FRACTAL ANALYSIS APPROACH IN THE CHARACTERISATION OF CEREBROVASCULAR COMPLEXITY IN ASYMPTOMATIC CEREBRAL SMALL VESSEL DISEASE

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by

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LIST OF SYMBOLS

b	Regression coefficient
B_o	Strength of external magnetic field
D_f	Fractal dimension
ε	Scale
H^+	Hydrogen ion
H_2O	Water
Hz	Hertz
kg	Kilogram
m^2	Square meter
MHz	Mega Hertz
mm	Millimetre
mmHg	Millimetre of mercury
ms	Millisecond
N	Average counts
O	Oxygen atom
T	Tesla
t	Time
R^2	Coefficient of determination
r	Pearson correlation
r_s	Spearman correlation coefficient
s/mm^2	Seconds per square millimetre
µm	Micrometre
ω_o	Precession frequency
γ	Gyromagnetic ratio

LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
ACA	Anterior cerebral artery
ANOVA	Analysis of variance
APO E	Apolipoprotein E
BA	Basilar artery
BBB	Blood brain barrier
BMI	Body mass index
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CARASIL	Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy
CBF	Cerebral blood flow
CoW	Circle of Willis
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CSVD	Cerebral small vessel disease
CT	Computed tomography
CVR	Cerebrovascular reactivity
DICOM	Digital Imaging and Communications in Medicine
DIP	Digital image processing
DSA	Digital subtraction angiography
DTI	Diffusion tensor imaging
FLAIR	Fluid attenuated inversion recovery
FLIRT	Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool
FMRIB	Functional Magnetic Resonance Imaging of the Brain
FOV	Field of view
FSRP	Framingham Stroke Risk Profile
HDL	High density lipoprotein
HRMRI	High-Resolution Magnetic resonance imaging

HTRA1	High-temperature requirement A serine protease 1 gene
HUSM	Hospital Universiti Sains Malaysia
ICA	Internal carotid artery
ICBM	International Consortium of Brain Mapping
ICC	Intraclass correlation coefficient
IL-6	Interleukin-6
JEPeM	Jawatankuasa Etika Penyelidikan Manusia
JPEG	Joint Photographic Experts Group
LBC	Lothian Birth Cohort
LR	Linear regression
MCA	Middle cerebral artery
MIP	Maximum intensity projection
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MSS	Mild Stroke Study
NAWM	Normally appearing white matter
NIfTI	Neuroimaging Informatics Technology Initiative
PAI	Plasminogen activator inhibitor
PCA	Posterior cerebral artery
PET	Positron emission tomography
RF	Radiofrequency
RF^+	Presence of cerebral small vessel disease risk factors
RF^-	Absence of cerebral small vessel disease risk factors
RF^+ & WMH^+	Asymptomatic subjects with both cerebral small vessel disease risk factors and cerebral white matter hyperintensity
RF^- & WMH^+	Asymptomatic subjects with cerebral white matter hyperintensity without the evidence of cerebral small vessel disease risk factors
RF^+ & WMH^-	Subjects with cerebral small vessel disease risk factors only
RF^- & WMH^-	Subjects without both cerebral small vessel disease risk factors and cerebral white matter hyperintensity
SBP	Systolic blood pressure
sICAM-1	Soluble intercellular adhesion molecule -1
SLR	Simple linear regression
SPSS	Statistical Package for the Social Sciences

STRIVE	Standards for reporting vascular changes in neuroimaging
sVCAM-1	Soluble vascular cellular adhesion molecule-1
SVCI	Subcortical vascular cognitive impairment
SVD	Small vessel disease
TE	Echo time
TIFF	Tagged Image File Format
TOF	Time-of-flight
TR	Repetition time
TWS	Trainable Weka Segmentation
USM	Universiti Sains Malaysia
VA	Vertebral artery
vWF	Von Willebrand factors
WEKA	Waikato Environment for Knowledge Analysis
WMH	White matter hyperintensity
WMH^+	Asymptomatic subjects with cerebral white matter hyperintensity
WMH^-	Subjects without cerebral white matter hyperintensity

LIST OF APPENDICES

ANALISA FRAKTAL SEBAGAI PENDEKATAN UNTUK PENCIRIAN KOMPLEKSITI SEREBROVASKULAR DALAM PENYAKIT PEMBULUH DARAH KECIL SEREBRUM (TANPA SIMPTOM)

ABSTRAK

Penyakit pembuluh darah kecil (CSVD) merupakan penyakit yang dikenalpasti melalui simptom dan imej yang menunjukkan perubahan tidak normal pada otak yang diakibatkan oleh kerosakan pada pembuluh darah kecil. Kebanyakan pesakit mempunyai hiperintensiti jirim putih (WMH) pada imej otak dan dikenalpasti setelah mempunyai simptom. Pengenalpastian pada perubahan salur darah mungkin dapat membantu memantau risiko dan takat penyakit ini lebih awal memandangkan perubahan pada salur darah berlaku sebelum kecederaan pada bahagian otak berlaku. Analisa fraktal membolehkan kita mengukur kompleksiti serebrovaskular secara kuantitatif. Perubahan pada kompleksiti serebrovaskular merupakan indikator pengaliran darah yang tidak efektif yang boleh mengakibatkan kecederaan pada otak. Kajian ini dijalankan untuk mencari biopenanda awal CSVD tanpa simptom melalui pencirian kompleksiti "circle of Willis" dan salur darah yang berkaitan. Suatu kajian penerokaan keratan rentas melibatkan 22 subjek berumur 25 - 75 tahun yang mempunyai "QRISK2 score" rendah atau sederhana telah dijalankan. Subjek telah diperiksa melalui pengimejan resonans magnet / angiografi. Subjek yang mempunyai dan tidak mempunyai WMH telah dimasukkan ke dalam kajian ini. Analisa fraktal telah digunakan sebagai pendekatan pencirian kompleksiti pembuluh darah. Purata fraktal dimensi (D_f) pembuluh darah ini telah dibandingkan antara subjek yang mempunyai WMH (WMH^+; n = 8) dan subjek yang tiada WMH (WMH^-; n = 14). Purata D_f pembuluh darah ini juga telah dibandingkan antara subjek yang mempunyai

risiko untuk mendapat CSVD dan WMH (RF^+ & WMH^+; n = 6), subjek yang mempunyai risiko untuk mendapat CSVD sahaja (RF^+ & WMH^-; n = 5), dan subjek yang tidak mempunyai sebarang risiko untuk mendapat CSVD dan tiada WMH (RF^- & WMH^-; n = 9). Regresi linear mudah (SLR) telah dilakukan antara skor QRISK2 dan D_f pembuluh darah tersebut. WMH^+ mempunyai purata D_f pembuluh darah yang lebih rendah daripada WMH^-. Purata D_f pembuluh darah juga didapati lebih rendah di kalangan RF^+ & WMH^- dan RF^+ & WMH^+ berbanding dengan RF^- & WMH^-. Selain daripada itu, SLR telah menunjukkan yang kenaikan skor QRISK2 dapat meramalkan penurunan D_f pembuluh darah. Kajian ini menunjukkan penurunan dari segi kompleksiti pembuluh darah dapat dilihat pada subjek yang mempunyai risiko untuk mendapat CSVD dan juga pada subjek yang mempunyai WMH. SLR menunjukkan bahawa skor QRISK2 dapat meramalkan D_f pembuluh darah. Secara keseluruhannya, dapatan kajian ini menunjukkan potensi D_f pembuluh darah sebagai biopenanda awal CSVD. Walaubagaimanapun, kajian pada skala yang lebih besar perlu dijalankan untuk melihat potensi penggunaanya di dalam populasi yang lebih meluas.

FRACTAL ANALYSIS APPROACH IN THE CHARACTERISATION OF CEREBROVASCULAR COMPLEXITY IN ASYMPTOMATIC CEREBRAL SMALL VESSEL DISEASE

ABSTRACT

Cerebral small vessel disease (CSVD) refers to a spectrum of clinical and neuroimaging findings caused by pathological damage of small vessels of the cerebral parenchyma. Cerebral white matter hyperintensity (WMH) is one of the commonest neuroimaging findings of CSVD. Often, CSVD is diagnosed once the symptoms developed. Detection of the underlying vascular structural changes might facilitate early disease risk stratification and disease monitoring as vascular alteration precedes cerebral parenchymal injury. Of interest, fractal analysis allows us to quantitatively measure the complexity of the cerebral vascular structure in terms of fractal dimension (D_f). The cerebral vascular D_f changes are indicative of inefficient tissues perfusion which renders the cerebral parenchyma vulnerable to damage. The aim of this study is to explore a novel vascular neuroimaging marker of asymptomatic CSVD by characterising the complexity of the circle of Willis (CoW) and its tributaries as measured by D_f. An exploratory cross-sectional study was conducted involving 22 subjects of age between 25 - 75 years old with low to moderate QRISK2 score who underwent magnetic resonance imaging/angiography (MRI/MRA) examination. These subjects presented with or without WMH. The cerebral vascular complexity of the MRA image was characterised using D_f. The cerebral vascular D_f was compared between asymptomatic subjects with (WMH^+; n = 8) and without cerebral WMH (WMH^-; n = 14). Furthermore, cerebral vascular D_f was also compared between asymptomatic subjects with both CSVD risk factors and cerebral WMH (RF^+ &

WMH^+; n = 6), subjects with CSVD risk factors only (RF^+ & WMH^-; n = 5), and subjects without both CSVD risk factors and cerebral WMH (RF^- & WMH^-; n = 9). Simple linear regression (SLR) was performed between QRISK2 score and cerebral vascular D_f. Mean cerebral vascular D_f was significantly lower in the WMH^+ group than WMH^- group. Moreover, the mean cerebral vascular D_f of the RF^+ & WMH^- and RF^+ & WMH^+ groups were significantly lower than RF^- & WMH^- group. The SLR model had indicated that increased QRISK2 score significantly predicted reduction in cerebral vascular D_f. The cerebral vascular D_f was reduced in the subjects with CSVD risk factors and asymptomatic CSVD subjects with WMH. The SLR model had indicated that QRISK2 score significantly predicted cerebral vascular D_f. The results indicate that cerebral vascular D_f is a promising biomarker of asymptomatic CSVD subjects with WMH. Larger-scaled studies are required to explore its potential in a broader population setting.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

CSVD refers to a spectrum of clinical and neuroimaging findings resulting from pathological processes of various aetiologies affecting small vessels of the cerebral parenchyma (Wardlaw, Smith, & Dichgans, 2013)(Pantoni, 2010). Generally, CSVD shares similar risk factors with large vascular diseases which include ageing, increased body mass index (BMI) and hypertension (Staszewski et al., 2017). Thus far, the pathogenesis of CSVD is still not well understood. Nonetheless, it has been suggested that oxidative stress, inflammation, endothelial dysfunction, pathological vascular structural changes, blood brain barrier (BBB) impairment, and cerebral hypoperfusion are involved in the pathogenesis of CSVD (Pantoni, 2010)(De Silva & Miller, 2016)(Cuadrado-Godia et al., 2018).

Importantly, CSVD imposes a major public health challenge by contributing up to 45% of dementia and 25% of ischemic stroke (Kolominsky-Rabas et al., 2001)(Boulos et al., 2011)(Shi & Wardlaw, 2016). Often, the diagnosis of CSVD is based on incidental neuroimaging findings as there are only subtle clinical manifestations exhibited by the patients (Vermeer et al., 2007)(Fanning, Wesley, et al., 2014). In fact, CSVD is mostly found in the elderly and frequently detected in its advanced stage (A. S. Das et al., 2019). Nevertheless, it is of importance to note that CSVD patients of younger age could also present with varying degrees of clinical manifestation, from subtle mood disorders (Clancy et al., 2021) to cognitive dysfunction (Pavlović et al., 2011). Early disease screening is limited due to the silent and insidious nature of the disease (Shi & Wardlaw, 2016)(Peng et al., 2019). WMH is one of the commonest neuroimaging features of CSVD. It is important to note that

WMH is reflective of the cerebral parenchymal changes rather than the cerebral vessels itself. Detection of the underlying vascular structural changes might enable us to stratify the risk of developing CSVD at an earlier stage as vascular alteration precedes cerebral parenchymal injury.

Physiologically, blood vessels exhibit fractal patterns as the structural network is represented by larger blood vessel that progressively branched into smaller scaled blood vessels. The fractal patterns of the vascular network is essential for efficient blood flow and tissues perfusion (Losa et al., 2005). Of interest, fractal analysis, an image analysis technique based on fractal geometry allows us to quantitatively measure the fractal patterns of the vascular network in terms of D_f (Heymans et al., 2000)(Lopes & Betrouni, 2009). The D_f was measured on the segmented vascular images obtained from the vascular imaging technique.

D_f is a unitless metric which measures the complexity of irregular and self-similar structures such as blood vessels (Mandelbrot, 1983) (Di Ieva, 2016). Complexity of the vascular network describes its branching pattern which results from the underlying physiological or pathological processes. Fundamentally, reduced D_f is reflective of reduced vascular complexity of the vascular network under investigation, vice versa (Liew et al., 2011). Of relevance, cerebral vascular structural alteration and cerebral hypoperfusion has been implicated in the pathogenesis of CSVD. In the context of CSVD, deviation from the optimal cerebral vascular fractal pattern as depicted by reduced cerebral vascular D_f would indicate inefficient cerebral blood flow (CBF) and tissues perfusion. In effects, this would render the cerebral parenchyma vulnerable to damage as evidenced by cerebral WMH. Therefore, it is conceivable that we would be able to discern the D_f between the asymptomatic CSVD subjects and control. Hence, this study intends to explore a novel vascular neuroimaging marker of

asymptomatic CSVD subjects with WMH by characterising the complexity of the cerebral vascular network that encompass CoW and its tributaries as measured by D_f.

1.2 Rationale and significance of the study

To date, early disease risk stratification and disease monitoring remains as the major challenges in the management of CSVD. Ongoing research have been focusing on the search of imaging and systemic biomarkers of CSVD (Blair et al., 2017)(Pantoni et al., 2019)(Peng et al., 2019). This might open a new paradigm in understanding the pathogenesis, risk profiling, and monitoring of disease progression as well as treatment response in CSVD. Synergistically, both imaging and systemic biomarkers might enable us to detect, stratify the risk, and monitor disease development at an earlier stage. Of interest, early disease risk stratification and evaluation of extent of disease involvement might provide a longer window of opportunity to effectively implement therapeutic and preventative strategies. This is particularly important considering the clinical and economic burden caused by CSVD. Figure 1.1 illustrates the research problem of this study.

Recently, a growing body of literatures have explored the role of retinal vascular complexity as CSVD biomarkers in view of the shared embryological origin of the eye and brain structures (Baker et al., 2008). In the context of the studies, retinal vessels changes were thought to represent the current state of the cerebral small vessels. Several studies had found reduced retinal vascular D_f in CSVD subjects (Cavallari et al., 2011) (Cavallari et al., 2015) (Ong et al., 2013). Significant association was also noted between reduced retinal vascular D_f and CSVD neuroimaging markers (Doubal et al., 2010)(Hilal et al., 2014) (McGrory et al., 2019). Of interest, past evidences had suggested retinal vascular D_f as a potential biomarker

of CSVD (Cavallari et al., 2011)(McGrory et al., 2019)(Pantoni et al., 2019). In complement to the studies on the retinal blood vessels, this study seeks to explore whether there is a discernible change in the complexity of larger-sized cerebral blood vessels (i.e. CoW and its tributaries) of the CSVD subjects in comparison to controls. Importantly, CoW and its tributaries are part of the vascular network responsible in perfusing the cerebral parenchyma.

To our best knowledge, no previous study had used fractal analysis approach in the characterisation of cerebral vascular complexity in asymptomatic CSVD subjects. Recently, it was suggested that both small and larger vessels damage might occur together and interact dynamically in CSVD (Cuadrado-Godia et al., 2018)(Xu, 2014). It has been suggested that increased large arterial stiffness inflicted damage to the cerebral small vessels through increased flow load and pulsatile pressure (Cuadrado-Godia et al., 2018) (van Sloten et al., 2015)(O'Rourke & Safar, 2005)(Webb et al., 2012).Whereas, vascular changes in the small vessel disease (SVD) lead to hemodynamic alteration that precipitate vicious cycle of small and large vessels injuries (Brisset et al., 2013). Considering that previous studies had shown significant association between large vessels disease and CSVD (van Sloten et al., 2015)(Moroni et al., 2016), this study intend to explore a novel vascular neuroimaging marker in asymptomatic CSVD subjects with WMH by characterising the complexity of the CoW and its tributaries to address the research gap (Figure 1.2). This study focused on asymptomatic CSVD subjects with low to moderate QRISK2 score. The study subjects were recruited among the local population of Kelantan, Malaysia. In this study, the image of cerebral parenchyma and cerebral blood vessels (i.e. CoW and its tributaries) were captured by a 3.0 Tesla (T) three-dimensional MRI and (3D)-time-of-flight (TOF) MRA respectively. Of clinical relevance, 3.0 T 3D-TOF MRA is a non-

invasive, radiation free vascular imaging technique which is available in both research and clinical setting. The image of the CoW and its tributaries was segmented from the surrounding brain tissues and structures using a semi-automated approach, a combination of machine learning and manual segmentation technique. Subsequently, the complexity of the CoW and its tributaries as measured as D_f was analysed using fractal analysis. In this study, cerebral vascular D_f refers to the complexity of the CoW and its tributaries. Figure 1.2 shows the research gap of this study.

Essentially, a disease biomarker should be discernible between the diseased and control groups. Therefore, this study aimed to compare the cerebral vascular D_f between the WMH^+ and WMH^- groups. In the context of this study, the evidence of cerebral vascular D_f alteration in the CSVD subjects would indicate that vascular structural changes extend beyond cerebral small vessels. In addition, cerebral vascular D_f was also compared between RF^+ & WMH^+, RF^+ & WMH^-, and RF^- & WMH^-. Fundamentally, the evidence of cerebral vascular D_f alteration in the RF^+ & WMH^- may suggests that the subjects is at higher risk of developing CSVD. Besides, the cerebral vascular D_f was correlated with CSVD risk factors (i.e. age, BMI, systolic blood pressure (SBP), and total cholesterol / high density lipoprotein (HDL) ratio), and QRISK2 score, a well-established 10-year cardiovascular disease risk prediction score. Furthermore, this study had also investigated whether the QRISK2 score is predictive of the cerebral vascular D_f. Distinct from WMH and QRISK2 score, cerebral vascular D_f offers a quantitative measure of the current state of the underlying CoW and its tributaries. Figure 1.3 summarizes the rationale and significance of the study.

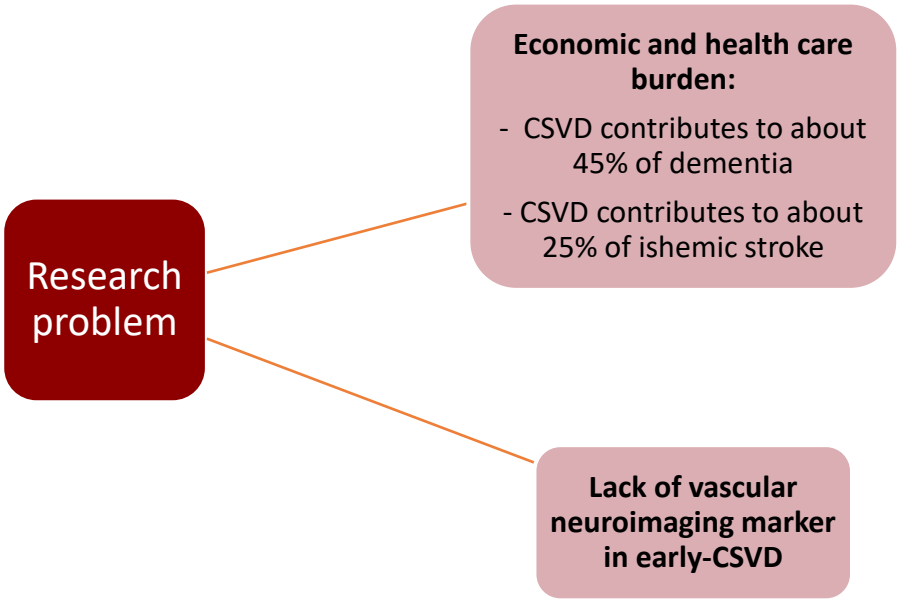

Figure 1.1 The research problem.

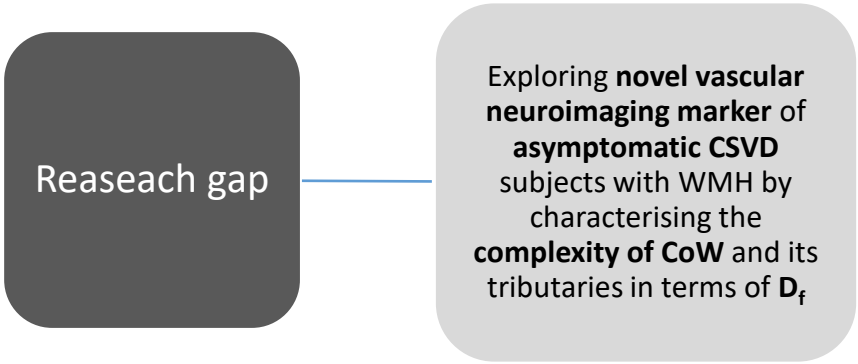

Figure 1.2 The research gap.

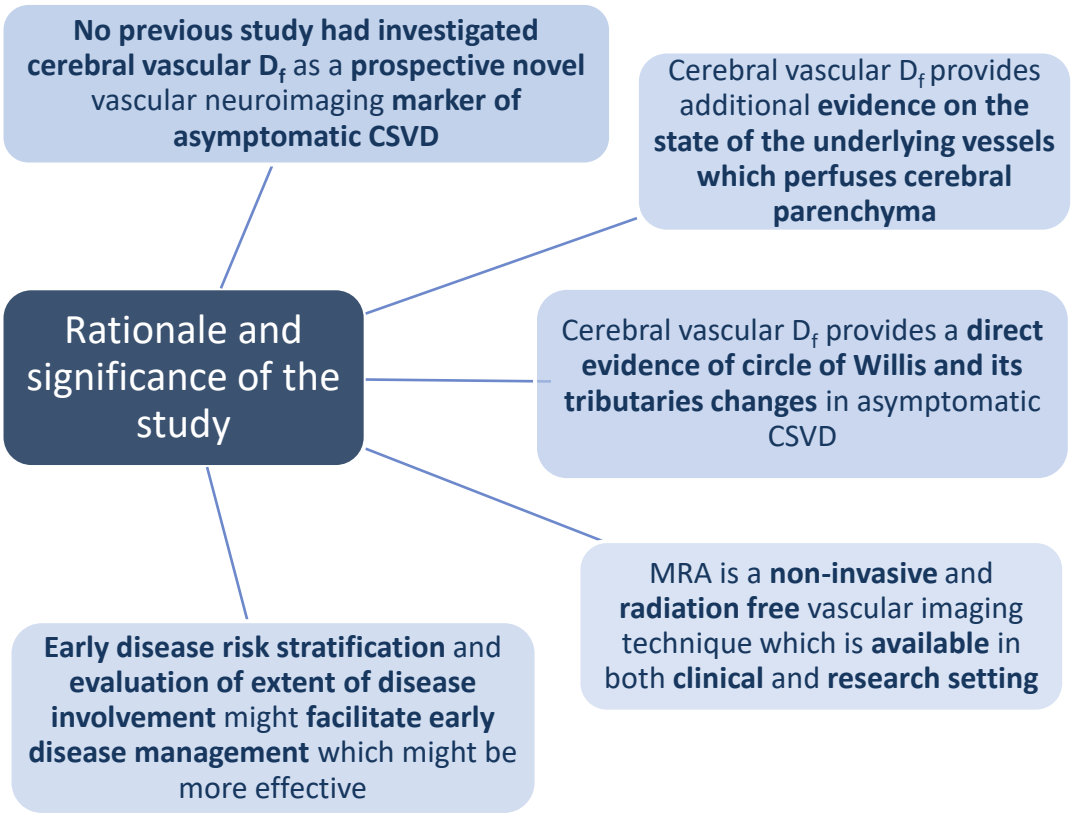

Figure 1.3 The rationale and significance of the study.

1.3 Research questions

1. Is there any significant agreement in terms of the cerebral vascular D_f measurement between the two independent researchers segmenting the cerebral blood vessels image?
2. What is the distribution (i.e. mean and standard deviation) of the cerebral vascular D_f in the asymptomatic subjects with cerebral WMH (WMH^+) and subjects without cerebral WMH (WMH^-)?
3. Is there any significant difference in terms of the cerebral vascular D_f between the WMH^+ and WMH^-?
4. What is the distribution (i.e. mean and standard deviation) of the cerebral vascular D_f in the asymptomatic subjects with both CSVD risk factors and

cerebral WMH (RF^+ & WMH^+), subjects with CSVD risk factors only (RF^+ & WMH^-), and subjects without both CSVD risk factors and cerebral WMH (RF^- & WMH^-)?

5. Is there any significant difference in terms of the cerebral vascular D_f between the RF^+ & WMH^+, RF^+ & WMH^-, and RF^- & WMH^-?
6. What is the correlation between CSVD risk factors and cerebral vascular D_f ?
7. What is the correlation between QRISK2 score and cerebral vascular D_f ?
8. If there is a linear relationship between QRISK2 score and cerebral vascular D_f :
   i) What is the proportion of the variation in the cerebral vascular D_f explained by QRISK2 score?
   ii) How much of the cerebral vascular D_f changes for one unit of change in the QRISK2 score?

1.4 Research hypotheses

1. There is no significant agreement in terms of the cerebral vascular D_f measurement between the two independent researchers segmenting the cerebral blood vessels image.
2. There is no significant difference in terms of the cerebral vascular D_f between WMH^+ and WMH^-.
3. There is no significant difference in terms of the cerebral vascular D_f between RF^+ & WMH^+, RF^+ & WMH^-, and RF^- & WMH^-.
4. There is no correlation between the CSVD risk factors and cerebral vascular D_f .

5. There is no correlation between the QRISK2 score and cerebral vascular D_f.
6. QRISK2 score does not contribute to the proportion of the variation in the cerebral vascular D_f
7. QRISK2 score is not predictive of cerebral vascular D_f.

1.5 General objective

To explore a novel vascular neuroimaging marker of asymptomatic CSVD subjects with WMH by characterising the complexity of the cerebral vascular network that encompass CoW and its tributaries in terms of D_f.

1.6 Specific research objectives

1. To test the reliability of the cerebral vascular D_f measurement between the two independent researchers segmenting the cerebral blood vessels image.
2. To determine and compare the cerebral vascular D_f between WMH^+ and WMH^-, and their association with risk factors (presence, RF^+ or absence, RF^-), respectively.
3. To determine the correlation between CSVD risk factors and QRISK2 score with cerebral vascular D_f , respectively.
4. To determine the proportion of the variation in the cerebral vascular D_f with respect to QRISK2 score.
5. To determine the value of the cerebral vascular D_f for a one unit change in the QRISK2 score.

1.7 Summary

To conclude, early disease risk stratification and evaluation of extent of disease involvement remains as the major challenges in the management of CSVD. Considering the economic and health care burden caused by CSVD, more recent attention has been paid on the search for early disease biomarkers. To address this gap, this study intends to explore the potential of cerebral vascular D_f as a novel vascular neuroimaging marker of asymptomatic CSVD subjects with WMH by characterising the complexity of the CoW and its tributaries. Fundamentally, this study aimed to delineate whether the cerebral vascular D_f is discernible between the subjects with WMH, CSVD risk factors and control. Furthermore, this study aimed to investigate the relationship between the CSVD risk factors and cerebral vascular D_f and the predictive value of QRISK2 score on cerebral vascular D_f. The scope of the study is the age-related and vascular risk factors related CSVD subjects from local population of Kelantan that presented with low to moderate QRISK2 score. 3.0 (T) MRI/MRA were used to diagnose WMH and to capture the image of CoW and its tributaries.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In chapter 2, section 2.2 till 2.8 provide an introduction on the current updates of CSVD, the pathogenesis of CSVD which build the foundation of CSVD biomarkers and the current perspective on CSVD biomarkers. Section 2.9 till section 2.15 elaborate on the background and literature review related to the prospective biomarker of interest; the cerebral vascular D_f. Section 2.16 summarizes on the proposed theoretical and conceptual framework related to this study.

2.2 Cerebral small vessel disease (CSVD)

CSVD refers to a spectrum of clinical and neuroimaging findings resulting from pathological processes of various aetiologies affecting arterioles, perforating arteries, capillaries, and the venules of the cerebral parenchyma (Wardlaw, Smith, & Dichgans, 2013)(Pantoni, 2010). Notably, the pathological damage of cerebral small vessels might impair the perfusion of the brain (Pantoni, 2010)(Hakim, 2019).

The disease is commonly found in the elderly and recognized in its advanced stage as it is usually asymptomatic at the early stage of the disease (A. S. Das et al., 2019). The silent nature of the disease is attributed to the small sized and location of the cerebral vascular pathological changes which generally spare the language centre, motor cortex, and cranial nerves (A. S. Das et al., 2019). Several cardiovascular disease risk factors such as hypertension (Staals et al., 2014)(Shi & Wardlaw, 2016) (Han et al., 2018), ageing (Staals et al., 2014)(E. E. Smith et al., 2015)(Shi & Wardlaw, 2016)(Han et al., 2018), diabetes (Longstreth et al., 1998)(Shi & Wardlaw, 2016) , and

smoking (Staals et al., 2014)(Shi & Wardlaw, 2016) have been implicated in the disease development.

Due to its silent nature, it is rather difficult to investigate the true burden of the disease in the population. However, it has been estimated from a number of population-based studies that approximately 5 to 28% of subjects have neuroimaging features suggestive of silent cerebral infarction (Price et al., 1997)(S. Lee et al., 2000)(R. R. Das et al., 2008). In recent years, there has been an increasing amount of literature discussing on the contribution of CSVD to cognitive impairment (Vermeer et al., 2007)(Blum et al., 2012)(Ferro et al., 2014)(Fanning, Wesley, et al., 2014), psychiatric disorders (Sachdev & Reutens, 2014), and subtle motor deficits such as gait and balance impairment (Baezner et al., 2014). Studies had also shown that those with CSVD are at higher risk of developing overt stroke (Vermeer et al., 2007)(Fanning, Wong, et al., 2014) which is a major cause of mortality and morbidity (Vos, T., Lim, S. S., Abbafati, C., Abbas, K. M., Abbasi, M., Abbasifard, M., ... & Bhutta, 2020). Overall, this implies that CSVD imposes significant clinical and economic burden on the health care system.

Despite of current advancements in the medical field, there has been few developments in the management of CSVD (A. S. Das et al., 2019). Notably, the silent nature of the disease and unstandardized neuroimaging reporting in earlier researches hinders deeper understanding of its pathogenesis and development of effective therapeutic measures (Wardlaw, Smith, Biessels, et al., 2013). Consequently, a recent consensus had proposed a standard approach for reporting neuroimaging findings in CSVD based on the STandards for Reporting Vascular changes in nEuroimaging (STRIVE)(Wardlaw, Smith, Biessels, et al., 2013). The collaborative group of researchers had agreed on the minimum standard requirement for image acquisition,

minimum standard requirement for image analysis and common terms and definitions used to describe neuroimaging features of CSVD on MRI (Wardlaw, Smith, Biessels, et al., 2013). The proposed term and definitions include (i) WMH of presumed vascular origin ; (ii) recent small subcortical infarct ; (iii) lacune of presumed vascular origin ; (iv) cerebral microbleed ; (v) perivascular space ; and (vi) brain atrophy (Wardlaw, Smith, Biessels, et al., 2013) (Figure 2.1).

Nonetheless, it is of importance to note that in the clinical practice, the diagnosis of CSVD is based on the observed cerebral parenchymal changes per se rather than the small vessels itself (Pantoni, 2014). This is due to the limited ability of standard imaging techniques in visualization of the small vessel of interest (Pantoni, 2014). Regardless, it should be noted that cerebral microinfarct might be visible on 7.0 T MRI in the absence of cerebral parenchymal changes on 1.5 T MRI (A. S. Das et al., 2019). This signifies the challenges faced in diagnosing and establishing the extent of CSVD in an individual patient (A. S. Das et al., 2019).

To date, early disease screening is limited due to the insidious and silent nature of CSVD (Shi & Wardlaw, 2016)(Peng et al., 2019). In recent development, more attention has been paid on the search of neuroimaging and systemic biomarkers intended for risks stratification, monitoring of disease progression and treatment response in CSVD (Blair et al., 2017) (Cuadrado-Godia et al., 2018)(Peng et al., 2019)(Mustapha et al., 2019). All in all, in view of the economic and healthcare burden that it incurs, a collaborative multidisciplinary approach is required to expand current understanding on the pathogenesis, clinical biomarkers, and therapeutic approaches of CSVD.

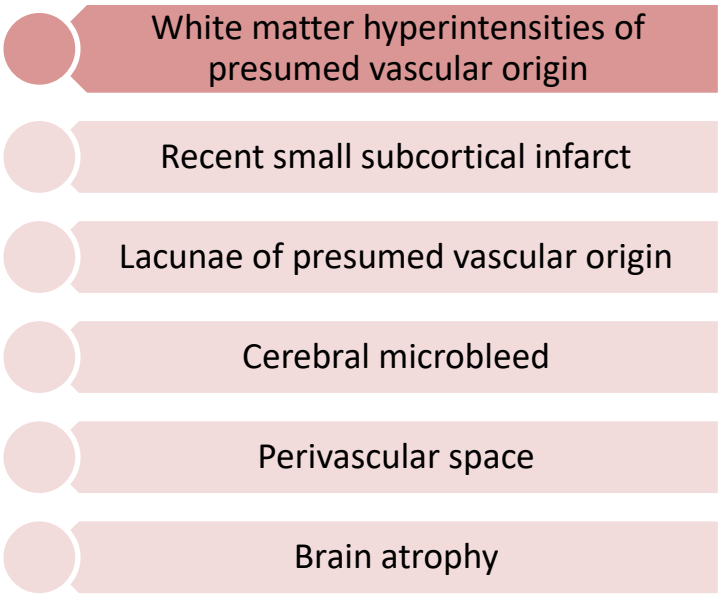

Figure 2.1 The common terms used to describe the neuroimaging features in CSVD based on STRIVE. The neuroimaging features of interest in this study is white matter hyperintensities of presumed vascular origin.

2.3 Aetiopathogenic classification of CSVD

CSVD is classified into six (6) types based on its aetiology and pathological characteristics (Pantoni, 2014) (Figure 2.2). These include (i) type 1: arteriolosclerosis ; (ii) type 2: hereditary and sporadic cerebral amyloid angiopathy ; (iii) type 3: genetic or inherited SVD unrelated to cerebral amyloid angiopathy ; (iv) type 4: immunology and inflammatory mediated SVD (v) type 5: venous collagenosis ; and (vi) type 6: SVD of other causes (e.g. post radiation angiopathy) (Pantoni, 2010). Among the six types of CSVD, arteriolosclerosis and cerebral amyloid angiopathy are more frequently encountered in the clinical setting. Of interest, this study focuses on the type 1 CSVD, which is also known as age-related and vascular risk factors related SVD (Pantoni, 2014).

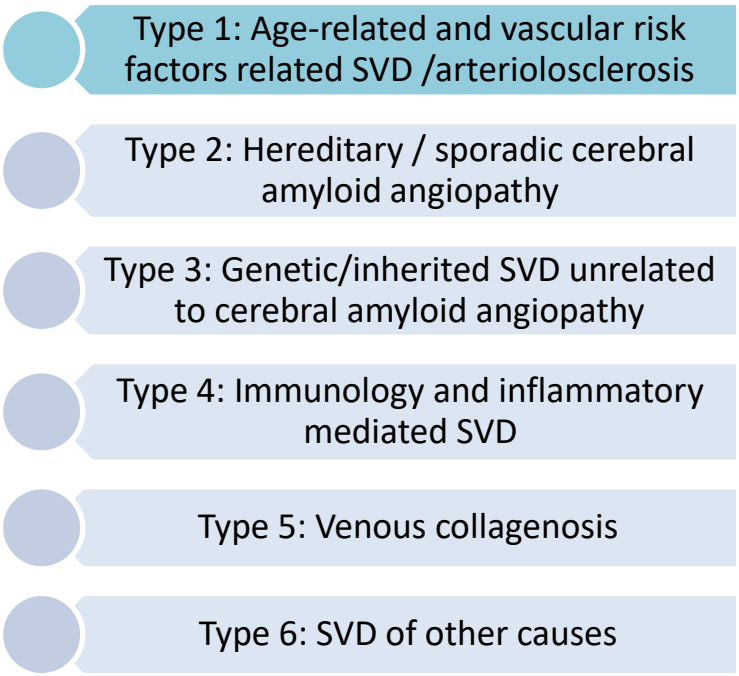

Figure 2.2 The aetiopathogenic classification of CSVD. The focus of the study is the age-related and vascular risk factors SVD which is also known as arteriolosclerosis.

2.4 Pathogenesis of cerebral parenchymal damage in CSVD

In general, CSVD shares similar risk factors with large vascular diseases (Staszewski et al., 2017). The CSVD risk factors could be divided into two groups, the modifiable CSVD risk factors and non-modifiable CSVD risk factors (Figure 2.3). The modifiable CSVD risk factors include hypertension (Staals et al., 2014)(Shi & Wardlaw, 2016) (Han et al., 2018), diabetes (Longstreth et al., 1998)(Shi & Wardlaw, 2016) , smoking (Staals et al., 2014)(Shi & Wardlaw, 2016), and dyslipidaemia (U. Khan et al., 2007) (Mok et al., 2014)(Shi & Wardlaw, 2016)(Sorop et al., 2017). In addition, recent evidence had demonstrated significant association between increased visceral fat area $\geq$100 cm^2,(Yamashiro et al., 2014), increased BMI (Gouw et al., 2008), increased waist diameter (Park et al., 2014), increased waist-to-hip ratio (Lampe et al., 2019), and CSVD. Similarly, significant association were also noted

between added dietary salt intake (Heye et al., 2015), lower physical activity (Moniruzzaman et al., 2020), and CSVD burden.

The non-modifiable CSVD risk factors include ageing (Mok et al., 2014) and genetic components (Choi, 2015). Previous studies had suggested the role of single-gene disorders in the development of hereditary CSVD such as Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL) and Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) (Choi, 2015). Inconsistent findings were noted in terms of sex predilection and the occurrence of CSVD. Apparently, both male and female sex has been found to be associated with CSVD (Staals et al., 2014)(de Leeuw et al., 2001). Several studies had documented no significant differences in terms of the prevalence of CSVD in both sexes (D. Liao et al., 1997)(Poels et al., 2010). Therefore, both sexes were not regarded as the defining criteria of subjects with CSVD risk factors in this study.

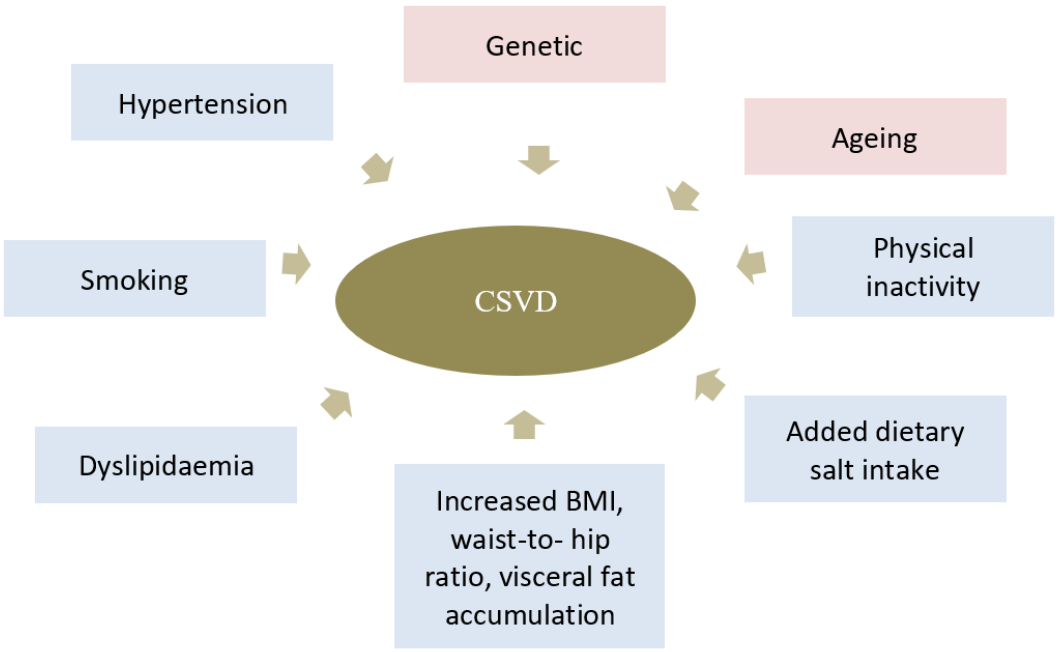

Figure 2.3 The modifiable (blue boxes) and non-modifiable (pink boxes) CSVD risk factors.

Collectively, almost all of the risk factors induced oxidative stress (Schiffrin, 2002)(Grochowski et al., 2018) and inflammatory responses (Grochowski et al., 2018). In addition, ageing and chronic hypertension cause large artery stiffening which results to an increase in flow and pulsatile pressure in the cerebral small vessels (Cuadrado-Godia et al., 2018)(Xu, 2014). As the innermost layer of the vascular structure, endothelial cell is rather susceptible to the injuries inflicted by chronic changes of the hemodynamic force (Renna et al., 2013)(Bleakley et al., 2015), oxidative stress (Sena et al., 2018)(Grochowski et al., 2018) and inflammatory reactions (Schleicher & Friess, 2007)(Sena et al., 2018)(Grochowski et al., 2018).

Noteworthy, impaired endothelial cell function might exacerbate oxidative stress by augmenting the release of superoxide anion production and a decrease in nitric oxide bioavailability (Grochowski et al., 2018). Indeed, a number of studies have reported the evidence of endothelial dysfunction markers such as increased soluble intercellular adhesion molecule-1 (sICAM-1) (Rouhl, Damoiseaux, et al.,

2012)(Cuadrado-Godia et al., 2018), vascular cellular adhesion molecule-1 (sVCAM-1) (Rouhl, Damoiseaux, et al., 2012) (Cuadrado-Godia et al., 2018) , and serum neurofilament in CSVD (Gattringer et al., 2017)(Duering et al., 2018)(Cuadrado-Godia et al., 2018). The ongoing endothelial dysfunction results in impaired vascular tone regulation (Grochowski et al., 2018)(Sena et al., 2018), BBB impairment (Wang et al., 2018), and maladaptive vascular remodelling (Goodwin, 2018). Maladaptive vascular remodelling resulted to pathological vascular alteration (Goodwin, 2018). In arteriolosclerosis, the diseased small vessels are pathologically characterised by (i) fibrinoid necrosis ; (ii) lipohyalinosis ; (iii) microatheroma ; (iii) segmental arterial disorganization ; and (iv) microaneurysms (Pantoni, 2010)(Ogata et al., 2014). Ultimately, the vascular wall changes might lead to reduction of vascular lumen diameter, vessels wall thickening, loss of vascular smooth muscle cells, and vessels rarefaction (Pantoni, 2010) (De Silva & Miller, 2016). These structural changes might be accompanied by the loss of vessels autoregulation and increased BBB permeability (Pantoni, 2010). Insight from both experimental and observational studies had suggested BBB leakage as one of the key factors involved in the pathogenesis of CSVD (Wardlaw, 2010)(Topakian et al., 2010)(Schreiber et al., 2013)(Wardlaw et al., 2017). Indeed, ageing, one of the prominent CSVD risk factors has been associated with increased BBB leakage (Wardlaw, 2010)(Wallin et al., 2017).

Importantly, increased BBB permeability allows toxic fluctuations of ionic concentration and incites inflammatory reaction in the brain parenchyma by allowing infiltration of immune cells and pathogens (Rajani & Williams, 2017)(Cuadrado-Godia et al., 2018). It was hypothesized that a subsequent chronic, diffuse and subclinical cerebral ischemia would lead to an incomplete cerebral infarction (Pantoni, 2010)(Q. Li et al., 2018). The concomitantly observed WMH on T2-weighted MRI

sequences is representative of oligodendrocyte death and neuronal demyelination (Pantoni, 2010). Figure 2.4 illustrates an overview of the pathogenesis of cerebral WMH development in CSVD. Whereas, it was projected that an acute, severe, and localized ischemia would lead to acute lacunar infarction as evidenced by cavitation in the cerebral white matter or deep grey matter areas on FLAIR or T1-weighted MRI sequences (Pantoni, 2010).

Recently, a research had found a significant association between lower CBF and increased BBB leakage in the cerebral WMH and normally appearing white matter (NAWM) areas (Wong et al., 2019). As both BBB permeability and CBF are regulated by the neurovascular unit these findings might suggest deterioration of the neurovascular unit in CSVD (Wong et al., 2019). Synergistically, both mechanisms might accelerate oligodendrocyte loss in the cerebral parenchyma. On the other hand, amyloid deposition and microaneurysms have been associated with the risk of vascular rupture and microbleed which appeared as microhypointense lesions on the gradient-echo MRI sequences (Pantoni, 2010).

All in all, it is apparent that the heterogeneity of the clinical features is reflective of the diverse mechanisms involved in the development of CSVD. Considering the dynamic nature of CSVD, further studies are required to explain the mechanism of the disease progression and regression (Shi & Wardlaw, 2016). Fundamentally, an in-depth knowledge pertaining the pathogenesis of CSVD would enable us to construct a more efficient and targeted approach in the management of CSVD.

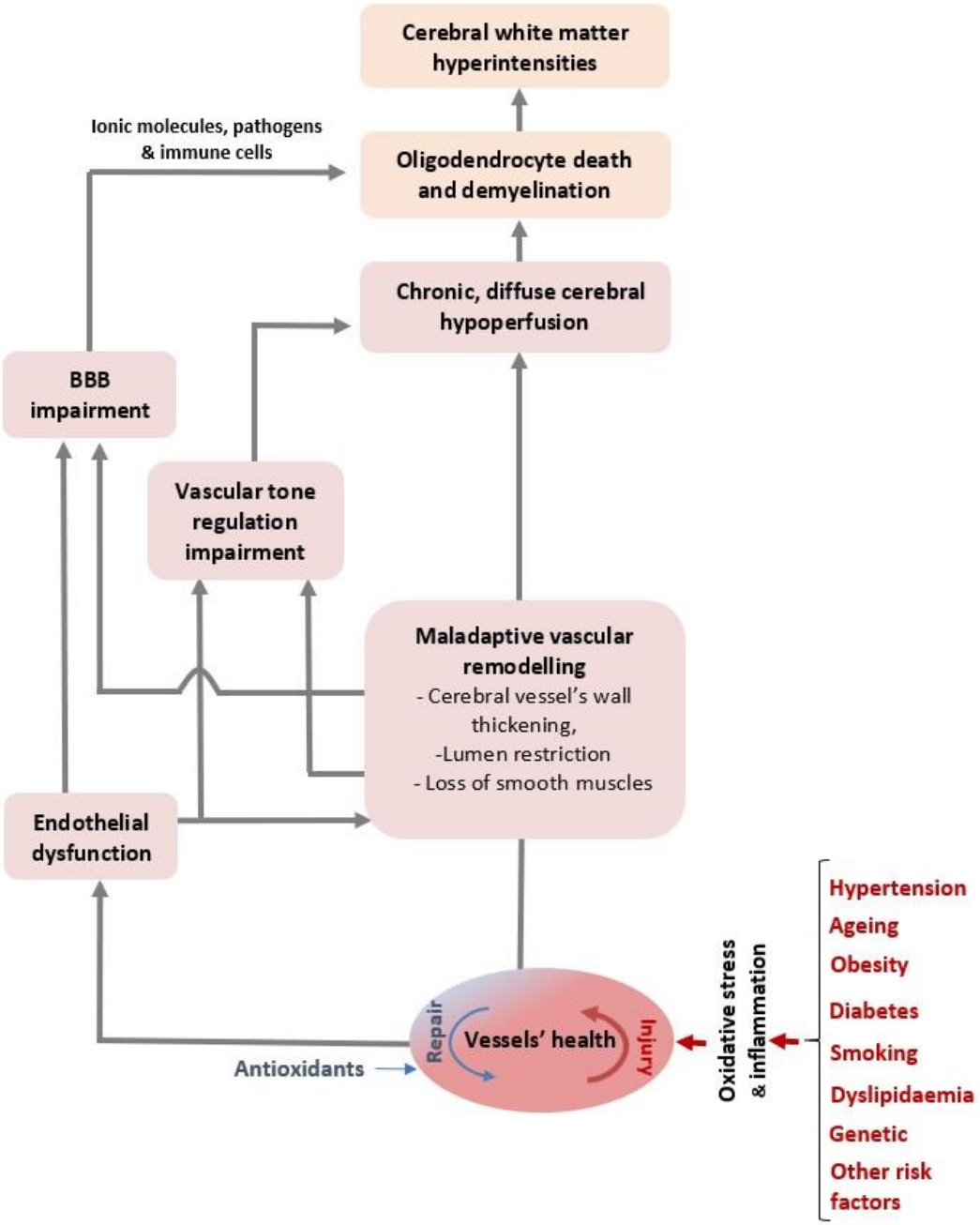

Figure 2.4 An overview of the pathogenesis of cerebral white matter hyperintensities development in CSVD. Repeated or prolonged exposure to CSVD risk factors tilted the intricate balance of the vascular injury and repair processes. This would subsequently lead to maladaptive vascular remodelling, chronic and diffuse cerebral hypoperfusion, demyelination, and cerebral white matter hyperintensities development.

2.5 Current perspective on CSVD biomarkers

In the past years, we had observed evolutionary changes in terms of the definition of biomarkers in line with the scientific and clinical advancements (García-Gutiérrez et al., 2020) . Recently, the Food and Drug Administration - National Institute of Health Biomarker Working Group had collaboratively agreed to concisely define biomarker as "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" (FDA-NIH Biomarker Working Group., 2016)(García-Gutiérrez et al., 2020). Fundamentally, a biomarker could be used as a tool in disease risk stratification, diagnostic criteria, monitoring disease progression, predicting the prognosis and even in early drug development (García-Gutiérrez et al., 2020).

In view of the clinical and socioeconomical burden imposed by CSVD, there have been an increasing amount of literature investigating potential biomarkers of CSVD. In general, the biomarkers of CSVD could be classified into two (2) main groups, namely molecular biomarker and imaging biomarkers. At the molecular level, numerous biomarkers have been investigated by the board of researchers. These include, oxidative stress biomarkers, inflammatory biomarkers, coagulation biomarkers, urinary albumin, cerebrospinal fluid (CSF) / serum albumin quotient, homocysteine, neurofilament, and genetic biomarkers. Of note, certain molecular biomarkers might appear in more than one group due to the interwoven link of various pathological processes involved in CSVD. For instance, apart from being regarded as vascular inflammatory biomarker, sICAM-1 is also classified as an endothelial dysfunction biomarker. The imaging biomarkers could be further divided into two categories namely retinal imaging and neuroimaging biomarkers. Retinal imaging biomarkers of CSVD include retinal artery wall thickness, retinal vessel tortuosity,

retinal vessel diameter, and retinal vascular complexity (Biffi et al., 2022). Whereas, aside from its diagnostic values, neuroimaging biomarkers have been used to assess the severity of CSVD, vascular function, and microstructural integrity (Blair et al., 2017).

It is of importance to note that several of the biomarkers listed above are non-specific to CSVD itself (Grochowski et al., 2018). Therefore, it is critically important to interpret the biomarkers finding within the clinical context. Indeed, further research is warranted to find an early disease biomarker of CSVD. Ideally a disease biomarker should be easily accessible in both research and standard clinical practice. Together, both neuroimaging and molecular biomarkers might enable us to seek a better understanding on the pathological and therapeutic aspect of the disease. An overview on several molecular and neuroimaging biomarkers that have been investigated and yielded encouraging results in CSVD research field will be provided in section 2.6 till 2.8.

2.6 Molecular biomarkers in CSVD

Oxidative stress is a state of imbalance between prooxidant molecules and antioxidant defences in favour of prooxidants (Pizzino et al., 2017). Recent evidences suggests that oxidative stress might be one of the factors responsible for cerebral vascular alteration in CSVD (De Silva & Miller, 2016). Several studies had investigated the relationship between antioxidant status such as carotenoid level, tocopherols, and vitamin C and CSVD. In 2001, the Rotterdam Scan Study had revealed significant association between reduced serum carotenoid level and severe periventricular white matter lesion (Den Heijer et al., 2001). Similarly, reduced serum

tocopherol levels and vitamin C level had been found to be associated with deep white matter lesion (Ohshima et al., 2013).

Endothelial cells dysfunction is characterised by impaired vascular function as evidenced by the presence of endothelial dysfunction biomarkers. Endothelial dysfunction biomarkers are represented by certain inflammatory biomarkers, coagulation biomarkers, plasma homocysteine, urinary albumin, and CSF / serum albumin quotient (Cuadrado-Godia et al., 2018)(Peng et al., 2019). Interleukin-6 (IL-6), intercellular adhesion molecules-1 (ICAM) and C-reactive protein (CRP) are among inflammatory biomarkers that have been studied in CSVD (Poggesi et al., 2016)(Cuadrado-Godia et al., 2018). In 2016, a systematic review on biologic markers of endothelial dysfunction in CSVD had concluded that both ICAM-1 and IL-6 have been consistently associated with sporadic CSVD (Poggesi et al., 2016). In contrary, there have been conflicting results regarding the association between CRP and CSVD (Poggesi et al., 2016). Meanwhile, several studies had attempted to investigate the association between coagulation biomarkers such as plasminogen activator inhibitor (PAI), D-dimer, von Willebrand factors (vWF), and CSVD. Of interest, it was suggested in a systematic review that among these biomarkers, PAI have been consistently associated with sporadic CSVD (Poggesi et al., 2016).

In addition, a recent meta-analysis had found that CSVD patients had higher homocysteine level in comparison to the control subjects (Piao et al., 2018). Further analysis demonstrated that the highest level of homocysteine was found in the subjects with cerebral white matter lesion which was followed by the subjects with silent brain infarction and lacunar infarction respectively (Piao et al., 2018). Interestingly, a recent retrospective study had shown that apart from significant association with the neuroimaging markers of CSVD (i.e. burden of lacunes, WMH , enlarged perivascular

spaces, and lobar cerebral microbleeds), total plasma homocysteine level was also found to be an independent predictor of cognitive dysfunction in the CSVD patients (Ji et al., 2020).

Besides, a recent meta-analysis had demonstrated independent association between albuminuria and several neuroimaging markers of CSVD which include lacunar infarcts, cerebral microbleeds, WMH, and enlarged perivascular spaces in the centrum semiovale and basal ganglia (Georgakis et al., 2018). Importantly, these findings also indicate the presence of microvascular pathological changes in the kidney of CSVD patients. This signify the importance of evaluating other systemic microvascular disease biomarkers in CSVD patients (Georgakis et al., 2018).

In line with these findings, a recent review had concluded that increased CSF to blood albumin quotient, and increased CSF neurofilament could be promising biomarkers of vascular cognitive impairment related to subcortical SVD (Wallin et al., 2017). Of importance, CSF/serum albumin quotient has been regarded as the gold standard assessment of BBB integrity (Wallin et al., 2017). An increase in CSF/blood albumin quotient is reflective of an increase in BBB permeability (Wallin et al., 2017). Meanwhile, increased in CSF or serum neurofilaments is regarded as a marker of neuroaxonal damage (Wallin et al., 2017)(Cuadrado-Godia et al., 2018). Apart from increased in CSF neurofilaments, past studies had reported increased in serum neurofilament in CSVD patients as compared to controls (Gattringer et al., 2017)(Duering et al., 2018)(Cuadrado-Godia et al., 2018). The serum neurofilament was also significantly associated with neuroimaging features of CSVD and processing speed performance (Duering et al., 2018).

Several genetic markers have been implicated in the development of hereditary CSVD. For instance, CADASIL, is characterised by mutation in the NOTCH3 genes