DETERMINATION OF PLASMA METABOLITES, ITS RELATED METABOLOMIC PATHWAYS AND CORRELATION WITH CLINICAL PARAMETERS OF COGNITIVE FRAILTY AND MILD COGNITIVE IMPAIRMENT

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by

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أعوذ بالله من الشيطان الرجيم

بسم الله الرحمن الرحيم

﴿ ۞ ٱللَّهُ ٱلَّذِي خَلَقَكُم مِّن ضَعۡفٖ ثُمَّ جَعَلَ مِنۢ بَعۡدِ ضَعۡفٖ قُوَّةٗ ثُمَّ جَعَلَ مِنۢ بَعۡدِ قُوَّةٖ ضَعۡفٗا وَشَيۡبَةٗۚ يَخۡلُقُ مَا يَشَآءُۚ وَهُوَ ٱلۡعَلِيمُ ٱلۡقَدِيرُ ﴾ (الروم: 54)

صدق الله العظيم

I seek Allah's protection from the rejected Satan

In the name of Allah most Compassionate Most Merciful

Allah is He Who created you in (a state of) weakness, then gave you strength after weakness, then after strength gave (you) weakness and grey hair. He creates what He wills. And it is He Who is the All-Knowing, the All-Powerful (i.e. Able to do all things), Surat Al-Rum

ALLAH Almighty has spoken the truth

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LIST OF ABBREVIATIONS

BI	Barthel Index
CHS	Cardiovascular Health Study
CKD	Chronic Kidney Disease
CDR	Clinical Dementia Rating
CF	Cognitive frailty
EFS	Edmonton Frail Scale
EMS	Elderly Mobility Scale
ELSA	English Longitudinal Study of Aging
FFP	Fried Frailty Phenotype
GDS	Geriatric Depression Scale
IANA	International Academy on Nutrition and Aging
IAGG	International Association of Gerontology and Geriatrics
MCI	Mild Cognitive Impairment
MoCA	Montreal Cognitive Assessment
MMSE	Mini-Mental State Examination
NMR	Nuclear Magnetic Resonance
SPPB	Short Physical Performance Battery
TUG	Timed Up & Go

PENENTUAN METABOLIT PLASMA, LALUAN METABOLOMIK YANG BERKAIT DAN KORELASI PARAMETER KLINIKAL BAGI KELEMAHAN KOGNITIF DAN KETAKMAMPUAN KOGNITIF RINGAN

ABSTRAK

Kelemahan kognitif (CF) telah berkembang sebagai konsep sejak beberapa tahun kebelakangan ini, pada mulanya digunakan untuk menggambarkan kejadian bersama gangguan kognitif ringan dan kelemahan fizikal, tanpa kehadiran demensia. Penuaan populasi berlaku di seluruh dunia dan Malaysia mempunyai populasi warga tua yang paling pesat berkembang. CF pada usia tua mungkin menjadi semakin penting. Metabolomik adalah disiplin saintifik baru yang mungkin menyediakan kaedah baru untuk mengenalpasti CF dengan menggunakan teknik sensitif dan khusus seperti resonans magnetik nuklear (NMR). Tujuan kajian ini adalah untuk mengenal pasti cap jari metabolik yang boleh digunakan untuk membezakan subjek dengan CF daripada MCI dan sihat, untuk mengenal pasti cap jari metabolik yang boleh digunakan untuk membezakan subjek dengan MCI daripada sihat, untuk meneroka laluan metabolit yang dikenal pasti, dan untuk meneroka korelasi antara penanda biologi yang dikenal pasti dan data klinikal. Sampel darah dikumpulkan daripada 56 CF (purata umur: 72.6 tahun), 75 MCI (purata umur: 65.1 tahun), dan 78 teguh (purata umur: 63.3 tahun). Sampel plasma dipisahkan dengan pengemparan, kemudian sampel plasma dicampur dengan penimbal fosfat dan kemudian dianalisis menggunakan spektroskopi NMR. Analisis data dilakukan menggunakan analisis multivarian termasuk analisis komponen utama (PCA) dan analisis perbezaan ortogon separa kuasa dua terkecil (PLS-DA). Untuk diskriminasi antara CF dan sihat, model PLS-DA menunjukkan kepekaan, spesifikasi dan ketepatan model masing-masing adalah

66.1%, 67.9% dan 65%. Metabolit yang dikenal pasti dalam kumpulan ini ialah asparagin, trimethylamine N-oxide (TMAO), asid metilmalonik, kreatinin, asid suksinik, asid asetik, dan alpha D-glukosa. Untuk diskriminasi antara CF dan MCI, model PLS-DA menunjukkan kepekaan, kekhususan dan ketepatan model masing-masing adalah 62.5%, 61.3%, dan 69.5%. Metabolit yang dikenal pasti dalam kumpulan ini ialah butanol, kreatinin, di-methyl-acetamide, beta D-glukosa, alanin, alpha D-glukosa, asid asetik, dan methlyhistamine. Untuk diskriminasi antara MCI dan sihat, model PLS-DA menunjukkan kepekaan, kekhususan dan ketepatan model masing-masing adalah 76%, 61.5% dan 72.5%. Metabolit yang dikenal pasti kumpulan ini ialah alpha D-glukosa, asparagine, TMAO, asid metilmalonik, beta D-glukosa dan asid hidroksi-isovalerik. Kebanyakan laluan metabolit yang dikenal pasti berkaitan dengan CF atau penyakit kronik. Kebanyakan korelasi antara metabolit yang dikenal pasti dan data klinikal tidak menunjukkan korelasi yang tinggi. Kajian ini menunjukkan bahawa teknik metabolomik plasma yang digunakan dapat membezakan antara CF, MCI, dan sihat. Metabolomik berasaskan NMR juga dapat mengenal pasti penanda biologi baru dalam plasma yang boleh berguna untuk mendiagnosis CF pada masa hadapan.

DETERMINATION OF PLASMA METABOLITES, ITS RELATED METABOLOMIC PATHWAYS AND CORRELATION WITH CLINICAL PARAMETERS OF COGNITIVE FRAILTY AND MILD COGNITIVE IMPAIRMENT

ABSTRACT

Cognitive frailty (CF) has evolved over recent years, initially used to describe the co-occurrence of mild cognitive impairment (MCI) and physical frailty without dementia. Population ageing is occurring globally, and Malaysia has the most rapidly growing older population. CF in old age is likely to become of increasing importance. Metabolomics is a novel scientific discipline that may provide a novel method for diagnosing CF using a sensitive and specific technique such as nuclear magnetic resonance (NMR). This study aims to identify metabolic fingerprints that can be used to distinguish subjects with CF from MCI and robust (healthy group), to explore the pathway of the identified metabolites, and to explore the correlations between the identified biomarkers and the clinical data related to CF and MCI. Blood samples were collected from 56 CF (mean age: 72.6 years), 75 MCI (mean age: 65.1 years), and 78 robust (mean age: 63.3 years). Plasma was separated by centrifugation, and then plasma samples were mixed with phosphate buffer and analyzed using NMR spectroscopy. Data analysis was done using multivariate analysis, including principal component analysis (PCA) and partial least square discriminate analysis (PLS-DA). For discrimination between CF and robust, the PLS-DA model showed sensitivity, specificity, and accuracy of 66.1%, 67.9%, and 65%, respectively. The identified metabolites of this group were asparagine, trimethylamine N-oxide (TMAO), methylmalonic acid, creatinine, succinic acid, acetic acid, and alpha D-glucose. For

discrimination between CF and MCI, the PLS-DA model showed sensitivity, specificity, and accuracy of 62.5%, 61.3%, and 69.5%, respectively. The identified metabolites of this group were butanol, creatinine, di-methyl-acetamide, beta D-glucose, alanine, alpha D-glucose, acetic acid, and methylhistamine. For discrimination between MCI and robust, the PLS-DA model showed sensitivity, specificity, and accuracy of 76%, 61.5%, and 72.5%, respectively. The identified metabolites of this group were alpha D-glucose, asparagine, TMAO, methylmalonic acid, beta D-glucose, and hydroxyl-isovaleric acid. Most of the pathways of the identified metabolites were related to CF or a chronic disease. Most correlations between the identified metabolites and the clinical data did not show a high correlation. This study showed that the application of metabolomics technique could differentiate between the CF, MCI, and robust. NMR-based metabolomics also identified potential biomarkers in plasma, which can help the diagnosis of CF in the future.

CHAPTER ONE

INTRODUCTION

1.1 Background

The prevalence of frailty among those aged 65 years and over is estimated at 17% (WHO, 2015). Cognitive frailty (CF) is a potential risk factor for dementia, functional decline, disability, poor quality of life, and mortality (Rivan et al., 2020). Specifically, this CF may help identify individuals with cognitive impairment caused by physical and non-neurodegenerative conditions and promote interventions that can improve the quality of life among older adults. CF is a clinical construct introduced by the International Academy on Nutrition and Aging (IANA) and the International Association of Gerontology and Geriatrics (IAGG), defined in 2013 as a heterogeneous clinical manifestation characterized by the comorbidity of physical frailty operationalized with the Cardiovascular Health Study (CHS) phenotypic model and cognitive impairment diagnosed with a Clinical Dementia Rating (CDR) scale of 0.5 among older adults without a concurrent diagnosis of Alzheimer's diseases (AD) or other dementias (Rivan et al., 2020). CF is divided into either comorbid physical frailty (>1 Fried criteria) and mild cognitive impairment (Petersen criteria) (Rivan et al., 2020). Frailty is conceptually defined as a clinically recognizable state in which the ability of older people to cope with every day or acute stressors is compromised by an increased vulnerability brought by age-associated declines in physiological reserve and function across multiple organ systems (WHO, 2017). Mild cognitive impairment (MCI), or mild neurocognitive disorder, is defined as a decrement in cognitive functioning that goes beyond normal ageing but does not yet meet the criteria for dementia or major neurocognitive disorder (Sachs-Ericsson & Blazer, 2015). However, this represents an

early clinical stage during which there may be an opportunity to preserve function and prevent further cognitive decline (Gallagher, Fischer, & Iaboni, 2017).

CF is selected to identify individuals with reduced cognitive reserve, a potentially reversible consequence of frailty rather than the result of neurodegenerative disorders (Shimada, Lee, Makizako, Chen, & Arai, 2018). The most common neurodegenerative include Alzheimer's disease, Parkinson's disease, prion disease, Amyotrophic lateral sclerosis, motor neuron disease, Huntington's disease, spinal muscular atrophy, and spinocerebellar ataxia; the non-neurodegenerative could include cardiovascular diseases and osteoporosis (Hague, Klaffke, & Bandmann, 2005; Harding et al., 2015; Klockgether, Mariotti, & Paulson, 2019; Martin, 1999).

CF is reported to increase the incidence of dementia, and it is associated with adverse health outcomes. However, this calls for the urgent formulation of a preventive action plan against the development of CF among older adults (Esteban-Cornejo et al., 2019; Ng, Feng, Nyunt, Larbi, & Yap, 2014; Rivan et al., 2019). The global prevalence of CF was reported to be between 1.0%–9.8%, respectively. It is important to note that prevalence is just a snapshot of a figure for a condition at a particular point in time (Rivan et al., 2020).

CF can be influenced by several risk factors, including vascular, lifestyle, physical activity, smoking status, psychosocial factors, and potential effects of poor nutritional status. Moreover, although some emerging biomarkers can adequately capture the risk of future physical and cognitive decline individually, they may not be precise for CF (Kameda, Teruya, Yanagida, & Kondoh, 2020). Therefore, it is necessary to identify possible biomarkers that can better determine the risks of CF and can potentially be used as a molecular signature for targeted interventions (Chen, Mao, & Leng, 2014; Looman et al., 2018). Human ageing is a highly complex biological process exhibiting

significant individual variation, and until now, its metabolic basis has been little understood (Kameda, Teruya, Yanagida, & Kondoh, 2020). For frailty diagnosis, Kameda and colleagues applied the Edmonton Frail Scale (EFS) and the Japanese version of the Montreal Cognitive Assessment (MoCA-J) and Timed Up & Go (TUG) test for the motor ability to evaluate cognitive aspects of frailty (Kameda et al., 2020). Rivan and colleagues conducted a community-based longitudinal study (the longitudinal study on a neuroprotective model for healthy longevity (LRGS TUA)) to determine the incidence and the predictors of CF among Malaysian older adults (Rivan et al., 2020). A Malaysian study in 2020 successfully reported that the incidence rates of CF and dementia among Malaysian older adults were 100 per 285 person-years at a five-year follow-up. Advancing age, depression, slow processing speed, lower performance in the timed up and go (TUG) test, low intake of vitamin D, and physical frailty were predictors in the development of CF among Malaysian older adults. Older age, a lower niacin intake, lack of social support, depression, and lower functional status were significant factors associated with cognitive frailty among older Malaysian adults. Malonaldehyde (MDA) and telomerase activity can be used as potential biomarkers to identify CF (Rivan et al., 2020).

Although the theoretical framework of frailty is well established, its operationalization and clinical implementation are still debated. Moreover, frailty is an important pre-disability syndrome with a multifactorial origin and needs to be monitored and treated. Nutritional aspects, such as the anabolic resistance to proteins, the inflammatory and oxidative status, and nutritional risk, are strong determinants of frailty and are suggested to be part of the framework (Zukeran & Ribeiro, 2017).

The phenotypic heterogeneity of frailty, the multisystem derangements that underlie its pathophysiology, and the fluctuations of individuals across severity states are

significant obstacles to a comprehensive appraisal of the condition (Cesari, Calvani, & Marzetti, 2017). Hence, a prospective investigation to determine the magnitude of CF as indicated by the incidence rate and the predictors of CF among multi-ethnic Malaysian older adults is essential to formulate public health strategies for healthy longevity (Stolz, Mayerl, & Freidl, 2019). Metabolomics is one of the systems' biology disciplines that comes in a chain after genomics, transcriptomics, and proteomics. It identifies and quantifies small molecular metabolites in the living organism's metabolic profile (metabolome) (Corona, Rizzolio, Giordano, & Toffoli, 2012). Each organism's metabolome is highly affected by internal or external environmental factors. Therefore, the variation in metabolome might be associated with a particular phenotype, specific nutrition, drugs, and diseases. This variation can be considered as the metabolic fingerprint or surrogate biomarkers of medical conditions or diseases. Metabolomics also helps in the ability to understand the pathways that lead to changes in the levels of body metabolites due to disease, a drug with/without environmental effect (George G. Harrigan, Maguire, & Boros, 2008). The reason for the use of metabolomics in the exploration of medical conditions in recent medical approaches is because it does reflect not only the variation in genetics, transcriptomics, and proteomics, which might be associated with the condition but also the environmental factors associated with the condition (Guţiu et al., 2010).

1.2 Application of Metabolomics in Disease Diagnosis

Different medical conditions can have distinct metabolic fingerprints (metabotype) on the metabolome due to the body's pathophysiological changes that usually reflect the metabolome. The metabotype is a group of specific metabolites that, either by their presence or absence, increased or decreased concentration, are distinctive for a specific

clinical status or disease (Semmar, 2012). Thus, researchers have investigated the use of metabolomics techniques to explore different medical conditions, particularly conditions with no definite or clear-cut diagnosis that need further invasive procedures. Metabolomics analyses can be used to understand these host–pathogens' interactions and screen host samples for biomarkers' characteristics of a specific state. Those biomarkers can then be used for disease diagnosis, prognosis, staging, and the assessment of new drugs with applications in viral, bacterial, and parasitic infections. Moreover, studying the metabolomics of the host immune response to infection can provide meaningful insights into global pathway differentiation. At the same time, its implementation in biomarker discovery is a valuable tool in studying infectious diseases with applications in disease diagnosis, staging and assessment of treatment efficacy (Tounta, Liu, Cheyne, & Larrouy-Maumus, 2021). A figure of metabolomics' place in the hierarchy of systems biology is presented in Figure 1.1.

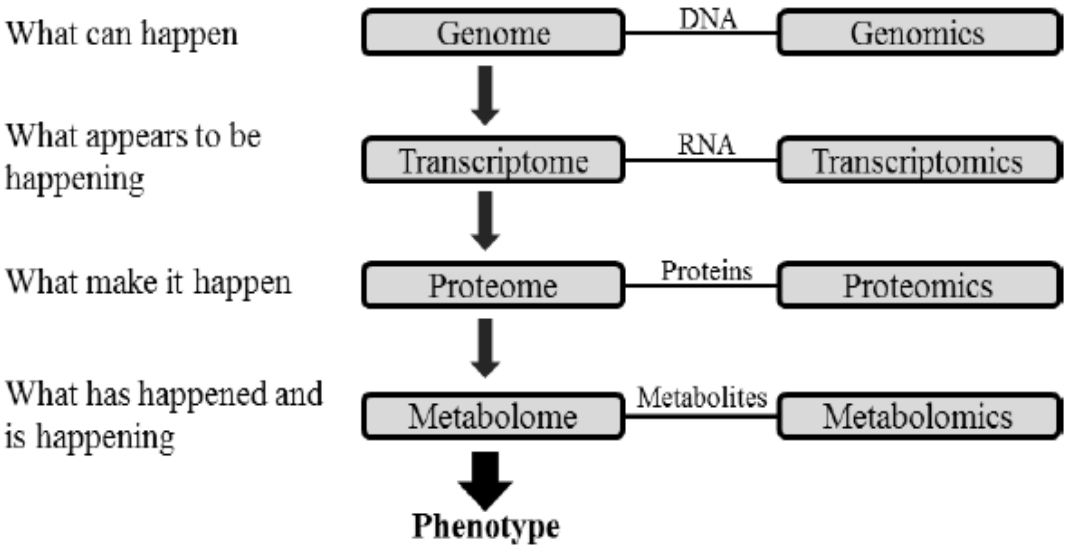

Figure 1.1: The hierarchy of systems biology. Modified from Dettmer et al. (Dettmer, Aronov, & Hammock, 2007)

As shown in Figure 1.1, transcriptomics, proteomics and genomics can provide relevant information regarding the genotype but deliver limited information about phenotype.

Metabolites are the closest link to phenotype, and their concentration levels reflect a snapshot of the physiological status of the cells, which shows how the metabolic profile of a complex biological system is changed in response to stress like adverse factors and environmental alterations (Fuhrer & Zamboni, 2015; Khoomrung et al., 2017; Patel & Ahmed, 2015; B. Peng, Li, & Peng, 2015; X.-X. Peng, 2013; D. G. Robertson, 2005; J. Zhang et al., 2014).

1.2.1 Application of Metabolomics Techniques to Identify Novel-Biomarkers of Diseases

Metabolomics studies aim to find a metabotype with the diagnostic or classifying value among patients and explore and measure the metabolites that will further assist in diagnosing a disease or its classification (Lindon, Nicholson, & Holmes, 2007).

Many metabolomics studies have been conducted on biological fluid samples to identify biomarkers of diseases such as liver disease, diabetes, asthma, cancer, and critical illnesses (Serkova, Standiford, & Stringer, 2011; Wang et al., 2012). These studies have identified metabotypes that can discriminate diseased from non-diseased subjects.

Some metabolomics studies aim to investigate metabolic changes consequent to exposure to environmental factors or toxins, such as smoking and other harmful substances. For example, in an approach to explore the effects of smoking and smoking cessation, researchers have used metabolomics techniques to quantify 140 metabolites in the fasting serum of three groups, namely current smokers, non-smokers, and quitters (who have quitted during the follow-up period of the study) in a longitudinal analysis (Xu et al., 2013). It was discovered that 21 smoking-related metabolites significantly differed from those of current smokers and non-smokers. Interestingly, the study discovered that 19 of 21 metabolites were reversible in quitters (Xu et al., 2013). In

another study of the systemic toxic effect of welding fumes on humans, Wei and colleagues used liquid and gas chromatography-MS to investigate the plasma metabolome of boilermakers' pre-welding and post-welding fumes exposure in a two stage-study (Wei et al., 2013). The study's first stage (stage one) was conducted in 2011 on 11 boilermakers. The second stage (stage two) was conducted in 2012 on eight boilermakers; five participated in stage one, and three were newly recruited boilermakers. The results showed that high exposure to high metal welding fumes causes a decrease in unsaturated fatty acids (Wei et al., 2013). As mentioned above, metabolomics studies are part of a continually growing body of evidence on the preeminent role that metabolomics can play in disease diagnosis and the pathogenic understanding of diseases and variable medical conditions. Consequently, this will help monitor, guide, and evaluate the current therapy and help find new drug targets for future drugs.

However, Kameda et al. (2020) and colleagues applied untargeted, comprehensive LC-MS metabolomic analysis to human blood from 19 frails and non-frail elderly patients who were clinically evaluated using the Edmonton Frail Scale, the MoCA-J for cognition and the TUG for mobility. Among 131 metabolites assayed, 22 markers for frailty, cognition, and hypomobility were identified, most of which were abundant in blood. Frailty markers included 5 of 6 markers related to cognition and 6 of 12 associated with hypomobility. These overlapping markers included metabolites related to antioxidation, muscle or nitrogen metabolism, and amino acids, most of which are decreased in frail older adults. Five frailty-related metabolites were decreased-1,5-anhydroglucitol, acetyl-carnosine, ophthalmic acid, leucine, and isoleucine- have been previously reported as markers of ageing, providing a metabolic link between human ageing and frailty. The findings indicate that metabolite profiles efficiently distinguish

frailty from non-frailty. Notably, the antioxidant ergothioneine, which decreases frailty, is neuroprotective. Thus, oxidative stress resulting from diminished antioxidant levels could be a critical vulnerability for the pathogenesis of frailty, exacerbating illnesses related to human ageing (Kameda et al., 2020).

1.3 Rationale

Diagnosis of CF takes much time and many questionnaires, which may cause some inconveniences to the older adults. In addition, due to inter-individual differences in metabolizing capacities, the metabolism rates of CF differ primarily among any given population. Consequently, the reaction rates are jointly limited by enzyme capacity and metabolite concentration (Fendt et al., 2010).

The early diagnosis of CF is imperative to start the optimum treatment and prevent the detrimental effects of chronic treatment consumption, which might take years to be manifested clinically. Indeed, this detection might encourage individuals suffering from CF to seek treatment for their illness. Nevertheless, this will have a positive impact on CF individuals themselves and their families, and society. Therefore, this study proposes using metabolomics to explore and identify CF compounds and discriminate subjects with CF from those with mild cognitive impairment (MCI) as well as from robust (healthy) subjects.

1.4 Hypothesis

This study hypothesized that metabolomics techniques could identify a metabolic fingerprint to discriminate CF individuals from MCI and Robust.

In this study, the proton nuclear magnetic resonance (^{1}H-NMR) metabolomics technique is used to investigate the plasma of individuals with CF and MCI as well as healthy individuals to test the hypothesis of novel biomarkers that can predict CF.

1.5 Aim of the Study

Using the metabolomics approach, this study aimed to determine plasma metabolites, their related pathways and their correlation with clinical parameters of cognitive frailty (CF) and mild cognitive impairment (MCI).

1.6 Objectives

The objectives of the current study were listed below

1. To identify metabolic fingerprints that can be used to distinguish subjects with CF, MCI, and robust.
2. To explore the metabolomics pathway analysis for diagnosis of CF and MCI.
3. To explore the correlations between the identified metabolites and the clinical data.

CHAPTER TWO

LITERATURE REVIEW

2.1 Physical Frailty

2.1.1 Definition

Frailty is common in healthcare research on older adults (Clegg, Young, Iliffe, Rikkert, & Rockwood, 2013). It has been explored across broad contexts, including falls (Lan, Li, Wang, & Chen, 2020), oncology (Ethun et al., 2017), recovery following surgery (Lin, Watts, Peel, & Hubbard, 2016), and in recent times, access to care in some European countries during the COVID-19 pandemic (Polidori, Maggi, Mattace-Raso, & Pilotto, 2020). Frailty has been found to predict the utilization of many healthcare services (Roe, Normand, Wren, Browne, & O'Halloran, 2017) Therefore, it has become a prevalent topic given its economic consequences.

Most of us have used the word "frail" to describe some older people we know. Although it means different things to different people, frailty is most commonly equated to being old and weak. Indeed, the Oxford Dictionary defines frailty as "the condition of being weak and delicate". To begin with, it is worth reiterating the key features of frailty that explain the amount of attention it has received in recent years and constitutes the basis for its choice as the subject for this research. Firstly, frailty is a common problem for older people, which grows rapidly in the numbers affected. Secondly, frailty bears adverse consequences, which are significant at both the individual and societal levels. Finally, frailty may be prevented or its adverse impact mitigated at least partially by appropriate interventions.

Given that the ultimate purpose of studying frailty is to understand how physicians may influence its development and effects, a detailed examination of its pathways is

essential. Early attempts to create graphical representations mainly focused on biological pathways. Fried proposed a cycle of frailty that assembles physiological and clinical factors in a feedback loop (Fried et al., 2001). For example, the effects of ageing, disease, and under-nutrition are linked to loss of muscle mass, known as sarcopenia. However, this, in turn, manifests as decreased strength, physical inactivity, and low energy expenditure, leading back to under-nutrition. The cycle is then repeated as a feedback loop.

However, to the scientific community, frailty has two different, albeit related, meanings. The first has its roots in demography and refers to the unobservable heterogeneity distribution in mathematical models for survival (Hogan, 2018), which have come to be known as frailty models. These models have been applied to mortality and ageing to establish the common ground it shares with the second meaning, which is the subject of this thesis. Here, frailty denotes the multidimensional loss of an individual's reserves that occurs with greater probability in the face of advancing age. This loss is vulnerable to developing adverse outcomes such as hospitalization, functional dependency, and death (Espinoza & Walston, 2005; Lally & Crome, 2007; Mohandas, Reifsnyder, Jacobs, & Fox, 2011; Pel-Littel, Schuurmans, Emmelot-Vonk, & Verhaar, 2009). Within medical circles, frailty is widely considered a clinical syndrome with an underlying biological basis and is thought to be a transitional state between robustness and functional decline (Lang, Michel, & Zekry, 2009). A key underlying concept is that multiple body systems are involved (Strawbridge, Shema, Balfour, Higby, & Kaplan, 1998). Historically, frailty was first conceptualized as involving multiple domains by Strawbridge almost two decades ago in his seminal work on older respondents in the Alameda County Study. He proposed viewing frailty as a syndrome involving

deficiencies in two or more of physical, nutritive, cognitive, and sensory domains (Strawbridge et al., 1998).

One widely referenced explanation is Rockwood and Mitnitski (2007), who state that frailty is an age-associated vulnerability to accumulated deficits that leads to increased risk when exposed to a stressor. Frailty is characterized by increased dependence, diminished strength, lowered endurance, and reduced physiological function (Keevil & Romero-Ortuno, 2015; Morley et al., 2013). Frailty syndromes include falls, decreased mobility, delirium, and confusion; frail older people are more likely to be admitted to the hospital (Parker et al., 2018). Although experts lack consensus on the exact mechanisms of frailty, it is accepted that stressors can exacerbate frailty, leading to further decline (Clegg et al., 2013). Frailty is treatable (Sahota et al., 2017), but its management depends on the individual. To treat frailty, it must first be identified appropriately (Ellis et al., 2017), including primary care, emergency departments, acute hospital wards, and day hospitals.

Fried et al. (2001) define the Fried Frailty Phenotype (FFP) as a clinical syndrome where three or more are present: unintentional weight loss, exhaustion, low physical activity levels, slow walking speed, and weak grip strength. FFP showed that frailty rates from 5,317 participants over a seven-year follow-up were predictive of adverse health outcomes, including hospitalization, falls, disability, and mortality (Fried et al., 2001).

The Irish Longitudinal Study on Aging (TILDA) states that one in ten Irish adults over 65 years old are frail. Accordingly, this increases to two in five Irish adults over 80 years old (Donoghue et al., 2018). In addition, data analyses from the TILDA study found that 16% of older Irish adults had attended an emergency department and had an inpatient stay in a hospital (Roe, Normand, Wren, Browne, & O'Halloran, 2017).

Inpatient hospital stays were the most common reason for the increased management cost of frail older adults (n=1,636) in a German longitudinal study over eight years (Hajek et al., 2018). Based on the five criteria of the FFP (Fried et al., 2001), the cost of healthcare increased by up to 54% if the older adult was positive for three criteria and by 101% if they were positive for four to five criteria. This study was adjusted for confounding by co-morbidity, as comorbidity may also increase the cost of healthcare (Hajek et al., 2018).

As the population ages and life expectancy increases, there will be a higher demand for healthcare resources, and many older people may require institutionalization. Notably, the number of Irish adults over 65 increased by 19.1%, while nursing home residents increased by 8.6% from 2011-2016 (Murphy, Ashwick, Palmer, & Busuttil, 2019). Accordingly, this means that a more significant proportion of older adults are living at home. Up to 73% of Irish adults over 55 years old live with a spouse (Donoghue et al., 2018). As adults age and develop frailty, they may become dependent on others for activities of daily living (ADLs). Spouses can be the first to support their partners in need, but many have limitations. Having an unwell spouse can lead to a decline in the health and wellbeing of the other spouse (Valle, Weeks, Taylor, & Eberstein, 2013), which may lead to an additional risk of frailty. However, there is little evidence that explicitly treating frailty will reduce health service costs (Hajek et al., 2018). Frailty is confounded by the nature of the individual requiring support from acute services and in the community. For many older adults living at home or returning home from the hospital with a change to their function, this support may come from spouses. These spouses might also be frail and require support. The spouses' ability to provide care may be a factor in the success of a hospital discharge.

The ageing phenomenon has gained attention in Malaysia since this country was estimated to be an ageing nation for the coming years. Frailty syndrome is an alarming geriatric syndrome nowadays because of its adverse societal impacts, such as hospitalization, institutionalization, morbidity, and mortality. It is a dynamic process and potentially reversible if detected early (Fried et al., 2001). Thus, early detection of modifiable risk factors such as coronary artery disease (CAD), type 2 diabetes mellitus (T2DM), and alcohol intake frequency might be essential for early interventions in the primary setting. Frailty is a recognizable health state associated with multi-system deterioration (e.g., mobility, cognition, function, endurance). Not all older adults are frail, but it typically impacts the geriatric population. In their study, Lawson et al. state that around 10% of people over 65-year-old experience frailty. This number is even more significant (between 25%-50%) for those over age 85. Persons who experience frailty are more likely to experience adverse events such as falls, hospitalization, disability, dependence, placement in long-term care, and death (B. Lawson et al., 2017). Frailty is also a major risk factor for disability (Dudzińska-Griszek, Szuster, & Szewieczek, 2017). According to Griszek, typical physical symptoms of frailty include exhaustion, weakness (assessment based on the handgrip strength measurement), unintentional weight loss, slow gait, and decreased physical activity. The degree of frailty is assessed with the clinical frailty scale presented in Figure 2.1. Based on the severity of the clinical symptoms of frailty and patient condition, Lawson presents nine different ageing stages from very fit to terminally ill.

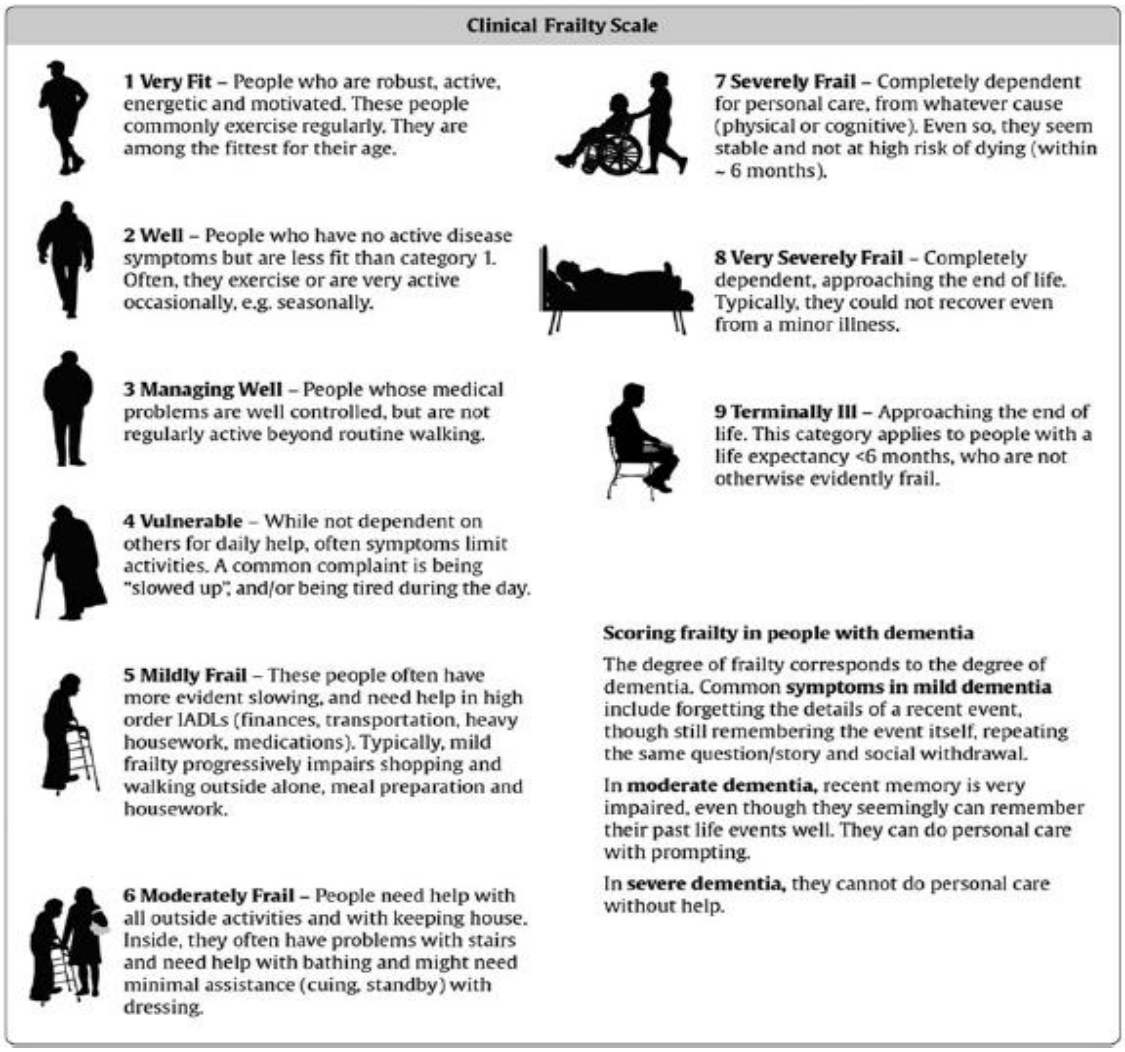

Figure 2.1: Clinical frailty scale (B. Lawson et al., 2017)

2.1.2 Epidemiology

Population ageing is a global phenomenon. Global, the number of adults aged 65 years and older is expected to rise from 841 million to 2 billion by 2050, while in Australia, the number is projected to rise from 2.4 million in 2007 to 6.4 million by 2056 (Wilson, 2012). Moreover, by 2056 almost 50% of older Australians will be classified as the 'old' old as they will be aged 85 years and over (Wilson, 2012). Furthermore, developing countries in Asia and Africa are expected to have the most significant burden from population ageing of these developing countries; Malaysia will be one of the most affected, with the Malaysian population aged ≥ 65 years expected to reach more than six million by 2035 (Figure 2.2) (S. T. Chen, Ngoh, & Harith, 2012;

Indonesia, 2013; Tsunami, 2017). As a result, Malaysia will be labelled as an ageing nation by 2035, with an estimated 15% of its population comprising adults aged 65 and above (Sabri, 2016).

Furthermore, those advanced in age tend to suffer from chronic diseases, sleep disruption, psychological problems, and cognitive decline. In addition, they are likely to be physically frail and have higher functional disabilities than younger people. Because of these conditions, the elderly require specific geriatric care and counselling, which are still lacking in Malaysia (Loh et al., 2015).

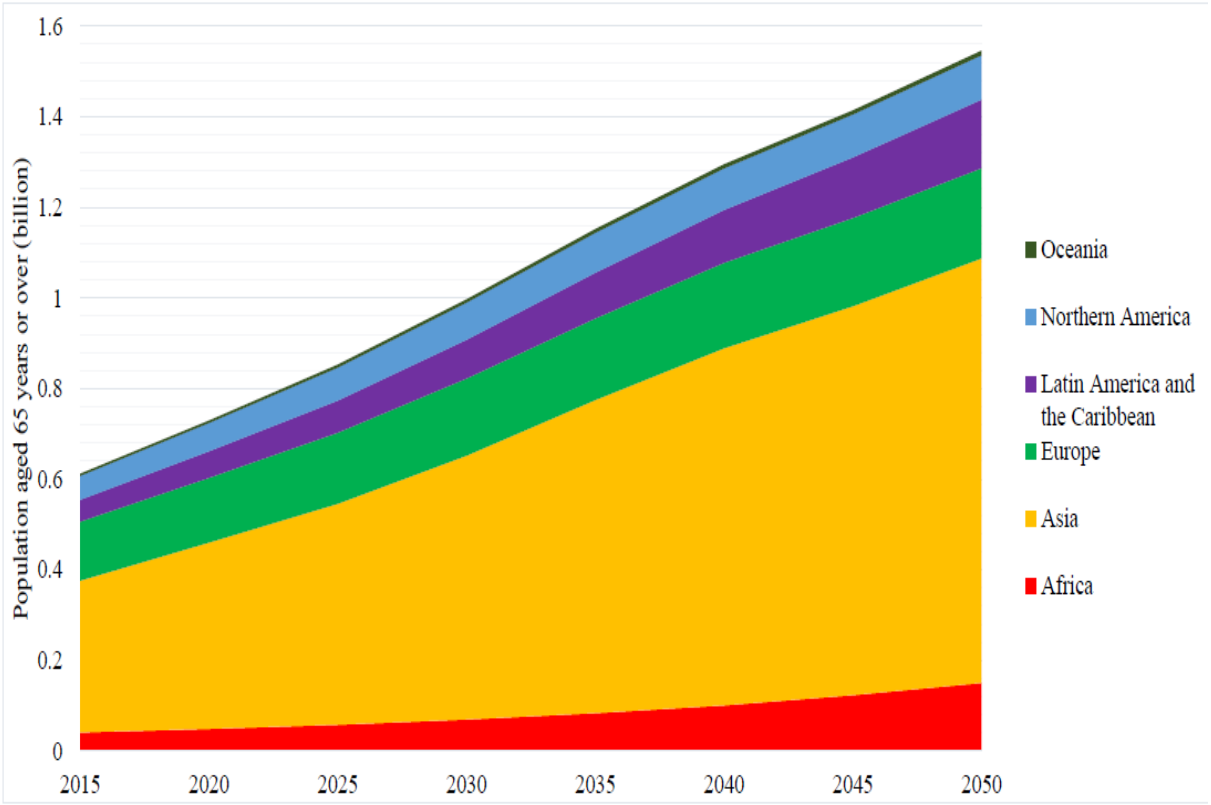

Figure 2.2: Older adults' population growth projection from 2015 to 2050. It is adapted from the (UN, 2015).

A frail person risks rapid deterioration when exposed to external stressors, such as acute illness (Ferrucci et al., 1996; Fried, Ferrucci, Darer, Williamson, & Anderson, 2004), illustrated in Figure 2.3. However, frailty is a dynamic syndrome where deterioration is

common, but the individual course of frailty varies, and the degree of frailty can be improved even in old age (Clegg et al., 2013; Kojima, Taniguchi, Iliffe, Jivraj, & Walters, 2019).

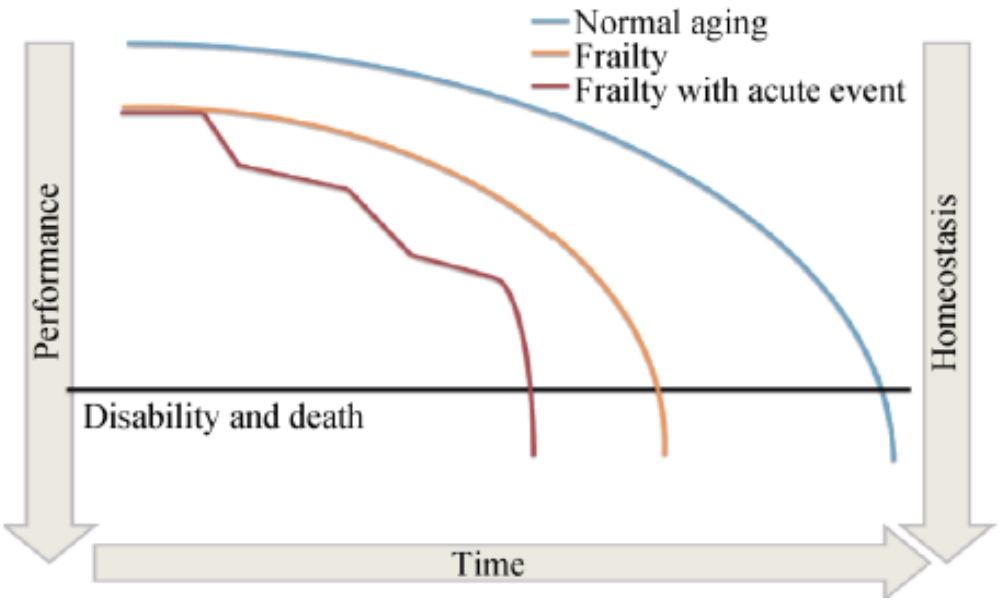

Figure 2.3: Fall to disability and death: comparison between normal ageing, frailty and frailty associated with an acute event (Tonet, Pavasini, Biscaglia, & Campo, 2019)

2.2 Cognitive Impairment

All healthy adults share the same basic cognitive processes (Nisbett, Peng, Choi, & Norenzayan, 2001; Park, 2002). These cognitive processes involve perception, attention (focusing, sustaining, shifting, and dividing attention), visuospatial ability (drawing, construction, and visual search), and expressive and receptive language (Cumming, Marshall, & Lazar, 2013; Cumming, Tyedin, Churilov, Morris, & Bernhardt, 2012). Nevertheless, cognitive impairment represents one of the most common geriatric syndromes among older adults. Between 16% to 20% of older adults in the USA have mild cognitive impairment (MCI) (Yaffe et al., 2010), which is characterized by a deterioration in cognitive functioning in at least one cognitive domain (memory,

executive functioning, attention, language, and/or visuospatial ability) juxtaposed with the absence of impaired functioning in everyday tasks (Tamura et al., 2011).

Additionally, individuals with MCI tend to experience more subjective cognitive concerns, functional impairment, self-rated health problems and psychopathology (Ganguli et al., 2019). For instance, those with MCI are approximately twice as likely to have depressive symptoms compared to similarly aged healthy adults, with more severe levels of cognitive impairment predicting more severe depression (Clément, Belleville, Bélanger, & Chassé, 2009; Shahnawaz et al., 2013). Therefore, continued research into the early detection and intervention of MCI is vital for the future of our elderly populations (Yang et al., 2023).

MCI is increasingly recognized as a prodromal phase of many types of later-life dementias—a chronic state of severe cognitive impairment (Aditi Gupta et al., 2017; Hucker et al., 2017; Jindel, Joseph, Morris, Santella, & Baines, 2003; McAdams-DeMarco et al., 2017; Tamura et al., 2011; Yaffe et al., 2010). Cognitive function has been argued to be one of the important components of frailty and is negatively associated with frailty risks (Rodríguez-Mañas et al., 2013). Cognitive function can be represented as a composite score of four cognitive function tests conducted during the primary interview (Verbal-fluency task, letter-cancellation task, immediate word-recall task, and delayed word-recall task) (Steptoe, Breeze, Banks, & Nazroo, 2013) which cover three important critical domains of cognitive functioning (executive function, processing speed, and memory) (Batty, Deary, & Zaninotto, 2016). All four scores in the English Longitudinal Study of Aging (ELSA) were normally distributed without evidence of floor and ceiling effect (Zaninotto, Batty, Allerhand, & Deary, 2018). A higher score indicates better cognitive function (Zaninotto, Batty, Allerhand, & Deary, 2018). This composite score has been used by a previous ELSA paper and was validated

in association with mortality (Batty et al., 2016). Additionally, cognitive difficulties are associated with poorer quality of life (R. A. Lawson et al., 2014; Schrag, Jahanshahi, & Quinn, 2000), lower life satisfaction (Rosqvist et al., 2017), higher levels of depression (Fernandez et al., 2009; Santangelo et al., 2009), and increased apathy (Butterfield, Cimino, Oelke, Hauser, & Sanchez-Ramos, 2010). The main risk factor for cognitive impairment is ageing. Other risk factors include diabetes, high blood pressure, high cholesterol, tobacco use, alcohol use, depression, and diet and exercise (Moyer, 2014). The relationship between increasing age and cognitive impairment is well-known and has been explored (Lobo et al., 2000; Prince et al., 2013). Cognitive impairment is also associated with higher usage of health and social services (Ganguli et al., 1996) and anxiety (Lee, Tsai, Gauthier, Wang, & Fuh, 2012; Ryder et al., 2002; Zahodne, Marsiske, & Bowers, 2013). More importantly, disruption of fluid and electrolyte homeostasis causes hypertension and cardiovascular dysfunction, while the build-up of waste products leads to impaired cognitive, gastrointestinal, and sexual function (Salerno, Parraga, & McIntyre, 2017).

Chronic kidney disease (CKD) progresses in significant functional limitations, substantial disabilities, and a decline in the health-related quality of life of those with the condition (Broers et al., 2017; Intiso, 2014; Painter & Roshanravan, 2013). These functional restrictions are mainly caused by CKD complications such as anaemia, malnutrition, metabolic dysfunction, impaired cognitive function, and poor muscle strength (Intiso, 2014).

Cognitive impairment and physical inactivity are linked together in the process of ageing. For example, dementia positively correlates with decreased physical activity, fear of falling, and associated pain (Bherer, Erickson, & Liu-Ambrose, 2013). In addition, Erickson et al. showed that regular physical exercises effectively reverse

hippocampal volume loss, which is connected to improved memory function. Accordingly, the personal mobility device should tackle both issues (Erickson et al., 2011). Accordingly, considerable evidence suggests a high cerebral and physical training efficiency combined. For example, cognitive function training combined with task-oriented physical activity can reduce the risk of falls among older adults.

Moreover, dual-tasking exercises are critical healthcare needs to improve balance and gait (Silsupadol et al., 2009). Both cognitive decline and physical frailty independently lead to increased disability, falls, mortality, increased health service needs, and high direct/indirect costs to healthcare, often long-term care and hospitalization (Adriaensen et al., 2014; Aguilar et al., 2014). Individuals with physical frailty and cognitive impairment may have a higher risk for disability than those with isolated physical or cognitive impairment. Nevertheless, most research groups have historically excluded older adults with cognitive impairment from frailty studies (Adriaensen et al., 2014; Prusaczyk, Cherney, Carpenter, & DuBois, 2017; Taylor, DeMers, Vig, & Borson, 2012). The International Consensus Group organized by the International Academy on Nutrition and Aging (I.A.N.A) and the International Association of Gerontology and Geriatrics (I.A.G.G) convened in 2013 to identify related domains of physical frailty and cognition and termed the phenomenon "cognitive frailty" (Adamis et al., 2014; Kelaiditi et al., 2013).

2.3 Cognitive Frailty

The Institute of Medicine Report on Cognitive Aging described a need to develop an operational definition of cognitive frailty (CF) for research, clinical detection, and public health surveillance (Liverman, Yaffe, & Blazer, 2015). A model for detecting CF could provide practitioners with the tools needed for early detection and secondary

prevention. Currently, the instrumental assessments for CF are time-consuming, expensive, and require extensive training, and the clinical translation properties are not precise (Kelaiditi et al., 2013). Furthermore, the translation of the CF construct into the clinical setting is limited by the lack of consensus on an operational definition and considerable heterogeneity and complexity in the diagnostic criteria. The International Consensus Group (I.A.N.A. /I.A.G.G.) report acknowledges the need to focus research efforts on a clinical condition characterized by the co-occurrence of physical frailty and cognitive impairment in the absence of overt dementia diagnosis or underlying neurological conditions (Kelaiditi et al., 2013).

The CF construct is considered a heterogeneous clinical syndrome in older adults with evidence of 1) physical frailty and cognitive impairment (Clinical Dementia Rating score of 0.5); and 2) exclusion of a clinical diagnosis of Alzheimer's Disease (AD) or other dementia (Kelaiditi et al., 2013).

CF has evolved as a concept over recent years, initially used to describe the co-occurrence of MCI and physical frailty without dementia (Facal et al., 2019). The characteristics of CF have since been developed so that it is now viewed as including the potentially reversible state of subjective cognitive decline and pre-physical frailty (at which stage it is thought preventative interventions could be targeted). Conceptually, it is thought of as a state of reduced cognitive reserve differentiated from "normal" brain ageing by potential theoretical reversibility (Ruan et al., 2015). Despite this, no practical interventions for these potentially reversible states have been published (Facal et al., 2019). Facal et al. conclude by explaining that neither physical nor cognitive areas of functioning are subordinated to each other and recommend that the operational definitions of physical and CF also incorporate social and psychological variables (for example, reading habits and social environment) given that these factors are likely to

influence an individual's cognitive reserves as they age. Interestingly, these authors seem to be arguing for a broader more multi-component approach to (cognitive) frailty (Facal et al., 2019). This emerging convergence between concepts can be evidenced by the fact that operational frailty instruments increasingly include a component screening for cognitive impairment: in a systematic review of 94 frailty tools, 46% included a cognitive measure, a significant majority of these papers being published after 2010 (Azzopardi et al., 2018). Several population-based studies estimated the prevalence rate of CF to be in the range of 1.0% to 12.0% (Feng et al., 2017; John, Tyas, Griffith, & Menec, 2017; Solfrizzi et al., 2017), whereas, in clinical settings, the figure was much higher at 10.7% to 40% (Delrieu et al., 2016; Jha et al., 2016; Montero-Odasso et al., 2016). Meanwhile, a study in Malaysia in 2020 found that the five years' cumulative incidence of CF was 35.5% (Rivan et al., 2020). Nevertheless, CF can be influenced by a number of risk factors, including vascular, lifestyle, physical activity, smoking status, and psychosocial factors as well as potential effects of a poor nutritional status (Fougère et al., 2017).

The extent to which physicians can predict CF using biomarkers depends on our behavioural markers' accuracy in early identification. Screening for detecting cognitive decline (i.e., neuropsychological) and frailty is determined by the identification tools for defining individuals with CF. Individuals with CF present with a unique neuropsychological profile, scoring worse on executive and attention tests. Individuals with three or more of the frailty criteria are more impaired than individuals with only one of the frailty criteria (Delrieu et al., 2016). Consequently, CF is defined as individuals with cognitive decline and one or more frailty criteria (Delrieu et al., 2016).

2.4 Diagnostic Methods

2.4.1 Physical Frailty

2.4.1(a) Frail Scale

The primary research tool was the FFP (Fried et al., 2001). Accordingly, this has been used in similar studies investigating frailty in caregivers (Potier et al., 2018; Santos-Orlandi et al., 2017). The Frail scale is a self-report measure of frailty that was proposed by the International Association of Nutrition and Aging and consisted of five items: fatigue, resistance, ambulation, illnesses, and loss of weight (G Abellan Van Kan et al., 2008). Each item is scored either zero or one to give a maximum score of five (Morley, Malmstrom, & Miller, 2012). This tool examines five different domains of frailty, and the participant was classed as robust (0/5 positive domains), pre-frail (1-2/5 positive domains), or frail (3 or more positive domains). Aside from formal definitions of frailty, knowing its opposite meaning can assist in achieving a better understanding of this seemingly vague concept. In this case, an antonym of frail is “robust”, which has been adopted to categorize health states (Fried et al., 2001). Table 2.1 shows the summary of the diagnostic method of the frail scale. A study conducted by Ravindrarajah et al. in the European Male Aging Study Group explored the association between frail scale and all-cause mortality (Ravindrarajah et al., 2013). The study demonstrated that frail men had an increased mortality risk compared to robust men, as defined by the frail scale. The frail scale has also been validated for older women (Gardiner, Mishra, & Dobson, 2015). Several other measures of frailty are used in research among older people. One such example is the Fried frailty scale. Fried et al. defined frailty as a clinical syndrome with three or more criteria: unintentional weight loss, self-reported exhaustion,

weakness (grip strength), slow walking speed, and low physical activity (Fried et al., 2001). A study among community-dwelling older people using the above frailty phenotype showed that the measure was independently predictive of incident falls, worsening mobility, hospitalization, and death (Fried et al., 2001). A study by Bieniek et al. examined the use of the Fried frailty index among older inpatients and found that completion of all five Fried frailty assessment criteria was possible in only 65% of studied patients (Bieniek, Wilczyński, & Szewieczek, 2016).

Another commonly used frailty scale is the Clinical Frailty Scale (CFS). The CFS is a subjective frailty assessment tool validated in acute hospital setting (Juma, Taabazuing, & Montero-Odasso, 2016). In a study by Rockwood et al., the 7-point CFS was used to measure the frailty of older patients who were followed up for five years to determine its ability to predict death or need for institutional care (Rockwood et al., 2005). The study found that each category increment in the CFS scale significantly increased the medium-term risks of death (21.2% within about 70 months) and entry into an institution (23.9%). The updated version of the CFS consists of a 9-point scale ranging from ‘Very Fit to ‘Terminally Ill’ (Alfaadhel et al., 2015; Mendiratta & Latif, 2020; Rockwood & Mitnitski, 2007). The CFS is a straightforward and practical way of stratifying frailty, but the CFS's disadvantage is observer subjectivity. The Fried frailty scale and CFS are commonly used in research and clinical practice (Dent, Kowal, & Hoogendijk, 2016).

2.4.1(b) Barthel Index

The Barthel Index (BI) measures functional abilities and is commonly used in clinical and research settings (Sainsbury, Seebass, Bansal, & Young, 2005). It has been recommended by the Royal College of Physicians and the British Geriatrics Society for