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PhD Thesis

THE RELATION BETWEEN MEDITERRANEAN DIET, INFLAMMATION AND FRALITY

IN THE ELEDERLY PEOPLE OF THE MOLI-SANI STUDY

Tutor:	**PhD Student:**
Prof.	**Salvatore Dudiez**
Licia Iacoviello	**158084**

Coordinator:
Prof.
Marco Sarchiapone

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Abstract

Frailty is a condition of increased vulnerability to stressors due to age-related declines in physiologic reserves across neuromuscular, metabolic and immune systems. Frailty is considered a medical geriatric syndrome with multiple causes and contributors and it is characterized by diminished strength, power and endurance. In the definition of the frail phenotype a key role could be played by nutrition and inflammation. The aim of this PhD thesis was to screen elderlies from the Moli-sani Study population to identify frail individuals and evaluate a possible relation between frailty, mediterranean diet (MD) and inflammation. The Moli-sani Study is an ongoing, prospective, population based cohort of 24,325 men and women (aged ≥ 35 years) who were randomly recruited from the general population of the Molise region, between March 2005 and April 2010, with the purpose of investigating genetic and environmental risk factors in the onset of cardiovascular, cerebrovascular and tumor diseases. The recall phase of the Moli-sani Study started in March 2017 and a subsample of partecipants aged ≥65 years were selected for the purpose of this PhD thesis. Finally, 226 elderly participants (mean age 72.7±5.5 years) attended the follow-up visit from May 2018 to May 2019. To assess frailty, the Survey of Health, Ageing and Retirement in Europe-Frailty Instrument (SHARE-FI) was used. Two SHARE-FI calculators, one for women and one for men, were used to categorize the population studied into three categories: non-frail, prefrail and frail. Dietary information were collected through the European Prospective Investigation into Cancer and Nutrition Food Frequency Questionnaire (EPIC-FFQ). Adherence to MD was calculated using the Trichopoulou score. Cognitive impairment and health-related quality of life were studied using two questionnaires: Montreal Cognitive Assessment (MoCA) and Short Form Health Survey (SF-36). Biochemical markers for cardiovascular diseases (total cholesterol, HDL-cholesterol, tryglicerdides, glucose) were tested on serum samples. Additionally, blood inflammation and coagulation biomarkers were determined: high sensitive C Reactive Protein (hs-CRP), blood cell count and D-dimers. Taking into account the inflammation biomarkers, a low-grade inflammation score (INFLA-score) was also calculated. The prevalence of non frail, pre-frail and frail individuals was 78.8%, 15.9% and 5.3%, in the Moli-sani elderly cohort, respectivelly. Pre-frail and frail categories were collapsed to create a dichotomous variable having just two categories: "pre-frail/frail" vs "non frail" individuals. Women were more pre-frail/frail than men (28.2% vs 14.7%, P=0.015). Pre-frail/frail individuals were older than non-frail individuals (mean ±SD: 75.1±6.0 vs 72.1±5.2 years, P=0.0008). Pre-frail/frail elderly people showed a higher prevalence of cardiovascular disease and hypertension.

There was no difference between normal and pre-frail/frail elderly people in the adherence to MD. However, considering participants aged 65-69 years (N=88), pre-frail/frail subjects showed a higher prevalence of a lower adherence to MD than non-frail elderlies (50.0% vs 21.6%, P= 0.054). There was no significant difference in dietary habit between pre-frail/frail and non-frail participants, except for legume (P=0.010), fruits and nuts consumption (P=0.047), whose consumption was lower in pre-frails/frails. Pre-frail/frail elderlies showed a higher cognitive impairment (MoCA test, mean ±SD: 22.1±4.1 vs 23.6±3.5, P=0.023) and aworse perception of physical health-related quality of life (SF-36 physical dimension score, mean ±SD: 41.8±7.1 vs 45±5.8, P=0.0057) than non-frails. Pre-frail/frail individuals had lower levels of serum total cholesterol than non-frails (mean ±SD: 185.6±38.9 vs 210±43.8mg/dL, P=0.0004), which could be associated to higher use of lipid-lowering drugs (43.2% vs 29%, respectively). High blood levels of hs-CRP was found in pre-frails/frails as compared with no-frail elderly people (median (IRQ): 2.5 (1.0;4.8) vs 1.4 (0.8;3.5) mg/L, P=0.043). D-dimers plasma levels were also higher in pre-frails/frails compared to the non-frails (median (IRQ): 298 (227;460) vs 254 (226;328) ng/mL, P=0.053). Pre-frails/frails showed higher levels of blood inflammation (hs-CRP) and coagulation (D-Dimers) biomarkers which confirm the coexistence of the two processes, but not of cellular inflammation biomarkers (WBC, platelets). These findings may be relevant to promote interventions controlling inflammatory pathways to achieve important improvements in the elderly population.

Chapter 1: Introduction

1.1 Frailty in elderly people: definition and characteristics

In the last century the average life expectancy at birth has been remarkably increased from roughly 45 years in the early 1900s to approximately 80 years today. By 2050, over 21% of the global population, or two billion persons, will be over 60 years of age [1]. This will lead to an increase in many age-related conditions, including frailty [2]. Frailty literally expresses the property of some materials that, without deforming, break when they are subjected to impacts. The same word is used to describe the characteristics of weakness and delicacy, referring to things or people. The term frailty started to appear in the geriatric and gerontology literature about 50 years ago, proposed as a predictive model for dependency, physical and mental decline in older persons [2,3]. Prior to this, other terms such as *chronic sick*, *debilitated*, *disabled*, *sedentary institutionalized*, *incapacitated*, or *functionally dependent elderly* were used to describe older person at risk of mortality or other adverse outcomes [4]. In 1974, Charles F. Fahey and the Federal Council on Aging (FCA) of the United States introduced the term *frail elderly* for a special group of older adults with *"physical debilities and emotional impairment, as well as debilitating physical and social environments"* who needed additional attention [4-7]. By 1978, the FCA defined the frail elderly as "*persons, usually but not always over the age of 75 who, because of an accumulation of various continuing problems, often require one or several supportive services in order to cope with daily life"* [4]. A significant increase in publications about frailty has been observed since the beginning of the 1990's. At this time, frailty was often defined as functional dependence for the activities of daily living, associated with chronic diseases. As an example, Pawlson LG [8] focused on multiple illness in frail persons, MacAdam M et al. [9] referred to them as *"elderly with chronic conditions"* and Williams FM et al. [10] defined them as *"requiring long-term hospital care owing to chronic debilitating diseases"*. Woodhouse KW and colleagues [11] gave the following description of frail persons in their article *"Who are the Frail Elderly? The frail elderly are individuals, over 65 years of age, dependent on others for activities of daily living, and often in institutional care. They are not independently mobile – whilst they do not have overt cardiac, respiratory, hepatic, renal or metabolic disease, minor abnormalities may be revealed on laboratory investigation. They may require regular prescribed drug therapy. Conditions contributing to frailty commonly include Alzheimer's disease, multi-infarct cerebrovascular disease, Parkinsonism, osteoporosis, osteoarthritis, and healed fracture events"*.

In 1992, Buchner DM and Wagner EH revised the definition of frailty [12]. They defined frailty as a reversible condition of reduced physiologic reserve associated with decreases in physiologic capacity in neurologic control (indicated by diminished ability to perform complex tasks), mechanical performance (e.g. diminished strength), and energy metabolism (e.g. decreased aerobic status due to cardiac or pulmonary diseases or both). This increased attention to frailty during the last 30 years was accompanied by the rapid growth of different concepts and definitions of frailty. Most commonly, frailty is now defined as a state of decreased reserve and decline in multiple physiological systems that results in an increased risk of adverse outcomes such as falls, decreased mobility, slow recovery from any illness, reduced independence and increased hospitalization, disability, and death [2,13-19]. It is very important to distinguish frailty from comorbidity and disability, because they are often used interchangeably to identify the physically vulnerable subset of older adults requiring enhanced care. Recent researches supports geriatricians' perceptions that these are distinct clinical entities, although interrelated, and that clinical management of each of these has its own unique content and challenges. Disability is defined as difficulty or dependency in carrying out activities essential to independent living, including essential roles, tasks needed for self-care and living independently in a home, and desired activities important to one's quality of life. Physical disability is mostly diagnosed by self-report of difficulty in specific tasks, but objective and performance-based tests of function also exist. It is recommended by several organizations that clinicians screen for disability in self-care tasks (Activities of Daily Living, ADL) and tasks of household management (Instrumental Activities of Daily Living, IADL) on an annual basis in persons aged older than 70 years. Instead, comorbidity is the concurrent presence of two or more medically diagnosed diseases in the same individual, with the diagnosis of each contributing disease based on established and widely recognized criteria [20-22]. In this sense, the concept of comorbidity could be viewed as an interface between the geriatric paradigm of health and the more traditional medical definition of disease [22]. Frailty is distinct from, but overlapping with, both comorbidity and disability. In addition, both frailty and comorbidity predict disability, adjusting for each other; disability may well exacerbate frailty and comorbidity, and comorbid diseases may contribute, at least additively, to the development of frailty. Early data from the Cardiovascular Health Study (CHS) also suggest that the presence of disability or frailty could contribute to development or progression of chronic diseases, possibly through the lower activity levels associated with the former two conditions, or through other pathways affecting some basic biological mechanism essential to the maintenance of homeostasis, such as inflammation, or sympathetic–parasympathetic equilibrium [23-25].

These causal relationships provide explanation for the frequent co-occurrence of these conditions, and suggest the clinical importance of differentiating them so as to identify appropriate interventions. There are causal interrelationships that can help explain why these three entities are likely to co-occur. A clinical manifestation of this co-occurrence is the high likelihood of finding a greater proportion of frail persons among those who are disabled than among the nondisabled. This is supported in data from the Women's Health and Aging Study, in which 28% of this moderately to severely disabled population of women aged 65 years and older living in the community were frail, compared to 7% of a healthier subset of older women in the CHS [26]. Despite numerous contemporary theories and models, the causes of frailty are not yet fully understood. Numerous functional tests, questionnaires and indexes to categorize frailty have been proposed. Nevertheless, validity, reliability and agreement, responsiveness and the interpretability for general elderly populations remained unclear, so that geriatricians cannot yet diagnose frailty. Most frailty models cover seven major domains as reported in table 1.

Table 1 – The seven major domains of frailty and their operationalization.

FRAILTY DOMAINS	OPERATIONALIZATION
1. Nutritional status	Body weight, appetite, Body Mass Index (BMI)
2. Physical activity or mobility	Level of physical activity, leisure time physical (group) activity, difficulty or needing help walking/moving in and around the house, gait speed
3. Strength or energy	Tiredness, energy level (for example exhaustion/fatigue), lifting an object that weighs over 5 kg, weakness in arms and/or legs, performing chair stands, climbing stairs, grip strength, calf muscle circumference
4. Cognition and mood	Memory problems, diagnosed dementia or cognitive impairment, depression/depressed mood, sadness, anxiety, nervousness
5. Social relations/social support	Social recourses, emptiness/missing people around
6. Comorbidity	Presence of different diseases, medication
7. Problems of sense	Vision and hearing problems

The phenotypic definition of frailty as a geriatric syndrome was first proposed by Fried LP et al. [2], and was tested in the CHS. A phenotype of frailty is identified by the presence of three or more of the following components:

1. Shrinking: weight loss, unintentional, of ≥10 pounds in prior year or, at follow-up, of ≥5% of body weight in prior year (by direct measurement of weight).

2. Weakness: grip strength in the lowest 20% at baseline, adjusted for gender and body mass index (BMI).

3. Poor endurance and energy: as indicated by self-report of exhaustion. Self-reported exhaustion, identified by two questions from the Center for Epidemiologic Studies Depression Scale (CES-D), is associated with stage of exercise reached in graded exercise testing, as an indicator of VO_2 max, and is predictive of cardiovascular disease.

4. Slowness: the slowest 20% of the population was defined at baseline, based on time to walk 15 feet, adjusting for gender and standing height.

5. Low physical activity level: a weighted score of kilocalories expended per week was calculated at baseline, based on each participant's report. The lowest quintile of physical activity was identified for each gender.

The Fried criteria [2] revolve around the age-related loss of muscle mass and strength known as sarcopenia. According to recommendations by the European Working Group on Sarcopenia in Older People (EWGSOP), sarcopenia is a syndrome characterized by the progressive and generalized loss of skeletal density, muscle mass, and strength, leading to a risk of adverse outcomes such as physical disability, poor quality of life and death [27]. The EWGSOP recommends using the presence of both low muscle mass and low muscle function (strength or performance) for the diagnosis of sarcopenia. As shown in figure 1, weakness and low physical function are cornerstones for the diagnosis of sarcopenia as well as for frailty. Sarcopenia has more frequently been the object of research in basic science, whereas in clinical practice much more attention has been given to frailty [28]. However, looking more closely at the pathogenesis of both these conditions, we find a lot of common details [2,29]. Both sarcopenia and phenotype frailty have been identified as predictors of similar adverse outcomes [16,17,30]. In addition, many of the adverse outcomes of phenotype frailty, such as increased risk of falls and fractures, disability, and loss of functionality and independence are probably mediated by sarcopenia. Thus, sarcopenia is a central biological substrate of frailty [28,29,31-32].
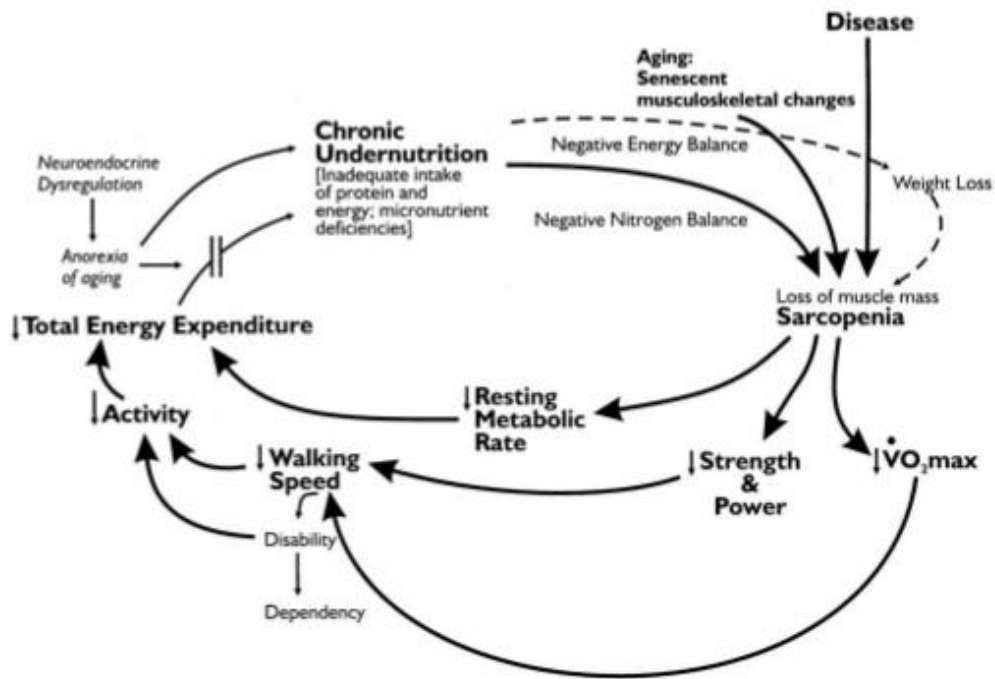
Figure 1 – Cycle of frailty hypothesized by Fried LP et al. Chroinc undernutrion due to an inadequate intake of protein and energy, weight loss and aging determines a condition of sarcopenia which results in a diminished strength, power, resting metablocic rate and walking of speed. All these factors could contribute to a frail phenotype [2].

From the biomedical and clinical perspective, frailty is broadly defined as a vulnerable condition in which multiple bodily systems are impaired [33-34]. With increasing age, there comes some physical and cognitive decline, such as hearing loss, diminished bone density, and poorer memory [35]. Frailty differs from the natural course of ageing although its prevalence increases with age. Frailty is not an inevitable feature of later life. First of all, not every older adult becomes frail even in extreme age. Secondly, compared to other older adults, frail older people display the degradation of many physiological systems that are responsible for healthy adaptation to the physical, social, and psychological demands of life [36]. Those older adults considered frail are particularly vulnerable to harmful or undesirable outcomes, such as hospitalization, institutionalization, injurious falls, or death [37]. Thus, frailty is a better indicator for those in need of intervention for their health and well-being than chronological age [38]. For these reasons, frailty is related to, yet also distinct from, natural ageing, represents those adults at the greatest risk of adverse outcomes, and it is heterogeneous [39]. Clinical definitions put an emphasis on the quick and efficient screening of patients. These definitions use the presence of chronic disease, hospitalization, physical functioning measures, and other biologically based measures (e.g., weight loss or walking speed) to identify frailty.

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While clinicians use a frailty classification to treat and care for older people, ageing researchers identify frailty to gain a better understanding of the ageing process and health experiences of older adults [40]. They separate and compare the different experiences of robust (i.e., not classified as frail) and frail older adults to determine the different factors involved (e.g., genetic factors and biological systems). By investigating these factors, researchers aim to identify the underlying mechanisms and to develop interventions for prevention, treatment, and rehabilitation of frailty. Alternatively, sociological and gerontological perspectives argue that frailty is socially constructed and influenced by sociocultural forces on healthcare practices and the meanings of the experiences related to frailty (e.g., disability and impairment). Due to the biomedicalization of ageing [41], frailty is considered a medical problem and so it falls to the healthcare community to identify and treat [42]. The term's increasing use in these clinical and medical contexts is what led Kaufman SR (1994) to report frailty as socially constructed. Using case studies, she described how frailty is constructed through social interactions in a clinical setting. In these interactions, older adults' lived experiences became lists of health or functioning problems (also known as deficits) and changes they needed to make in their lives. She proposed that frailty was a label imposed upon older people as a result of their interactions with the health care system and society [43]. More recently, Gilleard C and Higgs P (2010) have suggested that people's beliefs about frailty are shaped by societal and cultural views of health and ageing [44]. They theorized a "fourth age" resulting from the conflicting cultural views of old age; that is, one view of ageing in which older people live active, disease-free lives (the third age) and one in which they are dependent and have poor health (the fourth age). They suggested that the biomedical perspective views frailty (termed the fourth age) as negative due to its associations with decline and disease. Furthermore, broader society reflects these views by equating terms like *successful* with *healthy* ageing. Gilleard C and Higgs P proposed that the term frailty refers to the social representation of a weak old person associated with a marginal and vulnerable population without status in Western society. Frailty, or the fourth age, hence represents the feared and discouraged view of ageing [44]. Research concerning older people's own conceptualizations of frailty illustrates their beliefs and how these differ from biomedical models and include social views of ageing. By analyzing the different definitions of frailty from the Oxford English Dictionary, research literature, and older women's accounts of their lived experiences, Grenier A and Hanley J (2007) asserted that discourses and definitions of frailty revealed not only a perceived association with incapacity but also one of powerlessness, with implications of blame, highlighting feared aspects of ageing (such as decrepitude, dependency, and decline).

Frailty was discussed as an observable physical state, through the experience of vulnerability and negative social assumptions, and its associations with crisis events [45]. Supporting Grenier's and Hanley'findings [45], the qualitative investigation of Dutch older adults' (both men and women) meanings of frailty, by Puts et al. [46], showed that frailty was seen as a state of general physical impairment (commonly reflected in appearance and mobility) and disease. However, older adults also described frailty as a condition with delineable social and psychological ill-effects: reduced ability to cope with physical limitations; negative emotions (e.g., fear); impaired cognitive functioning; and poor social interactions [45]. These two qualitative studies show that older adults' own understandings of frailty are nuanced [45,46]. Older people include social and psychological aspects (e.g., negative emotions, poor social interactions, and fear) in their descriptions and discourses of frailty. To them, frailty is not just a physical state related to ageing or physical weakness as some biomedical definitions imply (e.g., phenotype of frailty). In a previous qualitative study, Grenier A [47] considered how older women negotiate their own views and experiences of frailty with the biomedical or clinical conceptualizations. By interviewing older women, she found that older women made a strong distinction between *being* and *feeling* frail. *Being frail* was imposed on them from medical or functioning classification, while *feeling frail* was associated with emotional experiences of traumatic events (e.g., loss of a loved one), disability, or physical impairment (e.g. incontinence). None of the women labelled themselves as frail, but they did reflect on moments in their lives when they felt frail. Feeling frail was more accepted because emotions were regarded as temporary states and consequently did not threaten their identity. However, admitting to being frail would require a change in their view of who they were – an identity shift. This work revealed how older adults negotiate this conflict between their self-view with the biomedical label of being frail [47]. Within the biomedical discipline, there are two commonly-used conceptual models (phenotype and accumulation of deficits) of frailty. Even with the increased application of these two models to the study of frailty, there is little to no agreement about what frailty actually is or how to measure it [22,48]. The two models conceptualize frailty as a physical syndrome caused by an underlying condition like sarcopenia (i.e., loss of skeletal muscle mass), which can be measured by the Fried phenotype [2] or the frail scale [49], and as a risk state secondary to accumulated deficits of illnesses, functional impairments, sensory impairments, and symptoms measured using a Frailty Index (FI) [32,50]. Therefore, phenotypic frailty proposes to be distinct of disability and comorbidities, while FI counts deficits regardless of their nature. Depending on the conceptual model, frailty is attributed to different sources and includes a variety of factors.

1.2 Determinants of frailty

From an epidemiological point of view, a 2010 UK study investigated the prevalence of frailty amongst 638 community-dwelling people aged 64-74 years [51]. The frailty prevalence rate was 8.5% for women, and 4.1% for men. The Fried investigators recorded a frailty prevalence rate of 6.9% in a cohort of 5201 men and women aged 65 years or more. Frailty rates of 3.2%; 9.5%; and 25.7% were recorded in age groups 65-70; 75-79; and 85-89 years respectively [2]. Frailty is self-perpetuating; its development results in a spiral of decline that leads to greater frailty and consequent risk of development of disability in older age. Recently, preliminary evidence has been reported that social factors, including social networks and socioeconomic status may also have a relationship with frailty [52]. However, it remains significant uncertainty regarding the contribution of social vulnerability to the frailty syndrome and further work to characterise the key elements that comprise social vulnerability [53] and the relationship between social vulnerability and frailty is required. Importantly, not all older people become frail as a result of the ageing process. A resistance to the development of frailty and a dissociation between chronological and biological age is often evident in older people [54]. In a proportion of vulnerable older people, the interaction of a number of processes can promote the development of frailty. Gobbens JJ et al. suggest ten life course determinants of frailty (eg, sex, income, lifestyle, life events) and the adverse outcomes (disability, health care utilization, death) [55]. The core of the model is formed by physical, psychological, and social frailty and their associated components. These components were selected based on the literature study and consultation of 17 experts in the field of frailty. Social vulnerability and frailty multiple social vulnerability factors including neighbourhood deprivation, social isolation and poor socioeconomic status have been associated with important adverse health outcomes including depression, cognitive impairment, admission to long-term care and death [56-58]. More recently, evidence to support an association between social vulnerability and frailty has been reported in two large observational studies [59,60]. In order to operationalize social vulnerability according to a deficit accumulation approach, investigators from the Canadian Study of Health and Ageing (CSHA) analysed data from 10,263 study participants aged ≥65 years. The social vulnerability index included items such as communication, social support, social engagement and socioeconomic status. Social vulnerability increased with age and was greater in women. A moderate correlation between frailty and social vulnerability was reported. Both frailty, identified using the FI, and social vulnerability independently contributed to increased risk of mortality suggesting that, although related, frailty and social vulnerability are likely to be distinct [53].

A 2009 cross-sectional analysis of data from 4,818 participants in the English Longitudinal Study of Ageing (ELSA) identified an independent association between individual socioeconomic status, neighbourhood deprivation and frailty. In a multivariate analysis adjusted for important potential confounding variables, lower individual wealth and greater neighbourhood deprivation were independently associated with frailty, identified using the FI [61]. Epigenetic mechanisms regulate the differential expression of genes in certain cells but do not result in a change to gene sequence or structure. DNA methylation and histone modification are considered to be the key mechanisms involved in the epigenetic regulation of gene expression [62]. These mechanisms, which are responsive to physiological and environmental cues and demonstrate reversibility, may have particular importance in the determination of physical phenotype and ageing [63,64]. As epigenetic mechanisms are responsive to environmental cues, they provide an attractive potential link between environmental factors, ageing and disease [65]. Epigenetic mechanisms have been investigated in the post-mitotic cells of the brain and have been associated with the development of synaptic plasticity and memory [66]. A growing body of literature supports the presence of an association between epigenetics, brain ageing and cognitive impairment [67]. Although there is evidence to support a potential link between epigenetics and ageing, the role of epigenetics in frailty is currently unclear and represents an area for future research. Importantly, as DNA methylation and histone modification are potentially reversible, epigenetic mechanisms may represent a potential target for future pharmacological therapeutic intervention. In humans, the causes of premature mortality are rooted in demographic, medical, lifestyle, and psychosocial factors. Indeed, Thomas McKeown's determinants of health framework can be applied to"hit"and recovery processes [68]. On the one hand, hits can be caused by the environment (e.g., being struck by lightning), individual behaviours (e.g., smoking), genetic susceptibility (e.g., to sepsis), and health care-relatedevents (e.g., iatrogenic); on the other hand, recovery processes can be facilitated by nurturing environments, health-promoting behaviours, genetic prorecovery factors (e.g., to neural injury), and effective healthcare interventions. Thus, the determinants of health are double-edged swords within the "hit" and recovery model of frailty [69-71]. Recently, Hoogendijk EO et al. described that all older adults are at risk of developing frailty, although risk levels are substantially higher among those with comorbidities, low socioeconomic position, poor diet, and sedentary lifestyles [72]. Dent E et al. suggested that lifestyle and clinical risk factors are potentially modifiable by specific interventions and preventive actions [73], as showed in figure 2.
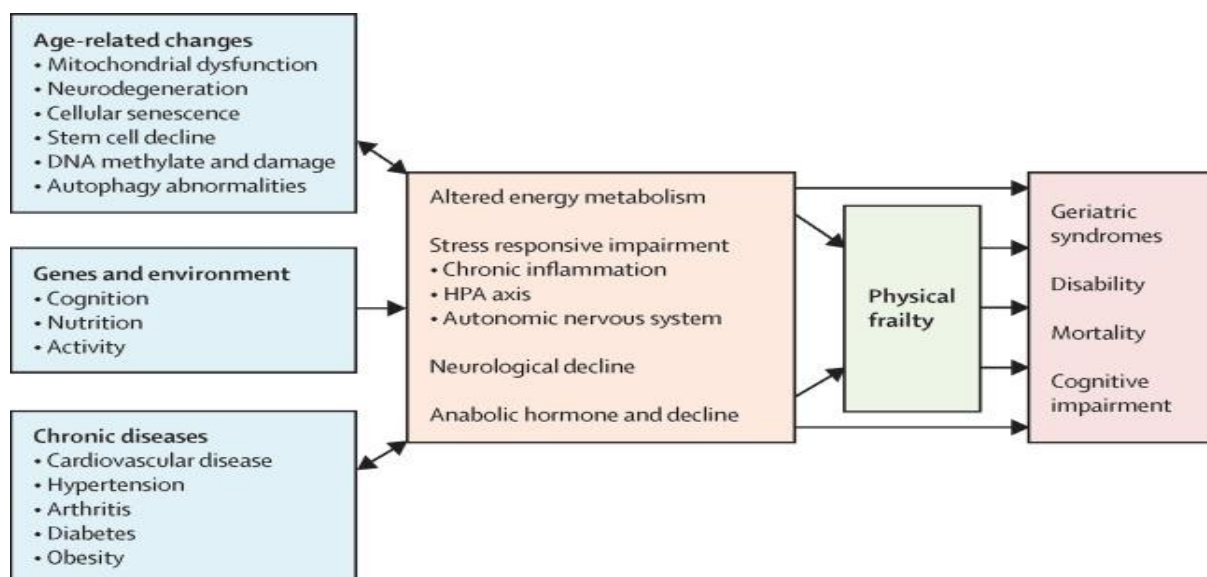
Figure 2 – Model pathway for physical frailty by Dent E et al. Several factors such as age-related changes, genes/environment and chronic diseases could cause frailty up to cause cognitive impairment, disability and mortality [73].

1.3 Nutrition, Mediterranean Diet and frailty

Nutrition is another factor closely related to the frailty syndrome; indeed, it is thought to influence both its etiology and treatment. Several studies have examined the association between intake of nutrients or nutrient biomarkers with the frail phenotype [74]. Chronic undernutrition, insufficient protein and energy intake lead to weight loss and sarcopenia, which may, in turn, negatively influence eating behavior and, thus, the nutritional status [74]. According to the definition of Fried et al., unintentional weight loss is a susceptibility criterion for frailty [2]. Therefore, undernutrition is frequent among frail populations [75,76]. Although undernutrition and frailty are two distinct entities, according to the studies, up to 90% of malnourished elderly people are also more frequently frail [75-77]. Indeed, although apparently in satisfying health due to overweight, elderly individuals are also likely to become sacopenic, by losing muscle mass and strength [78]. It is noted in the majority of cross-sectional studies that frail participants have the lowest energy intakes [76,79,80]. Recent studies have shown an association between frailty and nutritional status, showing a close relation between nutritional status assessed by Mini Nutritional Assessment (MNA) and frailty [75,81]. Nutritional risk assessed by MNA was also associated with incident disability in elderlies [82].

Anorexia of aging is generally defined as a loss of appetite or reduced food intake and considered as a modifiable risk factor for frailty [83]. Epidemiological studies have shown that reduced caloric intake is associated with changes in body composition and decline of physical function in the elderly [84]. Low energy intake is a frequent condition in the older population. It may affect 11.3% of men living in the community and up to 34.1% in long term care facilities [85]. Food intake decreases by nearly 25% between the fourth and seventh decades of life. The decline of energy intake can result in a dramatic decline in muscle mass and strength, leading to weight loss and disability [86]. A minimum of 25 Kcal/Kg/days is required to meet the energy requirements of the elderly. Measured Resting Energy Expenditure (REE) has been calculated between 20 and 28 kcal/kg/day in the elderly [87]. Vellas et al. have shown, in a ten year longitudinal study among 304 free-living elderly, that women with energy intake below 25 kcal/kg/day were more likely to become frail or to die [88]. Protein intake is inversely associated with frailty, except in one study where the distribution of daily protein intake, rather than the quantity itself, could explain this result [79]. Animal or vegetable protein sources are not essential in the relationship between protein intake and frailty [89]. Among various factors, adequate dietary protein intake is required to maintain muscle mass and function, and delay sarcopenia [90]. The Health, Aging and Body Composition Study (Health ABC) observed, over a three years period, that healthy elderly volunteers in the highest quintile of protein intake lost nearly 40% less total lean mass than those in the lowest quintile [91]. However, this study also highlighted that even the subjects in the highest quintile lost muscle mass during the follow-up, suggesting that the process can only be slowed down but not completely prevented. Aminoacids can stimulate muscle protein synthesis (MPS). Most of the enhancing effects on MPS after a meal are attributed to aminoacids [92]. Dietary protein intake can stimulate MPS and inhibit protein breakdown both in young and elderly people inducing a positive protein balance [93]. Higher dose of proteins may be required because of different muscle protein metabolism in the elderly. Several hypotheses have been developed to explain the relative anabolic resistance to aminoacids. The daily requirement for dietary protein is defined as the minimum amount resulting in a whole body net nitrogen balance of zero [94,95]. The current Recommended Dietary Allowance (RDA) for protein is established at 0.8g/kg-BW/day for all adults [96]. However, several limitations have been discussed for nitrogen balance studies. Precisely measuring nitrogen intake is very difficult as well as detecting short term changes in muscle mass. Therefore, measures may result in underestimation of protein requirements [97]. Furthermore, around 15% of people older than 60 years have a protein consumption below than RDA [98].

Short-term nitrogen balance results do not suggest that requirements for total dietary proteins are different in younger and older healthy adults [99]. Although conflicting data are reported in the literature, most nutritionists agree that protein needs in the elderly should be higher than the RDA [100]. Insufficient protein intake also influences the decline of physical function and co-morbidities. These factors must be taken into account when determining protein requirements in older people. Higher amounts of protein intake seem to reduce the risk of adverse events and to prevent frailty. In the Women's Health Initiative (WHI) observational study, after adjustment for confounders, a 20% increase in protein intake was associated with a 32% lower risk of frailty [101]. These observational studies support the idea that protein supplementation can prevent or reverse sarcopenia and frailty. A recent meta-analysis concluded that protein supplementation could increase not only muscle mass but also strength gains, in addition to strength training both in younger and older subjects [102]. However, longer-term trials are scarce and are needed to define optimal protein intake in the elderly [103]. Finally, specific recommendations published recently from the PROT-AGE Study Group for healthy older people indicate that to maintain muscle, elderly should have a higher average protein daily intake than younger people, in the range of 1.0 to 1.2 g/kg-BW/day [104]. It should be considered that nearly 10% of frail elderly and 35% of institutionalized elderly have an average protein intake below these estimates, making them major targets for interventions [105]. However, there is no clear evidence till date for benefits of higher protein consumption in frail elderly, and several studies with protein supplementation alone have failed to show improvement on muscle mass or function [106,107]. Probably, an excessively sedentary lifestyle – a hallmark of frailty – or the presence of inflammatory cytokines in frail skeletal muscle have induced a blunted response of muscle protein synthesis to protein ingestion [108,109]. To date, several studies have focused on micronutrients in relation to frailty, with a particular interest in vitamin D. Older people often suffer from hypovitaminosis D. Vitamin D deficiency is generally associated with poorer physical performances and is predictive of incident disability in community elderly people [110]. Besides improvement of lower extremity function, the influence of vitamin D on immunity may also be an important challenge in frail elderly, since vitamin D supplementation studies have shown beneficial effects on immune function [111]. Diet rich in n-6 and poor in n-3 polyunsaturated fatty acids (PUFAs) may result in a pro-inflammatory environment that may be deleterious for muscle or other tissues. N-6 PUFAs are a precursor of arachidonic acid which is a substrate of cyclooxygenase and lipooxygenase enzymes [112]. Low intake of eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) is associated with poor mobility in men according to the results of a cross-sectional study involving 417 Japanese elderly [113].

Fish-oil supplementation enhanced the benefits of strength training for lower limb strength, in a randomized study in elderly women [112]. In the same study, a greater improvement for chair-rising performances was observed for the trained group supplemented with fish oil. In another interventional study, fish oil supplementation resulted in an improvement in walking speed in postmenopausal women [114]. These results suggest that fish oil supplementation has a favorable effect especially in addition with strength training and may represent a promising preventive therapeutic strategy. For other micronutrients, data from the Italian InChianti cohort, a Tuscany (Italy)-based prospective population study designed to investigate factors contributing to decline of mobility in late life, are among the most interesting: frail participants consume significantly less vitamin D, E, C and folate, regardless of energy consumption, than non frail participants [115]. Low levels of vitamin E were also associated with an increased risk of frailty over time [116,117]. In the Spanish study on nutrition and cardiovascular risk (Seniors-ENRICA cohort), also interested in the association between micronutrient intakes and the frailty risk, an analysis of more than 1,600 aged individuals reported that poor intake of several vitamins (B6, C, E and folates), and non-adherence to the recommended dietary allowances for thiamine, niacin and vitamin B6 were all independently associated with the frailty risk [118]. Micronutrient deficiencies are commonly observed among elderlies with inadequate intake of various nutrients. Such deficits were also observed for antioxidant trace elements such as zinc and selenium [120-126]. Frail elderly women are more likely to have multiple micronutrient deficiencies. In a cross sectional study of 754 women of the Women's Health and Aging Study (WHAS), Michelon E et al. have observed lower serum concentrations especially for carotenoids, and also for vitamin B6 and folates. Furthermore, frail women were more likely to have at least two or more micronutrient deficiencies [127]. Carotenoid levels are generally considered as an indicator of fruit and vegetable intake, and may thus be considered as a biomarker of fruit and vegetable consumption [128-130]. Semba RD et al. have shown in the WHAS that women in the lowest quartile for carotenoids were 57% more likely to develop severe walking disability [119]. Lauretani F et al. have examined the association between total plasma carotenoids and changes in muscle strength over a six years follow-up in 628 participants of the InChianti study [131]. After adjustment for confounders, volunteers with plasma carotenoids in the lowest quartile were at higher risk to develop lower hip or knee muscle strength. Mean serum carotenoids levels were also associated with mean walking speed in the WHAS. A diet rich in fruits and vegetables may then reduce frailty factors [132]. Vitamin E and C supplementation have been shown to improve antioxidant enzymes and muscle function in aged animals [133]. In the InChianti study, Ble A et al. found an association between low circulating levels of vitamin E and frailty [134].

From the WHAS, Bartali B et al. showed that lower vitamin C intake is independently related to frailty [135]. In this cohort a lower decline of physical performances assessed by Short Physical Performance Battery Score (SPPB) was related only to higher vitamin E blood level and not to any other antioxidant parameters. However, data from the WHAS study suggest inter-relationships between vitamin B6, B12, selenium and frailty [128]. Indeed, women in the lowest quartile of serum concentrations of vitamin B6, B12, and selenium, in the WHAS study, had significantly higher risk of disability in activities of daily living (ADL)[135]. In another cross sectional study involving 655 subjects, plasma vitamin C concentration was correlated with handgrip strength and walking speed after adjusting for confounding factors [136]. Beside these aspects related to chronic undernutrition or to the intake of single nutrient or micronutrient, diet quality overall seems to play an important role in frailty development and progression [137]. Indeed, Mediterranean diet (MD) adherence in the older population has been associated to a reduced risk of frailty [138-140]. Moreover, it has been associated with long-term effect on individual frailty markers, such as decrease in mobility and walking speed [141,142]. The traditional MD is an eating pattern typical of the Mediterranean basin. This dietary model was originally defined by the American scientist Ancel Keys who initially described the eating behaviors of the Mediterranean people living in Southern Italy. Keys was impressed by the observation that these people used to consume some specific foods more frequently compared to his fellows in US or to those living in the Northern countries of Europe [143]. The key-feature of the MD is a wide consumption of fruit and vegetables, non-refined grains, cereals, nuts, legumes, fish, olive oil as main fat source and moderate wine consumption preferably during the main meals [144]. Conversely, this pattern includes low intake of both dairy products and meat. Traditional lifestyle of Mediterranean people was also characterized by moderate to intense physical exercise mainly performed during the working hours that, in that time, were mostly of agricultural type. Thus, MD could also have beneficial effects applicable to frailty, in addition to the well-known benefits on cardiovascular health and longevity [145-147]. Several studies, including some recently published studies, confirmed that greater adherence to a diet of Mediterranean-type food was associated with a lower odds/risk of frailty over time [148-153]. Using data from the 3-City cohort, a population-based longitudinal study of the relation between vascular diseases and dementia in person ≥65 years, a significant 68% decreased risk of developing frailty in the next 2 years was observed in a representative sample of people aged ≥75 years, suggesting that, even at advanced ages, this eating behavior could be beneficial [154]. The 3-City cohort study also showed that following a Mediterranean-type diet was beneficial in terms of disability risk.

These last projects emphasized the importance of this dietary pattern in the phase that precedes the state of disability and suggested a long-term effect of the eating habits on health [155]. Results from studies on MD and risk of frailty were recently included in a meta-analysis, where participants with the strongest adherence to this dietary pattern had a significantly decreased risk of frailty by 56% [156]. More recently, a report observed that the benefit of a higher adherence to the MD on preventing frailty was also evidentin at-risk older women from the Nurses' Health Study suffering from type-2 diabetes [157]. In the participants of the InChianti Study adherence to a Mediterranean-style diet was computed from the European Prospective Investigation into Cancer and Nutrition Food Frequency Questionnaire (EPIC-FFQ). Frailty was defined as having at least 2 of the following criteria: poor muscle strength, feeling of exhaustion, low walking speed and physical activity. After a 6-years follow-up, higher adherence to a Mediterranean-style diet was associated with lower odds of developing frailty compared with those with lower adherence. A higher adherence to a Mediterranean-style diet at baseline was also associated with a lower risk of low physical activity and walking speed but not with feelings of exhaustion and poor muscle strength [158]. A study by León-Muñoz LM et al. analyzed how low intake of certain micronutrients and protein has been associated with higher risk of frailty. Additionally, this research examined the association between adherence to the MD and the risk of frailty in older adults showing that an increased adherence to the MD decreased the risk of frailty [164]. On the contrary, Chan R et al. failed to find such associations [165]. MD may also be considered as a key component for healthy aging and preventing strategies for age related disability [159,160]. It has been shown that adherence to the MD had positive effects on mobility after a 9-years follow-up of 935 men and women aged ≥65 years [161]. High adherence to the MD was associated with a lower risk of developing disability. In a recent study, nutritional characteristics of MD were associated with frailty criteria with significant lower dietary scores in frail elderly [162]. Talegawkar SA et al. observed the association between a Mediterranean-style diet with the risk of frailty in community-dwelling older persons [163]. The association between frailty and MD may be explained by several mechanisms. Firstly, antioxidants, consumed in large amounts in the context of the MD, have been found to protect from the high oxidative stress which can cause muscle atrophy and loss of muscle fibers [166]. In particular, polyphenols highly present in the MD might act as mediators of this association. So far, several intervention studies regarding frail elderly people have been published, most of them including both nutritional and exercise interventions, with conflicting results. However, none of them specifically tested the effect of MD or its polyphenol components in the development or progression of frailty or its main features.

1.4 The relation between inflammation, coagulation and frailty

A low grade inflammatory state is common in older people and activated lymphocytes and monocytes of healthy older people produce greater levels of the pro-inflammatory cytokine IL-6 [167-169]. Low-grade inflammation is a subclinical condition defined as *"the chronic production, but at a low-grade state, of inflammatory factors"* [170-172]. Low-grade inflammation does not come from an infection but several physiological mechanisms are involved. Concentrations of inflammatory factors in these conditions are overall slightly higher than in healthy populations, but still remain in the healthy ranges. It is therefore hard to determine whether a specific patient exhibits low-grade inflammation, but it can be better defined at the level of a group of patients. Frailty and aging in general could be associated with chronic low-grade inflammation, also referred to as inflammaging, i.e. a condition that epidemiological studies have found to be associated with age-associated diseases. [173,174]. Inflammaging is a complex biological phenomenon that still needs to be explored as a path of successful/unsuccessful aging. As individuals grow older, several stressors (poor nutrition, diseases, oxidative stress, and environmental factors) increase the inflammaging process leading to frailty. Indeed, in centenarians the increase of pro-inflammatory markers is balanced by an increase of anti-inflammatory markers, suggesting that the detrimental or beneficial effects of inflammaging are largely personalized [175,176] (Figure 3). Inflammation has an important pathogenic role in many age-related diseases, including Alzheimer's disease (AD), atherosclerosis, heart failure, osteoporosis and osteoarthritis [177-179]. It has also been implicated in the pathophysiology of sarcopenia, an important component of the frailty phenotype [180,181]. As mentioned previously, sarcopenia or loss of muscle mass occurs with aging and is an important component of the phenotype of frailty [182]. Mediators of inflammation contribute to the pathogenesis of sarcopenia. Although direct evidence is not yet available in humans, in certain animal models it is apparent that activated inflammatory pathways contribute to muscle loss after chemical toxin or neuronal injury. In animals in which inflammatory pathways were generally inhibited, muscle loss was significantly less and post-injury regeneration was enhanced [183]. In the Health ABC study, IL-6 and TNF-α levels in individuals aged 70 to 79 years were inversely associated with muscle mass as determined by computed tomography imaging and formal strength testing [184]. This association was present even after adjusting for anti-inflammatory drug use and the presence or absence of inflammatory-associated conditions. Similarly, in the InChianti Study, high levels of IL-6, IL-1 and C-Reactive protein (CRP) were significantly associated with poor overall physical performance and reduced muscle strength [185].
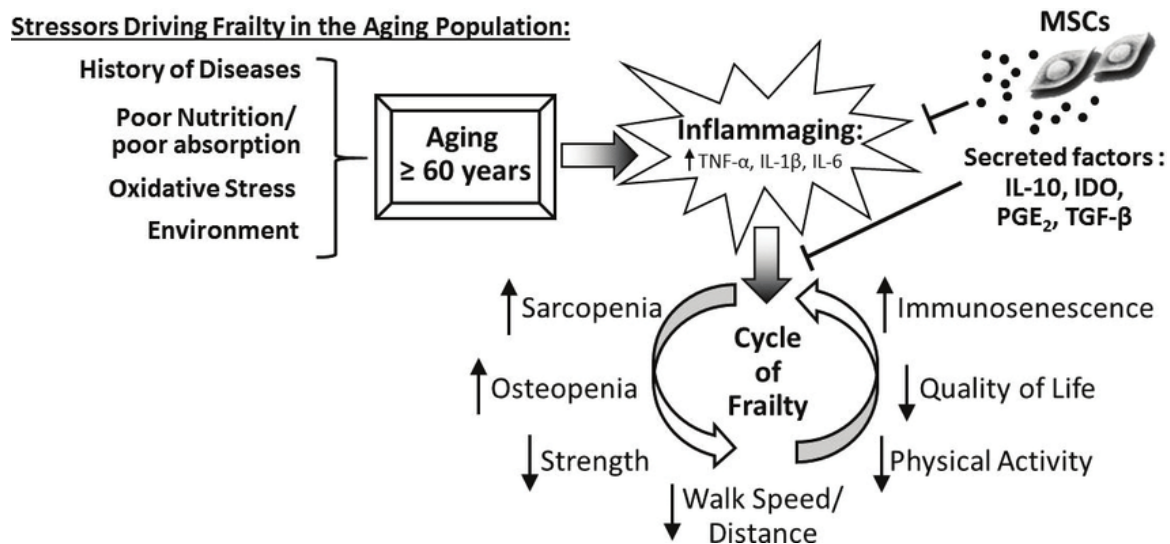
Figure 3 – The role of stressors, aging, and inflammation in frailty and the effects of mesenchymal stem cells (MSCs). As individuals grow older, several stressors (poor nutrition, diseases, oxidative stress, andenvironmental factors) increase the inflammaging process leading to frailty. MSCs secrete several factors that block or reverse the inflammaging process and ultimately reverses the effects of frailty [176].

There is substantial evidence to indicate that inflammation has a key pathophysiological role in frailty. A large prospective cohort study involving 5,201 participants reported an association between CRP and incident frailty as defined by the Fried criteria [2,186]. In a multivariate analysis adjusted for important covariates, every standard deviation increment in baseline CRP increased the five year incidence of frailty by 18%. A recent UK case-control study that included 101 participants reported results from a multivariate analysis demonstrating that TNF-α, IL-6 and CRP were independently associated with increasing frailty, FI and functional status [187]. A further cross-sectional study reported an independent association between increased white blood cell (WBC) counts, IL-6 and frailty in 558 older women [188]. Two additional studies that pre-dated current definitions of frailty reported that increased IL-6 and CRP were associated with increased risk of functional disability and death in community dwelling older people [189,190]. Of these, however, only CRP remained significant after adjusting for confounders. Finally, in the WHAS, higher WBC counts and IL-6 levels were independently associated with prevalent frailty [191]. Activation of the coagulation system and increase in procoagulant markers have been associated with the pathogenesis of atherosclerosis [192,193].

However, procoagulant markers, most notably D-dimers, fibrinogen, and factor VIII also increase with advancing age and may, in fact, correlate better with aging than with cardiovascular disease [192-195]. Pieper CF and colleagues, examining 1,729 participants aged ≥70 years in the Established Populations for the Epidemiological Study of the Elderly (EPESE) cohort, clearly demonstrated that increasing age was associated with high D-dimers levels. For example, 23% of the participants aged 90 to 99 years had high D-dimers levels (>600 μg/L) compared with 13% in the age group 80 to 89 years and 7% in the age group 70 to 79 years [196]. French investigators measured fibrinogen concentrations in healthy subjects aged 19 to 96 years and found levels to be significantly higher in participants aged more than 60 years when compared with younger subjects [197]. Hager K et al., examining healthy individuals across the life span, found that fibrinogen levels increased by 25 mg/dL per decade of life and that levels as high as 320 mg/dL were found in more than 80% of people aged more than 65 years [198]. Other markers of activated coagulation, such as plasminogen activating inhibitor-1 (PAI-1) and factor VIII, have been shown to be increased with age [199-201]. Thus, it is now apparent that frailty is associated with markers of activated coagulation in geriatric populations. For this reason, it has become apparent that both inflammatory and coagulation pathways are increasingly active with advancing age and to a greater extent in those who meet criteria for frailty. The coexistence of these factors suggests an interaction of inflammation and coagulation in the pathophysiology of frailty. It is known that inflammatory cytokines stimulate release of procoagulant factors in a wide range of cell types, and that the age-associated increase in these cytokines may thereby account for the associated activation of coagulation [191] (Figure 4). However, it should be recalled that adipose tissue expression of both prothrombotic factors and proinflammatory cytokines is high [202]. Thus, the relative increase in adiposity with advancing age, and particularly with frailty, may offer some explanation for the inflammatory and procoagulant profiles observed. The molecular mechanisms underlying increased levels of inflammatory cytokines in frailty are beginning to be elucidated. More recently, an inverse association between adherence to MD and chronic low grade inflammation has been observed in the Moli-sani Study, an Italian prospective cohort of 24,325 men and women, aged ≥35 years (*see methods for a full description of the Moli-sani Study*) [203-205]. In scientific literature there are still no works in which frailty is associated with MD and low-grade inflammation and coagulation. An apriori dietary pattern marker of foods and nutrients intakes associated with inflammation has been built in the Seniors-ENRICA cohort. The authors observed that the highest adherence to the Dietary Inflammatory index (DII) was associated with a higher risk for frailty (and slow gait speed) among 1,948 participants followed for up to 4 years [206].

All these observations will assume greater importance if interventions aimed at regulating inflammatory/coagulation pathways result in clinically important improvements, such as in physical or cognitive function.

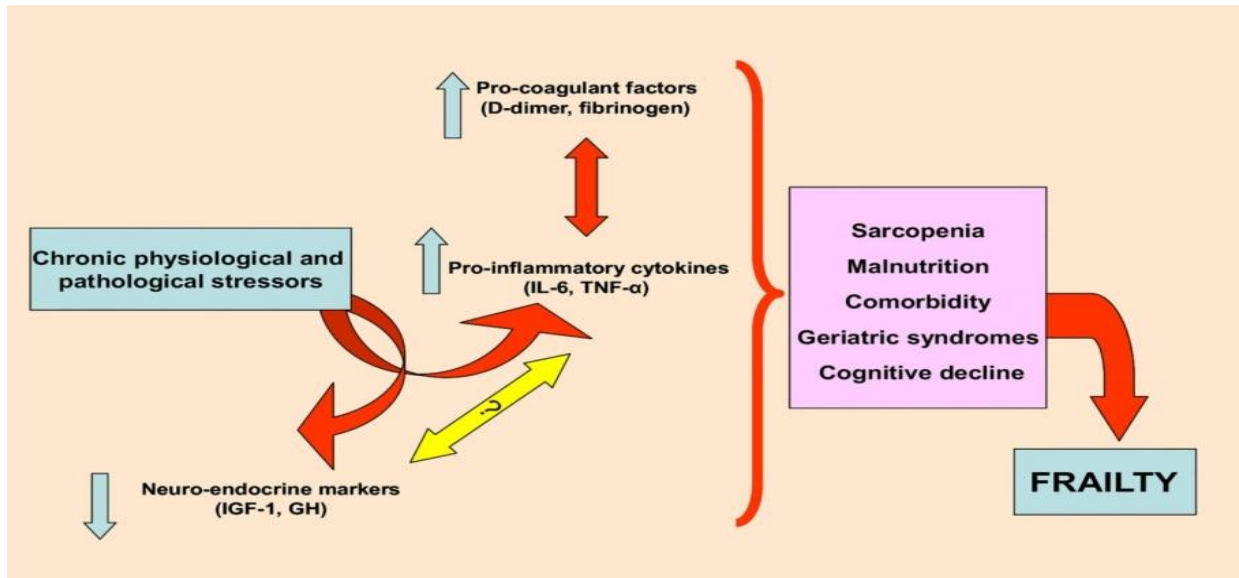**Figure 4 – Inflammation/coagulation pathway to frailty. Proinflammatory signals, including IL-6 and TNF-α, increase with age, as do markers of activated coagulation, including D-dimer, fibrinogen, PAI, and Factor VIII. To the extent that these are primary processes, they may contribute to a cycle of physiologic changes that, in composite, are described as frailty [191].**

1.5 Neuropsychological status and frailty

Attention for psychological frailty has been growing in recent years. Several studies have shown that ageing is often accompanied by an increase in psychological complaints and cognitive disabilities. Many older persons have symptoms of depression and anxiety which greatly impede their daily functioning [207,208]. In addition, many elderlies are lonely [209] or feel they are losing control of their lives [210]. Virtually all older people are also confronted with a decline in their cognitive abilities. Their memory becomes less reliable, their speed of thought reduces and their ability to do several things at the same time diminishes. The rate of this cognitive decline varies enormously from individual to individual, and is closely related to psychological and physical health. Older persons who are depressed, for example, often have problems with their memory or ability to concentrate, as do people who suffer a great deal of pain or severe fatigue. Cognitive disabilities can have an adverse effect on day-to-day functioning and are by no means always the result of dementia [211,212]. Studies of frailty which have also looked at psychological factors suggested that the psychological domain plays an important role in the concept of frailty [213,214]. As people get older, their chance of developing chronic diseases and functional disabilities increases. In addition, their social networks often shrink and they may lose their partner. To maintain a good quality of life and mental well-being, people must have a way of compensating for lost capacities [215]. In the neuropsychological domain, depression is a key component of frailty. Like frailty, depression is a highly prevalent condition among older adults up to 15% [216,217] and an important predictor of mortality in older adults [218]. Depression significantly decreases the quality of life, just like frailty lowers life satisfaction [219-221]. Furthermore, analogue to the concept of frailty, depression is a syndromal diagnosis. Depending on the definition and operationalization of frailty, both syndromes partially overlap, even if frailty is defined as a purely physical condition. For example, physical complaints such as fatigue, weight loss and poor endurance, may be related to depression, to frailty or to both. Despite similarities in several domains, there is a lack of research into the relationship between frailty and depression. Although similar biological mechanisms have been hypothesized to account for both syndromes-frailty and depression-for example, subclinical cardiovascular disease [222,223] and inflammation [224,225], it is unlikely that one mechanism is largely responsible for either or both syndromes in all individuals. In fact, although the overlap between depression and frailty is underexplored, evidence to date demonstrates that they are distinct constructs that share moderate overlap (similar to frailty and disability).

For example, Mezuk et B al. [226] used confirmatory latent class analysis and found that frailty and depression represented distinct constructs (a three-class solution for depression and a two-class solution for frailty with moderate correspondence between the two). The frailty phenotype is undoubtedly still the topic of lively debate and, although experts agree on a more comprehensive definition of frailty that includes, among other things, the assessment of mental health, it is also important to note that there is still no consensus as to the clinical presentation of frailty for practical screening purposes [227,228]. Several studies have also shown frailty to be closely related to Mild Cognitive Impairment (MCI) in prospective study cohorts [229,230]. MCI is a syndrome defined as cognitive decline greater than expected for an individual's age and education level but that does not interfere notably with activities of daily life (ADL). Prevalence in population-based epidemiological studies ranges from 3% to 19% in adults older than 65 years. Some people with MCI seem to remain stable or return to normal over time, but more than half progress to dementia within 5 years. MCI can thus be regarded as a risk state for dementia, and its identification could lead to secondary prevention by controlling risk factors such as systolic hypertension. The amnestic subtype of MCI has a high risk of progression to Alzheimer Disease (AD) and other types of dementia [231]. In order to encourage combined research in frailty and cognitive impairment, the International Academy on Nutrition and Aging (IANA) and the International Association of Gerontology and Geriatrics (IAGG) organized an International Consensus Group (ICG) to propose the operational definition of cognitive frailty [232]. After the consensus was published, researchers put more attention on these two critical geriatric syndromes. Although the prevalence of cognitive frailty in the community setting is low (1.0–1.8%), it has been associated with a high risk of disability, poor quality of life, and death [230]. Furthermore, researchers have also found a 50-item Frailty Index (FI) to be significantly associated with temporal and frontal cortical atrophy, detected by computerized axial tomography, which indicates that frailty and cognitive decline might share common pathophysiological mechanisms [233-235]. All of these findings show that frailty and cognitive impairment are closely related to each other. However, Shimada H et al. [236] found that only 2.7% participants displayed overlapping frailty and cognitive impairment, with the majority of the subjects (97.3%) devoid of the combined syndromes. The use of frailty and cognitive impairment parameters in predicting mortality has previously been investigated. The results, however, revealed several discrepancies among various reports; some found combined physical frailty and cognitive function assessment to enhance the likelihood of the prediction of individual's risk of death than either measurement alone, while some researchers found no statistically significant enhancement of the combined effect [236-241].

Furthermore, most participants in these studies were Caucasians and aged from 60 to 90 years. The characteristic of cognition or frailty among the oldest-old had been shown to be different with other age groups [242,243]. Thus, the role of cognitive impairment, frailty or the combination of both in predicting adverse outcomes need to be further classified, primarily, among the oldest-old (aged ≥90 years) and also other races. To date, no studies have focused on only cognitive impairment or frailty or the two syndromes combined in predicting mortality in advance late-life, and thus the combined effects remain unclear in the oldest-old population. Many studies have worked within research and clinical settings to identify target populations with frailty and MCI [244]. Participants with MCI in a community cohort were described by a group of investigators from the Mayo Clinic in 1999, who then produced a series of diagnostic criteria [245]. A conference of international MCI experts then revised these criteria [246], and the National Institute on Aging (NIA) joined the Alzheimer's Association (AA) to publish the new diagnostic guidelines for the AD (NIA-AA). In these guidelines, the workgroups identified Alzheimer's disease as a continuum with three distinct stages: preclinical, MCI and dementia. They also outlined the following factors for the identification of MCI [247]:

- concern regarding a change in cognition;
- impairment in one or more cognitive domains;
- preservation of independence in functional abilities;
- absence of dementia

Several cross-sectional studies have reported an association between physical frailty and cognitive function [248,249]. In addition, longitudinal studies have revealed that a higher level of physical frailty is associated with an increased risk of incident AD [250] and MCI [251]. These studies suggest that in some older adults, physical frailty is associated with the development of MCI. Older adults who show signs of both physical frailty and MCI may be more likely to exhibit functional decline than those with either frailty or MCI. However, the combined prevalence relationship between frailty and MCI has not been clearly established. The associations of specific physical and functional impairments of frailty with MCI are also unclear. In a population-based study of community dwelling, elderlies in the second Singapore Longitudinal Ageing Study (SLAS-2), it was compared the prevalence of the physical frailty syndrome, low lean muscle mass, low muscle strength, slow gait speed, exhaustion, low physical activity, impaired balance, impaired gait, and elevated fall risk between individuals with MCI and non-MCI individuals with normal (high and low) cognitive functioning.

Brigola AG et al. proposed a model of association between factors, frailty, cognitive impairment and their outcomes in older adults [252] (Figure 5).
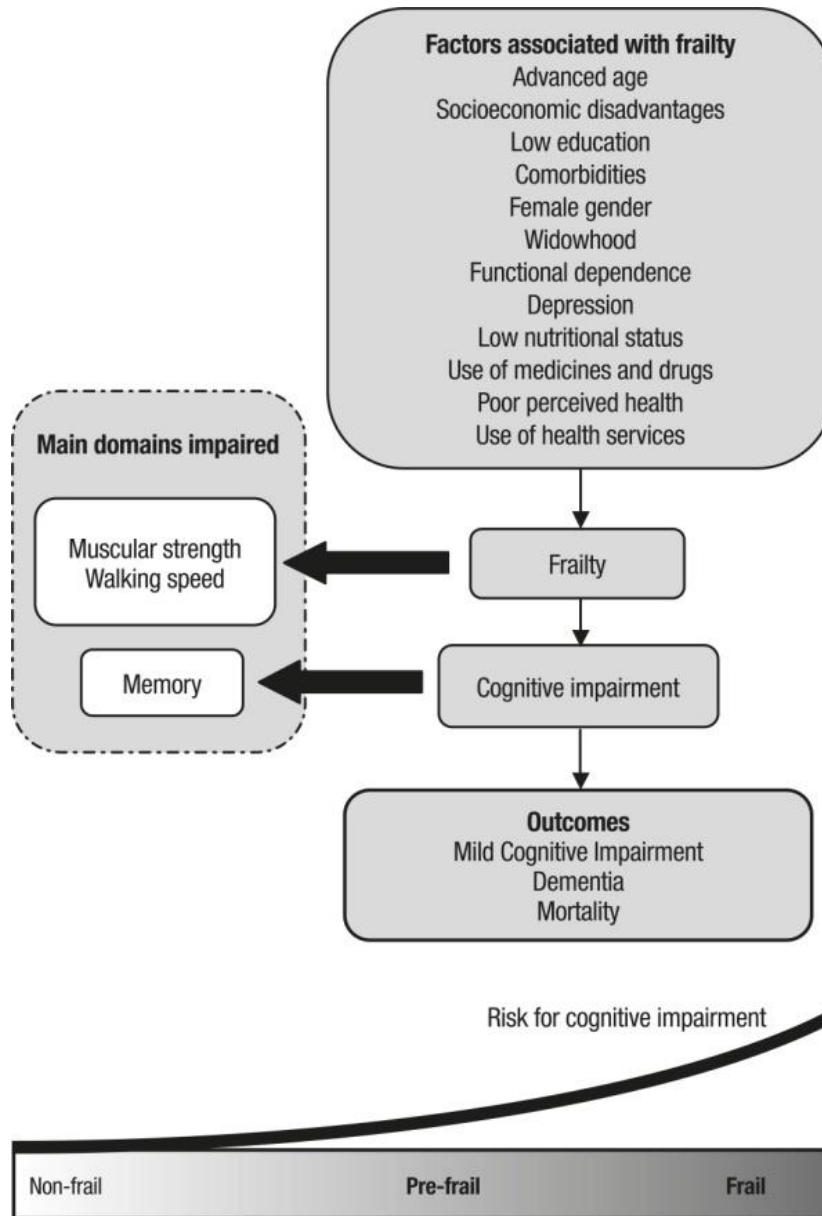

Figure 5 – Model of association between factors, frailty, cognitive impairment and their outcomes in older adults by Brigola AG et al. [252].

From a pathophysiological point of view, the etiology of the cognitive-frailty association appeared to be multifactorial and several mediators or possible pathways have been suggested such as hormonal and inflammatory processes, nutritional, vascular, neuropathological, and metabolic influences [253]. However, given that experimental evidence is lacking, other questions may address the underlying mechanisms and determine which is the most relevant component among the suggested mediators between frailty and cognition. The common consequences of aging may have an impact on age-related conditions such as physical frailty and cognitive decline. Aging mechanisms such as nutrition sensing signals, p53 activation, and subsequent telomere deletion and DNA damage, result in the physiological reserve declines of different organs. The long-term chronic stressor overload further accelerates the physiological reserve declines. Moreover, the different vulnerability of multiple organs, or different structures in same organ, such as brain, results in function-related homeostatic failure, and different phenotypes/diseases, physical frailty, or cognitive frailty. There is certainly the evidence for an association between frailty and cognitive impairment and it is desirable outlining some of the mechanisms that potentially underpin this relationship from brain neuropathology and hormonal dysregulation to cardiovascular risk and psychological factors [254]. These different mechanisms are not mutually exclusive, highlighting the need for further studies to better explicate the neurobiological basis of cognitive frailty [255].

Chapter 2: Aim of the study

The aim of this PhD thesis, carried out in collaboration with the Department of Epidemiology and Prevention of the I.R.C.C.S. Neuromed, Pozzilli, Italy, in the period May 2018 - May 2019, was to screen the over 65 years elderly population of the Moli-sani Study to identify frail individuals and evaluate a possible relation between Mediterranean Diet (MD), inflammation and frailty.

Chapter 3: Materials and Methods

3.1 The Moli-sani Study and cohort design

The cohort of the Moli-sani Study was originally recruited between 2005 and 2010 in the Molise region from city hall registries by a multistage sampling. First, townships were sampled in the major areas by cluster sampling; then, within each township, participants aged ≥35 years were selected by simple random sampling. Exclusion criteria were pregnancy at the time of recruitment, disturbances in understanding or willingness, current multiple trauma or coma, or refusal to sign the informed consent. Thirty percent of subjects refused to participate; based on a short questionnaire on risk factors and major diseases administered by telephone, those who refused were older and had a higher prevalence of cardiovascular disease and cancer. Subjects (n=24,325) from 30 Molise cities and villages of different sizes attended either of the two recruiting centers: the Catholic University in Campobasso (n=19,211; 79%) and San Timoteo Hospital in Termoli (n=5,114; 21%). The recruitment strategies were carefully defined and standardized across the two centers. The Moli-sani Study was approved by the Catholic University ethical committee [256]. On 2017 an active follow-up started at the clinics of the Epidemiology and Prevention Department of the I.R.C.C.S. Neuromed, Pozzilli, Molise, Italy based in Via Garibaldi 21, Campobasso, Molise, Italy, and a random subgroup of the Moli-sani participants were recalled to participate in the second wave recruitment. The study protocol of the active follow-up was approved by the Ethical Committee of the I.R.C.C.S. Neuromed on 29/03/2017. From May 2017 to May 2019 1,400 subjects were recruited. For this PhD thesis, men and women aged ≥65 years were selected among the participants to the re-call phase of the Moli-sani Study. From May 2018 to May 2019, 226 elderly participants were included in this PhD study.

Moli-sani follow-up visit

After signing the informed consent, trained research personnel took blood pressure (BP) and anthropometric measurements using methods standardized beforehand during preliminary training sessions. BP was measured by an automatic device (OMRON-HEM-FL31) [257] three times on the non-dominant arm and the last two values were taken as the BP [258,259]. Measurements were made in a quiet room with comfortable temperature with the participants lying down for at least 5 minutes.

Body weight and height were measured on a standard beam balance scale with an attached ruler, in subjects wearing no shoes and only light indoor clothing. Trained interviewers administered structured questionnaires to collect personal and clinical information including socio-economic status, physical activity, dietary habits, physio/pathological medical history risk factors for cardiovascular diseases (CVD) and/or tumor, family/personal history for CVD and/or tumor and drug use. Blood samples for laboratory analysis were also obtained from the participants who had fasted overnight and had refrained from smoking for at least six hours. Part of the samples were aliquoted and stored in liquid nitrogen in the Neuromed Biobanking Centre at the I.R.C.C.S. Neuromed of Pozzilli.

3.2 Frailty literature systematic review

To identify definition, determinants and measurement for frailty to be used in the cohort study, a systematic search of the literature was performed. For this purpose, PubMed database search was used. Search terms were broadly set as: *"frailty", "frail elderly", "geriatric assessment/methods", "frailty tests/measurement", "determinants of frailty", "frailty and nutrition", "frailty and Mediterranean Diet", "frailty and inflammation", "frailty and coagulation", "frailty, inflammation and coagulation", "frailty and psychological status", "frailty and depression", "frailty and MCI"*. The initial search was performed in February 2018 and all the studies about frailty were taken into consideration. The search was limited to English and Italian language articles. Only full research papers and review articles were considered. A lateral search was also performed, in which the citations of relevant articles were searched. Papers published before 2000 were also included if deemed relevant to the review. Original papers reporting studies conducted either on community dwelling or hospitalized/institutionalized participants (regardless of the reasons of admission) were eligible, provided that the findings could be extended to the general population, and not restricted to a segment affected by particular diseases or conditions.

The following criteria were also included in the literature review:

- population: aged ⩾65 years
- implementation/indicator: frailty objectively measured in either observational and cross-sectional trials
- outcome: frailty classification

- critiquing of frailty measurements:

  - time taken to perform the measurement

  - specialized equipment to measure frailty (for instance, a handgrip strength dynamometer)

  - requirement for assessor training

  - validity and reliability.

Reviews on frailty were initially consulted to determine the reliability and validity of frailty measurements. If no discussion of validity/reliability was included in these reviews, then relevant individual articles were searched.

Papers were included if they explicitly addressed frailty, irrespective of the definition or diagnostic instrument used. Some reasons for exclusion were: replicated data, letters to the editor, abstract only publications, conference proceedings, non systematic reviews, and editorials. *The Frailty of the elderly guidelines* proposed by the Regional Health Council of the Toscana Region were also consulted [260]. At the end of the research, we decided to use some questionnaires, tests and laboratory analysis, many of them already validated and widely used by geriatric clinicians and researchers to study frailty from different points of view:

1) **Frailty assessment**

   Survey of Health, Ageing and Retirement in Europe-Frailty Instrument (SHARE-FI)

2) **Dietary information**

   European Prospective Investigation into Cancer and Nutrition – Food Frequency Questionnaire (EPIC-FFQ)

3) **Neuropsychological status**

   Montreal Cognitive Assessment (MoCA)

   Short form health survey (SF-36)

4) **Blood inflammation and coagulation biomarkers**

   Hemocromocytometric analysis with leukocyte formula

   High sensitive C-Reactive Protein (hs-CRP)

   D-dimers

5) **Blood markers of cardiovascular disease (CVD) risk**

   Total cholesterol

   HDL cholesterol

   Triglycerides

   Glucose

3.3 Frailty assessment

The *Survey of Health, Ageing and Retirement in Europe - Frailty Instrument (SHARE-FI)* questionnaire by Romero-Ortuno R et al. [261], available on computer, detects the variables that, according to Fried et al. [2], characterize frailty and provides a quantitative sum value, using a specific algorithm that takes into account the gender difference. As showed in figure 6, this test, through different questions, allows to explore different aspects of frailty, such as fatigue, loss of appetite, weakness, walking difficulties and poor physical activity. The SHARE-FI was selected because it has sufficient construct and predictive validity, and it represents the first European research effort towards a common frailty language at the community level. *Jamar Plus+ digital handgrip dynamometer* was used to determine the grip strength at both hands, left and right (Figure 7). We decided to use Jamar Plus+ dynamometer because, as reported in several studies, it is the most widely cited instrument and appears to be generally accepted as the gold standard for its precision, accuracy and reproducibility [262]. The elderly subject was made to sit with the arms and hands extended along a (desk). The patient is asked to grip the handle of the dynamometer and to tighten as hard as possible, first with the left hand and then with the right hand. For each hand two tests were made and the measurements that appear on the display of the dynamometer were collected: grip strength (kg), average between the two measures made for each hand (AVG, kg), standard deviations (SD, kg) and coefficient of variation (CV, kg). The highest of the four was selected. This variable was kept continuous.

Two accessible via web SHARE-FI calculators *(see Additional files of reference [261])*, one for women and one for men, were used to obtain the total score and the related category frailty phenotype to which the patient belongs to [261].

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SHARE-FI

(Romero-Ortuno R et al., 2010)

1. EXHAUSTION
In the last month, have you had too little energy to do the things you wanted to do?
No
Yes

2. WEIGHT LOSS (DIMINUITION IN DESIRE FOR FOOD)
What has your appetite been like during this last month?
-it has been the same as usual and/or you have been eating as usual
-it has been less than usual and/or you have been eating less than usual
- it has been more than usual and/or you have been eating more than usual
The presence of the criterion was coded as 1 and its absence as 0.

3. WEAKNESS
Highest handgrip strength (Kg)
Right hand
Measurement 1:
Measurement 2:
Left hand
Measurement 1:
Measurement 2:

4. SLOWNESS
Because of a health or physical problem, do you have difficulty doing one of the following things? (exclude problems expected to last within 3 months)
Walking 100 meters:
No
Yes
Climbing one flight of stairs without resting:
No
Yes
One or two positive answers received the score of 1, and two negative answers received the score of 0.

5. LOW ACTIVITY
How often do you engage in activities that require a low or moderate level of energy such as gardening, cleaning the car, or doing a walk?
More than once a week
Once a week
One to three times a month
Hardly ever or never

</div>

Figure 6 – SHARE-FI by Romero-Ortuno R et al. is a validated and simple instrument for frailty screening which evaluates various aspects such as exhaustion, diminution in desire for food, weakness, difficulty in moving, low physical activity. Two accessible via web calculators (one for women and one for men) are used to obtain the total score and the frailty category to which the patient belongs to [261].

Figure 7 – Jamar Plus+ handgrip is a digital dynamometer used to determine the grip strength (weakness) at both hands, left and right. For each hand two tests are made and the measurements that appear on the display of the dynamometer are collected: grip strength (kg), average between the two measures made for each hand (AVG, kg), standard deviations (SD, kg) and coefficient of variation (CV, kg).

3.4 Dietary information

Food intake was determined by the validated Italian version of the *European project investigation into cancer and nutrition – Food Frequency Questionnaire (EPIC-FFQ)* [263,264]. The questionnaire, computerized with tailor-made software, allowed us to interview participants in an interactive way, including illustrations of sample dishes of definite sizes or by reference to standard portion sizes. The Nutrition Analysis of EPIC-FFQ (NAF) [264] was used to convert questionnaire dietary data into frequencies of consumption and average daily quantities of foods (g/day) and energy intake (Kcal/day). NAF was linked to the Italian food composition tables for energy assessment [266]. To simplify the interpretation of data and to minimize within person variations in intake of individual foods, 188 food items were classified into 45 predefined food groups on the basis of similar nutrient characteristics or culinary usage (Table 2). To study a possible relation between Mediterranean Diet (MD) and frailty, we evaluated the adherence to the MD by using the MD Score (MDS) developed by Trichopoulou et al. [265]. Briefly, scoring was calculated in a population free from CVD, cancer, or diabetes and was based on the intake of the following 9 items: vegetables, legumes, fruits and nuts, dairy products, cereals, meat and meat products, fish, alcohol, and monounsaturated to saturated fat ratio. For most items, consumption above the study median received 1 point; all other intakes received 0 points. For dairy products, meat, and meat products, consumption below the median received 1 point. Medians were gender specific. The possible scores ranged between 0 and 9, the latter reflecting the maximal adherence to the MD.

Table 2 – Food grouping used in the dietary analyses.

Foods or food groups	Food items
Potatoes	Potatoes
Cooked vegetables	Leafy vegetables, root vegetables, cabbages, onion, carrots, mushrooms, egg plants, artichokes, sweet peppers, spinach, pumpkins, canned vegetables in oil, picked vegetables
Raw vegetables	Raw leafy vegetables, raw tomatoes
Tomatoes (cooked)	Tomato sauces, tomatoes
Legumes	Lentils, beans, chickpeas, fava beans, green peas
Fruit	Apples, pears, kiwi, bananas, grapes, peaches, apricots, oranges, tangerines, plums, strawberries, melon, khaki, figs, cherries
Nuts and dried fruit	Peanuts, almonds, hazelnuts, walnuts, dried figs, dried dates, prune
Olives	Olives
Milk	Milk
Yogurt	Yogurt
Fresh cheese	Mozzarella, ricotta cheese, taleggio cheese, gorgonzola cheese, melted cheese slices, other soft cream cheese
Seasoned cheese	Fontina cheese, emmenthal, gruyere, parmesan, caciocavallo cheese, other seasoned cheese
Pasta and other grains	Pasta, yellow maize meal
Rice	Rice
Bread	White bread, bread with oil and other bread
Crisp bread, rusks	Breads sticks, crisp bread
Breakfast cereals	Breakfast cereals
Salty biscuits	Crackers
Red meat	Beef, pork, lamb, horse, game, other meats
White meat	Chicken, turkey, rabbit, veal
Processed meat	Sausages, ham, bologna sausage, dried beef, salami
Offal	Liver, offal
Canned fish	Canned tuna fish and other fish
Crustaceans, molluscs	Crustaceans, molluscs
Fish	Other fish
Egg	Eggs
Vegetables oils	Seed oils (except olive oils)
Olive oil	Olive oil
Butter	Butter
Margarines	Margarines
Animal fats	Visible fat from meat, poultry skin, fat from ham
Sugar & sweets	Sugar, honey, cakes, ice cream, confections, pastry, pudding
Fruit juices	Orange juice, grapefruit juices, other fruit juices
Soft drinks	Soft drinks
Coffee	Coffee
Tea	Tea
Other sauces	Dressing sauces for pasta other than tomato sauce
Mayonnaises	Mayonnaises
Soups	Vegetable soups
Bouillon	Meat and stock-cube broth
Snacks	Vegetable quiche
Pizza	Pizza
Wine	Red wine, rosé wine, white wine
Spirits	Alcoholic beverages other than wine or beer
Beer	Beer

3.5 Cognitive impairment and health-related quality of life assessment

Montreal Cognitive Assessment (MoCA)

The *Montreal Cognitive Assessment (MoCA)* was used as a rapid screening instrument for mild cognitive impairment (MCI). It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation [267]. Time to administer the MoCA is approximately 10 minutes. The MoCA evaluates different types of cognitive abilities (Figure 8). These include:

- **Visuospatial/executiveability**: these two abilities are assessed through the trail-making test B (TMT-B), which requires to draw a line to correctly sequence alternating digits and numbers (1-A, 2-B, etc.) and through a task which requires to draw a copy of a cube shape. **Clock-drawing test**:the person is asked to draw a clock that reads ten past eleven.

- **Animal naming**: three pictures of animals are shown and the individual is asked to name each one.

- **Memory and delayed recall**: five words are read and the test-taker is asked to repeat them. They are read again and asked to repeat again. After completing other tasks, the person is asked to repeat each of the five words again and given a cue of the category that the word belongs to if they are not able to recall them without the cue.

- **Attention**: the test-taker is asked to repeat a series of numbers forward and then a different series backwards.

- **Language abilities**: this task consists of repeating two sentences correctly and then listing all of the words that can be recalled that begin with the letter "F".

- **Abstraction**: the subject is asked to explain how two items are alike, such as a train and a bicycle.

- **Orientation:** the test administrator asks the person to state the date, month, year, day, place, and city, all referring to the day in which the test is peformed.

Figure 8 – MoCA test protocol. Several cognitive domains are explored: visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall and orientation [267].

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The total possible score of MoCa test is 30 points; a score of 26 or above is considered normal. The scoring breakdown is reported in table 3.

Table 3 – Score for each ability evaluated in the MoCA test.

MoCA ABILITIES	MoCA SCORE (points)
Visuospatial/executive ability	5
Animal Naming	3
Memory and delayed recall	5
Attention	6
Language	3
Abstraction	2
Orientation	6
Educational level	1 point is added to the test-taker's score if he or she has 12 years or less of formal education

Short form health survey (SF-36)

The *Short Form 36 (SF-36)* was used as a self-report measure of functional health and well-being [268]. The SF-36 is designed to be a brief yet comprehensive measure of general health status. The SF-36 questionnaire consists of eight scales yielding two summary measures: physical and mental health. The physical health measure includes four scales of physical functioning (10 items), role-physical (4 items), bodily pain (2 items), and general health (5 items). The mental health measure is composed of vitality (4 items), social functioning (2 items), role-emotional (3 items), and mental health (5 items). A final item, termed self-reported health transition, is answered by the subject but is not included in the scoring process. The SF-36 offers a choice of recall format at a standard (4 week) or acute (1 week) time frame. Likert scales and yes/no options are used to assess function and well-being on this 36-item questionnaire.

To score the SF-36, scales are standardized with an algorithm to obtain a score ranging from 0 to 100. Higher scores indicate better health status, and a mean score of 50 has been articulated as a normative value for all scales. During this PhD work, since SF-36 was self-reported to the elderly subjects, the official Italian version was used [269] (Figure 9).

SF 36 QUESTIONNAIRE
QUESTIONARIO SULLO STATO DI SALUTE

Codice del paziente_____

1. **In generale, direbbe che la sua salute è:**

   (indichi una casella)

   Eccellente ❏   Molto buona ❏   Buona ❏   Passabile ❏   Scadente ❏

2. **Rispetto ad un anno fa**, come giudicherebbe, ora la sua salute in generale?

   (indichi una casella)

Decisamente migliore adesso rispetto ad un anno fa	❏
Un po' migliore adesso rispetto ad un anno fa	❏
Più o meno uguale rispetto ad un anno fa	❏
Un po' peggiore adesso rispetto ad un anno fa	❏
Decisamente peggiore adesso rispetto ad un anno fa	❏

3. **Le seguenti domande riguardano alcune attivita' che potrebbe svolgere nel corso di una qualsiasi giornata. La Sua salute La limita attualmente nello svolgimento di queste attività?**

   Se SI, fino a che punto?

   (indichi per ogni domanda il numero 1,2 o 3)

		Si , mi limita parecchio	Si, mi limita parzialmente	No, non mi limita per nulla
a)	Attività' fisicamente impegnative, come correre, sollevare oggetti pesanti, praticare sport faticosi	1	2	3
b)	Attività' di moderato impegno fisico, come spostare un tavolo, usare l'aspirapolvere, giocare a bocce o fare un giretto in bicicletta.	1	2	3
c)	Sollevare o portare le borse della spesa.	1	2	3
d)	Salire qualche piano di scale.	1	2	3
e)	Salire un piano di scale.	1	2	3
f)	Piegarsi, inginocchiarsi o chinarsi.	1	2	3
g)	Camminare per un chilometro.	1	2	3
h)	Camminare per qualche centinaia di metri.	1	2	3
i)	Camminare per circa cento metri.	1	2	3
j)	Farsi il bagno o vestirsi da soli.	1	2	3

Figure 9 – First page of the Official Italian version SF-36 questionnaire. Through 36 multiple questions, different aspects are investigated such as physical activity, role and physical health, physical pain, health in general, vitality, social activities, role and emotional state, mental health.[269].

3.6 Blood inflammation, coagulation and CVD risk biomarkers

Venous blood sampling and treatment before laboratory analysis

Venous blood samples were obtained by a venipuncture using the *vacutainer system* between the hours of 7.00 and 9.00 a.m. from participants who had fasted overnight and had refrained from smoking for at least six hours. For hemocromocytometric analysis, an EDTA fresh tube was used. Inflammation, coagulation and CVD risk analytes were determined on frozen samples using the appropriately prepared serum or plasma, respectively:

- to obtain **serum**: the blood was taken in a tube with a separating gel and left at room temperature for about half an hour. Subsequently it was centrifuged at 3.000 rpm for 20 minutes, separating the serum;

- to obtain **plasma**: the blood was taken using tubes containing sodium citrate 3.8% in right proportion (1:9), mixed by gently inverting the tube and centrifuged at 3.000 rpm for 20 minutes at room temperature. The plasma was then separated from the corpuscular part to avoid alterations.

Laboratory analysis were performed in the centralized Moli-sani laboratory using commercial reagents and automatic analyzers.

Hemocromocytometric analysis with leukocyte formula

Clinical relevance: hemocromocytometric analysis evaluates the cell populations in the peripheral blood, both in terms of quantity and quality, including red blood cells (RBC), white blood cells (WBC) or leukocytes and platlets (PLT). The dosage of haemoglobin (Hb) and RBC indices are also included. The leukocyte formula provides quantitative and qualitative information on the five leukocyte populations: neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L) and monocytes (M) **Sample:** whole blood, EDTA

Dosage principle: the flow cytometry-based system uses light scatter, differential WBC lysis, and myeloperoxidase and oxazine 750 staining to provide a complete blood cell count. A cyanide-free method is used to measure Hb colorimetrically

Method: enzymatic using peroxidise

Haematological automated cell counter: all hemocromocytometric analysis were performed using the same cell counter ADVIA 120 (Siemens Healthcare Diagnostics Milan, Italy) within 1 h from blood collection. Quality Control intraday for ADVIA 120 was performed by using three different levels of standards: Abn I, Abn II and Normal.

Coefficient of variability (CV) was 2.9%, 2.5%, and 2.6% for WBC respectively for Abn I, Abn II and Normal; 4.2%, 2.4%, 2.8% for PLT respectively for Abn I, Abn II and Normal; 1.6%, 1.4% and 1.3% for RBC respectively for Abn I, Abn II and Normal

High sensitive C-Reactive Protein (hs-CRP)

Clinical relevance: protein that contributes to the activation of the complement system and to the acceleration of phagocytosis; it is an inflammation index

Sample: serum, separator gel

Dosage principle: frozen serum CRP causes the agglutination of latex particles coated with anti-CRP antibody

Method: immunoturbidimetric assay; wavelength = 570/750nm

Clinical chemistry instrument: ILab ARIES Instrumentation Laboratory, Milan, Italy. CV for hs-CRP was 7.3%

D-Dimers

Clinical relevance: D-Dimer is the degradation product of fibrin stabilized by coagulation factor XIIIa and therefore represents an index of fibrinolysis. Its clinical use is above all in the diagnosis of the exclusion of venous thrombosis and of pulmonary embolism. It can be used as a marker of fibrinolysis even in other clinical conditions such as the Disseminated Intravascular Coagulation (DIC). This analyte can be high even in other clinical conditions in which there may be hypercoagulability and consequently hyperfibrinolysis such as trauma, surgery, burns and tumors. A marked increase in its concentration occurs following pharmacological fibrinolytic treatment

Sample: plasma, sodium citrate

Dosage principle: D-Dimers levels were measured in a suspension of polystyrene latex particles of uniform size coated with a monoclonal antibody highly specific for D-Dimer domain included in fibrin soluble derivatives. When a plasma containing D-Dimer is mixed with the Latex Reagent and the Reaction Buffer, the coated latex particles agglutinate. The degree of agglutination is directly proportional to the concentration of D-Dimer in the sample and is determined by measuring the decrease of the transmitted light caused by the aggregates

Method: turbimetric immunoassay

Coagulation instrument: ACL9000 Instrumentation Laboratory Milan Italy. CV for D-dimer was 5.2%; 7.9% and 7.8% respectively for in-house plasma pool, low and high control

Blood markers of CVD risk

Serum lipids (total cholesterol, HDL-cholesterol, triglycerides and glucose) were assayed by enzymatic reaction methods using an automatic analyzer (ILab ARIES Instrumentation Laboratory, Milan, Italy). Quality control for lipids and glucose was obtained by a commercial standard (Ser 1 and Ser 2). The CV were respectively 4.2% and 4.2% for blood cholesterol; 3.4% and 5.1% respectively for HDL-cholesterol; 4.9% and 4.4% respectively for triglycerides, 3.1% and 2.9% respectively for blood glucose.

INFLA-score

The low-grade inflammation score (INFLA-score) had been used previously within the Moli-sani cohort and allows one to evaluate the possible synergistic effects of inflammation biomarkers. 10-tiles of each biomarker levels (CRP, WBC, platelets, G/L ratio) were generated. For all four components, being in the highest deciles (7 to 10) gave a score which increased from 1 to 4, while being in the lowest deciles (1 to 4) was negatively scored from -4 to -1. Being in the deciles 5 or 6 got zero points. In such a way, the INFLA-score ranged from between -16 and 16 and came up as the sum of the four biomarkers. An increase in the score represented an increase in low-grade inflammation intensity. For analysis purposes, quartiles of the INFLA-score were also generated [270,271].

3.7 Covariate assessment

History of cardiovascular disease (angina, myocardial infarction, revascularization procedures, peripheral artery disease and cerebrovascular events) was self-reported and confirmed by medical records and therapy. History of cancer was self-reported and confirmed by medical records. Hypertension, hyperlipidaemia and diabetes were defined by use of specific pharmacological treatment. Physical activity (PA) was assessed by a structured questionnaire (24 questions on working and leisure time and sport participation) and expressed as daily energy expenditure in metabolic equivalent task-hours. Leisure-time PA was expressed as daily energy expenditure in metabolic equivalent task-hours (MET-h/d) for sport, walking and gardening [272]. Height and weight were measured and Body Mass Index (BMI) was calculated as kg/m^2 and then grouped into three categories as *normal* (≤25), *overweight* (>25<30) or *obese* (≥30). Waist circumferences were measured according to the National Heart, Lung and Blood Institute guidelines [273].

Household net income categories were considered as *low* (< 25,000 Euro/year), *medium–high* (>25,000 ≤40,000 Euro/year) and *high* (>40,000 Euro/year). Education level was divided into three categories: *up to lower secondary*, *upper secondary* and *post-secondary*. Subjects were classified as *non-smokers* if they had smoked less than 100 cigarettes in their lifetime or they had never smoked cigarettes, *ex-smokers* if they had smoked cigarettes in the past and had stopped smoking for at least 1 year, and *current smokers* if they reported having smoked at least 100 cigarettes in their lifetime and still smoked or had quit smoking within the preceding year [274].

3.8 Statistical analysis

All continuous variables were tested for normality using Shapiro's test and were reported as means± standard deviation (SD). Categorical variables were reported as frequencies and percentages. Variables with positive skewed distribution (SHARE-FI score, triglycerides, glucose, hs-CRP, leucocytes, D-dimers) were natural log-transformed and were reported as median and interquartile range (IQR). SHARE-FI score was categorized in *non-frail*, *pre-frail* and *frail* separately for men and women, as reported by Romero-Ortuno et al. [261] (Figure 10).

Females
- If predicted DFS < 0.3151361243, NON-FRAIL
  - If predicted DFS < 2.1301121973, PRE-FRAIL
  - If predicted DFS < 6, FRAIL
Males
- If predicted DFS < 1.211878526, NON-FRAIL
  - If predicted DFS < 3.0052612772, PRE-FRAIL
  - If predicted DFS < 7, FRAIL

Figure 10 – Cut-offs for the SHARE-FI categorization by Romero-Ortuno R et al. [261].

Differences in the distribution of demographic (age, sex), socioeconomic (i.e. education, income), clinical (i.e. history of CVD, cancer, diabetes), biochemical (i.e. blood inflammation and coagulation biomarkers, lipids) and dietary (i.e. MDS, food groups, macronutrients) variables according to frailty categories were calculated using non-parametric Wilcoxon Two-Sample Test (for continuous variables) and Exact Fischer test (for categorical variables) in SAS software. Two-sided P-value <0.05 was considered as statistically significant. The data analysis was generated using SAS/STAT software, Version 9.4 of the SAS System for Windowsª2009. SAS Institute Inc. and SAS are registered trademarks of SAS Institute Inc., Cary, NC, USA. (*ref: SAS Institute Inc. Base SAS® 9.4 Procedures Guide: Statistical Procedures, 2nd edn. Cary, NC: SAS Institute, Inc; 2013*)

Chapter 4: Results

During this PhD thesis, from May 2018 to May 2019, 229 elderly subjects aged ≥65 were recruited during the recall phase of the Moli-sani Study at the recruitment centre of the Epidemiology and Prevention Department of the I.R.C.C.S. Neuromed, Pozzilli, based in Via Garibaldi 21, Campobasso, Molise, Italy. Finally, 226 people were analyzed because 3 subjects were excluded from the statistical analysis for unreliable questionnaires. We calculated the SHARE-FI score according to Romero-Ortuno R et al. [261] and determined distribution in the total sample (N=226). As showed in figure 11, we have a non-Gaussian distribution of the analyzed elderly population.
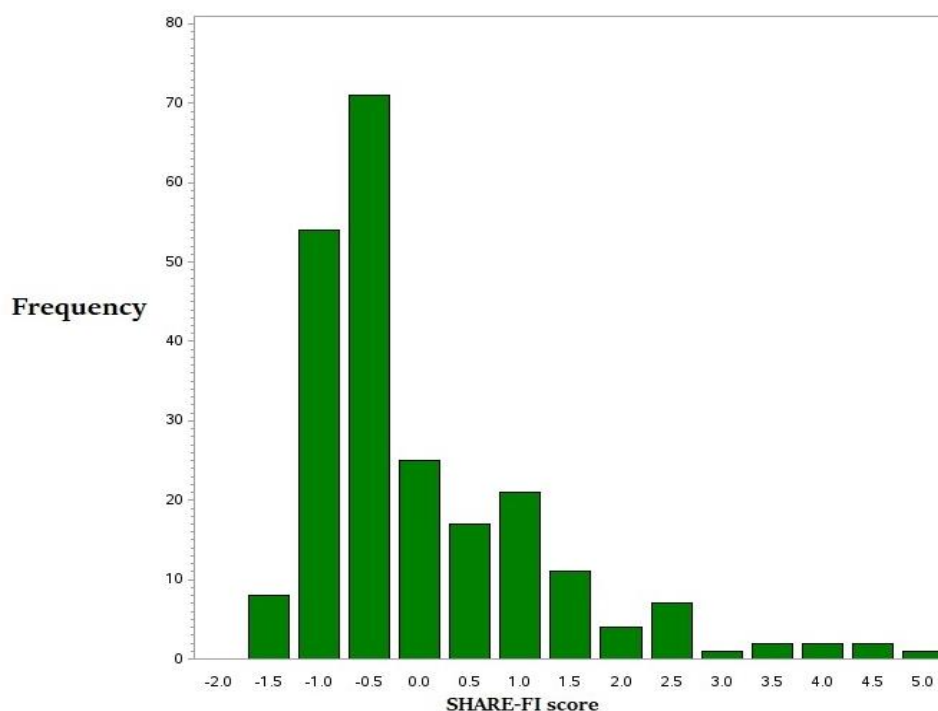
Figure 11 – SHARE-FI non-Gaussian distribution in the total sample (N=226).

The SHARE-FI score median (Interquartile Range, IQR) was the same both for men and women (median (IRQ): -0.43(-0.79;0.38) and -0.44 (-0.77;0.72), respectively). However, considering the distribution of the five components proposed by Romero-Ortuno R et al. [261], the prevalence of self-reported exhaustion (fatigue) was higher in women than in men (14.6% vs 4.3%, respectively; P=0.011). Moreover, the grip strength, determined using Jamar Plus+ digital dynamometer, was lower in women than in men (mean ±SD: 22.1 ± 6.1 vs 39.6 ± 8.6 Kg, respectively; P<0.0001) (Table 4).

Table 4 – SHARE-FI and its components distribution in men and women (N=226).

	Gender		
	Women	**Men**	**P value**
N (%)	110 (48.7)	116 (51.3)	
SHARE-FI score, median (IQR)	-0.43 (-0.79;0.38)	-0.44 (-0.77;0.72)	0.44
SHARE-FI score Components			
Fatigue,	14.6	4.3	**0.011**
Loss of appetite,	8.2	3.5	0.16
Grip strength, Kg mean±SD	22.1±6.1	39.6±8.6	**<0.0001**
Functional difficulties	10.9	17.2	0.19
Physical activity			0.74
hardly ever, o never	66.4	69.0	
one to three times a month	19.1	14.6	
once a week	4.5	3.5	
more than once a week	10.0	12.9	

p value from non-parametric Wilcoxon Two-Sample Test (continuous) and Exact Fischer test (categorical)

Values are presented as percentages unless otherwise stated

IQR= Interquartile Range

Using Romero-Ortuno R et al. categorization (*non-frail, pre-frail and frail*) [261], it was possible to divide the total sample (N=226) in the three above-mentioned groups. The prevalence of pre-frail and frail individuals was 15.9% and 5.3%, respectively. The prevalence of frail individuals resulted higher in women than in men (Table 5).

Table 5 – SHARE-FI categorization in the total sample (N=226).

	Non-frail	**Pre-frail**	**Frail**
Total sample, N (%)	**178 (78.8)**	**36 (15.9)**	**12 (5.3)**
SHARE-FI score	-0.61 (-0.82;-0.24)	1.20 (0.75;1.63)	3.51 (2.65;4.21)
Women, N (%)	**79 (71.8)**	**23 (20.9)**	**8 (7.3)**
SHARE-FI score	-0.65 (-0.89;-0.36)	0.80 (0.60;1.11)	3.06 (2.43;4.21)
Men, N (%)	**99 (85.3)**	**13 (11.2)**	**4 (3.5)**
SHARE-FI score	-0.54 (-0.80;-0.07)	1.65 (1.42;2.10)	3.80 (3.36;4.38)

Values are presented as median and Interquartile range unless otherwise stated

Abbreviation: IQR= Interquartile Range

Pre-frail and frail categories were collapsed to create a dichotomous variable having just two categories: *pre-frail/frail* vs *non frail* individuals. Women were more pre-frail/frail than men (28.2% vs 14.7%, respectively; P=0.015). The characteristics of the study population are showed in table 6. Pre-frail/frail individuals were older than frail individuals (mean ±SD: 75.1±6.0 vs 72.1±5.2 years, respectively; P=0.0008). Moreover, pre-frail/frail elderly people showed a tendency towards lower level of education (45.8% vs 36.7%, respectively; P>0.05) and household income ≤25,000 EUR/year (52.1% vs 41.6%, respectively; P>0.05) and a higher prevalence of history of cardiovascular disease (16.7% vs 10.2%, respectively; P>0.05). Pre-frail/frail elderly people showed a higher significant prevalence of hypertension than non fail individuals (72.9% vs 58.2%, respectively; P=0.068).

Table 6 – Characteristics of the study sample according to frail phenotype (N=226).

| | Frail phenotype | | | |
	Total	Non-frail	Pre-frail/frail	P value
N of subjects (%)	226 (100)	178 (78.8)	48 (21.2)	
Men	51.3	55.6	35.4	**0.015**
Age (years, mean±SD)	72.7±5.5	72.1±5.2	75.1±6.0	**0.0008**
Educational level				0.54
Up to lower secondary	38.7	36.7	45.8	
Upper secondary	39.6	40.7	35.4	
Postsecondary	21.8	22.6	18.8	
Household income (€/yr)				0.66
≤25,000	43.8	41.6	52.1	
>25,000≤40,000	32.7	33.7	29.2	
>40,000	15.9	16.9	12.5	
Unknown	7.5	7.9	6.3	
Smoking status				0.31
Non-smokers	53.1	50.6	62.5	
Current	10.6	10.7	10.4	
Former	36.3	38.7	27.1	
BMI (kg/m^2)				0.65
Normal weight	23.5	23.0	25.0	
Overweight	43.4	44.9	37.5	
Obesity	33.2	32.0	37.5	
History of CVD	11.6	10.2	16.7	0.21
History of Cancer	12.5	11.9	14.6	0.63
Diabetes	13.8	13.6	14.6	0.82
Hypertension	61.3	58.2	72.9	**0.068**
Hyperlipidaemia				0.14
Yes	30.1	27.5	39.6	
Unknown	5.8	5.1	8.3	

p value from non-parametric Wilcoxon Two-Sample Test (continuous) and Exact Fischer test (categorical)

Values are presented as percentages unless otherwise stated

Abbreviation: CVD: cardiovascular disease

Table 7 shows dietary habits of the study sample according to frail phenotype. For this analysis 222 individuals were considered, because 4 EPIC-FFQs were incomplete. There was no difference between normal and pre-frail/frail elderly people in the adherence to MD. However, considering the single MD components (food groups), we found a reduced consumption of fruits and nuts (P=0.047) and legumes (P=0.010) in pre-frails/frails compared to non frail individuals.

Table 7 – Dietary habit of the study sample according to frail phenotype (N=222).

	Frail phenotype		
	Non-frail	**Pre-frail/frail**	**P value**
N of subjects (%)	177 (79.0)	47 (21.0)	
Mediterranean diet score (0-9)	4.5±1.7	4.2±1.8	0.25
Mediterranean diet score, %			
Low (0-3)	27.7	34.0	0.55
Medium (4, 5)	41.2	42.6	
High (6-9)	31.1	23.4	
Energy intake (Kcal/d)	1826±456	1766±415	0.46
Food groups (g/d)			
Fruits and nuts	300±126	265±139	**0.047**
Vegetables	139±52	127±58	0.41
Legumes	32±17	25±14	**0.010**
Cereals	170±66	172±49	0.74
Fish	49±24	44±22	0.25
MUFAs/SFAs	1.33±0.27	1.26±0.20	0.18
Meat and meat products	75±29	78±36	0.67
Milk and dairy products	154±103	161±84	0.44
Moderate alcohol consumers, %	37.9	46.8	0.32
Macronutrients (g/d)			
Carbohydrates	211±62	206±54	0.74
Proteins	75±17	73±15	0.55
Animal protein	51±13	50±13	0.63
Vegetable protein	24±6	23±6	0.35
Fats	71±19	68±18	0.54
Monounsaturated FA	33.0±8	31.5±9	0.32
Polyunsaturated FA	7.7±2.0	7.6±1.9	0.82
Saturated FA	26±9	25±7	0.73
Fibre	19.0±4.3	17.6±5.0	0.12
Dietary calcium (mg/d)	893±282	859±254	0.25
Dietary sodium (mg/d)	2024±662	1982±519	0.99

Values are reported as means with standard deviation (SD) unless otherwise stated.
p value from non-parametric Wilcoxon Two-Sample Test (continuous) and Exact Fischer test (categorical)
Abbreviation FA: *fatty acids*

When participants aged 65-69 years, pre-frail/frail subjects had higher prevalence of a lower adherence to MD than non-frail elderly people (50.0% vs 21.6%, respectively; P=0.054, table 8).

Table 8 – Mediterranean Diet and frailty in participants aged 65-69 years (N=88).

	Frail phenotype		
Participants aged 65-69 yrs, N 88	**Non-frail**	**Pre-frail/frail**	**P value**
Mediterranean diet score,%			
low	21.6	50.0	**0.054**
medium	48.7	16.7	
high	29.7	33.3	

p value from non-parametric Exact Fischer test (categorical)

Pre-frail/frail elderlies showed a higher cognitive impairment (MoCA test, mean ±SD: 22.1±4.1 vs 23.6±3.5, respectively; P=0.023, table 9) and a worse perception of physical health-related quality of life (SF-36 physical dimension score, mean ±SD: 41.8±7.1 vs 45±5.8, respectively; P=0.0057, table 9) than non-frails.

Table 9 – Cognitive impairment (MoCA test, N=224) and health-related quality of life (SF-36, N=218) according to frail phenotype.

	Frail phenotype		
	Non-frail	**Pre-frail/frail**	**P value**
Short form health survey (SF-36), N 218			
N of subjects (%)	173 (79.4)	45 (20.6)	
SF36 mental score	45.9 (9.5)	44.2 (10.4)	0.31
SF36 physical score	45.0 (5.8)	41.8 (7.1)	**0.0057**
Montreal Cognitive Assessment (MoCA) test, N 224			
N of subjects (%)	177 (79)	47 (21.0)	
Total score	23.7 (3.5)	22.1 (4.1)	**0.023**

Values are reported as means with standard deviation (±SD)

p values from non-parametric Wilcoxon Two-Sample Test (continuous)

Abbreviation: SF-36= Short form health survey; MoCA= Montreal Cognitive Assessment

Pre-frail/frail individuals had lower levels of serum total cholesterol than non-frails (mean ±SD: 185.6±38.9 vs 210±43.8 mg/dL, respectively; P=0.0004, table 10), which could be explained by the higher use of lipid-lowering drugs (43.2% vs 29%, respectively).

Table 10 – Traditional markers of CVD risk (N=226).

	Frail phenotype		
	Non-frail	**Pre-frail/frail**	**P value**
N of subjects, (%)	176 (78.9)	47 (21.1)	
Total Cholesterol, mg/dL	210.0±43.8	185.6±38.9	**0.0004**
HDL-Chol, mg/dL	58.2±13.0	57.4±15.7	0.90
Triglycerides, mg/dL median (IQR)	120 (92.5;161.5)	97 (82.0; 155.0)	**0.075**
HCL drug, (%)	29.0	43.2	0.10
Glucose, mg/dL median (IQR)	103.0 (96.0;113.0)	109.0 (94.0;117.0)	0.29
MetS,%	40.3	48.9	0.32

Values are reported as means with standard deviation (±SD) unless otherwise stated.

 p value from non-parametric Wilcoxon Two-Sample Test (continuous) and Exact Fischer test (categorical)

Abbreviation: HCL=hypercholesterolemia;MetS=metabolic syndrome

High blood levels of hs-CRP was found in pre-frails/frails as compared with no-frail elderly people (median (IRQ): 2.5 (1.0;4.8) vs 1.4 (0.8;3.5) mg/L, respectively; P=0.043, table 11). D-dimers plasma levels were also higher in pre-frails/frails compared to the non-frails (median (IRQ): 298 (227;460) vs 254 (226;328) ng/mL, respectively; P=0.053, table 11).

Table 11 – Biomarkers of inflammation and coagulation (N=226)

	Frail phenotype		
	Non-frail	**Pre-frail/frail**	**P value**
N of subjects, (%)	178 (78.4)	48 (21.6)	
C-Reactive Protein (mg/L), median (IRQ)	1.4 (0.8;3.5)	2.5 (1.0;4.8)	**0.043**
Leukocyte count ($\times 10^9$/L), median (IRQ)	5.9 (5.2;6.9)	5.4 (4.8;7.0)	0.24
Granulocyte/lymphocyte ratio	2.00±0.99	2.14±0.94	0.42
Platelet count ($\times 10^9$/L)	236.2±53.3	236.7±69.0	0.93
INFLA-score (-16 to 16)	-0.086±5.24	0.63±7.20	0.63
D-dimer (ng/mL), median (IRQ)	254 (226;328)	298 (227;460)	**0.053**

Values are reported as means with standard deviation (±SD) unless otherwise stated.

 p value from non-parametric Wilcoxon Two-Sample Test

Chapter 5: Discussion

In our population of elderly Southern-Italy subjects, overall prevalence of pre-frailty was 20.9% in women and 11.2% in men, and the prevalence of frailty was 7.3% in women and 3.5% in men. Despite the small sample, this is in line both with the Cardiovascular Health Study (CHS) by Fried LP et al. [2], which showed a prevalence of frailty in the original cohort (1989-1990) of 7.3% in females and 4.9% in males, and with *Survey of Health, Ageing and Retirement in Europe – Frailty Instrument (SHARE-FI)* study by Romero-Ortuno R et al. [261] in which the prevalence of pre-frailty was 25.8% in women and 14.6% in men and the prevalence of frailty was 7.3% in female and 3.1% in male subjects. All these studies used the same instrument to measure frailty in the general population, confirming that the SHARE-FI represents a common language to evaluate frailty. As it currently stands, the main potential use of SHARE-FI is the screening and monitoring of frailty in the community or primary care setting in order to help decide who would benefit from secondary care referrals and/or early multidisciplinary case management. To that effect, the advantages of SHARE-FI are that it can be easily administered in the community by non-physicians (e.g. nurse, health visitor or other allied health professionals), and it is a brief instrument. If we consider the five components (exhaustion, weight loss, weakness, slowness and low activity) of the questionnaire individually, in this PhD work women experience more fatigue and have less handgrip strength. This result was also found in the study by Joosten E et al., in which the grip strength was higher in men than in women (23.1 ± 9.8 vs 14.7 ± 7.4 kg; $P < 0.001$) in a sample of 209 geriatric inpatients [275]. The fact that gender has a strongest influence on grip strength was also found in a multivariate linear regression analysis by Dudzińska-Griszek J et al. [276]. A recent systematic review investigating the prevalence of frailty [277] in 21 community based cohort studies, involving 61,500 older people found that, when the reported rates were restricted to the studies that used the phenotype model, the weighted average prevalence rate was 9.9% (95% Confidence Interval (CI) 9.6-10.2%) for frailty and 44.2% (95%, CI 44.2-44.7%) for pre-frailty. Moreover, frailty was statistically more prevalent in women (9.6%, 95% CI 9.2-10.0%) than men (5.2%, 95% CI 4.9-5.5%). Due to low number of frail persons in our study, for further analysis, pre-frail and frail categories were collapsed to create a dichotomous variable having just two categories: *"pre-frail/frail"* vs *"non frail"* individuals. We found that frailty increased with age and was higher in women than men. Also in other studies frailty increased steadily with age, e.g.: 65-69 years: 4%; 70-74 years: 7%; 75-79 years: 9%; 80-84 years: 16%; >85 years: 26% [278].

Rates appear to be higher in studies that employed the graded frailty index, which would count as frail some people whose increased risk is captured in the pre-frail category of the phenotype model [279]. Among the determinants of frailty, we can also include socio-economic status. As showed in our study, pre-frail/frail subjects had a lower level of education and household income. In scientific literature several studies reported that, for both men and women, adequacy of income was an independent determinant of frailty. This is compatible with the link between poverty and ill-health [280]. It is likely that frailty is an accompaniment of poorer health. Previous studies also suggested that socioeconomic factors contribute to differences in frailty and pre-frailty and that health inequalities as a result of education, occupational class, and wealth persist throughout old age [281,282]. Additionally, recent studies found an increased risk in the worsening of frailty over time in lower educated persons aged ≥55 years compared to higher educated persons [283-285]. This persisting inequality in old age can be partly explained by lifestyle and health, such as lower consumption of moderate amount of alcohol, higher sedentariness, higher obesity, higher chronic disease rates, and being depressed [286-290]. We know from the literature that there is a relationship between social factors and health. This relationship manifests itself in two ways. First, social integration (marriage, family relationships, friendships, church and civic communities, volunteering) is a good "therapy" for the health of the individuals because it prevents health problems, leads to more rapid recovery and extends life [291-293]. The more social relationships someone has, the fewer health problems they will suffer. This suggests that the onset of social frailty can lead to the onset or exacerbation of health problems. Older people with a low education level or a low income have a higher risk of mortality, being admitted to an institution and developing functional disabilities. These health differences among older persons suggest a systematic and chronic problem for the public health system, and indicate that the highest proportion of all health problems occurs in groups which are socially and economically underprivileged. If substantial socioeconomic differentials in frailty are found to exist among older persons, greater efforts need to be made to eliminate and reduce those differentials. In our study a higher prevalence of hypertension was found in pre-frail/frail elderlies, confirming previous evidence. Several studies, indeed, have assessed the association of frailty with hypertension, although the cause-effect relation is still debated. Four longitudinal studies examined the risk of incidence of frailty according to baseline hypertension status, providing conflicting results [294]. Two studies found that baseline hypertension did not significantly predict incidence of frailty [295,298], but Boullion K et al found that hypertension was associated with an increased incidence of the combined outcome prefrailty/frailty (P=0.009) [296].

Castrejón-Pérez RC et al. [297] found that hypertension was associated with incident frailty at univariate analysis (Hazard ratio (HR) 2.11, 95% CI 1.03 to 4.31), but this association was not confirmed in the multivariate analysis (HR 1.58, 95% CI 0.83 to 3.01). In older adults, it has been even suggested that frailty can explain the paradoxical relationship between lower blood pressure (BP) and increased mortality documented in several studies [366-369]; this could be the reason for which the higher hypertension prevalence is survived frail persons. For example, data from the *National Health and Nutrition Examination Survey (NHANES)* demonstrated an effect modification of hypertension according to frailty level in terms of walking speed [370]; in fit persons, elevated BP was associated with greater mortality, while in frail participants higher BP was associated with lower mortality risk. The *Systolic Blood Pressure Intervention Trial (SPRINT)* study showed that compared with standard BP control, intensive control reduces the incidence of cardiovascular events both in frail and non-frail persons, but this study did not show any effects of intensive BP control on risk of frailty-related outcomes, such as gait speed and mobility limitation [371,372]. The results of this PhD thesis showed that there is no difference in the Mediterranean diet (MD) adherence among non-frails and frails. However, consumption of individual food such as fruits, nuts and legumes was lower in pre-frail/frail elderlies. Our findings concur in part with those obtained in previous research [299,300]. Bollwein J et al. [299] conducted a cross-sectional study among 192 community-dwelling older volunteers (>75 years) in Nürberg (Germany), who were classified into quartiles of the alternate MED score, developed from the original score of Trichopoulou et al. and adapted for a non-Mediterranean population. In multivariable analyses, the frequency of frailty, based on the Fried criteria [2], was significantly reduced in the highest quartile of the MED score [299]. In the *Invecchiare in Chianti (InChianti)* study, Talegawkar SA et al. [300] selected 690 community-living persons aged ≥65 years in Tuscany (Italy), who were classified according to the MD score. Individuals were followed during 6 years to identify incident frailty, assessed with the Fried criteria [2] except for weight loss. After adjustment for confounders, higher adherence to a Mediterranean-style diet was associated with lower odds of developing frailty as compared with those with lower adherence. Although we did not find difference in the MD score, probably due to the low power of our study, our findings shows that the most important components of the MD may contribute to lower risk of frailty, namely legumes, fruits and nuts. Moreover, a significant tendency toward less frailty according to a high adhesion to the MD was observed in participants aged 65-69 yeras; this probably reflects an interaction between the MD and subclinical diseases in the older adults. In fact, there is evidence that frailty is associated with subclinical cardiovascular disease [301,302].

The effect of a high dietary quality on frailty might be mediated by a high fruit and vegetable intake and low intake of animal products as proposed in the MED score. Furthermore, vegetable foods are the main nutritional sources of carotenoids and vitamin C. There is evidence that these associations are mediated by tissue damage caused by oxidative stress [303] and inflammatory processes [304,305]. This is supported by the finding that MD score is strongly associated with lower plasma concentrations of inflammation biomarkers in middle-aged and older people [306]. The assumption of anti-inflammatory effects of some components of the MD score is supported by the findings of Lopez-Garcia E et al. [307] who detected a dietary pattern characterized by higher intakes of fruit, vegetables, legumes, fish, poultry and whole grains that was inversely associated with plasma concentrations of inflammation markers. Poor nutrition might contribute to frailty, but on the other hand frailty might also affect dietary intake, for example by impairments in going shopping, chewing, or swallowing. Based on our data, it is not possible to decide, whether a Mediterranean diet prevents frailty or whether the diet is altered by frailty or comorbidity which might contribute to nutritional risk. Another limitation is the small sample size, and the small group of frail participants, which limits the statistical power to detect significant associations, which we nevertheless detected. A strong point of our study is that all *European Prospective Investigation into Cancer and Nutrition – Food Frequency Questionnaires (EPIC-FFQs)* have been conducted in personal interviews by a single experienced nutritionist. Secondly, for all assessments, well-validated tools have been used, and professional personnel was engaged. In conclusion, our results are in line with existing evidence relating diet to frailty. About blood biomarkers inflammation, elevated levels of CRP and D-dimers were found in the frail elderly people of the Moli-sani Study. Higher hs-CRP levels were identified in the frail compared with the not frail group. In our study, as hs-CRP serum levels increased, D-dimers plasma levels also increased, so that the inflammatory/coagulation pathways have the potential to spiral into a self-perpetuating cycle, as several of the key factors feed back in a positive manner. For example, D-dimers are known to stimulate the synthesis and release of proinflammatory cytokines, including IL-6 form peripheral blood monocytes and mouse hepatic cells in vitro, as noted above [308,309]. IL-6 in turn, perpetuates the cycle by enhancing coagulation factor and platelet synthesis with microthrombi formation that, in turn, result in endothelial cell damage and further stimulation of both inflammatory and coagulation pathways. Thus, an increase in generalized thrombotic tendency seen in late life could be the result of the commonly acknowledged inflammatory dysregulation of aging. About biomerkers of CVD, we surprisingly found that total cholesterol serum levels were lower in pre-frail/frail people.

In an Italian study [310] the authors evaluated the association between serum cholesterol levels and social, clinical, and functional characteristics in 637 elderly hospitalized patients (mean age=79.1 years, range = 65-97) from the Geriatric Evaluation and Rehabilitation Unit (GERU) at P. Richiedei Hospital in Gussago, Brescia (Italy). Patients consecutively admitted to the GERU during an 18-month period underwent a multidimensional evaluation including information on demographics, cognitive status, physical health (number of chronic diseases and administered drugs), functional disability, and nutritional status. Mean cholesterol levels were significantly lower in men, persons living with others, older individuals, and individuals with cognitive impairment, poorer somatic health, higher disability, and a higher level of malnutrition. So that lower serum cholesterol levels may be considered an independent hematologic marker of frailty in elderly hospitalized patients [310]. In our case, low blood cholesterol levels were associated with the use of lipid-lowering drugs. In gene-specific models, which to some extent mimic the effects of long-term exposure of individuals to the modulation of lipid-lowering drug targets, most of the corresponding therapeutics (including statins) would be predicted to reduce frailty. A prospective cohort study of 383 residents aged ≥65 years found that the risk of mortality, all-cause hospitalizations, and incidence of falls during the 12-month follow-up were lower among statin users than non-users [311]. In contrast, an observational study of statin use and incident frailty in women aged ≥65 years failed to observe associations between current statin use, duration and potency of statin use and incident frailty. However, among users of low potency statins, longer duration of use was associated with reduced risk of frailty [312]. Our study was one of the few investigating frailty and Mild Cognitive Impairment (MCI). Growing evidence has indicated that there is a connection between frailty and cognitive impairment. Several studies have reported a longitudinal association between frailty and rate of MCI in elderly community-dwelling individuals. Boyle PA et al. [230] reported, in an assessment that used 12 years of annual follow-up data, that physical frailty was associated with a high risk of MCI, such that each 1-unit (grip strength, timed walk, body composition, and fatigue) increase in physical frailty was associated with a 63% increase in the risk of MCI. Although the criteria for determining frailty and MCI varied lightly between studies, our results were in accordance with previous findings, and thus add support to the association between frailty and MCI. Despite some methodological differences, most previous studies report prevalence figures for MCI or for cognitive impairment without dementia ranging from 11% to 23%. The *Women's Cognitive Impairment Study of Exceptional Aging (WISE)* used global and domain-specific cognitive measures and found that the prevalence of MCI or cognitive impairment without dementia was 23.2% in a sample of 1,299 participants aged ≥85 years [313].

The *Mayo Clinic Study of Aging (MCSA)* diagnosed 329 of 1,969 study participants (16.7%) with MCI or cognitive impairment without dementia using the Clinical Dementia Rating (CDR) Scale, a neurologic evaluation, and neuropsychological testing to assess 4 cognitive domains: memory, executive function, language, and visuospatial skills [314-315]. In the present study, we found the combined prevalence of frailty and MCI using *Montreal Cognitive Assessment (MoCA)* test and *Short Form Health Survey (SF-36)* self-administered questionnaire. MoCA test, and a state of low physical and mental health perceived by the subject through SF-36 questionnaire. These results suggest that frailty is linked to both conditions. Many researchers believe that the definition of frailty should include mental health as well as physical functioning. The *Frailty Operative Definition – Consensus Conference (FOD-CC)* project reported that experts agreed on the importance of a more comprehensive definition of frailty that should include assessment of physical performance, including gait speed and mobility, nutritional status, mental health, and cognition [17]. The results of the present study are in line with the new concept of frailty, which included cognition. Individuals with a co-occurrence of frailty and MCI may face a higher risk of incident disability than healthy older adults or older adults with either frailty or MCI. The *French Three-City Study* established that frail persons with a cognitive impairment were significantly more likely to develop disabilities in activities of daily living (ADL) and instrumental ADL (IADL) disabilities [316]. Moreover, the *Hispanic Established Populations for the Epidemiologic Study of the Elderly (HEPESE)* demonstrated that frailty and cognitive impairment affect mortality differently when they occur independently compared with when they are present together. For instance, individuals with cognitive impairment and frailty had higher mortality compared with individuals with either frailty or cognitive decline [317]. Further longitudinal studies are needed to clarify the ways in which frailty and MCI might affect vulnerability among older adults.

Chapter 6: Conclusions

Frailty is a practical and unifying concept in the care of older people that directs attention away from organ specific diagnoses towards a more holistic viewpoint of the patients and their predicament. It is a state of vulnerability to poor resolution of homeostasis following a stress and is strongly associated with adverse outcomes. Distinguishing older people who are frail from people who are not frail should therefore form an essential aspect of assessment in any health care encounter that might result in an invasive procedure or potentially harmful medication. Preventing frailty and its consequences is a challenge to an aging society, and there are important obstacles to such prevention. One is the need for a more precise characterization of the clinical syndrome of frailty and its natural history. Multiple, interacting diseases are common in frailty; the prevalence of such comorbidity increases as disability increases, but it is unclear how this relation should be interpreted. For example, some diseases (e.g. stroke, myocardial infarction, renal failure) may have similar mechanisms and may all be expressed as frailty. Likewise, frailty may be a final common path in the expression of unrelated diseases. In either event frailty may be merely a state through which many elderly people pass toward the end of life. Interventions should be targeted to the frail, and the evaluation of them should focus on the consequences of frailty. This PhD study provides a comprehensive overview of the components of frailty within several domains: nutritional, physical, psychological, social and biochemical. Although consensus on frailty measures remains elusive, this research describes the most commonly used frailty components and the corresponding indicators in contemporary publications. These findings can be used to guide the development of a theoretical framework of frailty in future studies. Clinicians can use these frailty components and their corresponding indicators to comprehensively identify frail older adults from physical, psychological and social perspectives to provide holistic care to meet the multidimensional healthcare needs of this vulnerable population. We used the *Survey of Health, Ageing and Retirement in Europe – Frailty Instrument (SHARE-FI)*, because it is a simple frailty screening instrument for primary care, it has sufficient constructand predictive validity, and it is readily and freely accessible via web calculators. Inadequate nutritional intake is frequent among older people and related to increasing dependence and care needs. The association between poor nutritional intake and frailty was demonstrated in numerous recent studies. Not only energy and protein intake has been linked to frailty and frailty-related parameters. Particularly the role of micronutrients in the developmentof frailty has been highlighted in recent publications.

Micronutrient intake has been found to be associated with frailty or related parameters of physical functioning, sometimes even independent from energy intake. In clinical practice and as part of the management of aging it should therefore be recommended to use a suitable tool for routine screening of nutritional risks. Consequently, the goal will be to raise the general dietary intake of high-quality food to provide sufficiently both macro and micronutrients. We found that adherence to Mediterranean Diet (MD) components was associated with lower risk of frailty. Although consumption of fruit, legumes and nuts showed a reduced risk of frailty, we can say that the association between MD and frailty is mostly due to the global dietary pattern instead of single foods. In the future, clinical trials should evaluate whether an intervention promoting MD is able to prevent frailty with its anti-inflammatory effect. There are several potential mechanisms underlying the association between greater adherence to a Mediterranean diet and lower risk of frailty. One possibility is the high intake of foods rich in antioxidants. Fruits and vegetables are rich in carotenoids, vitamins, and polyphenols. Oxidative stress is a risk factor for frailty and fruits and vegetables may decrease the risk of frailty by counteracting oxidative status. Frail individuals have higher levels of inflammatory markers, and inflammation is considered to be closely associated with frailty. A frequent intake of nuts has been associated with decreased levels of IL-6, CRP and fibrinogen and MD is associated with low levels of inflammatory markers and may reduce frailty risk through this mechanism. The fact that these beneficial effects are observed in old frail subjects suggests that it is never too late to change dietary habits for improving health status. Adherence to a MD pattern has been associated with better cognitive function, lower rates of cognitive decline, and lower risks of Alzheimer's disease and dementia. Both frailty and cognitive impairment are very common among nonagenarians and centenarians. The combined syndrome, and not frailty or cognitive impairment alone, is a significant risk factor among elderly people. This study evidenced the association between frailty and cognitive impairment. This topic is very recent and deserves further research in epidemiological and clinical studies. In addition, the data may support the debate and the elaboration of public policies focused on frailty syndrome and cognitive impairment, in order to deal with the demographic growth of the country and the higher life expectancy of the population. Moreover, it is the first study in which the *Short Form Health Survey (SF-36)* questionnaire was used to study the mental and physical health perceived by the same recruited subject with surprising results associated with frailty syndrome. Based on the results of studies in the scientific literature, we can also presume that depression mediates the relation between frailty and cognition, or inflammatory reactions. Because frailty is related to cognitive aging, several changes in mental abilities occur with increasing age.

Although experts disagree on the core underlying processes involved, one factor that links many factors associated with cognitive aging is neuroinflammation. Markers of inflammation are associated directly with deficits in cognitive function and with diseases that are risk factors for cognitive decline. Neuroinflammation is also associated with depression and may account for the complex interaction of depression and cognition in older adults. Interventions that reduce inflammation may improve cognition. Understanding how neuroinflammation affects cognition may provide directions for useful interventions to prevent or treat cognitive decline and frailty in older adults.

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