NEUROCOGNITIVE PROFILES AND THROMBOGENIC INHIBITORS (TAFI, TFPI) IN APPARENTLY ASYMPTOMATIC INDIVIDUALS WITH CEREBRAL SMALL VESSEL DISEASE

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PROFIL NEUROKOGNITIF DAN PERENCAT TROMBOGENIK (TAFI, TFPI) PADA INDIVIDU ASIMPTOMATIK DENGAN PENYAKIT PEMBULUH DARAH KECIL SEREBRAL

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LIST OF ABBREVIATIONS AND ACRONYMS

AD	Alzheimer's Disease
BBB	Blood Brain Barrier
CMB	Cerebral Microbleeds
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
CSVD	Cerebral Small Vessel Disease
CT	Computed Tomography
ELISA	Enzyme Linked Immunosorbent Assay
FLAIR	Fluid Attenuated Inversion Recovery
FMD	Flow Mediated Dilation
FSIQ	Full Scale Intelligence Quotient
HDL	High Density Lipid
HMWK	High Molecular Weight Kininogen
ICAM	Intercellular Adhesion Molecule
LDL	Low Density Lipid
ICV	Intracranial Volume
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
OCP	Overall Coagulation Potential
OHP	Overall Hemostasis Potential
PVS	Perivascular Space
RAVLT	Rey Auditory Visual Learning Test
RF	Radiofrequency
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TE	Time Echo
TFPI	Tissue Factor Pathway Inhibitor
tPA	Tissue Plasminogen Activator
TR	Time Repetition
VCAM	Vascular Cell Adhesion Molecule
VLDL	Very Low Density Lipid
WAIS	Wechsler Adult Intelligence Scale
WMH	White Matter Hyperintensities

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ABSTRAK

Penyakit pembuluh darah kecil serebral atau otak (CSVD) adalah istilah sering digunakan bagi menghuraikan pelbagai ketidakabnormalan otak di bawah persembahan pengimejan neuro. Hiperintensiti jirim putih (WMH) merupakan imej yang biasa dilihat pada populasi normal dan sihat, juga dipanggil sebagai persembahan normal jasad putih telah menjadi isu kerana ia menjejeaskan fungsi kognitif, keupayaan gerakan motor, sehingga menjadi suatu beban kepada komuniti terutama dari sudut ekonomi. Pelbagai faktor telah dihipotesiskan menyumbang kepada patogenesis penyakit senyap dan progresif ini, antaranya adalah tapak jalan koagulasi, inflamasi, transduksi isyarat, dan metabolisme lipid. Walaubagaimanapun, usaha bagi merungkai keseluruhan mekanisme masih diperlukan. Kajian ini bertujuan untuk mencari perhubungan antara CSVD pada individu asimptomtaik dengan penanda biologi darah terutamanya perencat trombogenik (TAFI dan TFPI) serta profil neurokognitif. Kajian ini merupakan kajian saringan melibatkan penyertaan dan pengambilan subjek melalui Klinil Rawatan Keluarga, Hospital Universiti Sains Malaysia. Suatu aplikasi pengiraan risiko kardiovaskular atas talian (QRISK2) telah digunakan sebagai metod saringan jangkaan risiko. Peserta yang memenuhi kriteria inklusi dan eksklusi telah dibawa menjalani pengimbasan MRI (Phillips 3-Tesla Achieva MR Scanner), persampelan darah dan ujian neuropsikologi berdasarkan prestasi Wechsler Adult Intelligent Scale IV (WAIS-IV). Imej MRI

peringkat awal telah digunakan sebagai kaedah pengumpulan berdasarkan kepada kehadiran WMH melalui pengskoran Skala Fazekas. Plasma darah telah digunakan bagi pengenalpastian faktor trombogenik (TAFI, TFPI) menggunakan kaedah ELISA dan tujuh subujian WAIS-IV iaitu Block Design, Matrix Reasoning, Visual Puzzle, Digit Span, Letter Number Sequencing, Coding dan Symbol Search telah digunakan sebagai ujian neuropsikologi bagi domain pemikiran persepsi (PRI), memori kerja (WMI), dan kelajuan pemprosesan (PSI). Analisis ujian statistik telah dilakukan menggunakan IBM SPSS Statistics versi 24.0. Sejumlah tiga puluh tiga (N=33) subjek telah direkrut berumur antara 25 hingga 62 tahun, dengan taburan jantina serupa iaitu sebanyak 30% lelaki dan 70% wanita pada peringkat awal dan kajian susulan. Sejumlah 15 orang telah dikenalpasti mempunyai WMH pada peringkat awal walaubagaimanapun hanya 11 orang hadir semula pada peringkat susulan. Purata umur telah dikekalkan pada umur 39 tahun pada kedua-dua waktu kajian. TFPI adalah lebih tinggi pada waktu susulan manakala TAFI adalah lebih tinggi pada peringkat awal bagi kedua-dua kumpulan. Walaubagaimanapun, hanya TFPI pada susulan menyumbang kepada model perkaitan kehadiran WMH [B=-3.964, Wald 5.106, OR= 0.021, $p<0.05$]. Membandingkan kedua-dua kumpulan, hanya TFPI susulan menunjukkan perubahan signifikan. Profil neurokognitif menunjukkan perkaitan antara PSI dengan umur, skor QRISK2 dan PRI dengan nilai p=0.003, 0.016, dan 0.018, namun tiada domain kognitif menunjukkan perkaitan atau nilai ramalan ke atas WMH. Selain itu, WMI dan TFPI pada susulan juga menunjukkan korelasi signifikan pada $p<0.05$. Keputusan ini menggambarkan hanya TFPI adalah berkaitan dengan kehadiran WMH, disertai perubahan pada PSI. Meskipun paras TAFI berkurang setelah setahun, peranan protein ini kemungkinan tidak ketara pada peringkat awal. Kajian ini juga membentuk kohort terpilih bagi individu

asimptomatik CSVD didalam populasi sebagai pemangkin kepada kajian di masa hadapan, termasuklah merungkai peranan penanda biologi novel dan semakin mendapat perhatian bagi CSVD.

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ABSTRACT

Cerebral small vessel disease (CSVD) is the term coined to address the many abnormal presentations of the brain under neuroimaging techniques. White matter hyperintensities (WMH), one of the most common appearance seen under normal healthy populations which often described as normal appearing white matter becomes a concern as it posed a detrimental effect on cognitive functions as well as gait and motor abilities, culminating a huge burden to the society economically. There are various causal factors suggested to contribute in pathogenesis of this silent yet progressive disease. Among the main pathways hypothesized are the coagulation/complement, inflammation, signal transduction and lipid metabolism. This current study aims to determine the relationship between CSVD in healthy individuals, and the selected blood biomarkers particularly thrombogenic inhibitors (TAFI and TFPI) as well as neurocognitive profiles. This was an exploratory study from random selection of subjects recruited from Klinik Rawatan Keluarga, Hospital Universiti Sains Malaysia. An online-based cardiovascular risk calculator (QRISK2) was also used for risk prediction. Those who met the inclusion and exclusion criteria were recruited and underwent MRI brain scanning (Phillips 3-Tesla Achieva MR Scanner), blood sampling and neuropsychological performance based on Wechsler Adult Intelligent Scale IV (WAIS-IV). The baseline MRI images were used to group the subjects based on the presence of white matter hyperintensities (WMHs) scored

by Fazekas scale. Blood plasma was used to determine the thrombogenic factors (TAFI, TFPI) by using ELISA and seven subtests of WAIS IV were taken for neuropsychological testing Block Design, Matrix Reasoning and Visual Puzzle; Digit Span and Letter-Number Sequencing; Coding and Symbol Search for three different main cognitive domains Perceptual Reasoning, Working Memory and Processing Speed, respectively. The analyses were done by performing statistical test using IBM SPSS Statistics version 24.0. A total of thirty-three (N=33) subjects recruited with QRISK2 score <20%, aged between 25 to 62 years old, with similar distribution of 30% male and 70% female at both baseline and follow up. Upon MRI scanning, 15 (45.5%) were detected with WMH at baseline however only 11 (40.7%) of them came back for follow up at one-year. The mean age was 39 years old (range from 25 to 62 year old. TFPI at follow up was seen to be significantly increased meanwhile TAFI was higher at baseline point. However, only TFPI at follow up contributed to the association model of WMH outcome [B=-3.964, Wald 5.106, OR= 0.021, $p<0.05$]. Comparing both groups together, only TFPI showed significant changes between WMH+ and WMH- at follow up. The neurocognitive profiling shows association between PSI with age, QRISK2 score and PRI with p value 0.003, 0.016 and 0.018 respectively, however no cognitive domains show any association or predictive value to the WMH outcome. Interestingly, WMI and TFPI at follow up show a significant correlation with $p<0.05$. The result suggests that only TFPI is associated with presence of WMH, coupled with changes in PSI. As for TAFI, despite a decreased level within a year, its role is probably not apparent in the early stage of CSVD. Finally, the study has also established a selected cohort of at-risk asymptomatic CSVD individuals within our local population for future studies, including to further elucidate the role of emerging or novel biomarkers for CSVD.

CHAPTER 1

INTRODUCTION

1.1 Study Background

Cerebral small vessel disease (CSVD) is defined as a heterogenous disorder with clinical and imaging findings that are thought to originate from pathologies in perforating cerebral arteries, capillaries and venules (Pantoni 2010). Recognised features of CSVD include white matter hyperintensities (WMHs) and lacunes of presumed vascular origin, cerebral microbleeds (CMB), perivascular spaces, and total cerebral atrophy (Wardlaw et al. 2013) with all types show significant association with incident ischaemic or haemorrhagic stroke (Rensma et al. 2018). In addition, multiple reviews suggested the causal factors with evidence of concurrent occurrence to ageing which then lead to hypothesis on vascular ageing as the root cause of problem, even in the absence of any neurological symptoms (i.e. asymptomatic) (Pantoni 2010, Abraham et al. 2016; Arvanitakis et al. 2016). Moreover, cross sectional studies have found increased circulating markers of endothelial activation for instance at autopsy in patients with symptomatic CSVD to have thickening and hyaline deposition of the small perforating end arterioles supplying white matter, whilst a more severe and larger symptomatic lacunar infarction microatheroma exhibited neuronal loss, ischemic demyelination and

gliosis. Supporting evidence shows that significant reductions of blood flows happen in CSVD. Hence endothelial dysfunction is taken into consideration that can be assessed by examining the soluble plasma markers (Poggesi et al. 2016).

Previous studies have shown that cross sectional assessment of circulating biomarkers is increased in symptomatic CSVD, although their significance in disease pathomechanism remains contentious (Johansson et al. 2002; Markus et al 2005). Notwithstanding, the consequent vascular damage in the brain can cause a wide degree of pathological presentations, with one impact in particular is recognised to be associated with vascular cognitive impairment and dementia, which has an increased incidence and prevalence similar to stroke with ageing.

Vascular damage in the brain can cause a wide degree of pathological presentations. The preclinical (or asymptomatic) state of both conditions have not been investigated widely enough to provide clarity on the disease spectrum and the common pathomechanism pathways. Hence, this study examined the preclinical (asymptomatic) individuals prospectively as defined by the imaging findings of CSVD (in specific, the WMH). Vascular dysfunction is said to be caused by various pathologies mainly the inflammatory pathways, coagulation/complement pathway, as well as lipid metabolism (Pogessi et al. 2016). The components of coagulation pathway particularly Tissue Factor Pathway Inhibitor (TFPI) and Thrombin Activatable Fibrinolysis Inhibitor (TAFI) have been shown to be altered in vascular disorders involving cardiovascular disease, stroke and haemophilia (Stavik et al. 2017; Orbe et al 2015; Foley et al 2013; Pan et al. 2009).

- Blood biomarker testing is important due to multiple occurrence of contraindication on MR imaging bared by patients.

Neuroimaging of WMHs is frequently done under MRI scanning as it provides clearer difference between normal and abnormal tissue, with no ionization radiation compared to Computed tomography (CT) scanning. The altered areas of white matter attenuation on CT was first discovered back in late 1980s which was then described as patchy low attenuation in periventricular and deep white matter, referred as leukoaraiosis (Hachinski et al. 1987). Upon investigations under MRI scanning, it was found to be more obvious and recognizable as abnormal areas of signal intensity on MRI due to its better sensitivity to soft tissue changes than CT, specifically on subtle water content alterations (Wardlaw et. 2013).

Damaged small vessels at cerebral area are mostly progressive leading to progressive functional decline that is correlated with affected brain area. In most cases, WMHs has been shown to predict the occurrence of vascular cognitive impairment (Wardlaw et al. 2013), supported by multiple experiments confirming the association of white matter lesions and cognitive decline in patient groups and in asymptomatic older adults (de Groot et al.. 2000). The pattern of cognitive impairment in CSVD rarely seen as deficits of episodic memory in mild form, however commonly presented with executive dysfunction. Executive function refers to higher cognitive processes requiring simultaneous multiple operation for enhanced performance (O'Sullivan et al. 2004). These arguments form the bases for the conceptual framework as summarised in Figure 1.1.

1.2 Rationale of the Study

In general, the study sought to examine the relationship of neurocognitive profiles and selected thrombogenic factors particularly TFPI and TAFI in healthy, asymptomatic individuals with the presence of white matter hyperintensities (WMH). Previous studies have replicated on the idea of CSVD correlation with cognitive decline, mostly in older adults either symptomatic or non-symptomatic, post stroke patients and adults with multiple risk factors in a community dwelling ageing population. Database commonly uses the Mini Mental State Examination (MMSE) Rey Auditory Verbal Learning Test (RAVLT) (Papma et al. 2013), Color Trails Test and Digit Cancellation Task (Biesbroek et al. 2016), with lesser evidence on Weschler Adult Intelligence Scale version IV (WAIS-IV). Subjective cognitive impairment is frequently seen in those with WMH that serves as a predictive global cognition decline/deficit in the span of 12 months interval (de Groot et al. 2001; Haley et al. 2009).

Various studies conducted to investigate the exact mechanism underlying white matter hyperintensity in CSVD with multiples pathways postulated. These include to measure the cerebral blood flow, inflammatory molecules (ICAM, VCAM, C-Reactive Proteins, Interleukines), coagulation proteins (t-PA, plasminogen activator inhibitor-1 (PAI-1)), with few laboratories focusing on TAFI and TFPI in mechanism of ischaemic stroke (Denorme et al. 2016, Kraft et al. 2010) with heterogenous results providing an ambiguous mechanistic information on its role in cerebral vascular disease in general (Durand et al. 2014; Mertens et al. 2017).

The complexity of CSVD brings challenges for an early diagnosis yet allows for possibility of development of disease modifying treatments for secondary prevention. Hence, it is important to seek biomarkers particularly at the preclinical phase or early phases as a preventive therapeutic measure, in order to move towards healthy aging. Hence, temporal relationship of neurocognitive function and memories with levels of thrombogenic blood biomarkers may provide further clue to the pathomechanism and as a potential biomarker to predict the outcome of CSVD in apparently asymptomatic individuals.

1.3 Objectives and Hypothesis

1.3.1 General Objective

To study the temporal relationship of the neurocognitive profiles with the potential thrombogenic inhibitors using TAFI and TFPI in apparently asymptomatic individuals with CSVD.

1.3.2 Specific Objectives

1. To determine the levels of thrombogenic blood biomarkers (TAFI and TFPI) with regards to the WMH findings at baseline and at 12 months follow-up.
2. To correlate the neurocognitive profiles with the presence of WMH at the baseline period and at 12 months follow-up.
3. To correlate demographic, QRISK2 and neurocognitive profiles with the levels of thrombogenic blood biomarkers (TAFI and TFPI).

1.3.3 Hypothesis

1. There is a significant difference in QRISK2 score with WMH presence at baseline and follow up.
2. There is a significant difference in neurocognitive profiles with WMH presence at baseline and follow up.
3. There is no significant relationship between thrombogenic inhibitors (TAFI and TFPI) and WMH presence at baseline and follow up.
4. There is no significant correlation between thrombogenic blood biomarkers with neurocognitive profiles and WMH presence at baseline and follow up.

1.4 Conceptual Framework

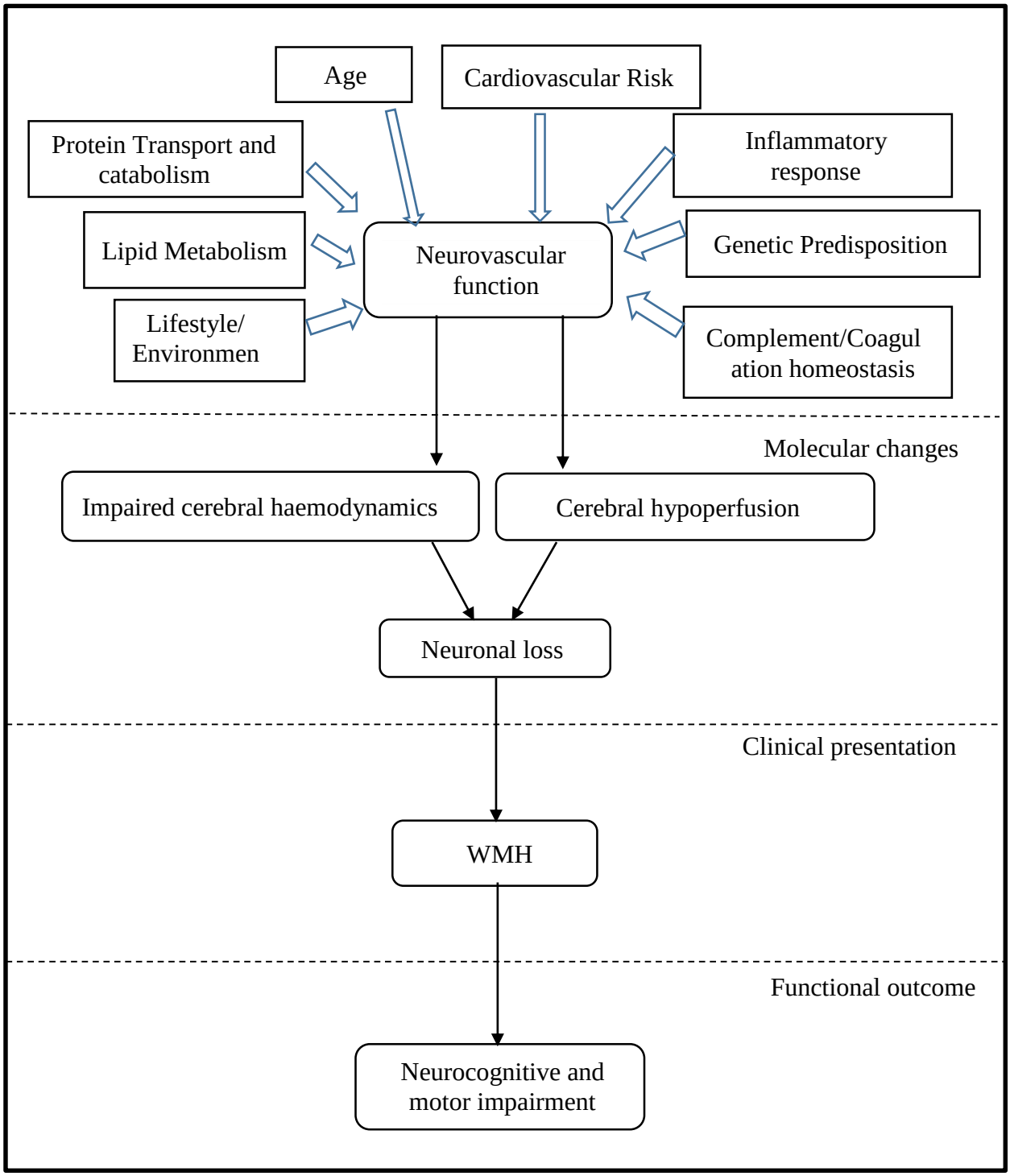

Figure 1.1 Conceptual Framework.
Adapted from Abraham et al. (2016), Poggesi et al. (2016) & Ritz et al. (2017).

CHAPTER 2

LITERATURE REVIEW

2.1 Cerebral Small Vessel Disease

Cerebral small vessel disease (CSVD) is the term commonly used to describe a syndrome of clinical, cognitive, neuroimaging and neuropathological findings thought to arise from disease affecting the perforating cerebral arterioles, capillaries, and venules, resulting in brain damage in the cerebral white matter (Pantoni 2010). CSVD covers a wide spectrum that broadly conceptualized from decreased brain perfusions (Weiner and Taimur 2015), and can be divided into various forms based on the imaging changes in the white matter and subcortical grey area including recent small subcortical infarct, lacunes, white matter hyperintensities (WMH), prominent perivascular spaces (PVS), cerebral microbleeds (CMBs) as well as atrophy (Wardlaw et al. 2013). These different types and features of CSVD are traditionally taken as different entities on their own, however recent reports suggest that some, if not all are correlated and more likely to share common diffuse intrinsic small vessel abnormalities, hence probably more dynamic compared to previous theories of segregating the presentations (Shi & Wardlaw 2016). According to Wardlaw et al. (2013), it was thought that the main key problem might be on the diffused cerebrovascular endothelial failure. CSVD penumbra shares common small