s); Pearson p-value 0.04 (Figure 4.20)
- LT CCA EDV (cm/s); Pearson p-value 0.01 (Figure 4.21)
- LT ICA EDV (cm/s); Pearson p-value 0.01 (Figure 4.22)

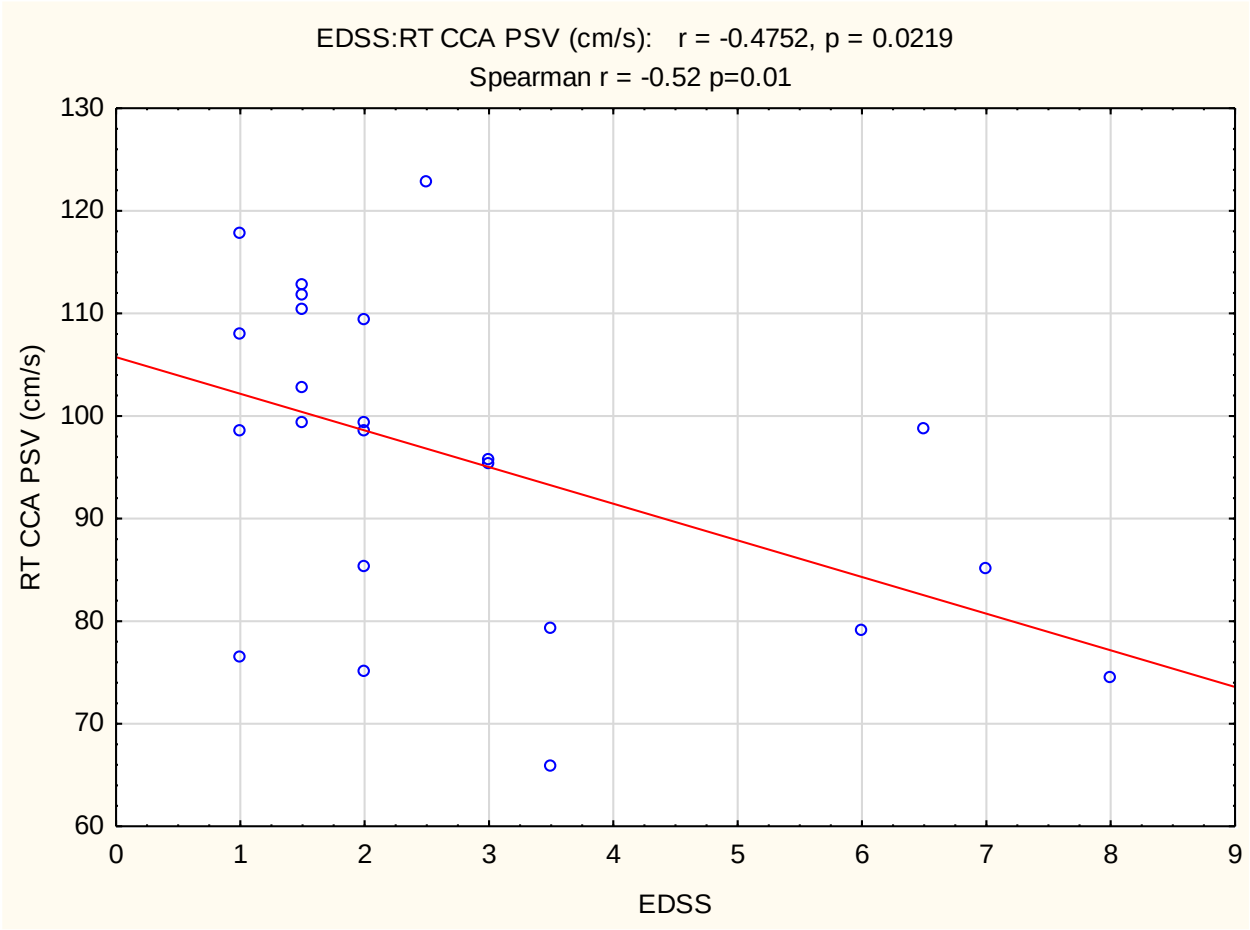

Figure 4.17: Significant negative association between disability and the PSV of the RT CCA

RT, right; CCA, common carotid artery; PSV, peak systolic velocity; EDSS, expanded disability status scale

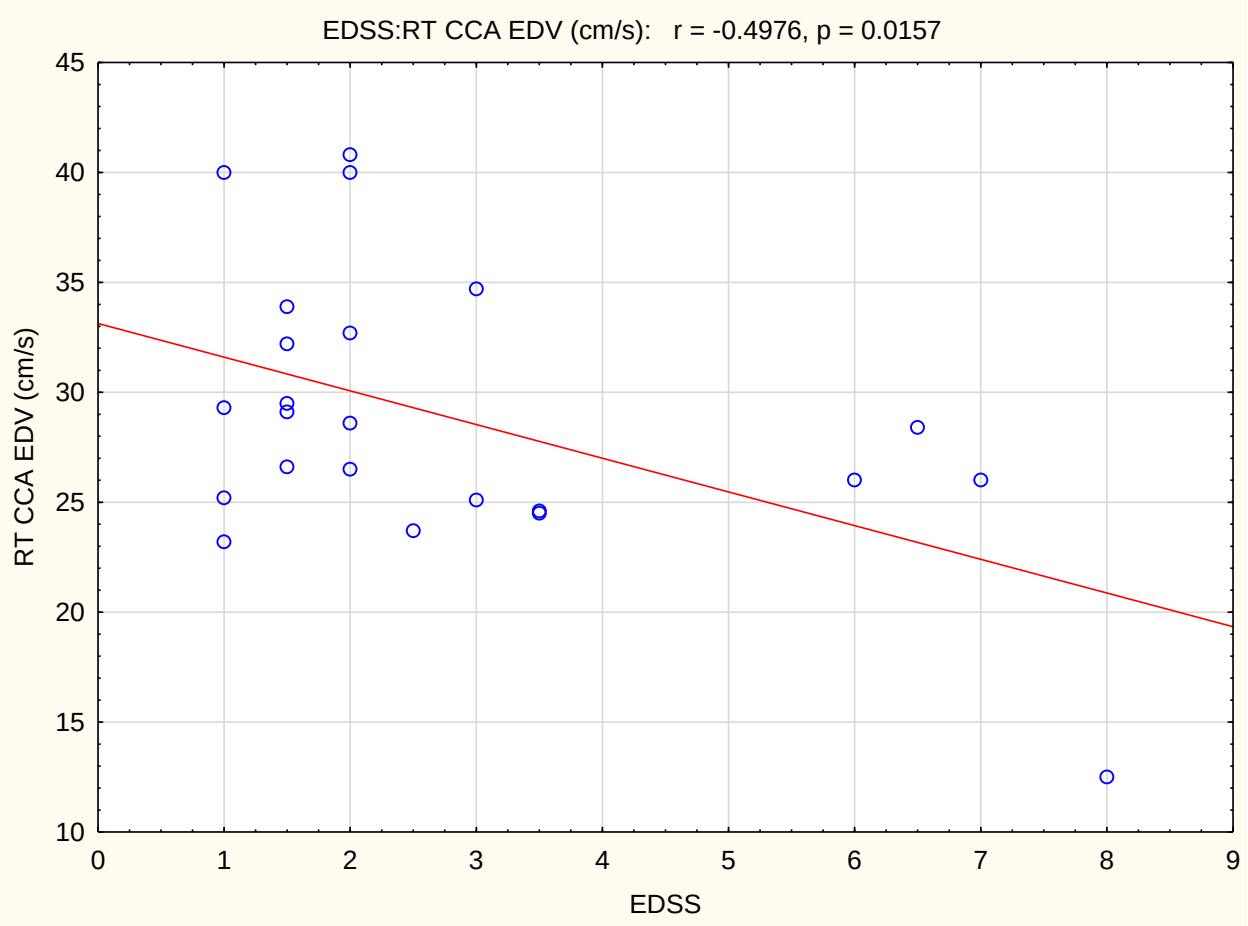

Figure 4.18: Significant negative association between disability and the EDV of the RT CCA

RT, right; CCA, common carotid artery; EDV, end-diastolic; EDSS, expanded disability status scale

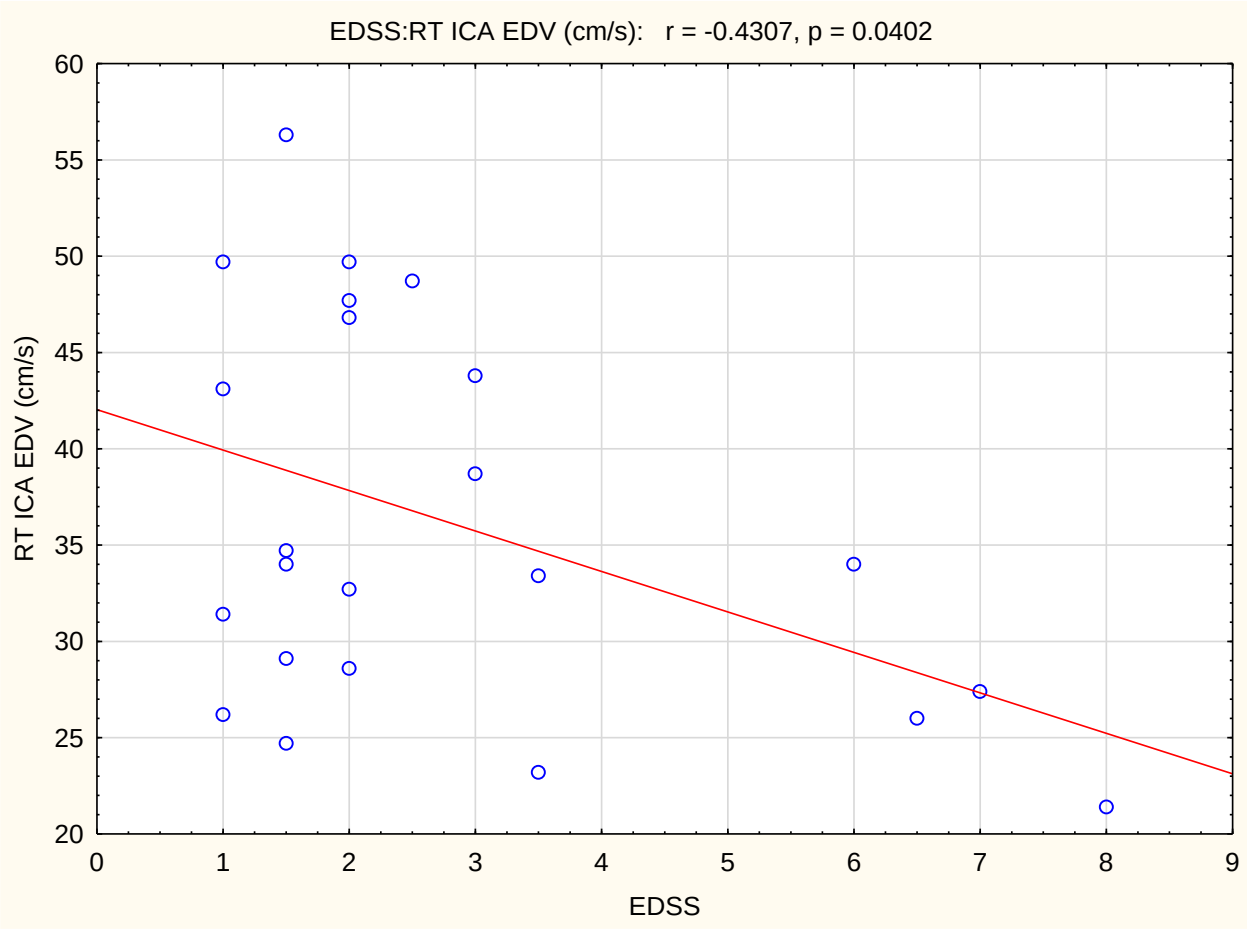

Figure 4.19: Significant negative association between disability and the EDV of the RT ICA

RT, right; ICA, internal carotid artery; EDV, end-diastolic velocity; EDSS, expanded disability status scale

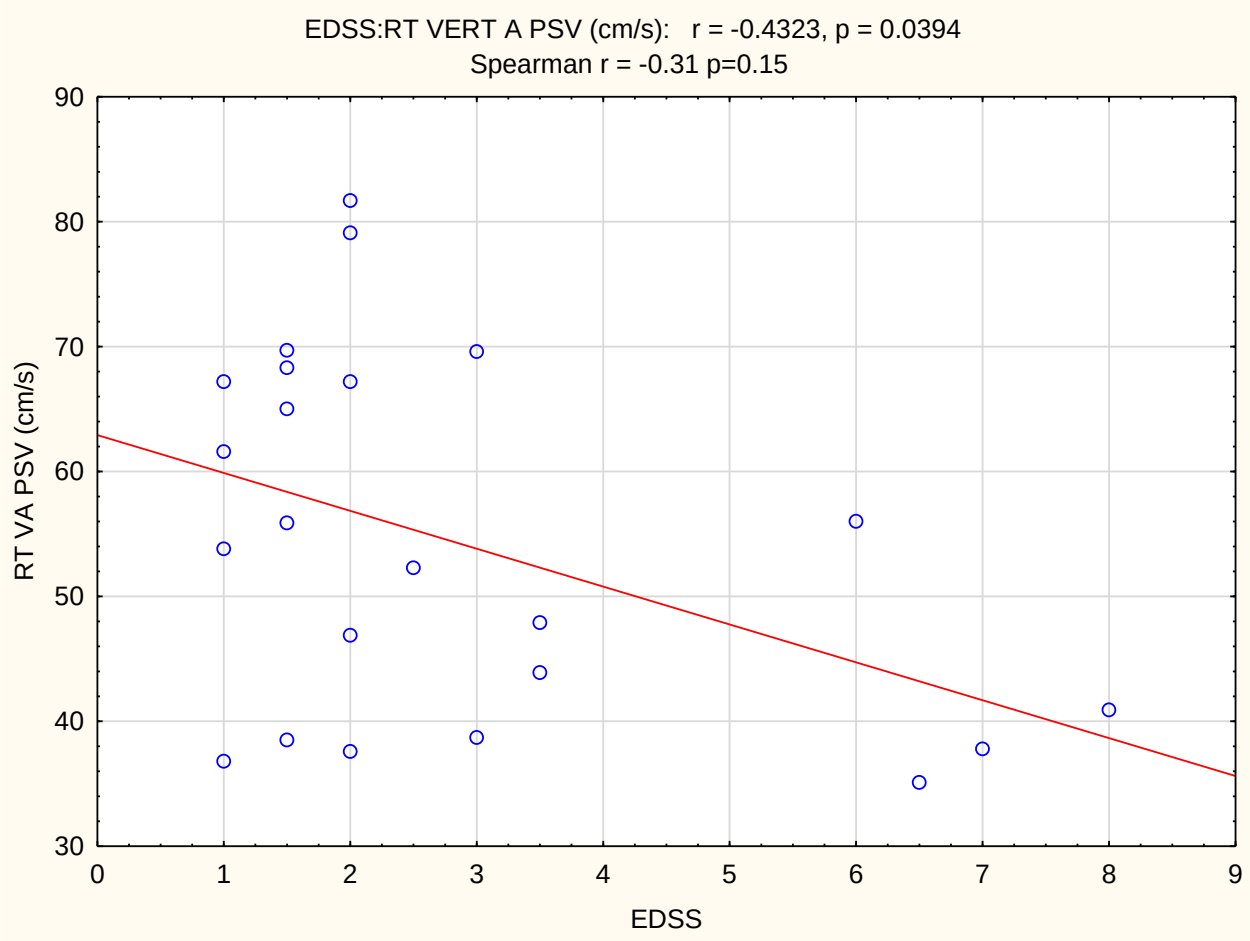

Figure 4.20: Significant negative association between disability and the PSV of the right vertebral artery

RT, right; VA, vertebral artery; PSV, peak systolic velocity; EDSS, expanded disability status scale

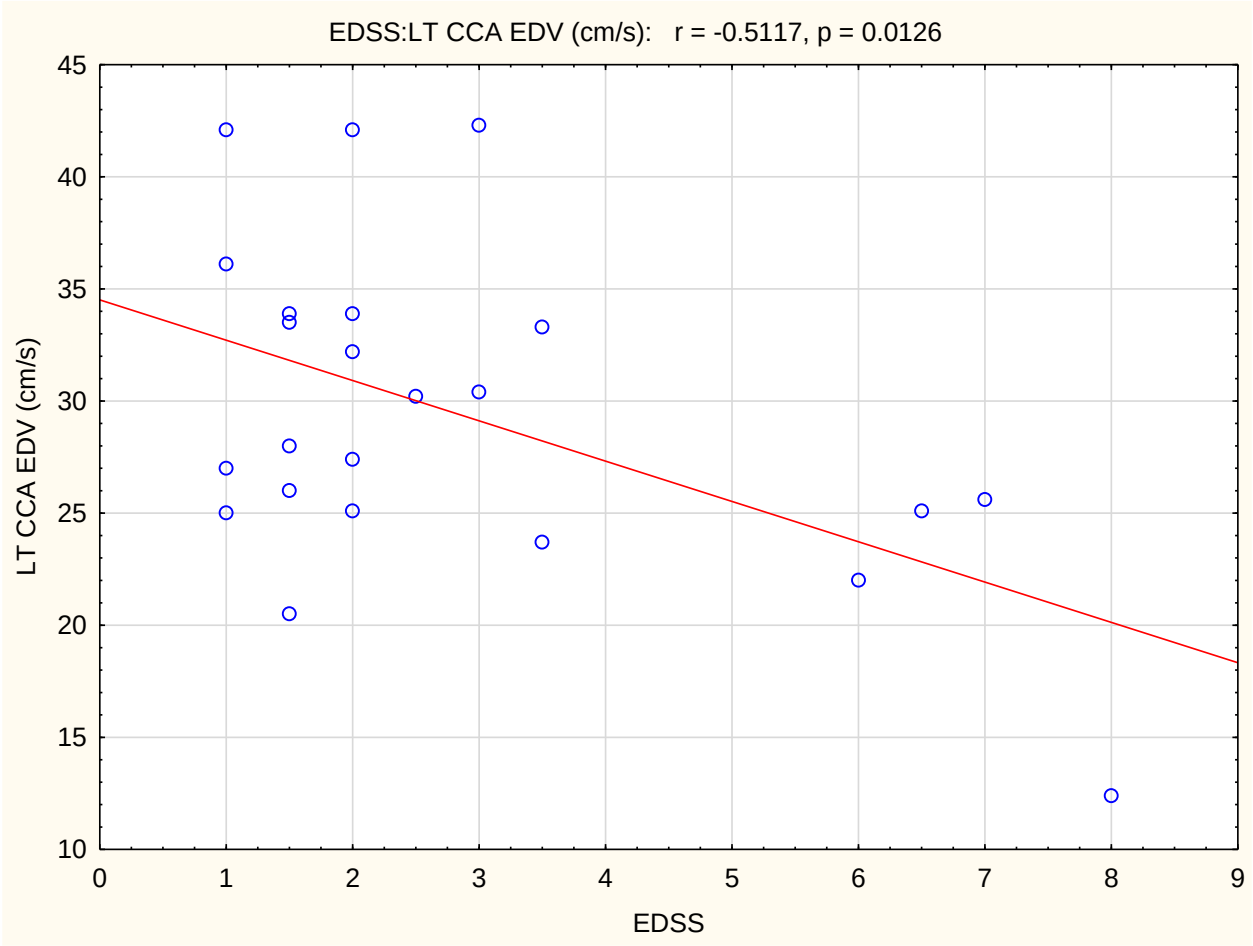

Figure 4.21: significant negative association between disability and the EDV of the left CCA

LT, left; CCA, common carotid artery; EDV, end-diastolic velocity; EDSS, expanded disability status

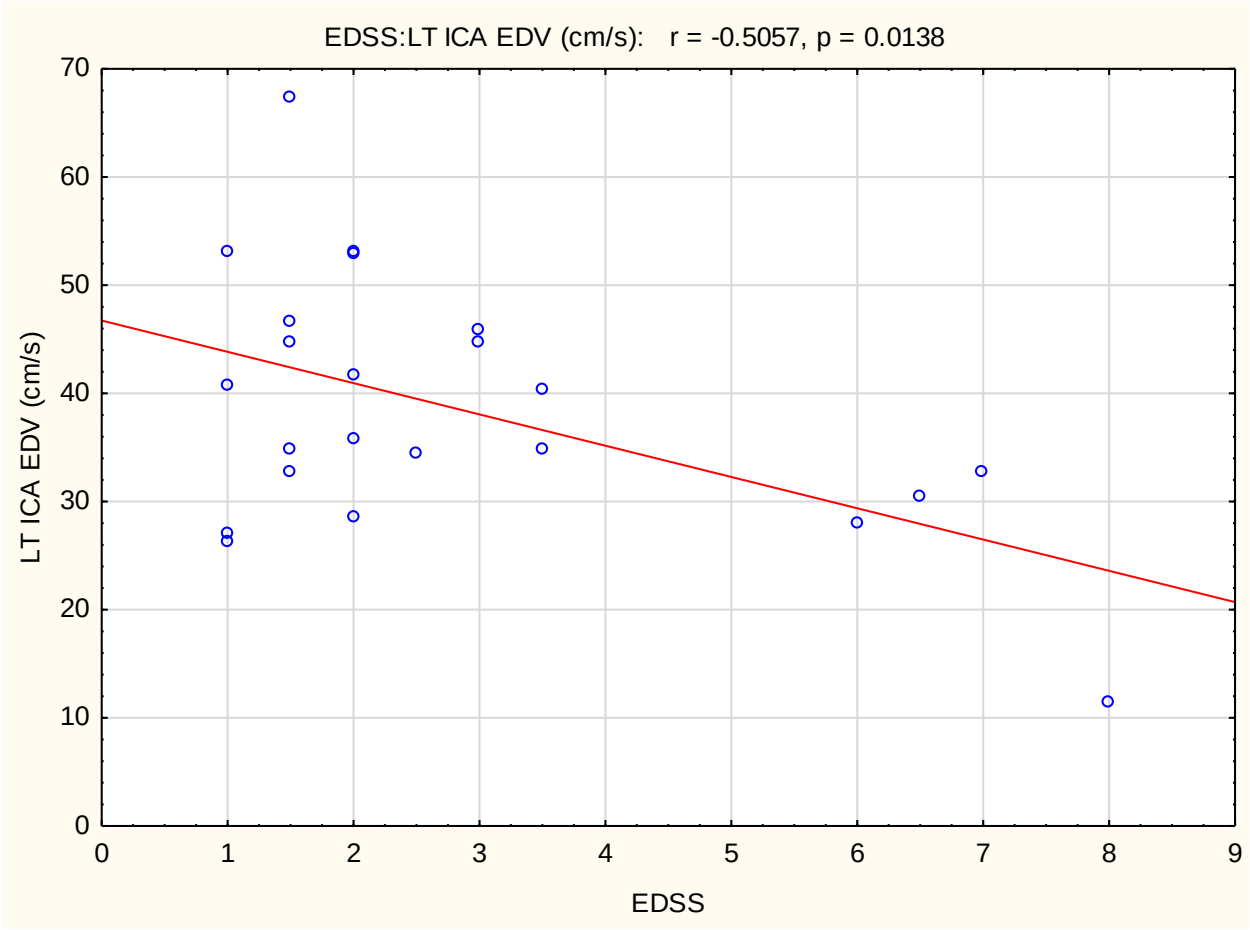

Figure 4.22: Significant negative association between disability and the EDV of the LT ICA

LT, left; ICA, internal carotid artery; EDV, end-diastolic velocity; EDSS, expanded disability status scale.

Table 4.46: Correlation between disability and the extracranial vascular ultrasound variables in the MS group

Disability parameter	Ultrasound variable	Pearson		Spearman		# cases
		r	p	r	p	
EDSS	RT CCA IMT (mm)	0.54	<0.01	0.39	0.07	23
EDSS	RT CCA PSV (cm/s)	-0.48	0.02	-0.52	0.01	23
EDSS	RT CCA EDV (cm/s)	-0.5	0.02	-0.36	0.09	23
EDSS	CSD PROX RT CCA (mm)	0.3	0.16	0.08	0.72	23
EDSS	CSD MID RT CCA (mm)	0.71	<0.01	0.55	<0.01	23
EDSS	CSD DIST RT CCA (mm)	0.65	<0.01	0.5	0.01	23
EDSS	RT ICA PSV (cm/s)	-0.14	0.53	0.07	0.73	23
EDSS	RT ICA EDV (cm/s)	-0.43	0.04	-0.3	0.17	23
EDSS	CSD PROX RT ICA (mm)	0.25	0.25	0.27	0.22	23
EDSS	PROX ECA RT PSV (cm/s)	0.14	0.51	0.11	0.63	23
EDSS	CSD PROX RT ECA (mm)	-0.01	0.95	0.16	0.48	23
EDSS	RT VA PSV (cm/s)	-0.43	0.04	-0.31	0.15	23
EDSS	RT VA EDV (cm/s)	-0.4	0.06	-0.35	0.1	23
EDSS	CSD PROX RT VA (mm)	-0.02	0.93	-0.1	0.65	23
EDSS	RT PSV ICA/CCA ratio	0.23	0.28	0.36	0.09	23
EDSS	RT EDV ICA/ECA ratio	0.04	0.84	0.06	0.79	23
EDSS	LT CCA IMT (mm)	0.42	0.04	0.4	0.06	23
EDSS	LT CCA PSV (cm/s)	-0.37	0.08	-0.32	0.14	23
EDSS	LT CCA EDV (cm/s)	-0.51	0.01	-0.32	0.14	23
EDSS	CSD PROX LT CCA (mm)	0.31	0.14	0.2	0.35	23
EDSS	CSD MID LT CCA (mm)	0.55	<0.01	0.54	<0.01	23
EDSS	CSD DIST LT CCA (mm)	0.34	0.11	0.29	0.19	23
EDSS	LT ICA PSV (cm/s)	-0.26	0.22	-0.38	0.07	23
EDSS	LT ICA EDV (cm/s)	-0.51	0.01	-0.26	0.23	23
EDSS	CSD PROX LT ICA (mm)	0.11	0.62	0.25	0.25	23
EDSS	PROX LT ECA PSV (cm/s)	0.21	0.34	-0.02	0.92	23
EDSS	CSD PROX LT ECA (mm)	0.21	0.33	0.22	0.32	23
EDSS	LT VA PSV (cm/s)	-0.25	0.25	-0.21	0.34	23
EDSS	LT VA EDV (cm/s)	-0.24	0.27	-0.1	0.65	23
EDSS	CSD PROX LT VA (mm)	-0.06	0.8	-0.13	0.56	23
EDSS	LT PSV ICA/CCA ratio	-0.02	0.94	-0.05	0.82	23
EDSS	LT EDV ICA/ECA ratio	-0.22	0.32	-0.12	0.57	23
EDSS	PV PROX RT IJV (cm/s)	0.09	0.69	0.18	0.4	23
EDSS	PV MID RT IJV (cm/s)	-0.12	0.6	-0.04	0.87	23
EDSS	PV DIST RT IJV (cm/s)	0.01	0.95	0.13	0.56	23
EDSS	CSA PROX RT IJV (mm^2)	-0.36	0.09	-0.24	0.28	23
EDSS	PV PROX LT IJV (cm/s)	0.03	0.89	0.04	0.87	23
EDSS	PV MID LT IJV (cm/s)	-0.26	0.23	-0.2	0.36	23
EDSS	PV DIST LT IJV (cm/s)	0.28	0.2	0.09	0.67	23
EDSS	CSA PROX LT IJV (mm^2)	-0.05	0.81	-0.12	0.6	23

EDSS, expanded disability status scale; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PV, peak velocity; CSD, cross-sectional diameter; PROX, proximal; DIST, distal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery; CSA, cross-sectional area; IJV, internal jugular vein

4.7 MATCH-MERGED DATA

The current case-control study data (Study B) and data of a previous study entitled: Ultrasound investigation of risk factors for extracranial vascular pathology in patients with multiple sclerosis (Study A), were matched and merged. The matching genetic, lifestyle, biochemical and ultrasound variables including disability status of the cohort of 23 MS participants for study A (excluding males) and 25 MS participants on study B, were merged and analysed. The merging of the study data created a larger cohort of MS participants (n=48) which improved the statistical power and correlations between the multiple variables.

4.7.1 ASSOCIATION BETWEEN BIOCHEMICAL PARAMETERS AND THE EXTRACRANIAL VASCULAR ULTRASOUND VARIABLES IN THE MERGED MS GROUP

Biochemical parameters were not available for all 48 MS participants in the merged group.

Table 4.47 shows the statistically significant correlations found between the following match-merged biochemical parameters and the extracranial ultrasound variables:

- Iron - positively correlated with the
    - EDV of the right ICA (Pearson p-value 0.01),
    - RT PSV ICA/CCA ratio (Pearson p-value 0.01, Spearman p-value 0.01)
    - RT EDV ICA/CCA ratio (Pearson p-value <0.01, Spearman p-value 0.04)

- Transferrin – positively correlated with the
    - RT CCA PSV (Spearman p-value <0.01)
    - RT PSV ICA/CCA ratio (Spearman p-value <0.01)
    - LT CCA PSV (Pearson p-value 0.03)

- % saturated transferrin – positively correlated with the
    - RT CCA PSV (Spearman p-value 0.04)
    - RT ICA EDV (Pearson p-value <0.01)
    - RT PSV ICA/CCA ratio (Pearson p-value <0.01, Spearman p-value <0.01)
    - RT EDV ICA/CCA ratio (Pearson p-value <0.01, Spearman p-value <0.01)

- Ferritin – positively correlated with the LT ICA PSV (Spearman p-value 0.04)

- 25 OH-Vitamin D – positively correlated with the
    - PSV of the LT CCA (Spearman p-value 0.02)
    - EDV of the RT ICA (Pearson p-value 0.04)
    - PSV of the RT CCA (Pearson p-value 0.02, Spearman p-value <0.01)

    - PSV of the LT VA (Pearson p-value 0.02, Spearman p-value <0.01)
- Homocysteine – positively correlated with the
    - RT PSV ICA/CCA ratio (Pearson p-value 0.03, Spearman p-value 0.02)
    - PSV of the RT CCA (Pearson p-value <0.01, Spearman p-value <0.01)

- Serum folate – positively correlated with the PSV of the right VA (Pearson p-value 0.02, Spearman p-value 0.03)

- Vitamin B12 – negatively correlated with the PSV of the left VA (Pearson p-value <0.01)

- Total cholesterol – negatively correlated with the
    - PSV of the LT CCA (Spearman p-value 0.03)
    - EDV of the LT CCA (Pearson p-value 0.02, Spearman p-value <0.01)
    - PSV of the LT VA (Pearson p-value <0.01, Spearman p-value <0.01)

- HDL – negatively correlated with left CCA IMT (Spearman p-value 0.03)

- Triglycerides – negatively correlated with the PSV of the left CCA (Spearman p-value 0.04)

Table 4.47: Significant correlations between the biochemical parameters and the extracranial vascular ultrasound variables in the match-merged MS group (Study A and B)

Biochemical maker	Ultrasound variable	Pearson		Spearman		# cases	Merged group
		r	p	r	p		
S-iron	RT ICA EDV (cm/s)	0.41	0.01	0.27	0.1	38	MS
S-iron	RT PSV ICA/CCA ratio	0.39	0.01	0.41	0.01	38	MS
S-iron	RT EDV ICA/ECA ratio	0.46	<0.01	0.33	0.04	38	MS
S-transf	RT CCA PSV (cm/s)	0.2	0.24	0.43	<0.01	38	MS
S-transf	RT PSV ICA/CCA ratio	0.04	0.81	-0.44	<0.01	38	MS
S-transf	LT CCA PSV (cm/s)	0.35	0.03	0.25	0.12	38	MS
% Tf sat	RT CCA PSV (cm/s)	-0.32	0.05	-0.33	0.04	38	MS
% Tf sat	RT ICA EDV (cm/s)	0.42	<0.01	0.32	0.05	38	MS
% Tf sat	RT PSV ICA/CCA ratio	0.53	<0.01	0.56	<0.01	38	MS
% Tf sat	RT EDV ICA/CCA ratio	0.48	<0.01	0.43	<0.01	38	MS
Ferritin	LT ICA PSV (cm/s)	0.17	0.31	0.34	0.04	38	MS
25 OH-Vit D	LT CCA PSV (cm/s)	0.24	0.18	0.39	0.02	34	MS
25 OH-Vit D	RT ICA EDV (cm/s)	0.36	0.04	0.3	0.09	34	MS
25 OH-Vit D	RT CCA PSV (cm/s)	0.4	0.02	0.44	<0.01	34	MS
25 OH-Vit D	LT VA PSV (cm/s)	0.4	0.02	0.47	<0.01	34	MS
H-cyst	RT PSV ICA/CCA ratio	0.36	0.03	0.38	0.02	38	MS
H-cyst	RT CCA PSV (cm/s)	-0.49	<0.01	-0.45	<0.01	38	MS
S-fol	RT VA PSV (cm/s)	0.38	0.02	0.36	0.03	38	MS
Vit B12	LT VA PSV (cm/s)	-0.41	<0.01	-0.22	0.19	38	MS
Total cholesterol	LT CCA PSV (cm/s)	-0.26	0.11	-0.34	0.03	39	MS
Total cholesterol	LT CCA EDV (cm/s)	-0.37	0.02	-0.42	<0.01	39	MS
Total cholesterol	LT VA PSV (cm/s)	-0.44	<0.01	-0.47	<0.01	39	MS
HDL	LT CCA IMT (mm)	-0.31	0.12	-0.43	0.03	26	MS
Trigs	LT CCA PSV (cm/s)	-0.32	0.11	-0.41	0.04	26	MS

S-iron, serum iron; S-transf, serum transferrin; % Tf sat, percentage transferrin saturation; 25-OH VIt D, serum 25-hydroxyvitamin D ; H-cyst, homocysteine; S-fol, serum folate; HDL, high-density lipoprotein; Trigs, triglycerides; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PROX, proximal;; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery

4.7.2 ASSOCIATION BETWEEN LIFESTYLE FACTORS AND THE EXTRACRANIAL VASCULAR ULTRASOUND VARIABLES IN THE MERGED MS GROUP

4.7.2.1 Saturated fat, chicken with skin and physical activity

Statistically significant correlations (p-value <0.05) are summarised in Table 4.48. Physical activity is found to be strongly correlated with multiple carotid artery ultrasound variables. A statistically negative association exists between physical activity and the IMT of the left CCA (Pearson p-value 0.03, Spearman p-value 0.04) as well as a significant positive correlation with the carotid artery blood flow velocities. Similarly, a significant positive correlation is found between saturated fat and the IMT of the right CCA; and consumption of chicken with carotid artery blood flow velocity (Table 4.48).

Table 4.48: Correlation between the lifestyle parameters and the extracranial vascular ultrasound variables in the merged MS group

Lifestyle parameter	Ultrasound variable	Pearson		Spearman		# cases	Merged group
		r	p	r	p		
Saturated Fat	RT CCA IMT (mm)	0.3	0.08	0.37	0.03	36	MS
Chicken with skin	PROX LT ECA PSV (cm/s)	0.38	0.02	0.24	0.16	35	MS
Chicken with skin	RT CCA EDV (cm/s)	0.39	0.02	0.37	0.03	35	MS
Physical activity	RT VA PSV (cm/s)	0.4	0.01	0.42	<0.01	37	MS
Physical activity	LT CCA IMT (mm)	-0.35	0.03	-0.34	0.04	37	MS
Physical activity	LT ICA PSV (cm/s)	0.41	0.01	0.4	0.01	37	MS
Physical activity	LT ICA EDV (cm/s)	0.43	<0.01	0.38	0.02	37	MS
Physical activity	LT VA PSV (cm/s)	0.37	0.02	0.42	0.01	37	MS
Physical activity	LT PSV ICA/CCA ratio	0.37	0.02	0.39	0.02	37	MS
Physical activity	LT EDV ICA/ECA ratio	0.34	0.04	0.34	0.04	37	MS

RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; PSV, peak systolic velocity; EDV, end-diastolic velocity; PROX, proximal; ICA, internal carotid artery; ECA, external carotid artery; VA, vertebral artery

4.7.2.2 Passive smoking

The passive smoking status results were available for 25 MS participants in the merged group. No significant associations were found between passive smoking and the extracranial ultrasound variables.

4.7.2.3 Alcohol intake

Further analysis examined the association between alcohol intake and the extracranial vascular ultrasound variables. Alcohol intake was categorised into scores 0, 1, 2 and 3 (0=abstain, 1= 1-2 units /week, 2= 3-13 units/week and 3= 14-21 units/week).

Figure 4.23 indicates that the IMT of the left CCA was significantly thinner (Kruskal-Wallis p-value 0.02) in MS participants with an alcohol intake score of 1 (n=17; 0.55 ± 0.07) and 2 (n=8, 0.56 ± 0.19) in comparison to those MS participants who abstained from alcohol (score 0) (n=12; 0.67 ± 0.18).

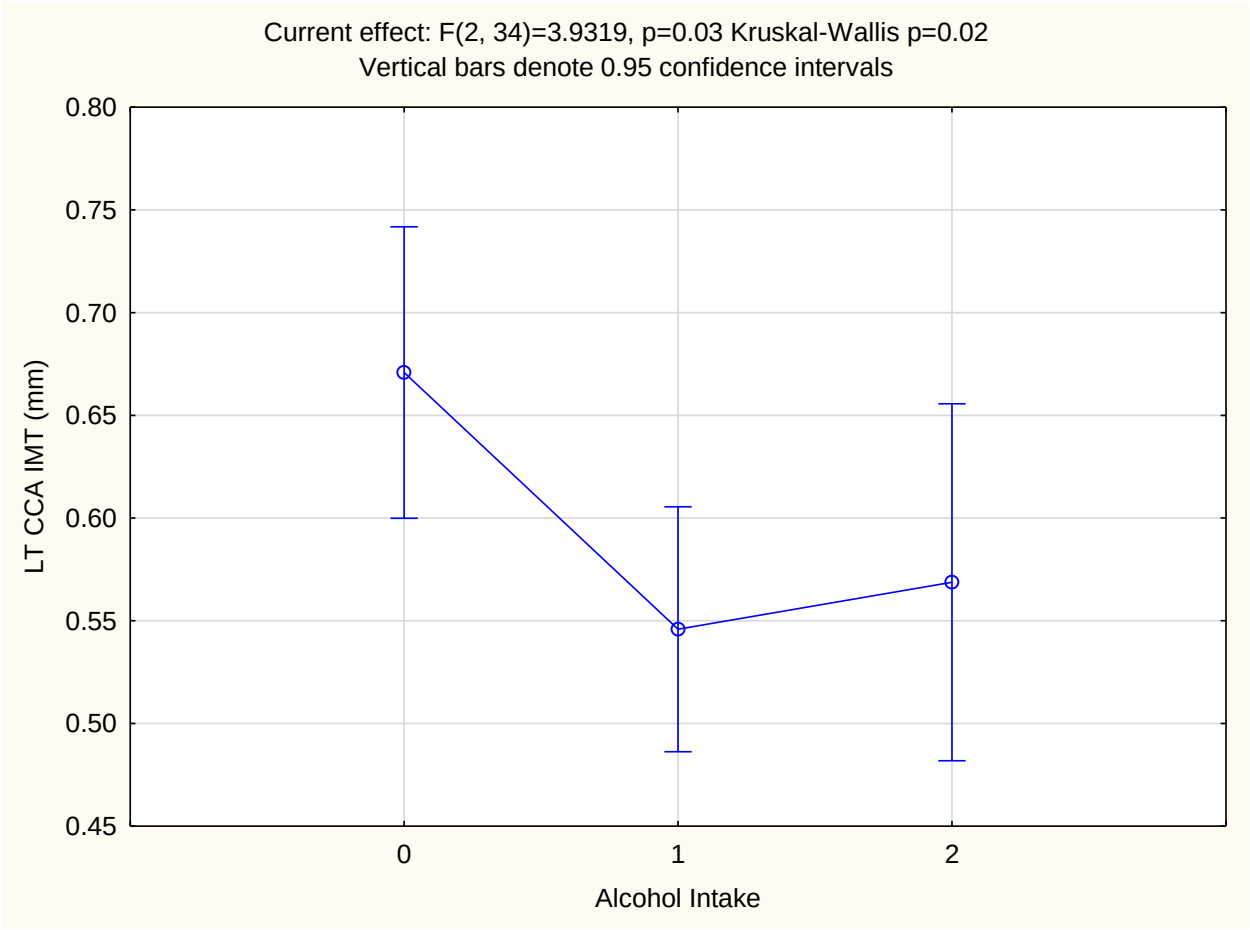

Figure 4.23: Significant difference in IMT of the left CCA between MS persons consuming alcohol and those abstaining from alcohol

LT, left; CCA, common carotid artery; IMT, intima media thickness; 0=abstain; 1= 1-2 units /week; 2= 3-13 units/week; 3= 14-21 units/week;

A significant difference (Kruskal-Wallis p-value 0.02) was also found between the left EDV ICA/CCA ratio in MS participants with an alcohol intake score of 1 and 2 and MS participants who abstained (score 0). The LT EDV ICA/CCA ratio was higher in MS participants with a score of 1 (n=17, 1.45 ± 0.34) and score of 2 (n=8, 1.31 ± 0.31) in comparison to those who had a score of 0 (n=12, 1.11 ± 0.25) (Figure 4.24).

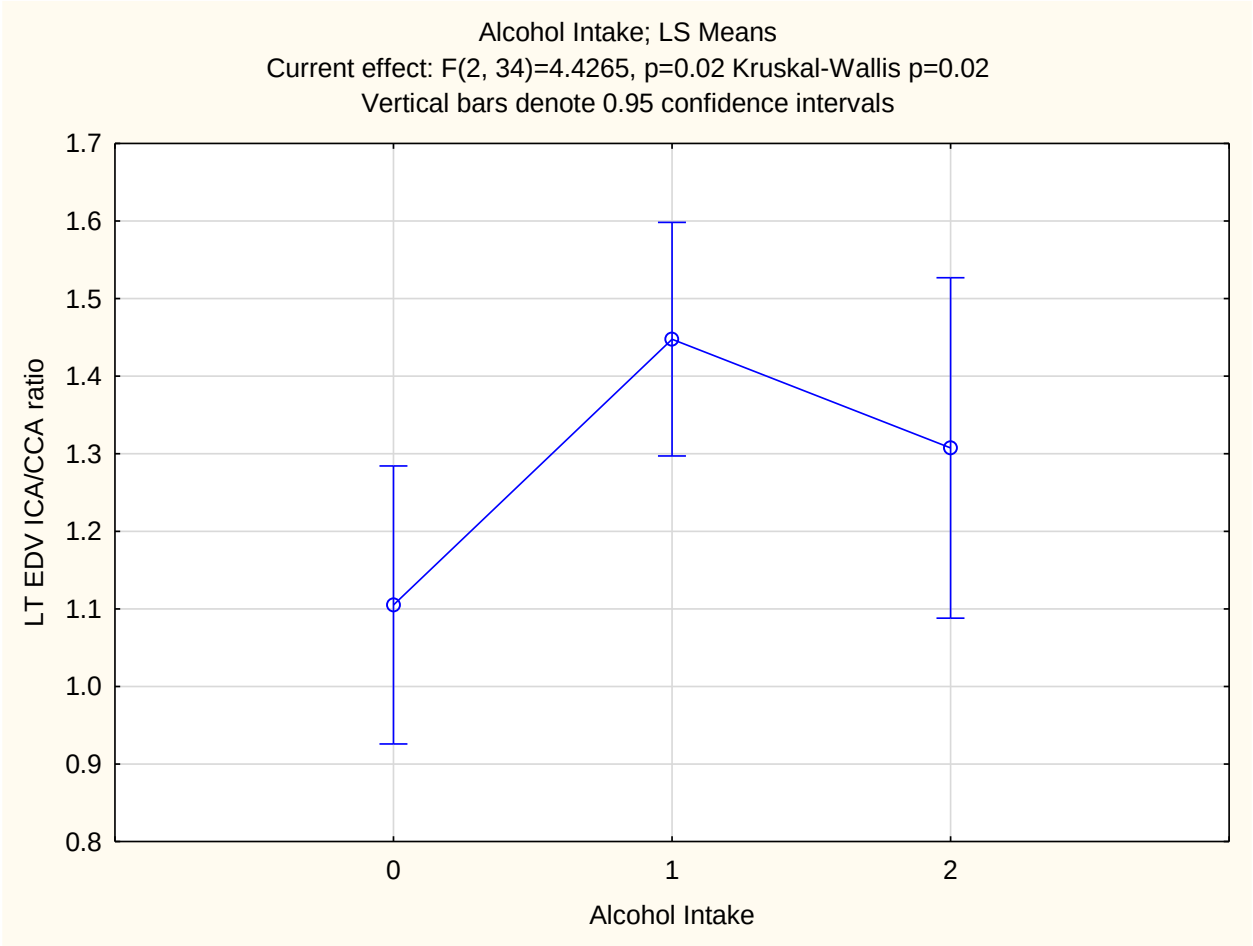

Figure 4.24: Significant difference in the LT EDV ICA/CCA ratio between MS persons consuming alcohol and MS persons abstaining from alcohol.

LT, left; EDV, end-diastolic velocity; ICA, internal carotid artery; CCA, common carotid artery.; 0= abstain from alcohol; 1 = 1-2 units/week; 2 = 3-13 units/week.

4.7.3 ASSOCIATION BETWEEN DISABILITY AND THE EXTRACRANIAL VASCULAR ULTRASOUND VARIABLES IN THE MERGED MS GROUP

The disability status was available for 36 MS participants in the merged group.

As seen in Table 4.49 and the scatter plot graphs (Figure 4.25 - 4.28) the EDDS had a significant positive association with IMT of the right CCA (Pearson p-value <0.01, Spearman p-value 0.03).

Similarly, a significant negative association was found between EDSS and the following ultrasound variables:

- EDV of the RT CCA (Pearson p-value 0.04) (Figure 4.25)
- PSV of the right VA (Pearson p-value 0.02, Spearman p-value 0.03) (Figure 4.26)
- EDV of the LT CCA (Pearson p-value 0.02) (Figure 4.27)
- EDV of the LT ICA (Pearson p-value 0.01) (Figure 4.28)

Table 4.49. Significant correlation between disability and the extracranial vascular ultrasound variables in the merged MS group

Disability parameter	Ultrasound variable	Pearson		Spearman		# cases	Merged group
		r	p	r	p		
EDSS	RT CCA IMT (mm)	0.48	<0.01	0.37	0.03	36	MS
EDSS	RT CCA EDV (cm/s)	-0.35	0.04	-0.26	0.13	36	MS
EDSS	RT VA PSV (cm/s)	-0.4	0.02	-0.37	0.03	36	MS
EDSS	LT CCA EDV (cm/s)	-0.37	0.02	-0.23	0.17	36	MS
EDSS	LT ICA EDV (cm/s)	-0.41	0.01	-0.22	0.19	36	MS

EDSS, expanded disability status scale; RT, right; LT, left; CCA, common carotid artery; IMT, intima media thickness; EDV, end-diastolic velocity; VA, vertebral artery; PSV, peak systolic velocity

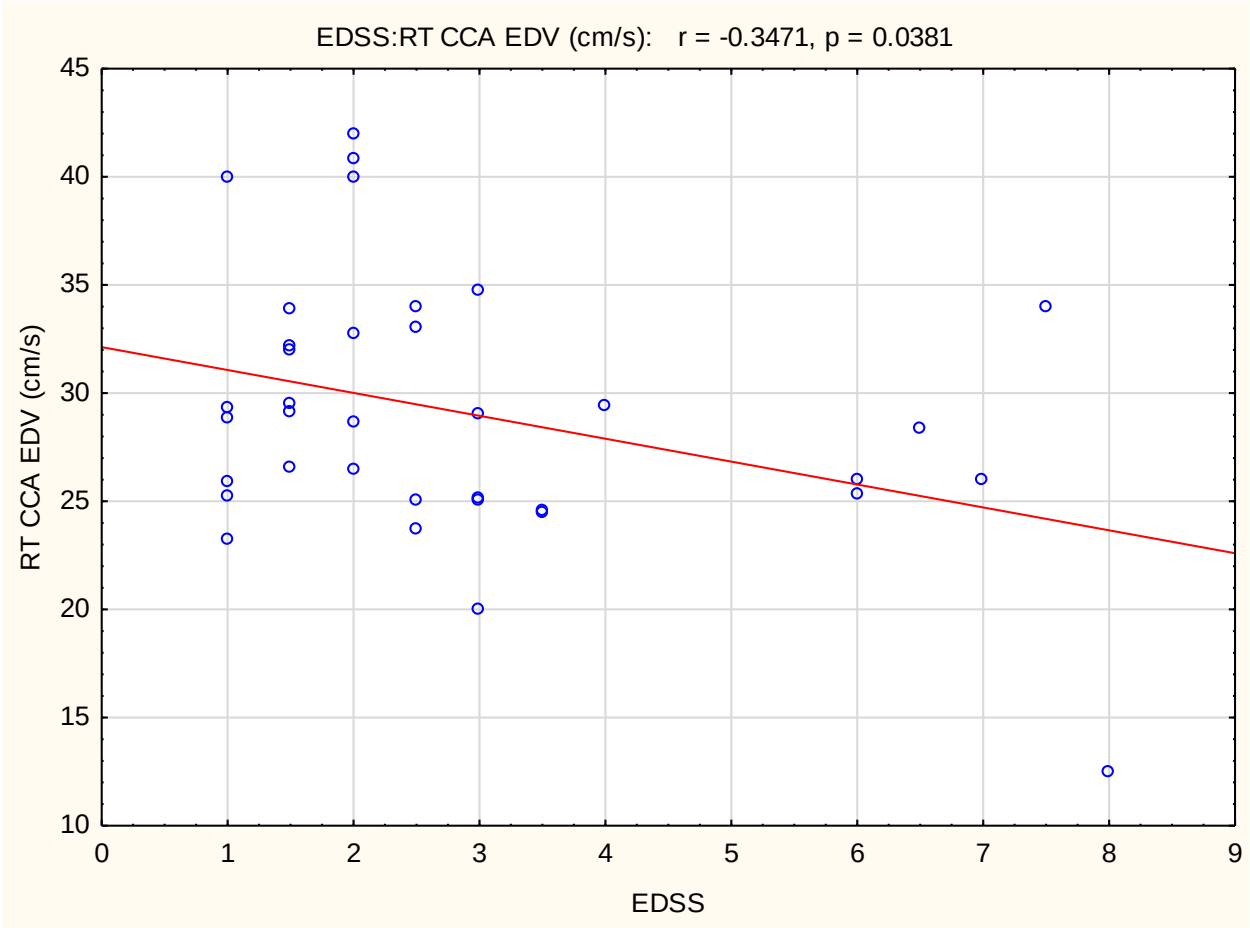

Figure 4.25: Significant association between disability and the EDV of the right CCA in the merged MS group

RT, right; CCA, common carotid artery; EDV, end-diastolic velocity; EDSS, expanded disability status scale

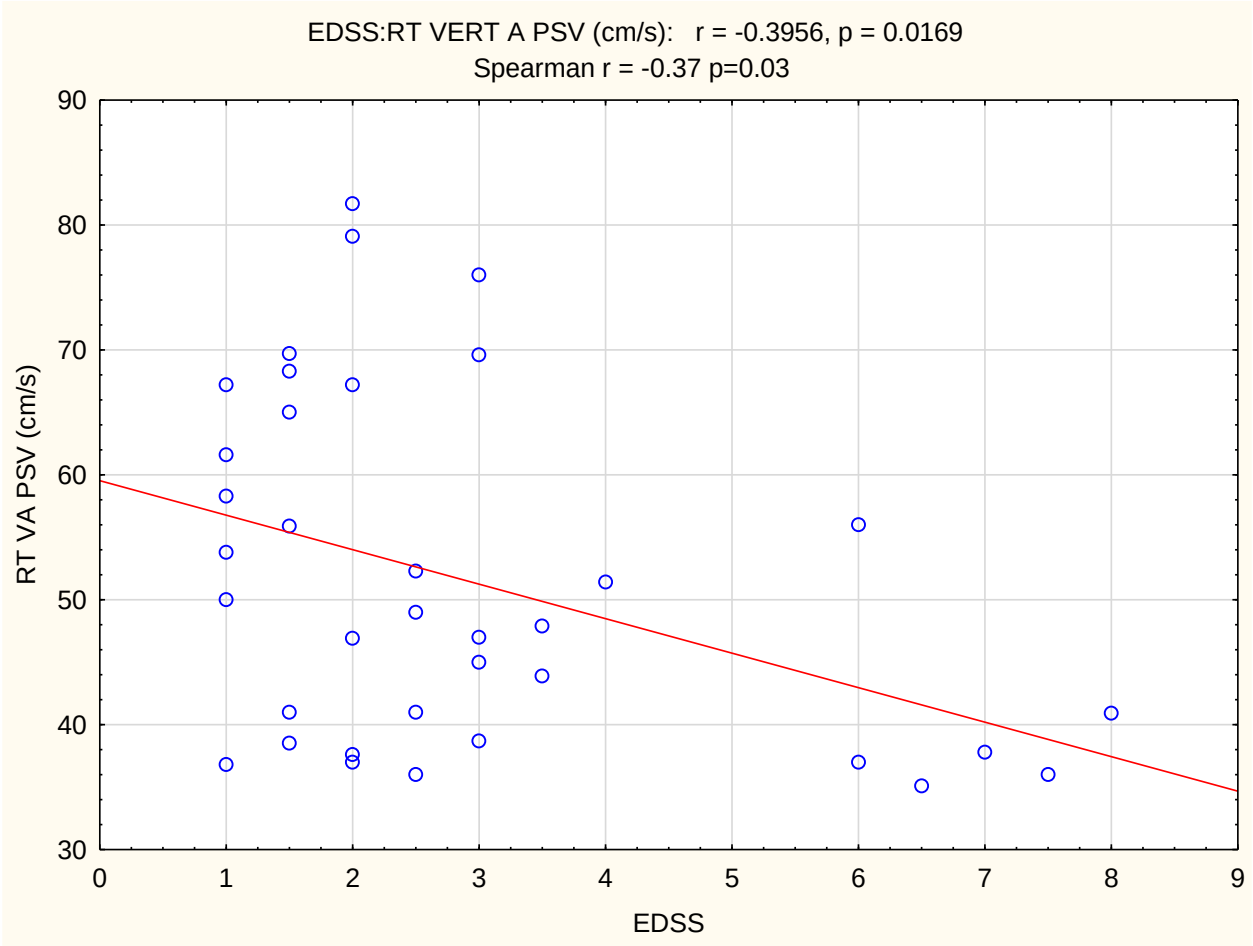

Figure 4.26: Significant negative association between disability and the PSV of the right VA

RT, right; VA, vertebral artery; PSV, peak systolic velocity; EDSS, expanded disability status scale

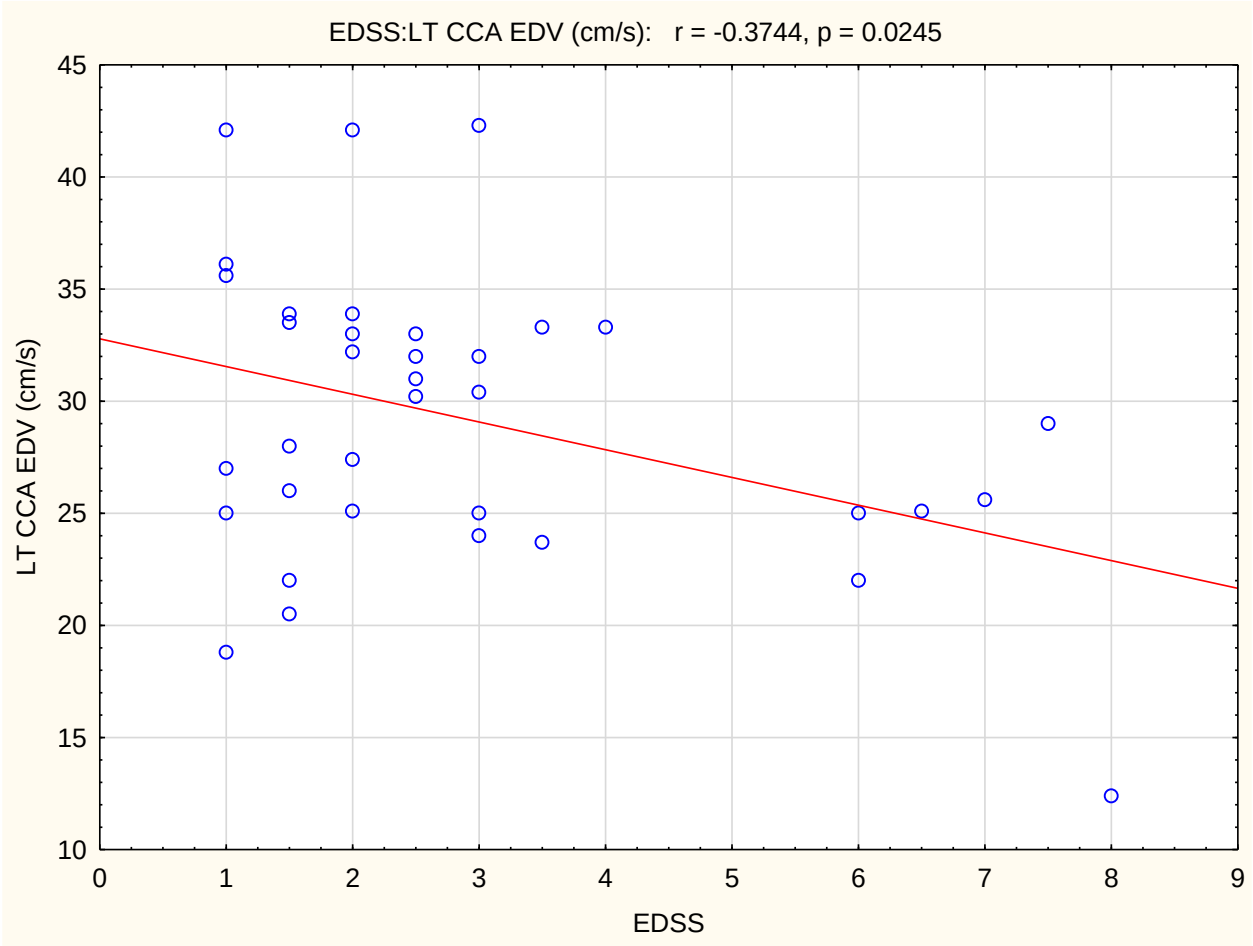

Figure 4.27: Significant negative association between disability and the EDV of the left CCA

LT, left; CCA, common carotid artery; EDV, end-diastolic velocity; EDSS, expanded disability status scale

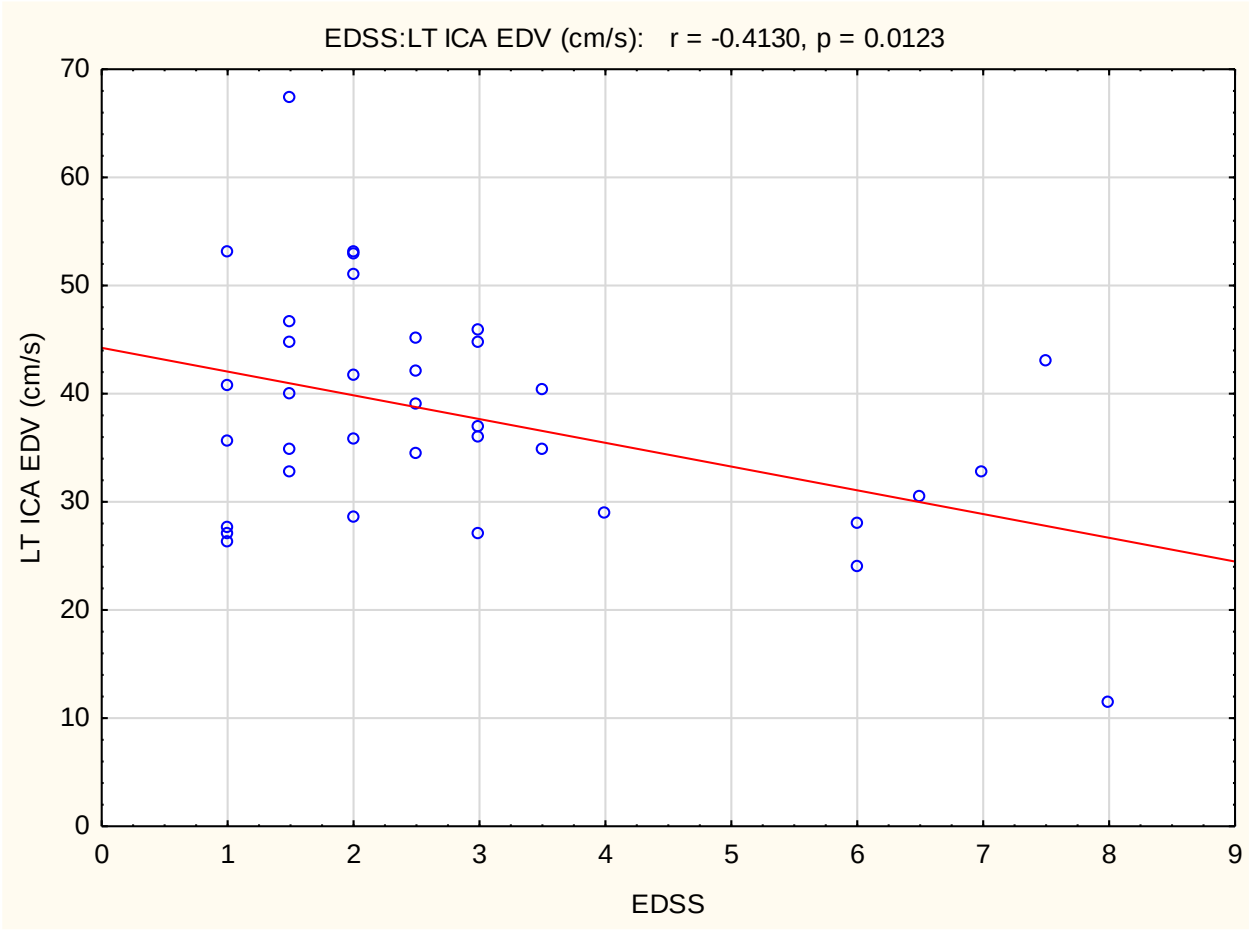

Figure 4.28: Significant negative association between disability and the EDV of the left ICA

LT, left; ICA, internal carotid artery; EDV, end-diastolic velocity; EDSS, expanded disability status scale

CONCLUSION

The study data provided a magnitude of significant and non-significant results which will be discussed and described in chapter 5.

CHAPTER 5
DISCUSSION

INTRODUCTION

Multiple sclerosis is described as a complex chronic inflammatory disease involving vascular, genetic, lifestyle and biochemical factors. This study demonstrated multiple significant associations between lifestyle, biochemical and disability parameters which unfold new hypotheses regarding the pathophysiological processes associated with MS. The discussion includes the associations in the match-merged cohort of MS participants (from Study A and Study B) and healthy controls. Study A is a previous ethically approved study titled: Ultrasound evaluation of the extracranial cerebrospinal venous system and carotid arteries in patients with multiple sclerosis; and study B is the current study titled: Ultrasound investigation of risk factors for extracranial vascular pathology in patients with multiple sclerosis (MS).

5.1. Vascular ultrasound parameters in the cohort of MS participants and controls

The internal jugular veins (IJVs) are the main venous drainage pathway for blood leaving the brain where venous outflow is more prominent in the supine position (Al-Omari & Rousan, 2010). According to Bos et al. (2016) and Czyzewska et al. (2015) the cross-sectional area (CSA) of the right IJV is larger than the left IJV in healthy persons. Stenosis of the IJV, defined as a reduction in the CSA of the IJV, is on one of the multiple CCSVI criteria in MS patients (Zamboni et al., 2009a, Zamboni et al., 2009b; Doepp et al., 2010). However this hypothesis of CCSVI and its association with MS has been disproved by Wattjes et al. (2011) and Gandhi et al. (2019) who state that CCSVI does not play a causative role in MS. In this study, one out of the 25 MS participants displayed a stenosis of the distal segment of the left IJV, however there was no evidence of significant venous reflux, valvular incompetence or septae. The patient therefore did not fulfil the criteria for CCSVI as this was an isolated finding. Pelizzari et al. (2018) conducted a 5 year longitudinal study aimed to investigate the evolution of the CSA of the major neck vessels in patients with MS and in healthy controls. Their findings indicated a significant consistent reduction of the IJV CSA with time, which may suggest that an association exists between IJV CSA and the disease course of MS. However, in this cross-sectional study there was no significant reduction in the IJV CSA in MS participants (n=25) compared to healthy controls (n=25).

According to Sethi et al. (2017), patients with MS exhibit reduced venous flow in the IJVs in comparison to healthy controls. On the contrary, in this study, peak velocity of the mid right IJV in the MS participants was significantly higher in comparison to the healthy controls ($p=0.02$, Figure 4.1, Table 4.4). There was no evidence of stenosis (reduced CSA) of the mid right IJV in the MS group which could cause the increased peak velocity.

5.2 Genetics and vascular ultrasound parameters

In this study, a significant association was found between the cross-sectional diameter (CSD) of the external carotid artery and genetic parameter (HLA DRB1*1501 allele) (p=0.03, Table 4.5). Although HLA DRB1*1501 has the strongest association with MS risk (Didonna & Oksenberg, 2015), no previous studies have been conducted to determine an association between genetics and carotid artery disease in MS. Therefore, this finding is novel and further research needs to be conducted to determine the effects of genetics on the extracranial vasculature in MS.

5.3 Biochemistry and vascular ultrasound parameters

Serum ferritin is described as a major iron storage protein and increased levels of ferritin are attributed to inflammation, infection and malignancy (Wang et al., 2010; Knovich et al., 2009). Kell and Pretorius (2014) confirm that serum ferritin is an inflammatory biomarker, which leaks from damaged cells and correlates with numerous inflammatory and degenerative diseases. Ma et al. (2015) conducted a study on serum ferritin levels and its association with carotid atherosclerosis in Chinese postmenopausal women and found that serum ferritin was independently associated with carotid atherosclerosis. The cIMT and prevalence of carotid plaque lesions were significantly increased in persons with increased levels of serum ferritin. These findings are further corroborated by Prats-Puig et al. (2016) who conducted a study to determine the association between circulating ferritin levels and carotid intima-media thickness (cIMT) in a cohort of healthy children and found a significant positive association between children's serum ferritin levels and cIMT (r=0.123; P=0.001). This current study demonstrated a significant positive association between cIMT and ferritin (Pearson p-value 0.01; Table 4.15) in the control group, which is keeping with findings of previous studies of similar context.

Folate is vital for neural tube development during infancy and is involved in maintaining the normal function of the nervous system in all ages as it plays an important role in the conversion of homocysteine to methionine (Horvat et al., 2016, Reynolds, 2006). Homocysteine, proven to correlate with the severity of coronary artery disease (CAD) (Shenoy et al., 2014), is an independent risk factor for atherosclerosis which is the most common pathological process that leads to cardiovascular diseases (Ganguly & Alam, 2015). Carotid intima-media thickness, measured by high resolution ultrasound, is accepted as the non-invasive marker for subclinical atherosclerosis (Basu et al., 2014). Increased levels of folate can reduce homocysteine levels to the normal range (Liebman & Weitz, 2014) which in turn can reduce the risk of cardiovascular disease. In addition, Durga et al. (2005) postulate that low concentrations of folate, which play a pathogenic role in atherosclerosis, are significantly associated with cIMT. However, this study found a significant positive association (Spearman p-value 0.02) between serum folate and cIMT in the cohort of MS participants; contradicting the results of previous

studies which demonstrated that serum folate is negatively associated with cIMT. This leads to question (I) why the finding is contradictory and (II) is this finding only specific to MS.

High-density lipoprotein (HDL), known to facilitate cholesterol uptake, plays an atheroprotective role by inducing the regression of atherosclerotic cardiovascular disease (Klancic et al., 2016; Fisher et al., 2012). The mechanism by which HDL is antiatherogenic is through reverse cholesterol transport. Through this pathway excess cholesterol is transferred from peripheral tissues, including arterial wall macrophages, to the liver for excretion (Rader, 2006; Tall, 2008). According to Stein (2004), risk factors for coronary artery disease (CAD) are the same as for cerebrovascular disease. In the current study, HDL had a significant negative association (Spearman p-value 0.03) with cIMT in MS participants. As per Tettey and colleagues (2014), low high-density lipoprotein cholesterol (HDL-c) levels are associated with increased cIMT, whereas higher levels are associated with lower acute inflammatory activity and lower disability in MS. A study by Liuba et al. (2003), which investigated whether acute infections in children could alter the carotid wall morphology and the lipid profile, demonstrated that acute infections in children appear to be complemented by enhanced oxidative modification of low-density lipoprotein (LDL) and a decrease in HDL-c. Carotid artery intima-media thickening may result from these lipid changes. Similarly, Tiozzo et al. (2016), postulate that HDL_2, a subfraction of HDL, is inversely associated with cIMT and may have a protective biological role against atherosclerosis. The findings of this study suggest that MS patients may be at increased risk of developing CVD.

In this study, significant associations were also found between the right and left carotid artery peak and end-diastolic blood flow velocities and biochemical parameters. Total cholesterol was negatively associated with end-diastolic velocities of the left CCA and ICA in the MS group and the peak systolic velocity of the left vertebral artery in the control group. An increase in cholesterol signifies a reduction in blood flow in diastole within the vessels. Farhoudi et al. (2011) report that a higher cholesterol level (LDL,180 mg/dL), decreases mean blood flow velocity of the internal carotid artery. A reduction in blood flow velocity results in low shear stress which induces a reduction in the vessel diameter and intimal hyperplasia (Papaioanno & Stefanadis, 2005). Lowering total cholesterol levels will therefore improve blood flow in MS and prevent intimal hyperplasia associated with early atherosclerosis.

Alíefendíoğlu and colleagues (2007) conducted a study to determine the effects on iron deficiency anaemia on cerebral blood flow in infants aged 6-36 months. Their findings showed a significant increase in cerebral blood flow velocities in children with low haemoglobin values. This may be due to increased cardiac output and decreased vascular resistance caused by anaemia. In this study, haemoglobin levels were negatively associated with the carotid artery

blood flow velocities in the MS cohort of participants (Table 4.16), where extracranial arterial blood flow was increased in MS participants with low haemoglobin values. This finding confirms the study findings of Alíefendíoğlu and colleagues (2007).

Iron deficiency and increased homocysteine levels in MS is associated with vascular damage (Van Rensburg et al., 2006). Serum iron levels in the MS group were positively associated with blood flow velocities in the right ICA and right ICA/ECA ratios, negatively associated with the peak systolic velocity of the right ECA and negatively associated with the peak systolic velocity of the right ECA in controls. The lack of literature on the association between blood flow velocities in the extracranial vascular systems and biochemical parameters in MS suggests that the significant associations found between carotid artery blood flow velocities and biochemical parameters in this study are novel and further prospective studies need to be undertaken to verify or contradict the current findings.

In this study, a significant association was also found between the cross-sectional diameter (CSD) of the carotid arteries and biochemical parameters (s-iron, s-transferrin, LDL, triglycerides, %Tf saturation, s-folate, homocysteine, CRP, 25 OH-Vit D and HDL). There's no evidence in literature which reports studies conducted on the assessment of the sonographically measured cross-sectional diameters of the carotid arteries in MS and its association with biochemical parameters.

Furthermore, this study also indicated a significant association between the CSA of the IJVs and biochemical parameters [%Tf saturation (negative association); homocysteine (positive association); 25-OH Vitamin D (negative association); HDL (negative association)].
The IJVs are the dominant drainage pathway for the cerebrovenous system in the supine position. Arterial inflow from the carotid arteries into the brain must equal venous outflow from the cranium (Mancini et al., 2014). In this study, significant associations were found between the peak velocities of the IJVs and biochemical parameters (s-iron, s-transferrin, %Tf saturation, ferritin, haemoglobin, vitamin B12, homocysteine, CRP, 25 OH-Vit D, HDL and triglycerides). The reason for the significance of these associations remains unanswered as literature on IJV blood flow velocity in MS and biochemistry has not been reported.

There are numerous questions that emanate from these new findings, thus the clinical relevance of the findings of IJV CSA and its significant association with biochemical parameters in MS in this current study need to be investigated further.

5.4 Lifestyle factors and vascular ultrasound parameters

There is evidence that intake of saturated fatty acids is associated with increased risk of coronary artery disease (Hu et al., 2001 Hooper et al., 2001). A significant positive association between cIMT and saturated fat (Spearman p-value <0.01) was found in the MS cohort of participants in the study conducted.

Reducing dietary intake of saturated fat is recommended for reducing CVD risk based on the hypothesis that saturated fat is positively associated with low-density lipoprotein cholesterol (LDL-c) which plays a role in the development of atherosclerosis and coronary heart disease (Liu et al., 2017; Sacks et al., 2017). Greater consumption of dietary pulses and lower saturated fat is significantly associated with lower cIMT and cholesterol, thus reducing the risk of CVD (Chiavaroli et al., 2017). Similarly, Petersen et al. (2014) conducted a study to review the correlation and association between dietary components and cIMT. They found that saturated fat is positively associated with cIMT and a Mediterranean style dietary pattern (high in fruits, wholegrains, fibre, olive oil and low in saturated fat) may reduce carotid atherosclerosis development and progression.

This current study also demonstrated a significant association between the CSD of the carotid arteries and lifestyle parameters (alcohol intake, consumption of chicken with skin and saturated fat). Lee et al. (2009) have shown that alcohol intake was significantly positively associated with the common carotid artery diameter in men and women. Significant associations were found between the right and left carotid artery peak and end-diastolic blood flow velocities and lifestyle parameters (intake of saturated fat, consumption of chicken with skin, fruit/vegetable/fibre intake, dietary folate, physical activity, passive smoking and alcohol intake). During physical exercise, cerebral blood flow increases, resulting in an increase in the blood flow velocity in the CCA, ICA and ECA (Sato et al., 2011). Similarly, in this study, a significant positive association was found between physical activity and peak and end-diastolic velocities in the right and left carotid arteries in MS participants. Hagg et al. (2005), Fragoso (2014) and Marck et al. (2016) postulate that physical exercise improves cardiac and vascular function by reducing cIMT and lowering the risk of CVD in MS. Participation in physical activity by MS patients may improve vascular function, slow down disability progression and improve quality of life in MS.

Passive smoking increases the risk of cardiovascular disease by approximately 30% and the effects thereof are nearly as damaging as those from chronic active smoking (Barnoya & Glantz, 2005). This current study excluded smokers, however still demonstrated a significant negative association between passive smoking and blood flow velocity in the internal carotid arteries in the MS and control groups. The blood flow velocity in the major neck arteries was significantly reduced in passive smokers in comparison to non-smokers. Kim and Gornik (2014) postulate that smoking increases blood viscosity in a dose-dependent manner. This

means that an increase in blood viscosity causes a reduction in flow rate due an in increase in friction between the blood and vessel wall. This could explain the study finding of reduced blood flow in the internal carotid arteries in passive smokers. Cessation of passive smoking will therefore not only reduce blood viscosity and improve blood flow, but reduce the risk of CVD in MS. Regarding passive smoking and its positive association with IJV CSA in MS persons and healthy controls, it can be questioned whether the increase in IJV CSA can be due to an increase in the IJV pressure.

A significant difference was found in carotid artery blood flow velocities between MS persons and healthy controls who drank alcohol and those who abstained from alcohol intake (MS persons; Kruskal-Wallis p-value 0.02 and controls; Mann-Whitney U test p-value 0.01). The blood flow velocities (cm/s) in the right CCA, right VA and left ICA were significantly higher in persons drinking alcohol (1-13 units/week) in comparison to those abstaining. According to Schminke et al., (2005) alcohol intake shows a protective effect against atherosclerosis. Gundersen et al., (2013) postulate that the vasoactive properties of social alcohol intake are dose dependent, suggesting that low doses of alcohol intake increases cerebral blood flow, which is congruent with the current study findings. Furthermore, long-term alcohol consumption results in cerebral hypoperfusion (Gundersen et al., 2013).

It can thus be concluded that modification of lifestyle and dietary habits, involving increased consumption of polyunsaturated fats and reduced consumption of saturated fats, may reduce the risk of CVD in persons with MS.

5.5 Disability and vascular ultrasound parameters

Multiple sclerosis (MS) is the most common cause of neurological disability in young adults (Kingwell et al., 2013; Marck et al., 2017). The primary purpose of early treatment in MS is to reduce neurological disability and slow down disease progression (Jokubaitis et al., 2015).
The disability status of MS persons is assessed using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). This scale quantifies disability in MS patients according to their signs and symptoms observed during a neurological assessment (Madras, 2014).
The associations between MS disability and carotid artery atherosclerosis is currently under-investigated, therefore literature on associations between cIMT and EDSS remains limited.
Talaat and colleagues (2015) investigated possible carotid artery changes and arterial stiffness in MS patients where the EDSS, pulse wave velocity, ankle brachial index and cIMT were measured in all participants. The study included 33 patients with MS and 22 healthy controls. Their findings showed that there were no significant correlations between cIMT and EDSS in MS patients. On the contrary, Nelson et al. (2014) investigated the prevalence of vascular

dysfunction and the effects of biochemical and lifestyle factors on carotid arteries and internal jugular veins (IJVs) in patients diagnosed with multiple sclerosis (MS); and found that EDSS was significantly associated with cIMT ($p<0.0001$). This is congruent with the findings of this current study which demonstrates that EDSS is significantly positively associated with cIMT in persons with MS (Pearson p-value <0.01). This present study also showed that the disability status of MS participants was significantly negatively associated with the blood flow velocities in the right and left CCA, ICA and right VA. The peak and end-diastolic blood flow velocities were lower in MS persons with a greater disability. This association suggests that due to greater disability, physical activity is limited, resulting in altered peripheral circulation and cerebral hypoperfusion.

Although more research is needed to determine associations between MS disability and carotid artery disease, it can be deduced that cIMT is significantly associated with MS disability. Reducing risk factors associated with carotid intima media thickening may reduce the risk of CVD and slow down disability progression in MS patients.

CONCLUSION

This cross-sectional observational case-control study has demonstrated, with vascular ultrasound, a plethora of significant positive and negative associations between extracranial vascular ultrasound variables and genetic, lifestyle, biochemical and vascular factors as well as MS disability. Some of these significant findings were determined in previous MS studies of similar context, however there are several novel findings which unfolded in this study. The new MS findings include significant associations between: 1) the carotid vessel diameters and biochemical and lifestyle parameters as well as the presence of the HLA DRB1*1501 allele; 2) IJV CSA in MS and biochemical and lifestyle parameters, specifically passive smoking; 3) MS disability and carotid artery blood flow velocities; and 4) carotid artery blood flow parameters and biochemical markers.

These encouraging findings indicate that the pathophysiological processes associated with MS and disability progression is complex and integrated, where vascular factors play an integral part in the disease process. Further studies are therefore needed to establish the clinical relevance of these new findings and determine treatment measures to reduce MS disability and improve quality of life.

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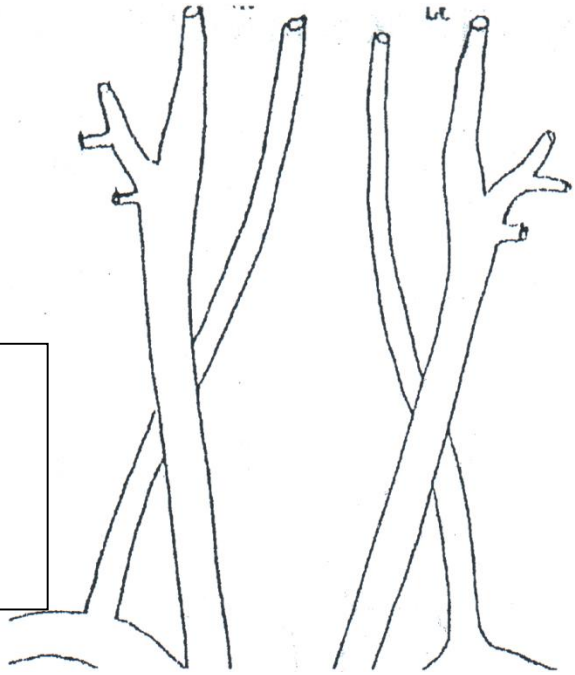
Appendix A : CAROTID DUPLEX REPORT

PATIENT NAME: DATE:

AGE: CONTROL/MS:



PLAQUE:
Calcified
Mixed
Fibrous
Soft
Ulcerated
Sub-plaque haemorrhage

PLAQUE:
Calcified
Mixed
Fibrous
Soft
Ulcerated
Sub-plaque haemorrhage

RIGHT LEFT

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

COMMENT:

(Adapted from the E22 vascular laboratory, Groote Schuur Hospital, Carotid Artery Duplex template)

Appendix B. DOPPLER SPECTRAL ANALYSIS

Diameter stenosis	Peak Systolic velocity	ICA/CCA Systolic ratio	ICA EDV
0-49%	< 125cm/s	<2	<40cm/s
50-69%	125-210cm/s	2-3	40-70cm/s
70-79%	>210cm/s	>3	70-100cm/s
80-99%	>280cm/s	>3.7	>100cm/s
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 C: INTERNAL JUGULAR VEIN (IJV) DUPLEX

PATIENT NAME: DATE:

AGE: CONTROL / MS

RIGHT		LEFT
Velocity (cm/s) =	PATENT Proximal IJV	Velocity (cm/s)=
Velocity (cm/s)=	Mid IJV	Velocity (cm/s)=
Velocity (cm/s)=	Distal IJV	Velocity (cm/s)=
	CSA proximal IJV (mm^2)	

Appendix D: BRAINBIOCHEM QUESTIONNAIRE

DATE:	PATIENT CODE:
FOLLOW-UP:	BRAINBIOCHEM CONSENT CODE:
VISIT: FOLLOW-UP	CONSENT FOR STORING DNA:
First Name	
Middle Name	
Surname	
Title	
Address	
Date Of Birth	
Age (Yrs)	
Marital status	
Number of children	
Highest level of education	
Profession	
Employment (Full time/Part time)	
Country of Residence	
Race	
Gender	
Home / Work Phone Number	
Email address	
Cell Phone Number	
Health Practitioners	
Referred by:	

MEDICAL HISTORY

Do you have a medical diagnosis or condition?

Please specify:	
Or control subject for research	

How old are you now?	
How old were you when the diagnosis was first made? (years of age)	
How old were you when you noted the first symptoms?	

Which symptoms are associated with your illness or condition?

Please Specify:	

In your opinion, what event(s) preceded the diagnosis that may have caused the condition?

Did you make a change in diet/lifestyle before diagnosis/relapse (e.g. did you start taking coconut oil)?

In your opinion, are you resistant to common infections such as colds and flu?	
Do you tend to push yourself harder than other people do?	
Are you a risk-taker?	
Would you neglect your own health to improve the health of other people?	

Height (in metres)	
Weight (in kilogrammes)	
Calculated BMI:	

Blood pressure:

Systolic	
Diastolic	

The following questions are meant to find out what dietary and lifestyle habits may have had an effect on your health
(1) at the time of diagnosis
(2) at follow-up, after you have implicated any lifestyle changes

DIET QUESTIONNAIRE
How many times a week have you consumed the following foods over the previous 3 months? E.g. 2x

Beef hamburgers / minced meat	
Red meat (e.g. beef, lamb, mutton)	
Fried chicken / cooked chicken with skin	
Hot dogs / processed meat / Meat pies	
Salad dressings (excludes 'Lite' versions)	
Butter and margarine (excludes 'Flora olive' / proactive versions)	
Eggs (excludes cooking and baking)	
Full cream milk and full cream dairy products	
Fried hot potato chips, potato crisps, corn chips, buttered popcorn	
Biscuits, cake, cookies, pastries, muffins	
SATURATED AND TRANS-FAT SCORE (Office Use)	

Fibre intake through Bread: White/Whole Wheat/ Brown/Rye/Other	

Fibre intake through Cereals: Cooked Oats	
Fibre intake through Cereals: Sorghum	
Fibre intake through Cereals: High fibre instant cereal	
Fibre intake through legumes (beans, peas, lentils)	
Potatoes (with the skin on) - no creamy sauces or butter added	
Five portions fruits and/or vegetables (whole, salad)	
FRUIT / VEG / FIBRE SCORE (Office use)	

Broccoli, cauliflower, mushrooms	
Avocado, spinach	
Citrus – oranges, grapefruit	
Turnips / artichokes	
Organ meats (e.g. liver, kidney, giblets)	
FOLATE SCORE (Office use)	

MEDITERRANEAN DIET

Fish and seafood	
Olives and olive oil on salads	
Nuts and seeds (flaxseeds, rapeseed, pumpkin, walnuts)	
Plant-based meals: fruits, vegetables, beans, nuts, legumes, seeds	
Herbs and spices instead of salt	
Red wine 1 glass/day or less (OPTIONAL)	
MEDITERRANEAN DIET SCORE (Office use)	

IMPROVING EYESIGHT

Red/orange/yellow peppers	
Kale, spinach, pumpkin, carrots, berries, mangoes, grapes, kiwi fruit	
LUTEIN / ZEAZANTHIN (MACULA/RETINA) SCORE (Office use)	

Coffee, Green Tea, Rooibos Tea	
Sweets or Chocolates	
Carbonated Drinks	
"Light" or "Diet" Carbonated Drinks?	
If you take Probiotics, how often?	
Please also provide the purpose that it is being used for:	
Please also provide the strain:	
What oil do you use for cooking?	
Food allergy/intolerance	

FOODS THAT PROMOTE GLYCATION

Beef or beef sausage fried over the fire, or stir-fried	
Crumbed fried chicken	
Chicken with skin roasted or fried	
Bacon fried until brown	
AGEs SCORE (Office use)	

PHYSICAL ACTIVITY
Which best describes your physical activity status? (Includes walking, swimming, cycling, attending exercise classes, each lasting more than 30 minutes)

Recreational sport occasionally or complete lack of exercise	
Recreational sport 1 time a week	
Exercise 2 - 3 times a week	
Exercise 4 or more times a week	

DAYTIME ACTIVITY
Which best describes your day-time or occupational activity?

Sedentary (desk, work, driving, etc.)	
Moderate (house work, gardening, walk often)	
Intense physical labour (building and construction work, etc.)	

ALCOHOL CONSUMPTION
How many units of alcohol do you consume on average per week? One unit alcohol equals 250 ml beer or lager, 1 glass (125 ml) of wine, 1 pub measure of spirits.

Abstain	
1 - 2 units occasionally only	
3 - 13 units	
14 - 21 units	
22 units or more	

BLOOD LOSS
Blood Donor?

No	
Current	
Previous	

IRON INTAKE

No		
Current		
Previous		

VITAMIN B12 INJECTIONS

No	
Current	
Previous	

VEGETARIAN/VEGAN

No	
Current	
Previous	

HRT AND CONTRACEPTIVES
FEMALES -

Are you pregnant	
Taking Hormone replacement therapy (HRT)	
Taking oral contraceptive pill (OCP)	
!!! NB: If relevant, please provide the name of the medication	

SMOKING OR DRUG EXPOSURE

This section captures your exposure to smoke or recreational drugs prior to diagnosis.
The information is completely confidential.

Have you ever smoked tobacco (cigarettes, cigars or pipe?)

NO, never smoked	
YES, previously	
CURRENT SMOKER	

Have you been exposed to significant PASSIVE SMOKING prior to diagnosis or relapse?

NO, never been exposed to tobacco smoke	
YES, previously	
YES, at the time of diagnosis	
Did your father/mother/other family member smoke when you were young?	

IF YES
Please provide the following information:

If you have ever smoked, what would the total number of years be that you did smoke if you added them up?	
If you did not smoke, how long were you exposed to passive smoking before diagnosis/relapse?	
What was your highest number of cigarettes per day?	
What was your average number of cigarettes per day?	
If you stopped smoking, when was the last time you smoked?	
If you smoked within the past 30 days, how many cigarettes did you smoke every day on average?	

*Have you ever taken recreational drugs (cannabis etc.)?*_____
(Please specify) _____
SLEEP
Do you sleep at least 7 hours per night?

IF NO

How many hours do you sleep at night on average?	
How many times do you wake up during the night on average?	
Do you turn on the light when you wake up?	
How long does it take you to fall asleep again on average?	
How many hours do you sleep during the day?	
Is your room dark at night?	
Is your bed comfortable?	

Do you have conditions that prevent you from sleeping peacefully? Please specify

Do you have pain? Please specify what type of pain, how long you have had the pain and what you are doing to alleviate it

Type?	
How long?	
Alleviation?	

More information:	

Do you have problems with cognition?	Age at Onset	Medication	Side effects
Memory			
Depression / Anxiety LOW MOOD			
Other neurological or psychiatric diagnosis?			

MEDICAL HISTORY CONTINUED
The questions that follow assess the way your body responds to nutrition and injuries.
This first section relates to the way your body absorbs, uses or loses iron.

	Age at Onset	Medication	Side effects
Anaemia			
Heamochromatosis (iron overload)			
Thalassaemia			
Restless Legs Syndrome			
Affected by Blood loss during Child Birth			
Trauma with blood loss (shock)			
Major Surgery CAESAREAN			
Blood Donation			
Heavy Menstruation			
Porphyria-like symptoms			
Unexplained episodes of SEVERE abdominal pain			
Sun induced rashes			
Miscarriages (accidental loss of pregnancy)			
Abortions (medical facilitated loss of pregnancy)			

This section relates to the way your body absorbs, uses or loses energy and nutrients.

	Age at Onset	Medication	Side effects
Obesity/Overweight			
Metabolic syndrome/Insulin resistance			
Low body weight			
Anorexia (weight loss)			
Recurrent fatigue			
Chronic Fatigue Syndrome/ Yuppie Flu (ME)			
Fibromyalgia			
Low Blood Sugar			
Low Blood Pressure			
Recurrent diarrhoea			
Irritable bowel disease/Crohn's disease/Ulcerative colitis			
Coeliac disease			

This section relates to the way your body responds to infections and allergens.

	Age at Onset	Medication	Side effects

Recurrent sinusitis			
Asthma			
Hay fever			
Recurrent cystitis (bladder infection)			
Inflamed gums (gingivitis or periodontitis)/ Teeth with root canals			
Serious prolonged infection (e.g. hospitalisation when you were young)			
Epstein Barr Virus/ Coxackie virus			
Candida			
Other Chronic Infections (please specify):			

Symptoms of Cardiovascular Disease

	Age at Onset	Medication	Side effects
Hypothyroidism			
Heart Attack / CVD			
Heart Failure			
Hypertension			
High cholesterol			
Stroke			
Thrombosis			
Varicose veins			
Metabolic Syndrome / Diabetes			

The following section relates to the possible influence of occupational, surgical, accidental, or deliberate exposures to any of the following on your health.
Please choose "YES" if any of the following conditions or events have ever applied

Heavy / occupational exposure to solvents, glues or volatile substances (like benzene, toluene, contact adhesives, paints, welding fumes, etc.)	
Significant exposure to toxins / poisons such as spraying fruit trees or insects	
Occupational exposure to anaesthesia gases (anaesthetist, dental hygienist, theatre sister)	
Exposure to general anaesthesia (including dental procedures) before diagnosis/relapse	
Exposure to nitrous oxide (Entonox/laughing gas) during labour	
If you have ever had general anaesthesia, how many times?	
Did you have dental fillings removed before diagnosis/relapse?	

MEDICATION

What prescription medication were you on when you were diagnosed/relapsed? (e.g. malaria prophylaxis)

What NON-PRESCRIPTION medication were you on when you were diagnosed/relapsed? (e.g. Panado)

What NUTRITIONAL SUPPLEMENTS (i.e., NOT INCLUDING NATURAL REMEDIES OR HERBS) - were you taking when you were diagnosed/relapsed? (i.e. off the shelf vitamins and minerals)

Were you taking any HOMEOPATHIC MEDICINE? (i.e. remedies prescribed by a neuropath, homeopath, acupuncturist, chelation therapist, etc. based on your own research)?

FAMILY MEDICAL HISTORY
In this page you are asked to enter any of the following illnesses or conditions in your family. Please provide the most accurate information that you can
Please select which, if any, of the following apply to THE CLIENT's genetic ancestry. This information is not essential, but will facilitate accurate interpretation of the test results:

African - South of the Equator	
African - North of the Equator	
European - Scottish	
European - Scandinavian	
European - Other	
Asian	
Indian	
South American	
Arabic	
Other (Please Specify)	

FAMILY SUBSTANCE ABUSE
Certain familial problems with alcohol and drug use or senility are relevant in evaluating risks for the CLIENT. Please provide the following information for this purpose if you experienced any of the following in your family home:

	Affected Family Member
Substance Abuse or Dependence: Alcoholism	
Substance Abuse or Dependence: Drug abuse	

MEDICAL CONDITIONS - Family History
Are any of your family members (e.g. grandparents, parents, siblings, uncles, aunts, nephews, or cousins) affected by any of the following conditions?

	Affected Family Member (s)	Age at onset
Multiple sclerosis		
Anaemia		
Alzheimer’s disease/dementia		
Hemochromatosis		
Restless Legs syndrome		
Porphyria		
Hypothyroidism		
Heart Attack / CVD		
Heart Failure		
Hypertension		
High cholesterol		
Stroke		
Varicose veins		
Recurrent pregnancy loss		
Thrombosis		
Obesity		
Metabolic Syndrome		
Diabetes Type 1		
Diabetes (adult onset/Type II)		

Crohn's Disease/Ulcerative colitis/IBD		
Coeliac Disease		
Recurrent Sinusitis		
Recurrent cystitis (bladder infection)		
Asthma		
Hay Fever		
Parkinson's Disease		
Migraine		
Recurrent Headaches		
Epilepsy		
Bipolar		
Stress/Depression/Anxiety		
Schizophrenia		
Lupus (SLE)/Rheumatoid arthritis		
Thyroid disease		
Cancer		
Other Diseases not included on this list		

Appendix E: LETTER OF PERMISSION FROM RESEARCH SITE

BERGMAN, ROSS & PARTNERS
RADIOLOGISTS

PR No 3803465
VAT No 4530146937

BERGMAN, ROSS & PARTNERS RADIOLOGISTS

DR J.W. BERGMAN
M.B.CHB(UCT), DCH(SA), FCP(SA)PAEDS, F.F.RAD(D)(SA)
DR J.L. ROSS
M.B.CHB(UCT), F.F.RAD(D)(SA)
DR B.A. COTTON
M.B.CHB(UCT), F.C.RAD(SA)
DR J.V. BEKKER
M.B.CHB(STEL), M.MED(RAD.D.)(STEL)
DR J.C. BASSON
M.B.CHB(STEL), M.MED(RAD.D.)(STEL)
DR Y. VADACHIA
M.B.CHB(UCT), M.MED(RAD.D.)(STEL)
DR M.A. HAYES
M.B.CHB(UCT), F.C.RAD(SA)
DR A.D. BRANDT
M.B.CHB(UCT), F.C.RAD(DIAG)(SA)
DR S.H. SY MINGTON
M.B.CHB(PRET), M.MED(RAD.D)(PRET)
DR A. SABAN - Radiologist Assistant
M.B.CHB(UCT), F.C.RAD(DIAG)(SA)
PROF A. SCHER - Radiologist Assistant
M.B.CH.B.(UCT),D.M.R.D(R.C.P&S.)(LONDON),F.C.RAD.(D)SA

26 February 2016

Dear Merlisa

Re: Approval to Utilize Bergman Ross & Partners Radiology Facilities for the Ultrasound Investigation of Risk Factors for Extra Cranial Vascular Pathology in Multiple Sclerosis (MS)

This letter hereby confirms that Bergman Ross and Partners (BRP) have approved the utilization of the N1 City Hospital and Kuilsrivier Hospital for your Doctoral Project mentioned above.

Bergman Ross and Partners wish you everything of the best in obtaining the qualification of Doctor of Radiography (DGRDGR).

We thank you for considering the use of our facilities and welcome the academic integration.

Kind Regards

Machelle Sadler

MACHELLE SADLER
Practice Manager
Bergman Ross & Partners
PO Box 12716
N1 City 7463
021 5952515

Appendix F: ETHICS 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
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

7 May 2018
REC Approval Reference No:
CPUT/HW-REC 2017/H4(renewal)

Faculty of Health and Wellness Sciences – Medical Imaging and Therapeutic Sciences

Dear Ms Kemp

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC on 30 March 2017 to Ms Merlisa Kemp – 196095433 for ethical clearance. This approval is for research activities related to student research in the Department of Medical Imaging and Therapeutic Science at this Institution.

TITLE: Ultrasound investigation of risk factors for extracranial vascular pathology in patients with Multiple sclerosis (MS)

Supervisor: Professor P Engel-Hills and Prof SJ Van Reusburg

Comment:

Data collection permission is required and has been obtained.

Approval will not extend beyond 8 May 2020. 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.

The investigator(s) should understand the ethical 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 HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Dr. Navindhra Naidoo
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Appendix G: INFORMED CONSENT (English and Afrikaans)

CEREBROSPINAL VENOUS AND CAROTID ARTERY ULTRASOUND STUDY
INFORMED CONSENT /
INGELIGTE TOESTEMMING VIR SEREBROSPINAAL VENEUSE EN KAROTIS ARTERIE ULTRAKLANK STUDIE

INVESTIGATORS STATEMENT/ VERKLARENDE ONDERSOEK:

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'.

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.

VOLUNTARY PARTICIPATION / VRYWILLIGE DEELNAME:

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.

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

BACKGROUND / AGTERGROND:

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 and clot formation as well as plaque formation in the arteries of the neck.

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.

THE PURPOSE OF THE STUDY / DIE DOEL VAN DIE STUDIE:
The purpose of this study is to use Ultrasound to investigate the presence of 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.
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.

ULTRASOUND EXAMINATION / ULTRAKLANK ONDERSOEK:
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 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. This examination is not painful. Some patients find this test relaxing.

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.

RISK AND BENEFITS / RISIKO'S EN VOORDELE:
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.

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.

OTHER INFORMATION / ANDER INLIGTING
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. Results of the study will be published where participants will be informed of the outcome.

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. Uitslae van hierdie studie sal gepubliseer word waar narvorsings onderwerpe ingelig sal word oor die resultate.

SUBJECT’S STATEMENT / ONDERWERP VERKLARING:
I understand what the procedure will be for this Ultrasound scan. I voluntarily agree to take part in this study. I have read and/or someone has read the information pertaining to the study to me. The information is in a language with which I am fluent and comfortable. I was given an opportunity to ask questions and all my questions have been answered to my satisfaction. I understand that taking part in this study is voluntary. I may choose to leave the study at any time.

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 en my vrae was beantwoord. Ek verstaan dat ek kan enige tyd van die studie kan onttrek.

Please complete the table indicating your voluntary participation in the study / Voltooi asseblief die tabel om U vrywillige deelname aan te dui.

SIGNED AT / GETEKEN TE	
DATE / DATUM	
NAME OF WITNESS / NAAM VAN GETUIE	
SIGNATURE OF WITNESS / HANDTEKENING VAN GETUIE	
NAME OF PARTICIPANT / NAAM VAN NAVORSINGS ONDERWERP	
SIGNATURE OF PARTICIPANT / HANDTEKENING VAN NAVORSINGS ONDERWERP	

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