Review Article

Evolution of Biomarkers for Frailty over the Last Decade : A Literature Review

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Aging is a Complex Physiological Process which involves remodeling of cellular components and processes, decline of functional reserve, adaptation of the body to these changes and finally senescence. An important concept in ageing physiology is Frailty which is a physiological, age-related condition characterized by a decrease in functional reserve across multiple organ systems. The phenotype of physical frailty has five components, that is, slowness, weakness, low physical activity level, unintentional weight loss and exhaustion It is crucial to mention "Inflammaging", a term that signifies the development of an age-related inflammation in the body wherein there is an increase in acute phase reactants and pro-inflammatory cytokines contributing independently to the pathogenesis of frailty. Inflammaging has been proposed to be associated with frailty and many studies suggest the existence of a relationship between age-related Frailty and inflammatory Biomarkers. The aim of this review is to study the evolution of our understanding of the role of inflammatory markers in development of Frailty in the older population.

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Frailty has been accurately defined by the International Association of Gerontology and Geriatrics (IAGG) Frailty Consensus as a reduced strength and physiologic malfunctioning that increases an individual's susceptibility to increased dependency, vulnerability and death[1]. Frailty develops when there is multi-system decline or failure. The number of systems involved contribute to the impact of frailty[2]. The implication of a person being labeled frail is that the severity of illness is likely to be more and the chances of recovery less than in pre-frail or individuals without Frailty.

Our understanding of Frailty has drastically improved with time. Aging, illness, poverty, immune status, endocrine factors are all believed to cause and affect frailty in an individual[2]. Inflammation is one of the pathways and an established causative factor for Frailty. Thus, Frailty is multifactorial and a single pathophysiological process cannot be ascribed to the genesis of Frailty. It is a result of cumulative cellular damage gathered over time with factors an interactive role[3]. In a robust individual, there are homeostatic buffers which prevent decline of function even after any insult to the body and retard progression towards debility. It is only when a limit is surpassed that frailty develops in an individual. This threshold is strongly determined by the age of an individual and decreases as one ages, because the homeostatic reserve of the body tends to with time[4].

Editor's Comment :

- Functional decline rather than the chronological age of the individual is more consistent with the concept of aging.
- Inflammaging plays a pivotal role in induction and progression of frailty in elderly.
- Frailty incident to increasing age is associated with higher levels of inflammatory markers.
- Early detection of frailty by use of biomarkers may prove useful in reversing the condition.

Frailty is thought to be reversible, so early detection may prove useful in returning the subject to state. Frailty can be measured using different scales. In 2001, Fried and colleagues designed a scale which used clinical parameters to assess and grade frailty. The five physical features of the Fried, *et al* criterion are: unintentional weight loss, exhaustion, weakness, slow walking speed and low physical activity[5]. Individuals can be categorized into being in one of the 3 states; robust or non frail, frail and dependent. The stages of Frailty, based on the Fried phenotype criterion are; A person is not frail if the score is 0; 1 or 2 score implies increased risk of becoming frail and are hence termed as pre-frail; A score of 3-5 suggests frailty. This was

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followed by another breakthrough when Rockwood and Mitnitski proposed another Frailty criterion which includes Psychosocial aspect of Frailty along with the physical aspect[6]. Both these scales are, today, the most commonly used scales for the measurement of frailty.

The current review includes studies which used either of these criteria for measuring frailty. Pro-inflammatory cytokines promote protein degradation and affect important metabolic pathways and thereby influence Frailty[7]. The review focuses on the evolution of using inflammation as the key risk factor for induction and progression.

Materials and Methods

Search strategy :

Studies were sought through an extensive bibliographic search making use of these databases: PubMed, Embase, CINAHL, Medline updated to January, 2021. The search terms used were (frailty) AND (inflammatory biomarkers) OR (cytokines) AND (elderly).

Eligibility criteria for selection of studies :

The studies included in this systematic review are cross-sectional, cohort, prospective studies or randomized clinical trials. Only the trials conducted on populations aged more than 65 years were selected. Frailty criterion used in studies was Fried Frailty phenotype criterion[5]. Another criterion used was Rockwood Frailty Index[6]. When we found papers published by the same author/s, we included only the complete ones and the most recent ones in the study. Only the trials and studies conducted on humans were included in the review. Important outcomes pertaining to the title of the review, that is, any significant rise in inflammatory markers, general inflammatory profile, levels of endocrine immune markers, racial and gender variations and topography were evaluated and relevant findings were taken into account. Only the studies and trials in English were considered. Studies having confounding factors such as other comorbidities were excluded. We tried to identify publications describing the relation of inflammatory Biomarkers as an index of Frailty in the elderly. 11 were selected for the systematic review out of the 53 relevant articles found.

Study characteristics :

The studies done were from multiple Countries in different Continents and thus provided a universal view-point. The studies were during different time periods and therefore, the evolution of our understanding of the concept of Frailty and its association with inflammation. We have summarized the relevant details of each study in a tabulated form (Table 1).

Data collection :

The information that was extracted from various studies included: Author, Year of publication, Country, Type of study, Sample size, Frailty criteria, Inflammatory biomarkers and Conclusion. The studies are presented in Table 1 along with their findings.

Discussion

This review shows that from 2013 to 2020, numerous markers were linked frailty. Conclusions of the studies varied across the studies, but it was uniformly observed that a multitude of inflammatory markers were associated with increased incidence of Frailty among elderly. In the beginning of the decade, a prospective study by Gale *et al* indicated that C-reactive protein and Fibrinogen levels were significantly associated with Frailty and this association was more pronounced in women[8]. Endocrine markers also emerged as causal factors for frailty. Baylis *et al* concluded that levels of White cell count, neutrophils, monocytes, ESR and dehydroepiandrosterone augmented the frailty status of an individual[18]. The study by Lai *et al* focussed on institutionalized men which were an unexplored subset of the elderly population at that time[10]. Their contribution revealed a strong correlation between IL-6 and incident Frailty in the population of older men above 65 years of age. However, contrary to the previous studies, no significant association was observed with CRP or TNF-alpha. The plausible explanation lied in the exclusion of persons with co-morbidities in this study; as authors argued that co-morbidities might have led to increased CRP and also Frailty in previous studies[10].

The year 2016 marked the beginning of a change in how we looked at “Frailty and Inflammation”. Lu *et al*, through their cohort of 76 participants found a direct association of Frailty with soluble glycoprotein 130 (sgp130) and IL-6 receptors[12]. As the levels of these markers are more stable these are considered more reliable indicators of Frailty. This study is consistent with the notion that changes in cytokine migration and chemokine response alter Frailty in elderly[12].

One of the significant findings in the year 2017 was a weak association of frailty with IL-8 levels, seen in the study done by Hsu *et al*[13]. Later in 2017, study by Langmann, *et al* on long-term-care female residents threw light on levels of inflammatory markers at 12 and 24 months following start of the trial and any subsequent development or progression of Frailty[14]. Their study shows functional decline with time in

Table 1 — *Studies showing association of frailty with inflammatory markers*

Authors	Year of publication/ Country	Type of study/ Sample size(n)	Frailty criteria	Inflammatory biomarkers associated
Gale CR *et al*[8]	2013/ U.K.	Prospective study/ 2146	Fried frailty phenotype criteria	For each SD unit increase in CRP and fibrinogen levels, an odds ratio greater than 1 was observed. implies the association is positive and significant.
Baylis D *et al*[9]	2013/ U.K.	Cohort study/ 254	Fried frailty phenotype criteria	A plethora of inflammatory markers consisting of WCC, T4, ESR, lymphocytes, neutrophils, monocytes and DHEAS was shown to be associated with increased risk of frailty.
Lai HY *et al*[10]	2014/ Taiwan	Prospective study / 386	Fried frailty phenotype criteria	Trends towards a high IL-6 with frail status was demonstrated. Association of TNF- alpha was seen both in adjusted and unadjusted for age trails.
Zhu Y *et al*[11]	2016/ China	Cross – sectional study/1478	Modified frailty phenotype criteria	Approximately a quarter of participants were categorized into high CRP group, one-eighth in low CRP and more than half in intermediate CRP group. From high to low CRP, hospital admissions increased linearly.
Lu Y *et al*[12]	2016/ Singapore	Cohort study/ 76	Rockwood frailty index and Fried frailty phenotype criteria	Positive associations of the Frailty Index with levels of soluble glycoprotein 130, I-309, B-cell attracting chemokine 1, RANTES, and leptin were observed. MCP-1 showed an inverse relation with the Frailty Index.
Hsu B *et al*[13]	2017/ Australia	Cross sectional study / 1705	Fried frailty phenotype, Rockwood frailty phenotype criteria	For each SD increase in levels of IL-6, men were at more risk of developing frailty when compared to robust individuals. For each SD increase in IL-8, males have an odds ratio of 1.16 more likely to be pre- frail and 1.28 for being frail. IL-8 continued to be associated with frailty when adjusted for age.
Langman GA *et al*[14]	2017/ U.S.A	Double blind RCT / 178	Modified Fried frailty index	Frail participants had higher levels of CRP, TNF- alpha- R1, TNF-alpha R2, IL-6, IL-6-R.
Marcos Perez D *et al*[7]	2018/ Spain	Cross sectional study/ 259	Fried frailty phenotype criteria	Levels of TNF-R2, TNF-alpha, IL-6 and CRP were seen to be high in frail participants.CD19+ cell concentration significantly decreased in the frail group.
Semmarath W *et al*[15]	2019/ Thailand	Cross sectional study / 526	Fried frailty phenotype criteria	Both inflammatory markers (CRP and IL-6) were increased in the frail group of patients, irrespective of the sex of the patient. IL-6 was reported to be more associated with frailty risk.
Marzetti E *et al*[16]	2019/ Italy	Cross sectional, case control/1200	SPPB	Physical frailty and sarcopeniacytokinome includes higher levels of CRP and lower concentration of MPO, IL-8, MCP -1, IF alpha, FGF-beta, TNF -alpha, MIP-1 beta, IL-17.
Palmer J *et al*[17]	2019/ U.S.A	Prospective analysis/100	Trauma specific frailty index	Frailty associated pro-inflammatory biomarkers observed were IL-1beta, IL-6 -2 alpha, TNF- alpha and endocrine biomarkers IGF-1 and GH.

SD: Standard deviation; CRP: C-reactive protein; WCC: White cell count; ESR: Erythrocyte sedimentation rate; DHEAS: Dehydroepiandrosterone; IL-6: Interleukin-6; TNF-alpha: Tumor necrosis factor- alpha; RANTES: Regulated upon activation normal T cell expressed and secreted; MCP-1: Monocytes chemoattractant protein 1; RCT: Randomized clinical trial; CD: Cluster of differentiation; SPPB: Short Physical Performance Battery-score; MPO: Myeloperoxidase; FGF-beta: Fibroblast growth factor-beta; MIP-1:Macrophage inflammatory protein; IGF-1: Insulin like growth factor-1; GH: Growth hormone

subjects having higher baseline levels of IL-6, CRP and TNF-alpha irrespective of baseline comorbidities.14 In the study by Marcos Perez D, *et al* explored immune markers in elderly and a slight decrease in CD19+ cells was observed in the frail group. An increase in the CD4+/CD8+ ratio in frail subjects was also significantly prominent[7]. The study also suggested that sTNF-RII levels may have clinical relevance in screening for Frailty since TNF-alpha gets shed and cleaved into sTNF-RI and sTNF-RII which can be measured more accurately in blood to look for significant TNF-alpha activities[7]. Marzetti *et al* found an inverse relationship between Frailty and levels of myeloperoxidase, platelet derived growth factor BB isoform (PDGF-BB), IL-8 and monocytes chemoattractant protein 1.16. Their study pivoted around the cytokinome in frail patients and hence entails the discussion regarding muscle atrophy and degeneration in a frail individual. Interferon gamma induced protein 10(IP-10) is implicated as a marker of muscle atrophy and hence leading to physical Frailty and Sarcopenia according to their study. P-selectin levels the walking speed and high levels were found in older frail women[16]. The studies support an association between age-related chronic inflammation and development of Frailty. These findings can be put into use for early identification of frailty by using certain Biomarkers as diagnostic tools and preventing progression of Frailty into severe functional disability. The individual contribution of the various Biomarkers is discussed at length below.

CRP and Frailty :

C-reactive Protein (CRP) is an inflammatory Biomarker whose production is upregulated in inflammatory and infectious conditions by the Liver and becomes detectable in the blood after 6 hours and peaks within 36-48 hours[19]. With increasing age, a chronic, low grade inflammation sets in, also termed as inflammaging, leading to decrease response to antigens. Studies by Kenny RA *et al* in the 1980s, first, described elevation of CRP in acute infections in the elderly population[20]. CRP can also be used as a major prognostic marker in most of the pathological states. CRP levels are directly proportional to the intensity of inflammation and, thus, higher CRP levels indicate a more severe disease and prognosis. CRP concentration increases during aging and may contribute to the pathogenesis of age-related Cardiovascular events and Diabetes[21]. It was seen that this association was far greater in women than in men. One potential explanation is that women have more adipose tissue which results in increased CRP production by the Hepatocytes in women. This also suggests that obesity triggers inflammation and has more influence Frailty in women[8]. Raised CRP levels ve detrimental effects on multiple organ-systems Sarcopenia, Anaemia, Glucose intolerance and Blood dyscrasias. All of this ultimately culminates into adverse health outcomes including falls, disabilities, dependencies and death.

IL-6 and Frailty :

Interleukin-6 is an inflammatory marker produced by many different cells. Levels of IL-6 increase with age and are also associated with many of the age related diseases. Increased IL-6 concentrations have been shown to be associated with increased risk of developing disability and reduced muscle strength[10]. Grip strength is also a component of Frailty criterion which is more specifically associated with increased levels of inflammatory cytokine, IL-6. This is consistent with the other studies where increased levels of IL-6 were found to lead to Frailty because they caused decrease in muscle mass and muscle strength that resulted in reduced grip strength; a specific weakness phenotype[22]. Some studies also found that IL-6 through its inhibiting effect on Erythropoietin also caused anemia. Cellular senescence is a major causal factor of Frailty which develops with increasing age. Senescence inducers cause mutations, DNA damage, reactive metabolites and proteotoxic stress which, by activating tumor suppressor genes, initiates the senescence response. Chronic inflammation and tissue dysfunction are the result of the infiltration and phenotypic changes of immune cells by the senescent cells[13].

TNF- alpha and Frailty :

TNF-alpha is a cytokine produced by macrophages and monocytes. It upregulates the production of IL-6 and mediates immune mechanisms both in normal as well as pathological states. Increased levels of TNF-alpha in frail patients have shown to increase the rate of skeletal muscle cells apoptosis. Serum levels of TNF-alpha were reported to be directly proportional to IL6 and CRP levels in geriatric population that suggests activation of the entire inflammatory cascade. Some studies also showed lower levels of TNF-alpha. One reason for the above statement could be that TNF-alpha induces IL-6 production but appear in circulation. Moreover, being a less stable marker; TNF-alpha is a relatively less reliable indicator of chronic inflammation[10].

Fibrinogen and Frailty :

Fibrinogen, a glycoprotein, consists of 6 subunits: two alpha, beta and gamma respectively. It is

synthesized in the Liver and is involved in the coagulation cascade. Levels of Fibrinogen have been found to influence the Frailty status of an individual[23]. Conversely, physiological changes subsequent to aging also affect the coagulation profile through their actions on fibrinogen[8]. Higher baseline concentration of fibrinogen was found to be associated with higher incidence of frailty among women[23]. The effect accentuated when the baseline concentration of CRP was also higher[23].

IL-8 and Frailty :

IL-8 is a Cytokine produced by Phagocytes and Mesenchymal cells under inflammatory stimulus and activates Neutrophils producing a plethora of effects. Activated Neutrophils undergo chemotaxis, exocytosis and respiratory burst. IL-8 is one of the meditators responsible for Neutrophil migration and accumulation at the site of inflammation[8]. IL-8 was found to be weakly associated with Frailty. For one SD increase in IL-8, there was increased odds-ratio for developing frailty in men. IL-8 showed positive association with frailty even when adjusted for age.

Other markers and Frailty :

Baseline levels of other markers; Neutrophils, WCC, Monocytes, Lymphocytes, ESR, freeT4, DHEAS, ratio of cortisol to DHEAS is found to be relevant in predicting Frailty as these markers, directly or indirectly, lead to development of the state of inflammaging[13]. The concept of inflammaging states that aging is associated with an increase in the inflammatory Cytokines and hence the Leukocytes and acute phase reactants.

Clinical relevance :

With the help of this literature review, we have reviewed the relationship increasing age marked increase in the levels of the inflammatory markers. Most of the evidence suggests that the levels of CRP, IL-6 and to some extent, TNF-alpha are consistent with the concept of inflammaging and that these markers can be used to assess the Frailty status of individuals, especially the elderly[24]. Measurement of Serum CRP levels is being considered as an appropriate screening test but its use is limited to the detection of pathological or various inflammatory states. These studies support the use of CRP and IL-6 as diagnostic tools for frailty in elderly which could help predict the susceptibility of an individual to disease and disability. To study the role of immune markers in the oldest old, more studies are required. There are some studies that provide contradictory evidence to the findings of the above studies and there are some that incriminate other inflammatory markers in Frailty. We still need confirmatory evidence so as to use these markers in real world settings to diagnose and delay or reverse Frailty wherever feasible. This is clinically very relevant as population over 60 years is expected to be more than 22% of the world population by 2050 and the clinician is bound to see significant multi-morbidity in the years to come[25].

Conclusion

Frailty incident to increasing age is associated with higher levels of inflammatory markers. After reviewing the pertinent literature, CRP and IL-6 can be strongly associated with the development of Frailty in the elderly. The elevated levels of CRP and IL-6 were consistent both in community and hospital settings and across geographical regions. However, the effects of other immune markers cannot be ignored. TNF-alpha and IL-8 have also shown to be associated with increased Frailty, besides Fibrinogen. Majority of the studies showed a generalized increase in the inflammatory Biomarkers favoring the concept of inflammaging.

In conclusion, the pathogenesis of Frailty is poorly understood; however, this review examines the evolutionary concepts and current evidence to a growing link between inflammation and Frailty. Chronic immune dysregulation or imbalance associated with inflammation is a better putative justification for the biologic basis of Frailty and functional decline rather than the chronological age of the individual. How this inflammaging can be reversed by modulating the inflammatory cytokine milieu is a matter of further research.

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