



## Review

## Altered immune system in frailty: Genetics and diet may influence inflammation

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## ARTICLE INFO

## Keywords:

Frailty syndrome  
Inflamm aging  
Immune system  
SNP  
Nutrients

## ABSTRACT

Frailty is a complex geriatric syndrome associated with biological vulnerability to stressors and decreased physiological reserve. Its etiology and pathogenesis are not completely understood, although various causes and complex pathways have been proposed. Immune system alterations (immunosenescence and “InflammAging”) have been suggested to contribute to frailty, but a precise causative role of such alterations remains to be determined.

Genetic studies support the suggestion of immune system involvement in frailty: genetic variants in genes involved in immune system function have been associated with the syndrome.

Interestingly, nutritional status, through its effects on cellular metabolism, may also influence the immune system, i.e. hormone and cytokine (mainly adipocytokine) levels, and immune cell populations and function, increasing inflammation and contributing to frailty.

This review aims to discuss the role of immune system alterations in frailty, analyzing the role of genetic factors in frailty onset and the impact of diet on inflammation and, in turn, on frailty.

## 1. Introduction

Recent years have brought a remarkable rise in the average age of the population, making caring for the elderly a growing challenge. A better understanding of the underlying mechanisms of aging will be essential to guide the development of therapies aimed at improving the health of older people. In this context, increasing attention has been paid to frailty as a potential explanation of the health diversity found in the elderly. Although a clear definition is still lacking, frailty is commonly understood as a geriatric state associated with increased vulnerability to internal and external stressors resulting from a significant loss of physiological reserve. Described as a disorder of multiple inter-related physiological systems, frailty is characterized by sedentariness, fatigue, weight loss and poor muscle strength, and it increases the risk of adverse outcomes, such as falls, disability, hospitalization and even death (Fried et al., 2001; Mulero et al., 2011; Clegg et al., 2013). In frailty, accumulation of cell damage accompanied by impairment of repair mechanisms (i.e. DNA repair, synthesis and control, detection and clearance of damaged proteins and lipids, clearance of abnormal organelles and cells), and defense against injury leads to loss of the

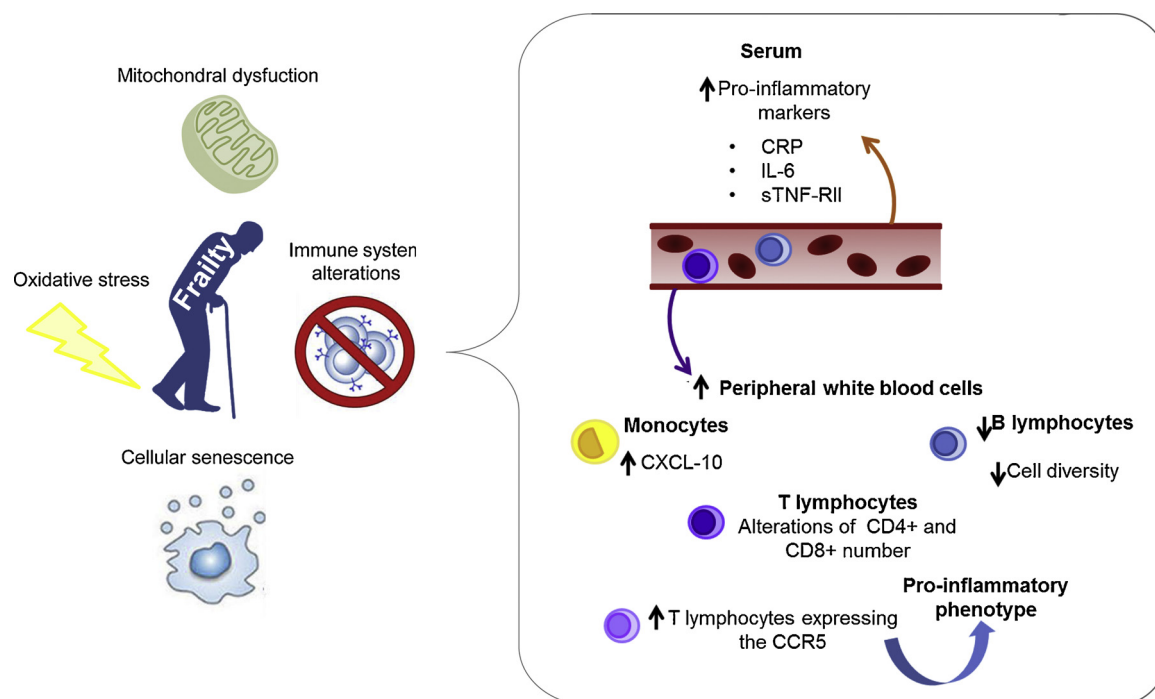
homeostasis of multiple systems and to physiological decline (Lang et al., 2009; Li et al., 2015). However, frailty is a dynamic process that may be delayed or even reversed (Xue, 2011; Clegg et al., 2013).

There are two main systems used to define frailty: the Frailty Phenotype (FP) developed by Fried and colleagues (Fried et al., 2001), and the Frailty Index (FI) created by Rockwood and collaborators (Rockwood et al., 1996; Rockwood and Mitnitski, 2007). The FP considers five physical criteria: weight loss, exhaustion, weakness, slow walking speed and low levels of physical activity. Frailty is diagnosed when at least three of these are met; a person meeting one or two criteria is classified as pre-frail, while a non-frail individual meets none of them (Fried et al., 2001). This system frames frailty as a distinct clinical entity, separate from disability. The FI, on the other hand, is computed by counting the number of deficits an individual accumulates over time (Rockwood et al., 1996; Drey et al., 2011). In this way, the presence of frailty, in this case considered a state reflecting dysfunction of physiological systems, can be evaluated on the basis of the individual's functional status, and the presence of diseases and physical and cognitive deficits. The FI, incorporating a wider spectrum of information, including cognitive status, is considered to predict death more precisely

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**Fig. 1.** Frailty is a syndrome linked to several pathophysiological factors, including oxidative stress, mitochondrial dysfunction and cellular senescence. Moreover, dysregulation of inflammatory processes seems to play a central role. Increased serum levels of inflammatory molecules, including CRP (C-reactive protein), IL-6 (interleukin 6) and sTNF-RII (75 kDa soluble TNF $\alpha$  receptor II), have been observed in frail and pre-frail elderly people. Frail subjects have elevated peripheral white blood cell counts. Monocytes from frail people show higher expression of CXCL-10 (CXC chemokine ligand 10), a pro-inflammatory mediator. The T and B cell repertoires appear to be affected. Alterations in the percentages of CD8<sup>+</sup> and CD4<sup>+</sup> T lymphocytes are suggested to play a role in frailty. However, additional studies are needed to better elucidate this role, as some studies reported an increase in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio, whereas others described increased CD8<sup>+</sup> and decreased CD4<sup>+</sup> cells percentages in frail subjects. Moreover, in frail elderly, higher levels of T lymphocytes expressing the CC chemokine receptor 5 (CCR5) has been reported in frail subjects: these cells have a type-1 pro-inflammatory phenotype. A reduced percentage of B lymphocytes and B cell diversity has been observed in the peripheral blood of frail people.

(Rockwood and Mitnitski, 2007; Avila-Funes et al., 2009).

Several pathophysiological factors, including dysregulation of inflammatory processes, oxidative stress, mitochondrial dysfunction and cellular senescence, underlie the frailty syndrome (Viña et al., 2016), and it is also influenced by many other factors, such as socio-demographic characteristics, psychological conditions, nutritional status, lack of physical activity and comorbidities (Dent et al., 2016). However, is not clear what drives frailty and little is known about the risk factors that contribute to the development of the syndrome. Environmental factors and individual genetics both influence the dysfunction of the biological mechanisms associated with frailty, but knowledge about the precise role of genetic factors in frailty is still fragmentary (Viña et al., 2016).

Genetic differences together with environmental factors can contribute to the dysfunction of physiological mechanisms associated with frailty, leading, in turn, to the dysregulation of multiple systems, including the immune system (Ruan et al., 2014). Furthermore, diet has been proposed to be a key element in the development frailty. Low intake of certain micronutrients and protein is associated with a higher risk of developing frailty (Bonney et al., 2015; Lorenzo-López et al., 2017). However, the effect of diet on frailty has only occasionally been explored: Bollwein and co-authors showed a relationship between frailty and the distribution of daily protein intake (Bollwein et al., 2013), while the Mediterranean diet has been linked to a decreased risk of frailty (Kojima et al., 2018).

In this review, we aim to summarize current knowledge on the involvement of the immune system in frailty, and on the influence of genetic factors on frailty risk and/or status, focusing in particular on genes involved in immune system function. We will also analyze the impact of diet on inflammation, in order to highlight not only the importance of genetic factors in frailty, but also the role of lifestyle that

might potentially contribute with aim view to preventing immune system alteration and, thus, frailty.

## 2. The immune system and frailty syndrome

Aging of the immune system leads to a low grade, chronic systemic inflammatory state, dubbed “InflammAging” by Franceschi and colleagues (Franceschi et al., 2000), and other authors have suggested the existence of a relationship between changes in inflammatory molecule levels and diseases and syndromes typical of old age (Frasca and Blomberg, 2016). The “InflammAging” inflammatory phenotype is characterized by elevated inflammatory molecule levels, and associated with increased morbidity and mortality in older adults (Roubenoff et al., 2003; De Martinis et al., 2006). Following its initial definition, the concept of “InflammAging” was further developed. It was suggested that “InflammAging” is a systemic physiological process that may involve one or several organs, leading to an increased risk of age-related chronic diseases and frailty (Cevenini et al., 2010). More recently it has been speculated that InflammAging is a dynamic auto-inflammatory process that can be amplified and propagated to neighbouring and distant cells and organs, thus accelerating and expanding aging processes both locally and systemically (Franceschi et al., 2017). Furthermore, the concept of anti-inflammation, understood as an active phenomenon, has also been introduced. The rising levels of pro-inflammatory molecules in aging stimulate an increase in levels of anti-inflammatory mediators, serving to neutralize the dangerous inflammatory processes. The balance between inflammation and anti-inflammation has been suggested to determine the rate of aging, the onset and severity of age-associated disorders, and the individual’s ability to achieve extreme longevity (Franceschi et al., 2007; Giunta and Sergio, 2008).

Inflammation is considered one of the seven pillars of aging, the others being adaptation to stress, epigenetics, macromolecular damage, metabolism, proteostasis, and stem cells and regeneration (Kennedy et al., 2014). For instance, alterations of the immune system are associated, in older subjects, with loss of muscle mass and strength, reduced levels of mobility, performance and physical activity, and depression, all key features in the definition of frailty (Walston et al., 2006) (Fig. 1).

### 2.1. Inflammatory molecules in frailty

Upregulation of cytokines, specifically interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF) has been related to increased morbidity and mortality in older adults (Cohen et al., 2003; Schlaudecker and Becker, 2014). Inflammation has been associated not only with an enhanced risk of morbidity, but also with functional decline in older adults, and it has been identified as a key element in the frailty syndrome (Kanapuru and Ershler, 2009).

Previous studies highlighted a strong relationship between frailty (as classified by Linda Fried in 2001) and inflammation: elevated serum IL-6, TNF- $\alpha$ , and C-reactive protein (CRP) levels have been related to impaired function and mobility (Ferrucci et al., 1999; Bruunsgaard et al., 2003; Puts et al., 2005). A more recent meta-analysis, including 32 cross-sectional studies and 23,910 older subjects, showed that frailty and pre-frailty were associated with significantly higher serum levels of inflammatory molecules, including CRP and IL-6, compared with the levels recorded in non-frail participants (Soysal et al., 2016) (Fig. 1).

IL-6 is a pleiotropic cytokine that fulfils different biological functions in immune regulation, hematopoiesis, inflammation and oncogenesis. IL-6 is commonly expressed at low levels. However, during infection, trauma, aging and/or other stress states, it can be secreted by endothelial cells, B cells, T cells, macrophages, dendritic cells and tumor cells (Dijsselbloem et al., 2004).

Circulating IL-6 levels have long been known to increase in older adults and in several pathophysiological processes, e.g. atherosclerosis, osteoporosis and sarcopenia, and raised IL-6 levels are associated with functional decline, disability and mortality in older adults (Ershler, 1993; Harris et al., 1999; Reuben et al., 2002; Maggio et al., 2006). Ferrucci and co-authors, in a 3.5-year follow-up in older women, suggested that increased IL-6 levels might predict a significantly higher risk of developing physical disability and reduced muscle strength and motor performance, which are considered key components of the frailty syndrome (Ferrucci et al., 2002). In 2002, Leng and colleagues, in a pilot study, reported an inverse correlation between IL-6 levels and hemoglobin/hematocrit in their frail group, and indicated chronic and low-grade inflammation as potential contributors to the decreased hemoglobin concentrations recorded in these frail subjects (Leng et al., 2002). Furthermore, after stimulation with lipopolysaccharide, peripheral blood mononuclear cells (PBMCs) of frail subjects showed higher IL-6 production compared with values recorded in non-frail individuals (Leng et al., 2004). In addition, in a cohort of elderly women, Leng and co-authors also demonstrated that white blood cell (WBC) count, a well-known cellular marker of systemic inflammation, and IL-6 levels were independently associated with prevalent frailty (Leng et al., 2007). More recently, a study performed in a Chinese elderly population confirmed the association between frailty and high serum IL-6 and adiponectin levels; these levels were also negatively linked to physical function (Ma et al., 2018).

Elevated serum CRP levels have been recognized to be associated with increased vulnerability to mortality in frail older individuals (Giovannini et al., 2011; Puzianowska-Kuźnicka et al., 2016). In a cross-sectional study, Walston et al. found that subjects with CRP levels greater than 5.77 mg/l had an odds ratio (OR) of 3.5 for prevalent frailty (Walston et al., 2006). However, some longitudinal studies did not find an association between higher inflammatory markers and increased risk of frailty, suggesting that additional studies are needed to better elucidate the role of pro-inflammatory molecules in frailty

(Walston et al., 2006). A 2005 study showed that only moderately elevated CRP levels were associated with frailty and no association was found for IL-6 (Puts et al., 2005). The Rancho Bernardo Study, a prospective cohort study with 1353 participants, reported an association between higher levels of inflammatory markers and reduced survival and shorter adult lifespan. Specifically, in men, higher IL-6 and CRP levels were associated with shorter survival time and lifespan independently of lifestyle, comorbidities and other cardiovascular risk factors; in women, CRP was not significantly related to survival time or lifespan (Wassel et al., 2010). Using data from The English Longitudinal Study of Ageing (ELSA), Gale and co-authors showed that higher baseline concentrations of CRP and fibrinogen were each independently linked to an increased risk of frailty in women; in men, no evidence of a relationship between higher concentrations of these inflammatory markers and increased risk of frailty was found (Gale et al., 2013). In addition, elevated CRP levels have been reported to be negatively associated with cognitive functioning and muscle quality in females but not in males (Canon and Crimmins, 2011). Although the above cited studies show an association between serum CRP levels and frailty in older subjects, several others reached different conclusions, failing to find any significant associations (Baylis et al., 2013; Lai et al., 2014). These discrepancies may reflect the difficulties in understanding inflammation, a process that involves multiple inflammatory proteins rather than a single, specific one.

TNF, like IL-1b, IL-6 and CRP, has been reported to show age-related upregulation (Zanni et al., 2003).

The Framingham Heart Study associated increased production of TNF and IL-6 by PBMCs with increased mortality in a cohort of community-dwelling elderly subjects (Roubenoff et al., 2003). Moreover, elevated plasma TNF levels were correlated with mortality in centenarians, independently of the presence of diseases, in particular dementia and cardiovascular disorders. The observation that high TNF levels act as a marker both for these pathologies and for frailty in very elderly people strengthens the possibility that TNF constitutes an important link between inflammation, cardiovascular diseases, dementia and frailty in the very old (Bruunsgaard et al., 2003). These data were further confirmed by Collerton and colleagues in 2012 (Collerton et al., 2012).

A very recent study analyzed lymphocyte subsets and concentrations of pro-inflammatory molecules in 259 older adults classified according to the FP system (Marcos-Pérez et al., 2018). The frail group showed a significant decrease in the percentage of CD19<sup>+</sup> cells (B lymphocytes) and an increase in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio. However, the study showed associations between frailty and the lymphocyte subsets that were of limited strength. On the other hand, the data obtained in relation to the inflammatory mediators lent further support to the involvement of “InflammAging” in the frailty syndrome. This study confirmed the involvement of chronic inflammation in frailty in later life; particularly strong associations were obtained for IL-6 and the 75 kDa soluble TNF- $\alpha$  receptor II (sTNF-RII). IL-6 showed a 70% increase in frail versus non-frail participants, while sTNF-RII showed a 19% increase in pre-frail and twofold increase in frail compared with non-frail subjects. Moreover, sTNF-RII may be a predictor of frailty: at concentrations higher than 3,461.3 pg/ml, frail subjects can be identified with quite high sensitivity and specificity (Marcos-Pérez et al., 2018).

On the contrary, other authors failed to find a significant association between biomarkers and frailty (Tsai et al., 2013), or reported mixed results (some markers were significant, others had no effect) (Lai et al., 2014; Namioka et al., 2017).

A 2009 study showed an association between frailty and upregulation of the expression of CXCL chemokine ligand 10 (CXCL-10), a potent pro-inflammatory mediator in monocytes (Qu et al., 2009). Frail subjects showed higher CXCL-10 expression levels than matched non-frail controls. Moreover, CXCL-10 expression correlated with IL-6 serum levels in frail subjects (Qu et al., 2009).

Taken together, the results of the above studies suggest that

inflammatory mediators IL-6, CRP and TNF might be used as biomarkers and could form the core of a dynamic model of the decreased physical functions and physiological processes contributing to frailty.

## 2.2. White blood cells in frailty

Immune system alterations are widely considered to be associated with the frailty syndrome, and on the basis of this premise, various studies have focused on lymphocyte counts. Some authors demonstrated a relationship between frailty and elevated peripheral WBC counts (Leng et al., 2007, 2009), and Soysal and co-authors confirmed this evidence in a 2016 meta-analysis including 32 cross-sectional studies and 23,910 older individuals (Soysal et al., 2016) (Fig. 1). A recent longitudinal study seeking to identify predictors of adverse health outcomes, such as hospital stays and mortality, analyzed the relationship between frailty and changes in WBC counts in older women (Fernandez-Garrido et al., 2018). The frailty score (measured using the FP system) was inversely associated only with lymphocyte count and not with total WBC or other blood cell subtype counts. Moreover, lower monocyte count and number of hospitalizations were significantly and inversely correlated. According to the authors, the reduced monocyte count recorded during a two-year follow-up was the only value significantly predictive of hospitalization (Fernandez-Garrido et al., 2018). Previous studies further reported that an increased WBC count is an independent predictor of cause of death (Margolis et al., 2005; Tamakoshi et al., 2007). Only a few studies have analyzed the different lymphocyte subpopulations. A 2005 study showed a significantly increased number of CD8<sup>+</sup> T lymphocytes and CD8<sup>+</sup>CD28<sup>-</sup> lymphocytes in frail versus pre-frail and non-frail women (Semba et al., 2005). CD28 is known to be the major costimulatory molecule required for T lymphocyte activation and survival; aging brings a progressive decline in CD4<sup>+</sup> and CD8<sup>+</sup> cells expressing CD28 (Boucher et al., 1998; Teteloshvili et al., 2018). Frail and non-frail subjects had similar total lymphocyte and total T cell count, but levels of CD8<sup>+</sup> and CD4<sup>+</sup> cells were significantly increased and decreased, respectively, in frail compared with non-frail individuals (De Fanis et al., 2008). Frail subjects had higher percentages and higher absolute counts of total CC chemokine receptor 5 (CCR5<sup>+</sup>), CCR5<sup>+</sup>CD8<sup>+</sup> and CCR5<sup>+</sup>CD45RO<sup>-</sup> (naïve) T lymphocytes compared with non-frail individuals (De Fanis et al., 2008), thus suggesting that expansion of specific T cell subsets with a type-1 pro-inflammatory phenotype may play an important role in frailty (Fig. 1).

In addition to the apparent alteration of the T lymphocyte repertoire, B cells, too, may be affected in frailty (Fig. 1). A study based on the Swedish NONA Immune Longitudinal Study showed an age-related decrease in B cell diversity in frail older individuals (Gibson et al., 2009); this was suggested to be a marker of global frailty rather than a finding specifically connected to immune frailty. Moreover, whether the loss of B cell diversity is a cause or a consequence of frailty remains an open question (Gibson et al., 2009). By performing high-throughput sequencing of immunoglobulin genes, Tabibian-Keissar and collaborators showed reduced diversity in peripheral blood and lymph node B cell repertoires from old people, compared with young subjects. Conversely, the diversity found in spleen samples from old subjects was larger than that found in samples from young individuals. These results suggest that age-related immune frailty may stem from altered B cell homeostasis, which leads to narrower memory B cell repertoires, rather than changes in somatic hypermutation (Tabibian-Keissar et al., 2016). These age-related deficits in the immune system may be caused by peripheral homeostatic pressures that limit bone marrow B cell production or migration to the peripheral lymphoid tissues, and may explain the reduced responses to infections and vaccines in the elderly (Ridda et al., 2009; Yao et al., 2011; Marttila et al., 2014).

## 2.3. The role of inflammation in frailty onset

Despite continued efforts to clarify it, the exact pathogenesis of frailty remains to be determined, even though inflammatory status is thought to play a crucial role. Cross-sectional studies, describing higher levels of circulating pro-inflammatory cytokines and acute phase proteins among frail older adults, suggest that systemic inflammation could be a potential mechanism in the pathophysiology of frailty (Singh and Newman, 2011; Gale et al., 2013). However, authors have advanced different interpretations of the association of inflammation with frailty, with some proposing that chronic, low-grade systemic inflammation may determine an enhanced physiological vulnerability in older subjects (Franceschi and Campisi, 2014), and others wondering whether inflammation may simply be a feature of frailty and of other aging phenotypes. Indeed, a 2016 meta-analysis failed to find a predictive role for CRP and/or IL-6 in three longitudinal studies (Soysal et al., 2016).

A recent work, published after the above cited meta-analysis, highlighted the role of midlife inflammation in promoting pathophysiological changes underlying frailty. An increased midlife inflammation composite score (calculated using four biomarkers: WBC count, fibrinogen, von Willebrand factor, and Factor VIII) was associated with a higher risk of frailty 24 years later (OR = 1.39). Moreover, subjects who maintained elevated CRP levels ( $\geq 3$  mg/L) or transitioned to a state of elevated CRP were more likely to subsequently develop frailty, compared with individuals who maintained low CRP levels (Walker et al., 2018). This finding clearly underlines the need to better understand the pathophysiology of frailty, and pinpoint when, exactly, risk factors exert their action. If the above findings are confirmed, efforts to target the drivers of inflammation earlier, rather than seeking to reduce inflammation later in life, could make an important contribution to frailty prevention.

## 3. Genetic factors that lead to frailty: focus on immune genes

The involvement of inflammation in the frailty syndrome is further supported by genetic studies. For example, a study by Dato and colleagues suggested the presence of a genetic influence on frailty variability and indicated that cluster analysis can define specific frailty phenotypes in different populations (Dato et al., 2012). In their study in Danish twin pairs, the genetic component of frailty was estimated to account for 43% of the overall robustness index ratio variability (Dato et al., 2012). Many studies have, in fact, investigated the impact, on frailty, of genetic determinants, highlighting the role of several single-nucleotide polymorphisms (SNPs) in a variety of different genes, including some related to the structure and function of skeletal muscle fibers, neuronal maintenance and signal transduction (Willems et al., 2017), apoptosis, and DNA methylation (Ho et al., 2011). Interestingly, SNPs located in genes important for immune system functioning have also been analyzed.

A candidate gene association study investigating the genetic components of frailty status, assessed in 3160 individuals using the FI system, described a role of inflammation and cholesterol transport in this syndrome (Mekli et al., 2015). The researchers analyzed 620 SNPs in genes involved in steroid hormone and inflammatory pathways. Five SNPs were found to be significantly associated with frailty: rs360722 in IL-18, rs4679868 and rs9852519 in IL-12A, rs1799986 in LRP, and rs6131 in SELP. These results demonstrate the importance of altered inflammatory processes in frailty status: IL-18 codes for a pro-inflammatory cytokine which plays a pivotal role in both local and systemic inflammation (Biet et al., 2002), while IL-12 is one of the major pro-inflammatory cytokines produced by antigen-presenting cells and is important in linking cellular and humoral immune responses (Metzger, 2010). SELP is also involved in inflammation; it codes for P-selectin, a cell adhesion molecule that takes part in the tethering, rolling and weak adhesion of leukocytes (Chen and Geng, 2006). LRP encodes the low



density lipoprotein receptor-related protein 1, a multi-ligand receptor involved in many cellular processes, such as intracellular signaling, lipid homeostasis and clearance of apoptotic cells (Lillis et al., 2008).

The same research group also reported an association between frailty and the SNPs rs1800629 and rs1566729, located, respectively, in the TNF and the protein tyrosine phosphatase receptor type J (PTPRJ) genes (Mekli et al., 2016). PTPRJ is a protein tyrosine phosphatase involved in the signaling for a variety of cellular processes, including cell growth and differentiation (Balavenkatraman et al., 2006). Moreover, it is expressed by all resting leukocytes and has been suggested to contribute to T cell activation (Lin and Weiss, 2003).

A very recent study, focusing on an Ashkenazi Jewish community, analyzed whether variants in the chromosome 9p21-23 locus may increase the risk of developing frailty in older adults (Sathyan et al., 2018). This specific region was chosen because many genome-wide association studies have identified several variants associated with age-related complex disorders, including cardiovascular diseases (Helgadottir et al., 2007; Samani et al., 2007), vascular dementia and Alzheimer's disease (Emanuele et al., 2011). In the aforementioned study by Sathyan and collaborators (Sathyan et al., 2018), the intergenic SNP rs518054, located between LOC105375977 and C9orf146, showed a strong association with FP (OR = 1.635), and this SNP also had functional relevance. Indeed, it is located in the enhancer region of the NFIB gene that plays an important role in lung maturation and brain development and also regulates chromatin accessibility. In this locus, there are other genes important for chromatin remodeling, for instance the non-coding RNA ANRIL. Indeed, the SNP rs2811712, located in the CDKN2B-AS1 gene, also known as ANRIL, that codes for a non-coding RNA, has already been analyzed in the context of physical function in older people and the minor allele was found to be associated with reduced physical impairment (Melzer et al., 2007).

All the above-mentioned candidate gene association studies obtained results that lose their significance after Bonferroni correction, but when the SNPs with the most significant associations were re-tested by ordered logistic regression using frailty status as the dependent variable, the association was confirmed. One hypothesis that could explain these conflicting results, namely the possibility that many genes, each with a small effect size and acting over a long period of time, together with environmental factors, may influence the development of frailty, brings us back to the fact that frailty is a complex syndrome. This hypothesis, which has never been demonstrated to date, therefore clearly needs to be investigated in the future. One way of doing this might be to use a polygenic risk score, which considers the contribution of the many genetic variants associated with the frailty syndrome (Sugrue and Desikan, 2019). Almeida and colleagues analyzed the CRP1846 G > A polymorphism, located in the CRP gene; they found, in older Australian men homozygous for the minor allele, that the odds for FP was 2.5 times greater than in those with the major allele (Almeida et al., 2012).

Variable number of tandem repeats (VNTR) polymorphisms in the IL-4 and IL-1RN genes have also been associated with FP: the A2 allele of the IL-1RN VNTR polymorphism as well as the combined IL-4low-IL-1Rahigh genotype are significantly associated with frailty in Mexican older adults (Pérez-Suárez et al., 2016). Ferrucci and Fabbri, in their recent review, described genetic variants and non-coding, single-stranded RNAs that influence blood levels of inflammatory mediators (Ferrucci and Fabbri, 2018). Analysis of the genetic risk factors for frailty is still only in its very early days, and future studies seeking to establish the genetics of frailty may provide useful information for clarifying the altered processes underlying this syndrome (Saedi et al., 2019). New genetic insights opening up the way for personalized preventive strategies could delay the occurrence of frailty and other related comorbidities as well as promote healthy aging.

#### 4. Diet, inflammation and frailty syndrome

As already mentioned, aging and frailty are associated with a significant rise in serum levels of pro-inflammatory markers (Soysal et al., 2016), and some studies have suggested that nutrients might be able to modulate systemic inflammation, impacting on frailty onset. Different associations between nutritional status, nutrient intake (mainly in terms of calories and protein) and frailty development are reported in the literature (Morley et al., 2013; Hernández Morante et al., 2019).

A recent study investigated whether a higher dietary inflammatory index (DII) is associated with an increased incidence of frailty (Shivappa et al., 2018). The DII is a literature-derived tool, used to assess the inflammatory potential of an individual's diet (Shivappa et al., 2014), and a close relationship between this index and higher plasma concentrations of inflammatory markers has been demonstrated (Tabung et al., 2015). The study by Shivappa and colleagues (Shivappa et al., 2018) showed that higher DII values were associated with higher incidence of frailty: subjects with a high DII score had a 37% higher risk of frailty. In particular, this association was significant in men, suggesting, also in this case, important sex differences. Some studies have shown differential effect in predicting chronic disease outcomes between women and men: the prognosis for females suffering from inflammatory conditions has been shown to be better than the prognosis for males, even though levels of inflammatory markers are usually higher in women (Casimir and Duchateau, 2011). The linear association between DII and serum markers of inflammation suggests that a healthier diet might be a means of modulating inflammation, and thus helping to prevent frailty (Fig. 2).

In this regard, a cross-sectional and longitudinal analysis in the population-based Rotterdam Study looked for associations between dietary patterns and general state of health, measured using the FI system (de Haas et al., 2017). Adherence to the national dietary guidelines was found to be associated with lower frailty at baseline, and high adherence was associated with lower frailty scores over time.

##### 4.1. Polyunsaturated fatty acids (PUFAS)

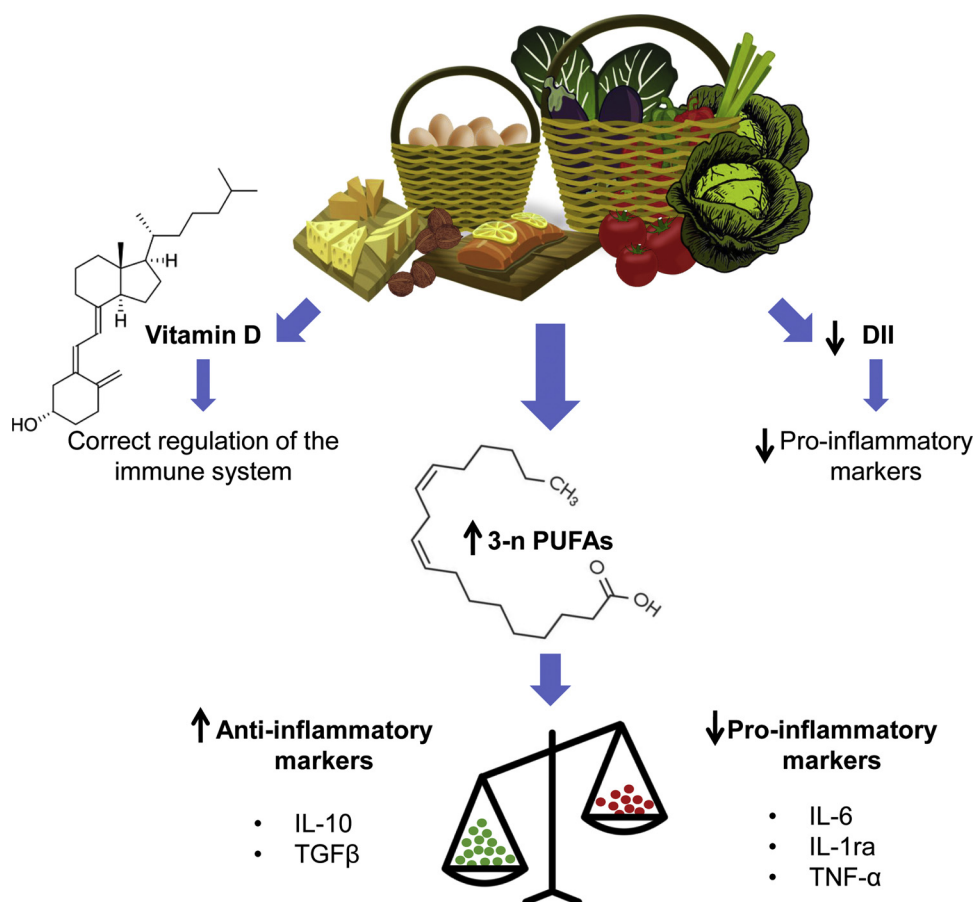
Dietary components that can exert their influence through the inflammatory state are n-3 polyunsaturated fatty acids (PUFAs).

##### 4.1.1. PUFAs and inflammation

It has long been known that n-3 PUFAs can decrease leukocyte chemotaxis and the expression of adhesion molecules, and thus reduce leukocyte-endothelial interactions, the production of eicosanoids, such as prostaglandins and leukotrienes, and the production of inflammatory cytokines (Sperling et al., 1993; Zhao et al., 2004; Rees et al., 2006; Yates et al., 2011). The possible association between n-3 PUFAs, considering both dietary intake and serum levels, and circulating inflammatory molecules has also been investigated in some studies.

**Dietary intake:** in healthy adults, higher n-3 PUFA intake inversely correlates with circulating levels of CRP and IL-6 (He et al., 2009). Important data from the Rotterdam Study showed that, after adjustment for possible confounding factors, higher intake of total PUFAs was associated with lower CRP levels. Similarly, intake of n-6 PUFAs was inversely related with CRP. No consistent trends were observed regarding n-3 PUFAs or n-3:n-6 PUFA ratio and CRP (Muka et al., 2015). A 15-year large prospective cohort study showed that high baseline consumption of n-3 PUFAs was associated with a reduced risk of death from inflammatory diseases in older women (Gopinath et al., 2011).

**Serum levels:** data from the Kuopio Ischaemic Heart Disease Risk Factor Study showed statistically significant inverse associations between total serum long-chain n-3 PUFA concentrations and CRP levels. The study also showed increased intake of fish in the higher serum total n-3 PUFA quartile, but this was not further analyzed in the article (Reinders et al., 2012). Conversely, high serum n-6 PUFA levels may promote inflammation, because the end-product of n-6 PUFA



**Fig. 2.** A good diet may be associated with a lower frailty risk and lower frailty scores over time; this relationship may be related to the impact of diet on inflammation. n-3 polyunsaturated fatty acids (PUFAs) and vitamin D are examples of nutrients involved in the inflammatory state. Serum n-3 PUFA levels are inversely associated with pro-inflammatory markers and positively associated with anti-inflammatory markers in the frail elderly population, while vitamin D has been associated with an increase in anti-inflammatory cytokines and a decrease in pro-inflammatory markers. Moreover, it is well established that this vitamin is fundamental for the correct regulation of both the innate and the adaptive immune system. In general, a higher dietary inflammatory index (DII) is associated with increased levels of inflammatory serum markers and a higher incidence of frailty.

metabolism, arachidonic acid, is a precursor of pro-inflammatory eicosanoids (Innes and Calder, 2018). However, a recent study on healthy men aged 42–60 years showed that serum total n-6 PUFA was weakly associated with lower CRP levels, with a stronger association found only for linoleic acid (Virtanen et al., 2018; Virtanen et al., 2018). As regards the elderly population, in a cohort from the INCHIANTI study, total serum n-3 PUFA levels were inversely associated with pro-inflammatory markers (IL-6, IL-1ra, TNF-α) and positively associated with anti-inflammatory markers (IL-10, TGFβ) (Ferrucci et al., 2006) (Fig. 2).

#### 4.1.2. PUFAs and frailty

Data from cross-sectional studies that tested the associations between PUFAs and only inflammatory or anti-inflammatory biomarkers present major limitations. Ultimately, the association between dietary n-3 and n-6 PUFAs and inflammation remains unclear, despite the contribution of well-designed studies such as the Rotterdam Study (Chowdhury and Steur, 2015). In older frail subjects, León-Muñoz et al. described a positive association between higher omega-3 intake and a lower risk of frailty development. Through a two-year longitudinal study in a large cohort, the authors described both the protective role of these fatty acids and the detrimental effect of a Westernized diet (León-Muñoz et al., 2015). Instead, in a survey from Taiwan (Lo et al., 2017), frailty was inversely associated with dietary intake of food rich in omega-3 fatty acids.

Strike et al., in a 2016 study, employed omega-3 supplementation to reduce frailty symptoms. The participants received a daily multi-component supplement composed of docosahexaenoic acid, eicosapentaenoic acid and other nutrients (phosphatidylserine, d-α tocopherol, folic acid, and vitamin B12) or a placebo. After a six-month intervention, the authors showed that supplementation improved mobility in frail females, underlining the positive effect of these fatty acids;

however, it cannot be ruled out that the other nutrients also played an important role in the observed effect (Strike et al., 2016). Inconsistent associations were found between n-3 PUFA dietary intakes and muscle function in two cross-sectional studies (Robinson et al., 2008; Rousseau et al., 2009).

A further study found baseline total plasma n-3 PUFAs (and DHA) to be associated with incidence of self-reported mobility disability after five years only in healthy older women (OR = 0.48, 95% CI: 0.25–0.93), suggesting that PUFAs may play a sex-specific biological role (Reinders et al., 2015).

#### 4.2. Vitamin D

In the elderly, 25-hydroxyvitamin D [25(OH)D] levels decline with increasing age (van Schoor and Lips, 2011; Gallagher, 2013; de Jongh et al., 2017).

The causes of this decline may include not only a reduction in nutritional intake, but also insufficient exposure to sunlight, chronic diseases, alterations in body composition resulting in a relative increase in fat mass, physical and cognitive disability, and polypharmacy (Nakamura et al., 2015).

##### 4.2.1. Vitamin D and inflammation

In addition to its well-known functions in the regulation of calcium homeostasis and bone mineralization, vitamin D is fundamental for the correct regulation of the immune system, since it controls both innate and adaptive immune responses (Wei and Christakos, 2015).

In previous studies, 25(OH)D has indeed been analyzed in relation to inflammatory markers. A 2012 study, in a cohort of asymptomatic adults, showed a statistically significant inverse relationship between 25(OH)D and CRP when 25(OH)D levels were lower than 21 ng/ml; above this threshold, 25(OH)D levels were associated with higher

serum CRP levels (Amer and Qayyum, 2012). A 2014 study detected the same U-shaped association between 25(OH)D and CRP: a linear decrease in CRP levels up to 25(OH)D concentrations of 21–25 ng/ml was detected, while an increase in CRP levels was reported in the presence of 25(OH)D concentrations higher than 21–25 ng/ml (Mellenthin et al., 2014), confirming previous data (Puts et al., 2005). However, not all the studies confirmed this association between vitamin D and CRP levels (Shea et al., 2008).

Few studies have been performed in elderly cohorts. De Vita et al., analyzing the role of vitamin D in older people (> 65 years of age) drawn from the InCHIANTI study, found that serum 25(OH)D levels were inversely associated with serum IL-6 levels and positively associated with serum IL-6 soluble receptor (sIL-6 r) levels (De Vita et al., 2014). Plasma levels of inflammatory molecules were also analyzed, but were not found to be associated with serum 25(OH)D levels, supporting the centrality of the IL-6 system in “InflammAging”. A similar study in an Irish community-dwelling sample (> 60 years of age) showed significantly higher concentrations of IL-6 and CRP, and higher IL-6/IL-10 and CRP/IL-10 ratios in individuals with deficient (< 25 nmol/L) serum 25(OH)D levels, compared with subjects showing sufficient (> 75 nmol/L) vitamin D levels (Laird et al., 2014). Together, these findings suggest that an adequate vitamin D status may be required for optimal immune system function, particularly in the elderly population (Fig. 2).

Consideration should be given to the hypothesis that the supposed anti-inflammatory activity of one nutrient may be influenced by another nutrients. For example, Itariu and colleagues found that the inverse association between vitamin D deficiency and systemic inflammation is overcome by treatment with n-3 PUFA supplements in severely obese adults, although vitamin D status remains unaffected (Itariu et al., 2013).

#### 4.2.2. Vitamin D and frailty

**Serum:** Several studies have shown reduced serum 25(OH)D levels to be associated with frailty (Wilhelm-Leen et al., 2010; Ensrud et al., 2011; Smit et al., 2012; Tajar et al., 2013; Zhou et al., 2016; Bruyère et al., 2017).

A systematic review and dose-response meta-analysis found a 25 nmol/L increment in serum 25(OH)D concentration to result in pooled risk estimates of 0.88 (95% CI = 0.82–0.95, I<sup>2</sup> = 86.8%) and 0.89 (95% CI = 0.85–0.94, I<sup>2</sup> = 0.0%) in, respectively, the six cross-sectional studies and four prospective cohort studies considered in the analysis (Ju et al., 2018).

The association between 25(OH)D and frailty has been shown to be only weakly significant in older women (Ensrud et al., 2010), confirming previous data from the InCHIANTI study (Shardell et al., 2009).

**Dietary:** Supporting the role of vitamin D in frailty, a recent study, analyzing the relationship between various nutritional parameters and frailty, found only low serum vitamin D levels to be associated with higher levels of frailty and a higher mortality risk across the frailty (Jayanama et al., 2018). Several observational studies have highlighted the importance of diet, particularly adequate intake of vitamin D-related nutrients, for the maintenance of good health in old age (Waern et al., 2015; Brouwer-Brolsma et al., 2016; Fernández-Barrés et al., 2016). In a cross-sectional analysis of the InCHIANTI study, Bartali and colleagues found that low intake of certain micronutrients (vitamins D, E and C, and folate) was significantly associated with frailty, independently of energy intake (Bartali et al., 2006). The authors suggested that evaluation of the quality of the individual's diet, as reflected in the intake of specific nutrients, should be included in frailty screening, diagnostic and treatment processes, as nutrition is a factor that significantly affects the health of older adults.

However, the exact role of vitamin D intake is still under investigation following the inconclusive results of an intervention study exploring the role of dietary supplementation in reducing inflammation (Ticinesi et al., 2016).

## 5. Conclusions

With study of the frailty syndrome still in its infancy, frailty analysis remains a major challenge. It is a challenge that needs to be overcome in order to shed light on the multiple mechanisms involved in the pathogenesis of this syndrome and the factors that contribute to its onset and/or status. Better knowledge of these aspects, allowing early diagnosis of frailty, can only improve the care of affected elderly people and is pivotal to efforts to prevent disability and adverse outcomes.

Although several mechanisms contribute to frailty, immune system alteration seems to play a central role: this syndrome is characterized by increased levels of pro-inflammatory markers and the resulting pro-inflammatory status can have negative effects on various organs. Future studies should aim to better clarify the immune system alteration in frailty, and seek to establish exactly when the inflammation appears.

It is also important to identify the possible risk factors for frailty, in order to understand whether any of them are modifiable and might therefore be targeted in efforts to prevent or at least delay the onset of the syndrome. Moreover, this type of study is essential to shed light on the altered processes that contribute to frailty. In this regard, the involvement of the immune system in frailty may be supported by genetic studies. To date, however, many studies in this field have given conflicting results, probably because the genetic contribution to frailty must be considered to be made by combinations of many genes, each with a small effect size and long-term action. Clearly, then, future analysis of genetic variants in the context of frailty must be based on the adoption of different approaches, such as the use of polygenic risk scores that, also taking into consideration environmental variables and lifestyle, such as diet, can provide personalized estimates of the risk of developing the syndrome.

Although current data are not conclusive, they nevertheless show that better understanding of the immune system alterations present in frailty, and of the effects of diet on the inflammatory status, would be valuable in order to plan specific diets. Dietary approach could be a feasible way to reduce “InflammAging” and to prevent and/or improve frailty management, leading to better care of the elderly.

## Funding

This work was supported by the Cariplo Foundation (Italy) - Association between frailty trajectories and biological markers of aging [FrailBioTrack 2017-0557].

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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