Ageing and immunity: unraveling the association between immunosenescence and frailty

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Biomarkers of the ageing immune system and their association with frailty – a systematic review

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ABSTRACT

Introduction

Ageing is associated with several physiological changes, including changes in the immune system. Age-related changes in the innate and adaptive immune system are thought to contribute to frailty. Understanding the immunological determinants of frailty could help to develop and deliver more effective care to older people. This systematic review aims to study the association between biomarkers of the ageing immune system and frailty.

Methods

The search strategy was performed in PubMed and Embase, using the keywords “immunosenescence”, “inflammation”, “inflammaging” and “frailty”. We included studies that investigated the association of biomarkers of the ageing immune system and frailty cross-sectionally in older adults, without an active disease that affects immune parameters. Three independent researchers selected the studies and performed data extraction. Study quality was assessed using the Newcastle-Ottawa scale adapted for cross-sectional studies.

Results

A total of 44 studies, with a median number of 184 participants, were included. Study quality was good in 16 (36%), moderate in 25 (57%) and poor in 3 (7%) of studies. The most frequently studied inflammaging biomarkers were IL-6, CRP and TNF-α. Associations with frailty were observed for increased levels of (i) IL-6 in 12 of 24 studies, (ii) CRP in 7 of 19 studies, and (iii) TNF-α in 4 of 13 studies. In none of the other studies were associations observed of frailty with these biomarkers. Different types of T-lymphocyte subpopulations were studied, but each subset was studied only once, and the study sample sizes were low.

Conclusion

Our review of 44 studies on the relation between immune biomarkers and frailty identified IL-6 and CRP as the biomarkers that were most consistently associated with frailty. T-lymphocyte subpopulations were investigated, but too infrequently to draw strong conclusions yet, although initial results are promising. Additional studies are required in order to further validate these immune biomarkers in larger cohorts. Furthermore, prospective studies in more uniform settings and larger cohorts are needed to further investigate the association with immune candidate biomarkers for which potential associations with ageing and frailty were previously observed, before these can be used in clinical practice to help assess frailty and improve the care treatments of older patients.

INTRODUCTION

The global population is ageing rapidly. Ageing is associated with several physiological changes, including changes in the immune system. Age-related changes in the innate and adaptive immune system are thought to contribute to frailty, but exact relations between immune parameters and frailty remain to be established. Frailty is highly prevalent and found in 20% to 30% of the older population over 75 years (1). A frequently used definition of frailty in geriatric medicine is a clinical state characterized by a decline in functioning across multiple physiological systems, accompanied by increased vulnerability to stressors, which results in high risk of poor health outcomes, including falls, incident disability, hospitalization and mortality (2). Mechanistically, frailty appears to be a multifaceted deregulation of several biological pathways and systems. Recent studies have addressed the correlation of biomarkers and the frailty clinical phenotype to a certain extent.

Among the possible mechanisms that contribute to the occurrence of frailty are the age-related changes that occur in the immune system. As the innate immune system serves as the first line of defense against injury and infections, it gives an immediate response to external stressors and, as a result, plays a crucial part in the development and shaping of immune responses, which, in turn, play a central role in inflammation and immune protection against infections (3). Emerging evidence suggests that the immune system is altered in frailty. These alterations are referred to as “immunosenescence” and “inflammaging”. These phenomena are characterized by age-related imbalances in immune responses and by alterations in the underlying cellular mechanisms. “Immunosenescence” refers to the decline of (predominantly) the adaptive immune system, and is characterized by reductions in the numbers as well as the antigen-recognition repertoire of naive T and B cells. These reductions in the adaptive immune system are thought to result from age-related declines in hematopoietic stem cell numbers and thymic involution. Inflammaging refers to an age-related over-activation of the innate immune system, resulting in a state of chronic, low-grade, sterile inflammation. Inflammaging is thought to be triggered both by the age-related declines in the adaptive immune system (as a compensatory response), as well as by the age-related accumulation of (immune-reactive) debris (4). Studies have demonstrated elevated CRP, cytokine and chemokine levels, and an abnormal white blood cell distribution in older adults, which were suggested to reflect a dysregulated inflammatory state related to advancing age and which have been linked to adverse outcomes of various diseases, such as cancer and COVID-19 (5).

It is, however, unclear whether these dysregulations in the innate and adaptive immune system are related to frailty. The relationship between the degenerated immune system and adverse outcomes could be based on underlying confounders

such as comorbidities or malnutrition, which can also lead to a state of chronic low-grade inflammation in older people. However, there is also evidence that chronic exposure to inflammatory mediators may be in part responsible for the development of chronic diseases (6). As immune changes may be one of the mechanisms underlying the development of frailty in older adults, understanding the immunological determinants for frailty may help to develop and deliver more effective care to older people. The aim of the present review is to study the association between biomarkers of the ageing immune system and frailty.

MATERIAL AND METHODS

Search strategy

The present systematic review was performed with the assistance of a trained librarian. This review was conducted following the PRISMA guidelines (http://www.prisma-statement.org/) for reporting and design of systematic reviews. We systematically queried PubMed and Embase for citations until December 31st, 2021. The search strategy only included MeSH terms and studies published in English. We used the keywords “immunosenescence”, “inflammation”, “inflammaging”, and “frailty”. The full literature search strategy can be found in Appendix 1.

Eligibility Criteria

Three authors (NdG, SPM, JP, FVdB and ETVH) independently selected the studies according to the following criteria. Any disagreement was resolved through discussion. Included studies were those (1) investigating frailty and immune biomarkers measurements at identical timepoint, (2) reporting blood biomarkers of the immune system or local tumor related immune biomarkers - such as tumor-infiltrating lymphocytes, as those cellular markers may reflect the inflammation status of frail patients - and (3) reporting frailty with standard tools, used in the medical research, assessing the different domains of frailty (unintentional weight loss, exhaustion, low energy expenditure, low grip strength, and/or slowed walking speed), with an available description of the scoring process. These instruments, of which the Fried Frailty Scale has been most used, have been validated against the gold standard Comprehensive Geriatric Assessment and are strongly predictive of mortality and other adverse events (7). Studies were excluded if these (1) comprised a population with an active disease with potentially major effects on both frailty and the immune profile at baseline (such as HIV, hemotologic malignancies, active infection,...) - we chose not to exclude studies investigating cancer patients, as cancer is often diagnosed in older populations and the impact of solid cancer on the immune

system is assumed to be relatively limited (in contrast to for example hematologic malignancy or HIV), (2) only investigated dementia and cognitive impairments, as cognitive functions comprise different pathways, (3) performed the measurements shortly after an intervention such as vaccination, or (4) did not use clear diagnostic criteria for frailty or used only one component or one geriatric impairment of the frailty phenotype (e.g only fatigability) for its diagnosis.

Data extraction

Three authors (NdG, SPM, ETVH) extracted data from the selected studies in a Microsoft Excel spreadsheet. The following information was extracted: (1) characteristics of the study population (including sample size, demographics, country in which the study was performed); (2) setting in which the study was performed; (3) diagnostic criteria for frailty; (4) immunological parameters assessed with corresponding methods of measurement; (5) measured biomarkers; (6) type and number of adjustments in the multivariate analyses; (7) main associations reported.

Description of analysis and presentation of data

We used a table to describe the studies and extract all the results. The table described the following information: author, country of the study performed, study design, type of population studied, aim of the study, inclusion and exclusion criteria, diagnosis (if applicable), type of treatment (if applicable), biomarkers, number of participants, age of participants, type of analysis, interpretation and conclusion.

From the extracted data, we counted the studies that investigated each biomarker. Furthermore, we determined studies demonstrating a statistically significant association with the biomarker.

Quality assessment

Study quality was assessed by three authors (NdG, SPM, ETVH) using the Newcastle-Ottawa Scale (NOS) adapted for cross-sectional studies (8). The NOS assigns a maximum of 10 points based on three quality parameters: selection, comparability, and outcome. Quality was assessed as poor if the score was below 5 points, moderate if the score was 5 or 6, good if the score was 7 or 8 and high if the score was 9 or 10. In case of disagreement between two authors, a consensus was reached after discussion.

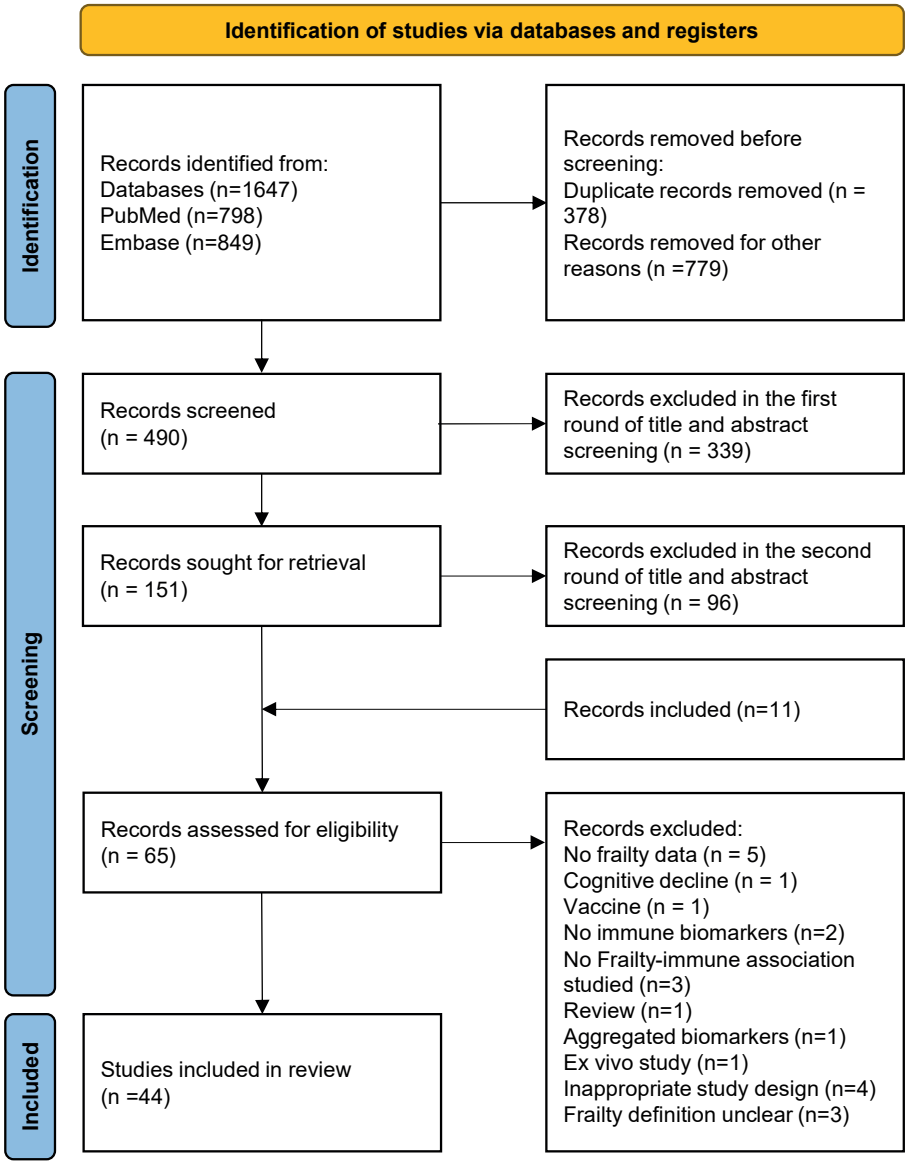
RESULTS

Data search results and characteristics of included studies

The flowchart of study selection is shown in Figure 1. The systematic searches resulted in a total of 1647 records. By the initial screening of titles and abstracts, 490 items were identified after removing duplicates and irrelevant records. After excluding 435 records for irrelevant source material that did not describe studies in accordance with the inclusion criteria, 65 records underwent full-text review. After reading the full text, 44 papers met the criteria for inclusion (9-52).

Table 1 shows details of the selected studies. The studies included a total of 18 419 participants, with a median of 184 participants. Older patients were over 60 years old. The included studies used different definitions of frailty; the Fried score was the most frequently used to measure frailty (in 24 studies, 55%). Studies were population-based (11 studies, 25%) or included older adults from hospitals or clinical research centers (8 studies, 18%, with 6 disease-specific studies), long-term care facilities (9 studies, 20%), general practice (6 studies, 14%) and 3 studies did not report the study population. The quality of the studies, assessed with a based New-Castle Ottawa Scale tool, was moderate in 25 studies (57%), good in 16 studies (36%) and poor in 3 studies (7%).

Figure 1. Study selection based on PRISMA methods.



In total, 143 unique biomarkers were studied. Figure 2 presents the 10 most studied biomarkers of the innate immune system. IL-6 was evaluated in 24 studies, CRP was reported in 19 studies, TNF-α was measured in 13 studies, and white blood cell (WBC) count was assessed in 10 studies. All other biomarkers of the immune system were

studied in less than 8 studies. Twelve out of 24 (50%) demonstrated statistically significantly higher IL-6 serum levels in frail individuals, and five studies observed non-significantly higher IL-6 serum levels in frail individuals. For instance, Adriaensen et al. performed a high-quality cross-sectional study on 394 community-dwelling older adults, with a mean age of 85 years (9). Adriaensen et al. measured frailty with a global functioning score, which was determined with the activities of daily living score (ADL), the short physical performance battery (SPPB), mini-mental state examination (MMSE) and Geriatric Depression Scale (GDS)-15. In total, 21% were frail, and 26% were mildly frail. IL-6 serum levels were significantly higher in patients with frailty. Compared to individuals with lower levels of IL-6, the study reported a higher odds of functional impairment occurring in individuals with slightly elevated IL-6 (adjusted OR (%95CI), 4.16 (1.6-10.9)) or highly elevated IL-6 levels (adjusted OR (%95CI), 4.35 (1.7-114)). Higher CRP levels were significantly associated with the presence of frailty in 7 of 19 studies (36%) and non-significantly in 5 studies (26%). Higher TNF-α levels were significantly associated with the presence of frailty in 4 out of 13 studies (30%) and non-significantly in 2 studies. The high-quality study from Collerton et al. investigated CRP and TNF-α serum levels in 811 older adults from the Newcastle 85+ study (12). The frailty status of the patients was evaluated using an approximation of the Cardiovascular Health study and Rockwood Frailty Index (RFI), assessing the MMSE and disability score from ADL. When models were fully adjusted for covariates, IL-6 and TNF-α were similarly associated with frailty. Lower basal IL-6 level (OR (%95CI), 0.50 (0.31-0.79)) and lower basal TNF-α levels (0.62 (0.39–0.98)) were associated with a lower risk of frailty and higher CRP levels (1.78 (1.12–2.85)) remained positively and significantly associated with a higher risk of frailty.

Figure 3 presents the 10 most studied biomarkers of the adaptive immune system and their association with frailty. The total lymphocyte count was investigated in 11 studies. In 8 out of 11 studies, the total lymphocyte count was not significantly associated with frailty. However, 3 studies observed a significant negative association of total lymphocyte count with frailty. For instance, Nunez et al. demonstrated in a high-quality study that low lymphocyte percentage was associated with a higher risk of frailty (p=0.001), and moreover, low lymphocyte percentages were also associated with risk of long-term mortality (40). WBC count was investigated in 10 studies, but only one study with a moderate quality, observed a significant positive association with frailty (9). T-cell subsets were only studied in two studies. The largest cross-sectional study, including 1072 participants, from Johnstone et al., demonstrated that higher percentages of naïve $CD4^+$ T-cells (p=0.001) and effector memory $CD8^+$ T-cells (p=0.02) were associated with a lower Frailty Index, whereas a higher percentage of $CD8^+$ central memory T-cells was associated with a higher Frailty Index (p =0.02) (19).

Table 1. Articles characteristics.

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Adriaensen, 2014 (9)	Belgium	415	84.5	Sum of 4 domains: ADL, GDS-15, SPPB, MMSE	ADL, GDS-15, SPPB, MMSE, ADL, SPPB	Population-based	IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-g, MCP-1 and CRP	IL-6 and CRP significantly positively associated with worse global functioning	Good
Alberro, 2021 (10)	Spain	356	Cohort 1: 79.77 Cohort 2: 76.98	Barthel index, Timed up-and-go (TUG), Gait speed (GS), SPPB, Tilburg frailty indicator (TFI), Gerontopole frailty screening tool (GFST)	TUG, GS, TFI, GFST	Community-dwelling	TNF-α, IL-6, CRP	IL-6, CRP, TNF-α elevated in older adults but no significant association reported with frailty	Moderate
Arauna, 2021 (11)	Spain	55	72	Fried Frailty Index	5 Fried criteria (5 FC): slowness, weakness, weight loss, exhaustion, low physical activity	Population-based	cMV from monocytes ($CD14^+/AV^{+/-}$), cMV from monocyte phenotype ($CD16^+/CD14^+/AV^{+/-}$), natural killers ($CD56^+/AV^{+/-}$). neutrophils (%) and monocytes (%) WBC ($x10^3$/uL), lymphocytes (%)	Significantly higher concentrations of cMVs from monocytes and NK in frail group but no association reported with frailty	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Collerton, 2021 (12)	UK	811	Inclusion at 85 years old, mean age of cohort not detailed	Fried Frailty status defined using an approximation of the Cardiovascular Health study methodology And Rockwood frailty index (RFI) computed from 40 potential deficits	MMSE, disability score from ADL	Older patients from general practice patients lists	WBC count, neutrophils, monocytes, eosinophils, basophils Lymphocyte count, CD4/ CD8 T cells ratio, memory/naive CD4 T cell ratio, memory/naive CD8 T cell ratio, memory/naive B cell, CD4/CD8 <1 IL-6, TNF-α, CRP	CRP, IL6, TNF-α, neutrophil count significantly positively associated with risk of frailty. Lymphocyte count, memory/ naïve B cell ratio and albumin significantly negatively associated with frailty	Good
Darvin, 2012 (13)	US	65	80.6	Fried Frailty Index	5 FC	Community-dwelling adults from a retirement community	IL-6	IL-6 significantly positively associated with frailty category and frailty score	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
De Fanis, 2008 (14)	US	26	83.8	Fried Frailty Index	5 FC	General clinical research center	T cell single marker: $CD4^+$, $CD8^+$, $CD45RO^+$, $CD45RO^-$, $CCR5^+$ T cell double markers : $CCR5^+CD4^+$, $CCR5^+CD8^+$, $CCR5^+CD45RO^+$, $CCR5^+CD45RO^-$	Higher levels of $CCR5^+$ T cell and $CD4^+$ T cell and lower levels of $CD8^+$ T cell levels observed in frail group but no association reported with frailty	Moderate
Fernandez Garrido, 2018 (15)	Spain	94	82	Fried Frailty Index	5 FC	Institutionalized older women	WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils	Lymphocyte count significantly negatively associated with frailty No association reported for total WBC, Monocytes, neutrophils, eosinophils	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Furtado, 2020 (16)	Portugal	358	83 (median)	Fried Frailty Index	5 FC	Institutionalized older women	Monocytes, granulocytes, WBC count Lymphocytes, IL-6, IL-1β, Ig-A, IL-10, IFN-g, CRP, TNF-α, TNF-α / IL-10 ratio	IL-6 significantly negatively associated with physical frailty score IL-10 (unadjusted), IL-1β, TNF-α and TNF-α/IL-10 (unadjusted) significantly positively associated with physical frailty score	Moderate
Gilmore, 2021 (17)	US	581	53.4	Fried Frailty Index	5 FC	Large nationwide cohort from Research Center	neutrophils, neutrophils-lymphocytes ratio, lymph-monocytes ratio, total WBC	Neutrophils, NLR, WBC significantly positively associated with post-chemotherapy frailty No association for lymphocytes, monocytes, LMR found	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Hammami, 2020 (18)	Tunisia	141	Very frail: 80 (median) Frail: 77 (median) Non-frail: 69 (median)	SEGAm score	CGA including Mini-GDS,mini-cog, MNA-SF, ADL (Katz score), TUG	Patients from hospital and nursing home	TNF-α, IL-6, IL-8, CRP	IL-6, IL-8, and CRP level significantly positively associated with frailty score; IL-8 and TUG significantly positively correlated; IL-6 and CRP significantly negatively correlated with MNA-SF; TNF-α, IL-6, CRP significantly negatively correlated with ADL score	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Johnstone, 2017 (19)	Canada	1072	86 (median)	Fried Frailty Index	5 FC	Patients from nursing home	$CD4^+$ and $CD8^+$ subsets (naïve, memory (central memory and effector memory), terminally differentiated, senescent), memory naïve $CD4^+$ ratio, memory naïve $CD8^+$ ratio, regulatory T cells, CD4/CD8 ratio, CD4/CD8 ratio<1 CMV-reactive $CD4^+$ and $CD8^+$ T cells	Higher levels of naïve $CD4^+$ T-cells and effector memory $CD8^+$ T-cells significantly associated with lower levels of frailty	Good
Kamijo, 2018 (20)	Japan	119	Non-sarcopenia: 65.3 Sarcopenia: 79.2 Non-frail: 65.4 Frail: 82.5	Clinical Frailty Scale	Assessment of cognition, mobility, function and comorbidities through direct examination and medical records	PD patients from Medical Center	IL-6, CRP	Elevated IL-6 and CRP values in frail group but no significant association reported with frailty	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Komici, 2020 (21)	Italy	128	69.2	Clinical Frailty Scale	Assessment of cognition, mobility, function and comorbidities through direct examination and medical records	HF patients admitted in Rehabilitation Unit	WBC count, CRP, Galectin-3	Gal-3 and CRP are independently significantly associated with frailty in elderly patients with systolic HF in a multivariable model	Moderate
Lai, 2014 (22)	Taiwan	386	81.5	Fried Frailty Index	5 FC	Residents from the long-term care facility	IL-6, TNF-α, CRP	IL-6 is significantly positively associated with frailty; No association found with TNF-α and CRP	Good
Laudisio, 2019 (23)	Italy	1035	NR	Fried Frailty Index	5 FC	Population-based	IL-6	IL-6 was significantly positively associated to frailty but not adjusted results	Moderate
Lee, 2016 (24)	Taiwan	946	65.5	Fried Frailty Index	5 FC	Community-dwelling adults	IL-6, ICAM-1	sICAM-1 and IL-6 significantly associated with frailty	Good

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Leng, 2002 (25)	US	30	Frail: 84.9 Non-frail: 81.3	Fried Frailty Index	5 FC	Population-based	WBC count IL-6	Frail subjects had significantly higher IL-6 serum levels and non-significantly higher WBC count but no significantly association reported	Moderate
Leng, 2009 (26)	US	558	Non-frail 72.8 Prefrail 77.0 Frail 80.0	Fried Frailty Index	5 FC	Population-based	monocyte count, eosinophil count, basophil count, neutrophil count lymphocyte count IL-6, CRP	IL-6 significantly associated with frailty No association reported with lymphocyte, eosinophil, or basophil count and frailty	Good

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	Country	Sample size	Age, yr (mean)	Frailty Definition	Geriatric assessments	Type of population			
Leng, 2011 (27)	US	133	84	Fried Frailty Index	5 FC	Outpatients' medical clinics, senior centers, residential retirement communities	IL-6, neopterin	Elevated neopterin levels IL-6-independently significantly associated with prevalent frailty IL-6 was not associated after adjustment for neopterin in multivariable model	Good
Lin, 2017 (28)	Taiwan	12	77.5	Chinese-Canadian Study of Health and Aging Clinical Frailty Scale (CSHA-CFS)	Grip power and the 6-minute walk test for physical activity	NR	Ig kappa protein, C7, C5	No association reported	Poor
Lorenzi, 2016 (29)	Spain	120	75.4	Fried Frailty Index, modified Rockwood FI	Katz's ADL, IADL, cognition by MMSE, mood by 15-item GDS, CIRS	Geriatric outpatient clinic	Htr1A	High Htr1a significantly associated with the presence of frailty	Good

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Lu, 2016 (30)	Singapore	76	68.41	Fried Frailty Index, Rockwood FI	MMSE, SF12-PCS for physical health, IADL, POMA, pulmonary function by ratio FEV1 to FVC	Population-based	Monocyte count WBC, lymphocytes, α/β T Cell ($CD3^+$, $CD27^+CD45RA^-$ %CD4, CD4/CD8 ratio of $CD28^+$, $CD45RA^+$ %CD8, $CD27^+CD45RA^-$ %CD8), γ/δ T Cell ($CD27^+$, $V/\delta2^+$ IFN-γ^+TNF-α^-, $V/\delta2^-$ IFN-γ^-TNF-α^+, $CD57^+$), B-cells (exhausted, $CD24^-CD38^-$, $CD24^-CD38^+$, $CD24^+CD38^+$, $CD24^{++}CD38^+$, IgM^+IgD^-), APC ($CD14^+CD16^+$ %$CD45^+$), sgp130, I-309, MCP-1, BCA1, RANTES, leptin IL6R, IL2RA	sgp130, IL-2Rα, I-309, MCP-1, BCA 1, RANTES, leptin, and IL-6R significantly associated with frailty; Frailty predicted by frequency of $CD8^+$ terminal effector and inversely predicted by $CD3^+$, $CD45RA^+$, central memory $CD4^+$	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Lu, 2021 (31)	Canada	887	Cohort 1: 67 Cohort 2: 72.7	Rockwood Frailty Index	GS, functional mobility using the POMA, MMSE score of global cognition, FI (Rockwood)	Community-dwelling adults	C3a CRP, TNF-α, sTNFR-I, sTNFR-II, IL-1RL1, sICAM-1, MIP-1a, MCP-2, IL-4, IL-5, IL-6, LIF, Galectin-3	Frailty Index and inflammatory cytokines positively associated	Moderate
Marcos-Perez, 2018 (32)	Spain	259	Non-frail 73.2 Pre-frail 77.05 Frail 85.8	Fried Frailty Index	5 FC	Population-based	Total lymphocytes ($CD3^+$) and lymphocyte subsets ($CD4^+$, $CD8^+$, $CD4^+/CD8^+$ ratio, $CD19^+$, $CD16^+CD56^+$(NK)) IL-6, CRP, sTNF-RII, TNF-α	IL-6, sTNF-RII $CD4^+/CD8^+$ ratio and $CD19^+$ significantly positively associated with frailty sTNF-RII had the strongest association with frailty	Good

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Marzetti, 2019 (33)	Italy	200	Frail: 77.6 Non-frail: 74.8	Physical Frailty + Sarcopenia (PF+S) = operational definition in the "Sarcopenia and Physical frailty in older people	Summary score of SPPB, low appendicular muscle mass on DXA scans, and 400-m walk test.	Population-based	Granulocyte-macrophage colony-stimulating factor (G-CSF) IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFNg, CCL (MCP-1), CCL3 (MIP-1a), CCL4 (MIP-1 β), CCL5, CCL11 (eotaxin-1), CXC motif chemokine ligand (IP10), TNF-α, CRP	Non-physical frail and sarcopenic controls had higher MPO, PDGF-BB, IL-8, MCP-1 Physical frail and sarcopenic patients had higher CRP But no significant association was reported	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Mathei, 2011 (34)	Belgium	567	Patients with CMV- titers: 84.5 Patients with CMV+ titers: 84.8	Individual components of frailty (ADL, MMSE)	SPPB, ADL, MMSE	Community-dwelling	IL-6, CRP	High CRP levels associated with low ADL and MMSE score; High IL-6 levels associated with higher number of frail individuals and low MMSE and ADL score But no significant association was reported	Moderate
Mekli, 2015 (35)	UK	3160	68.3	ELSA Frailty Index	Health-related problems (deficits) in a range of domains (Activities of daily living, cognitive function, falls and fractures, joint replacement, vision, hearing, chronic diseases, cardiovascular diseases, depression)	Population-based	Genes corresponding for IL-18, IL-12A, SELP, LRP	No association after Bonferroni correction	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Mustafaoglu, 2020 (36)	Turkey	61	65-74 years (n=43), 75-84 years (n=18)	Tilburg Frailty Score	25 questions of the Tilburg Frailty questionnaire	COPD patients at the outpatient clinic	Neutrophil-lymphocyte ratio (NLR) IL-6, IL-8, CRP	No significant association reported with frailty	Moderate
Navarro-Martinez, 2019 (37)	Spain	46	72.2	Fried Frailty Index	Charlson Comorbidity Index Ability to perform daily activities (Barthel index) Sleep quality (Athenas scale) Depressive symptoms (Yesavage scale) Cognitive function (MMSE test)	Patients with metastatic prostate cancer receiving antiandrogen therapy, enrolled in a clinical trial	WBC count, lymphocyte count, neutrophil count, monocyte count, eosinophil count, basophils count IL-6, TNF-α, IL- β, IL-8, CRP	IL-6 and fibrinogen significantly positively associated with frailty No association reported for lymphocyte count and CRP	Poor
Nevalainen, 2019 (38)	Finland	107	Nonagenarians (n=67), young controls (n=40) aged 19-29	Fried Frailty Index, 10-items Barthel Index (physical performance), Frailty Index	5 FC, assessment of activities required for daily living (mobility, dressing, feeding, toilet use, bowel, bladder control)	Community-dwelling adults from the Vitality 90+ study	B cells subsets: $CD19^+$ $CD27^+$ IgD^- (switch memory), $CD19^+$ $CD27^+$ IgD^+ (IgM memory), $CD19^+$ $CD27^-$ IgD^+ (naïve B cells), $CD19^+$ $CD27^-$ IgD^- (late memory) IL-6	IL-6 and $CD27^-$ IgD^- B cells were significantly associated with frailty but only in males	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	Country	Sample size	Age, yr (mean)	Frailty Definition	Geriatric assessments	Type of population			
Ng, 2015 (39)	Singapore	421	66.5	Fried Frailty Index	5 FC	Community-dwelling adults aged ≥55	CD4/CD8 ratio, $CD8^+CD28^+$ $CD27^-$, $CD8^+CD28^+$ $CD27^+$, $CD8^+CD28^-$ $CD27^-$, $CD8^+CD28^-$ $CD27^+$, $CD8^+CD28^-$, $CD8^+CD57^+$, $CD4^+CD28^+CD27^-$, $CD4^+CD28^+CD27^+$, $CD4^+CD28^-CD27^-$, $CD4^+CD28^-CD27^+$, $CD4^+CD27^-$, $CD4^+CD57^+$	$CD4^+$ $CD28^-$ $CD27^+$, $CD8^+CD28^-$, CD4/CD8 ratio were significantly positively associated with frailty and $CD8^+$ $CD28^-CD27^+$ was strongly predictive of frailty	Good
Nunez, 2020 (40)	Spain	488	78	Fried Frailty Index	Not clearly described	Patients aged ≥65 with acute coronary syndrome from single-center study	Lymphocyte count, WBC count	Lymphocyte count significantly negatively associated with frailty	Good
Palmer, 2019 (41)	US	100	77.1	Trauma-specific frailty index (TSFI) derived from the Rockwood frailty survey	Assessment of comorbidities, activities of daily living, social activity, nutritional status, general health attitude	Geriatric trauma patients	IL-1β, IL-6, IL-2Rα, TNF-α	TNF-α, IL-1 β, and IL-6 significantly positively correlated to frailty	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Ronning, 2010 (42)	Norway	187	80	Patients were classified as frail according to the CGA if they had either severe comorbidity or used more than 7 medications daily, were functionally dependent in personal activities of daily living, were malnourished, had reduced cognitive function or depression	Barthel Index, Nottingham Extended Activities of Daily Living Scale, TUG measures and grip strength to assess functional dependence; CIRS and MNA to assess comorbidity and nutritional status; MMSE and GDS to assess depression and cognitive function; EORTC QLQ-30 questionnaire	Colorectal cancer patients who were surgically treated	IL-6, TNF-α, CRP	CRP, IL-6, TNF-α significantly positively associated with frailty	Moderate
Samson, 2019 (43)	Netherlands	289	Men: healthy 70.2, intermediate 70.8, frail 70.9 Women: healthy 70.1, intermediate 70.5, frail 71.4	Frailty Index based on 36 'health deficits'		Population-based	Neutrophils, monocytes, lymphocytes, T cells, B cells, NK cells, CRP	CRP, neutrophils and monocytes count positively associated with frailty but only statistically significant in women	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Saum, 2015 (44)	Netherlands	2518	Non-frail: 67.8 Pre-Frail: 70.3 Frail: 73.7	Fried Frailty Index	CES-D, SPPB, grip strength, PAQE	Population-based	CRP	CRP significantly positively associated with frailty	Good
Speigl,2018 (45)	Germany	58	75	Balducci criteria of frailty, and according to the LOFS (Leuven Oncology Frailty Score)	GA, EORTC QLQ-C30 questionnaire, test G8, KP). Katz's Activities of Daily Living (ADL) and Lawton's IADL scales, fall history, self-perceived fatigue assessed by the Mobility–Tiredness test (MOB-T), MMSE, GDS-15, nutritional status by the MNA-SF, and comorbidity by the Charlson Comorbidity Index pain by VAS	Breast cancer patients from academics and hospital	Intra-tumoural T cells ($CD3^+$), granulocytic cells ($CD15^+$)	Intra-tumoral granulocytic cells ($CD15^+$) displayed significant inverse relationship with patient performance, contrary to T cells ($CD3^+$)	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Stanjek-Cichoracka, 2019 (46)	Poland	31	49.2	CSHA Clinical Frailty Scale		Patients waiting for lung transplantation	IL-6, IL-2, IL-18, IL-23, IL-12p70, IL-10, IL-7	No association reported between cytokines and frailty	Moderate
Su, 2017 (47)	China	306	70.5	Fried Frailty Index	5 FC	Participants from Comprehensive Geriatric Assessment and Health Care Service Study	MCP-1, MCP-3, MIP-1α, MIP-1β, IL-10	High concentrations of MCP-1 and MIP-1β were significantly associated with frailty	Good
Valdiglesias, 2018 (48)	Italy	180	74.98	Fried Frailty Index	Unintentional weight loss in the previous 12 months; poor endurance and energy; weakness, defined by poor grip strength; slowness, assessed via timed 4-m speed; and low physical activity level according to the Physical Activity Scale for elderly (PASE)	Geriatric outpatient clinic	CRP, tryptophan, kynurenine, Kyn/Trp ratio, neopterin, nitrite, tyrosine, phenylalanine, Phe/Tyr ratio	Neopterin, nitrite, tryptophan, CRP levels higher in frail patients but only nitrite is a predictor of frailty after multiple regression analysis	Good

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Verschoor, 2014 (49)	Canada	129	Adults (19-59 years, median): 34 Seniors (61-76 years, median): 69 Elderly (81-100 years, median): 89	Rockwood Clinical Frailty Scale	Assessment of cognition, mobility, function and comorbidities through direct examination and medical records	Community-dwelling healthy seniors, nursing home inhabitants, healthy young controls.	Monocytes subsets ($CD14^{++}$, $CD14^{++}CD16^{++}$, $CD14^{++}CD16^{+}$), Myeloid DCs CCR2, CX3CR1, TLR-2, TLR-4, TNF-α	Increase in the % of TLR-4 expressing classical monocytes in frail aged; Increased production of TNF-α and IL-8 (to a lesser extent) significantly higher in monocyte subsets from advanced-aged frail elderly; Classical ($CD14^{++}$)/ Intermediate ($CD14^{++}CD16^{+}$) monocytes ratio decreased in frail aged; Myeloids DC ($CD141^{++}$, $CD1c^{+}$) decreased in frail aged	Poor

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Wilson, 2020 (50)	UK	117	Healthy young: 26.0 Healthy older: 71.0 Frail older: 84.0	Frailty Index	Medical assessment (stroke, myocardial infarction, congestive, cardiac failure, diabetes mellitus, COPD, number of medications, continence, self-reported weight loss, BMI, physician assessment of Clinical Frailty Scale, food intake); Physiological assessment (self-reported mood); independence (bathing, dressing, transferring, feeding, toileting, finances, meal preparation, medications); cognition	Healthy young adults from staff and students from the University of Birmingham. Frail older were patients included from geriatric outpatient clinics and medically stable patients from the 1000 Elders cohort	IL-1ra, IL-4, CXCL8(IL-8), IL-9, IL-17, Eotaxin, IP10, MCP-1, MIP-1α, MIP-1β, RANTES	Frailty associated with reduced migratory accuracy toward CXCL8; CRP, IL-ra, IL-4, CXCL8, IL-17, Eotaxin, IP10, MIP-1α significantly higher in frail older; MCP-1 and IL-17 significantly lower in frail older	Moderate

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	Country	Sample size	Age, yr (mean)	Frailty Definition	Geriatric assessments	Type of population			
					(Addenbrooke's cognitive examination); physical function (walk speed, falls, effort, trouble getting going, handgrip)				
Yin, 2020 (51)	China	728	73.09	33-Item Modified frailty index (mFI), combining Rockwood, ethnicity, societal factors.	Not clearly described	Community-dwelling individuals	Tfh cells, Tfh2 cells, Tfh2/Tfh1 cell ratio, Tfh2/ Tfh17 cell ratio, $CD19^{+}$ B cells IL-6, IL-12, IL-21, TGF-β	TGF-β and IL-12 significantly associated with frailty; Tfh and Tfh2 significantly negatively associated with frailty. No significant association between B cell populations and frailty subtype	Good

Publication			Study population				Biomarkers studied for Frailty	Results	Quality assessment
Author	**Country**	**Sample size**	**Age, yr (mean)**	**Frailty Definition**	**Geriatric assessments**	**Type of population**			
Yousefzadef, 2017 (52)	US	63	81	Fried Frailty Index	5 FC	Not clearly described	MCP-1	MCP-1 significantly negatively associated with frailty	Good

Abbreviations: 5 FC= 5 Fried criteria (slowness, weakness, weight loss, exhaustion, low physical activity), ADL= Activities of Daily Living, GDS-15= Geriatric Depression Scale 15, SPPB= Short Physical Performance Battery, MMSE= Mini-Mental State Examination, TUG= Timed Up and Go test GS, TFI= Tilburg Frailty Indicator, GFST= Gerontopole Frailty Screening Tool

Figure 2. Graphic describing the most studied biomarkers of the innate immune system for frailty.

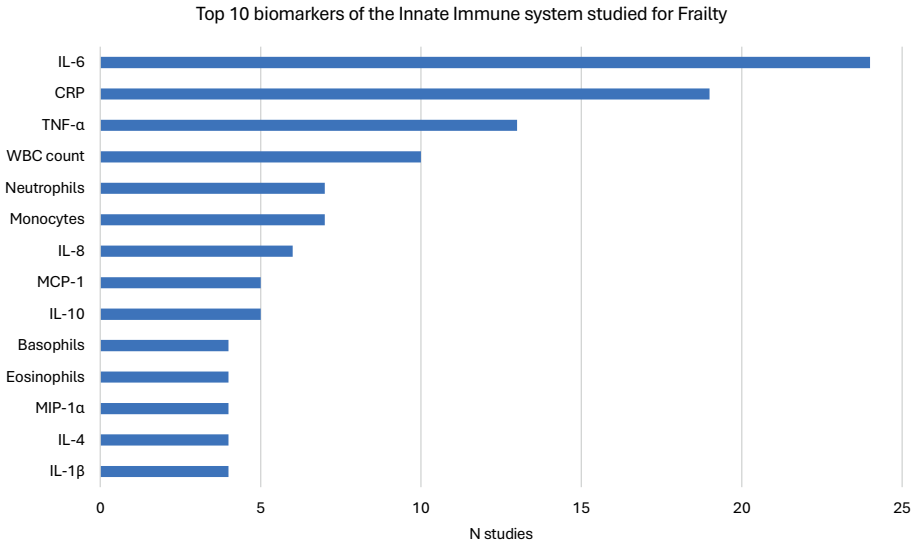
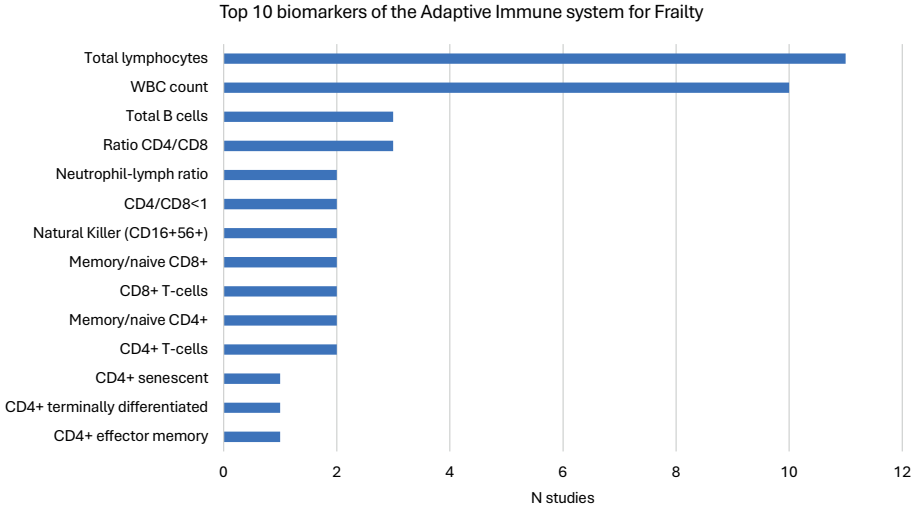


Figure 3. Graphic describing the most studied biomarkers of the adaptive immune system for frailty.



DISCUSSION

This systematic review shows that only 44 studies investigated the association of biomarkers of the ageing immune system with frailty. These studies reported many and diverse biomarkers of immunosenescence and inflammaging, especially cytokines and cellular biomarkers. Our review shows that elevated levels of IL-6 and CRP were consistently associated with frailty. Lymphocyte subpopulations were investigated too infrequently within small cohort studies only to draw any conclusions. Only one in three studies had high quality. A large body of literature recognizes IL-6, CRP and TNF-α as relevant biomarkers for the ageing innate immune system. Predictably, as IL-6 and TNF-α are predominantly monocyte-derived, Leng et al. have observed changes in the monocyte compartment with ageing (26). However, only a small number of studies reported those biomarkers as significantly associated with frailty. Our findings are consistent with the recently published systematic review by Xu et al., showing that peripheral inflammatory biomarkers are related to frailty (53). However, the review was restricted to a limited number of pre-specified biomarkers, including lymphocytes, IL-6, CRP, and TNF-α. Our review adds the open search for any biomarkers.

There are several explanations for the observed association between IL-6, CRP and TNF-α with frailty. CRP has been widely recognized as a marker for systemic inflammation. Walker et al. demonstrated in a prospective study that increasing CRP levels during midlife increased the risk of frailty later in life (54). Studies have shown that increases in CRP plasma levels are associated with increased risk of sarcopenia, cardiovascular diseases, disability, and cognitive decline in older individuals. Elevated CRP levels were also associated with increased risk of mortality in frail older patients (55-57). However, many diseases can cause increased CRP levels. Measurement of CRP plasma levels is a frequently used screening test in daily clinical practice. Clinicians use it as a tool to diagnose infections or clinical conditions closely associated with underlying inflammatory mechanisms. Therefore, it seems to be a rather unspecific biomarker of the ageing immune system.

There is growing evidence that IL-6 and TNF-α play a central role in the pathogenesis of chronic and age-related diseases. In acute inflammation, IL-6 promotes the expansion and activation of T cells and differentiation of B cells and modulates the synthesis of positive reactants such as CRP. Previous studies demonstrated an association with increased levels of proinflammatory serum markers in older adults and in individuals with dementia or Parkinson's disease. It has been suggested that IL-6 may advance the onset of age-related diseases.

Results for TNF-α are heterogeneous. TNF-α has previously been associated with exhaustion and chronic fatigue syndrome, which share a pathophysiological core

with the frailty condition (16, 58). Additionally, cytokine networks involving up- and downregulation of other cytokines may add additional layers to the heterogeneity of the immune responses among frail adults. Therefore, it may be important to consider patterns or profiles comprising numerous circulating cytokines rather than focusing on the effect of individual cytokines.

Total white blood cell counts and lymphocyte counts are the most studied biomarkers of adaptive immunity. Xu et al. discuss the relevance of leukocytes and lymphocytes in ageing (59). Emerging research shows that an elevated lymphocyte rather than leukocyte count has been associated with frailty and especially with low physical activity and grip strength (15). Lymphocyte subpopulations were investigated infrequently and in rather small cohort studies. Therefore, solid conclusions cannot be drawn. However, there are some promising results. During ageing, T lymphocytes are strongly affected due to changes in the proportion of T lymphocyte subpopulations that have undergone cellular senescence, such as a decrease in $CD4^+$ T cells and an increase in $CD8^+$ T cells (60). Moreover, cells of the innate (monocytes, macrophages, basophils, etc.) and adaptive ($CD4^+$ and $CD8^+$ lymphocytes, etc.) immune system can exhibit alterations in functions and phenotypes. Only a few studies reported an association of lymphocytes with frailty. For instance, Johnstone et al. demonstrated that lower levels of naïve $CD4^+$ T cells and higher levels of $CD8^+$ central memory T-cells were predictive of higher scores of the frailty index (19). Another study by Ng et al. investigated thoroughly T-cell subsets, demonstrating loss of CD28, an established hallmark of immunosenescence (61), as predictive of frailty (39). Only these two studies investigating lymphocyte subpopulations had a high quality. Johnstone et al. and Ng et al. included a large sample of elderly nursing home residents, increasing the power of the study to detect meaningful differences between groups (19, 39). The studies controlled for confounding factors, such as age, sex, comorbidities, and medication use, reducing the risk of bias in the results. The lack of association in other studies may be due to the vulnerability of frail individuals to acute and subacute diseases that affect inflammatory parameters. As a result, adjusting for these confounders in the analyses, particularly in low sample sizes, could decrease the significance of the results and explain the lack of association of the markers and frailty. In line with our results, studies were generally small and too few to draw any strong conclusion about the association of lymphocytes and frailty, based on currently available literature.

This review has several strengths. The review validates previous literature and additionally highlights the potential cellular biomarkers. To the best of our knowledge, this present study is the first to conduct an open search for any biomarkers, enlightening a large panel of cellular markers by describing the studies thoroughly and assessing their quality. We conducted an extensive literature search to identify

biomarkers and performed an adequate quality assessment for cross-sectional studies. Moreover, our study was systematically conducted, and the results were systematically reported. Results were described for each distinct marker, uncovering the research gap and opening the path for further investigations. However, we only included studies focusing on physical frailty, as cognitive-decline-related frailty covers a large spectrum of cognitive diseases, including a very wide panel of markers of interest. Therefore, other aspects of frailty, such as cognitive status (e.g., dementia), which is of relevance in frailty, were not considered. Further studies on cognitive decline should be required to understand frailty and inflammation. Furthermore, the heterogeneity of the studies did not allow to draw very strong conclusions. Non-standardized naming and measurements of the biomarkers make the comparison between studies more difficult; several studies defined subsets of CD4 and CD8 differently, but looked at the same marker; therefore, the total number of biomarkers found can be ambiguous. Underlying data or following standardized naming conventions could be used to improve the comparability between studies.

In conclusion, our review of 44 studies on the relation between immune biomarkers and frailty identified IL-6 and CRP as the biomarkers that were most consistently associated with frailty. T-lymphocyte subpopulations were investigated but too infrequently to draw strong conclusions yet, although initial results were promising. Additional studies are required in order to further validate these immune biomarkers in larger cohorts. Furthermore, prospective studies in more uniform settings and larger cohorts are needed to further investigate the association with immune candidate biomarkers for which potential associations with ageing and frailty were previously observed, before these can be used in clinical practice to help assess frailty and improve the care treatment of older patients.

DECLARATIONS

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Materials

Figure 1: Study selection based on PRISMA methods. Table 1: Characteristics of articles included and quality assessment. Figure 2: Top 10 biomarkers of the innate immune system studied for frailty. Figure 3: Top 10 biomarkers of the adaptive immune system studied for frailty. Figure S1: Biomarkers of the innate immune system studied

for frailty. Figure S2: Biomarkers of the adaptive immune system studied for frailty. Figure S3: Top 5 biomarkers and significant association found with frailty.

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SUPPLEMENTARY MATERIAL

Search strategy

Pubmed

("Immunosenescence"[Majr] OR "immune*"[tw] OR "immuno*"[tw] OR "immuni*"[tw] OR "inflammag*"[tw]) AND ("Aged"[Mesh:NoExp] OR "Aged, 80 and over"[Mesh] OR "elderly"[tw] OR "elder"[tw] OR "elders"[tw] OR "aged"[ti] OR "aging"[tiab] OR "ageing"[tiab] OR "oldest old"[tw] OR "older person*"[tw] OR "old person*"[tw] OR "older patient*"[tw] OR "old patient*"[tw] OR "older women"[tw] OR "old women"[tw] OR "older men"[tw] OR "old men"[tw] OR "old adult*"[tw] OR "older adult*"[tw] OR "Older individual*"[tw] OR "old people"[tw] OR "older people"[tw] OR "septuagenarian*"[tw] OR "octagenarian*"[tw] OR "octogenarian*"[tw] OR "nonagenarian*"[tw] OR "centenarian*"[tw] OR "senescence"[tw] OR "senescent"[tw] OR "geriatric"[tw] OR "geriatrics"[tw] OR "geriatrics"[MeSH] OR "older"[ti] OR "senior"[tw] OR "seniors"[tw] OR "older population"[tw]) AND ("Frailty"[Mesh] OR "frail*"[ti] OR "decline"[ti] OR "impairment"[ti] OR "deficit"[ti] OR "performance"[ti]) NOT ("Animals"[Mesh] NOT "Humans"[Mesh]) AND ("Biomarkers"[Mesh] OR "Biomarker*"[tw] OR "marker*"[tw] OR "index"[tw] OR "indices"[tw] OR "Hallmark*"[tw] OR "hall mark*"[tw] OR "endpoint*"[tw] OR "tool*"[tw]) AND ("2011"[Date - Publication] : "3000"[Date - Publication])

Embase

(exp *"Immunosenescence"/ OR "immune*".mp. OR "immuno*".mp. OR "immuni*".mp. OR "inflammag*".mp.) AND ("Aged"/ OR "elderly".mp. OR "aged".ti. OR "aging".ti,ab. OR "ageing".ti,ab. OR "oldest old".mp. OR "older person*".mp. OR "old person*".mp. OR "older patient*".mp. OR "old patient*".mp. OR "older women".mp. OR "old women".mp. OR "older men".mp. OR "old men".mp. OR "old adult*".mp. OR "older adult*".mp. OR "Older individual*".mp. OR "old people".mp. OR "older people".mp. OR "septuagenarian*".mp. OR "octagenarian*".mp. OR "octogenarian*".mp. OR "nonagenarian*".mp. OR "centenarian*".mp. OR "senescence".mp. OR "senescent".mp. OR "geriatric".mp. OR "geriatrics".mp. OR exp "geriatrics"/ OR "older".ti. OR "senior*".mp. OR "older population*".mp.) AND (exp "Frailty"/ OR "frail*".ti. OR "decline".ti. OR "impairment".ti. OR "deficit".ti. OR "performance".ti.) NOT (exp Animal/ NOT exp Human/) AND (exp biological marker/ OR "Biomarker*".mp. OR "marker*".mp. OR "index".mp. OR "indices".mp. OR "Hallmark*".mp. OR "hall mark*".mp. OR "endpoint*".mp. OR "tool*".mp.) NOT (conference OR conference abstract OR "conference review").pt. AND 2011:2022.(sa_year). NOT Alzheimer*.ti.

Figure S1. Graph describing the number of studies investigating the association between biomarkers of the innate immune system and frailty.

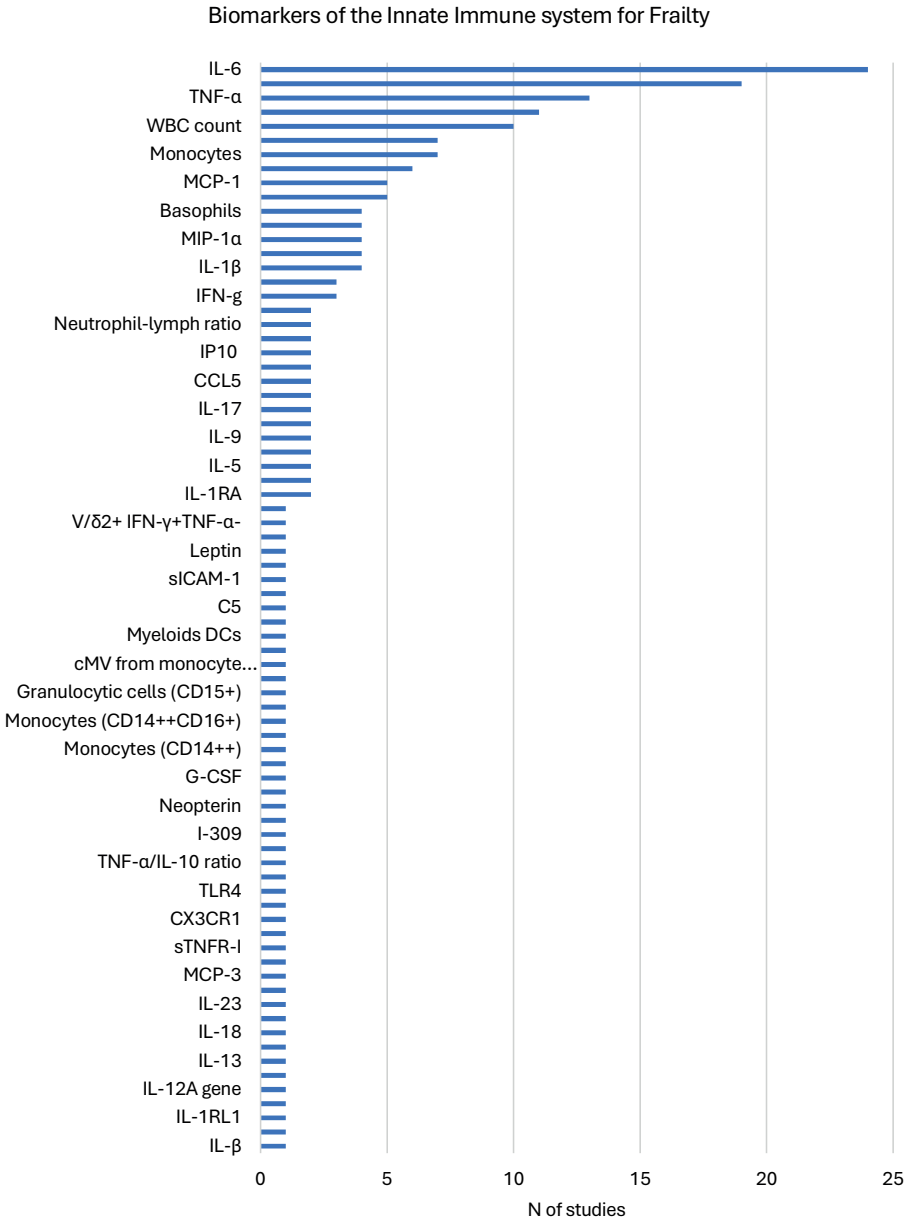

Figure S2. Graph describing the number of studies investigating the association between biomarkers of the adaptive immune system and frailty.

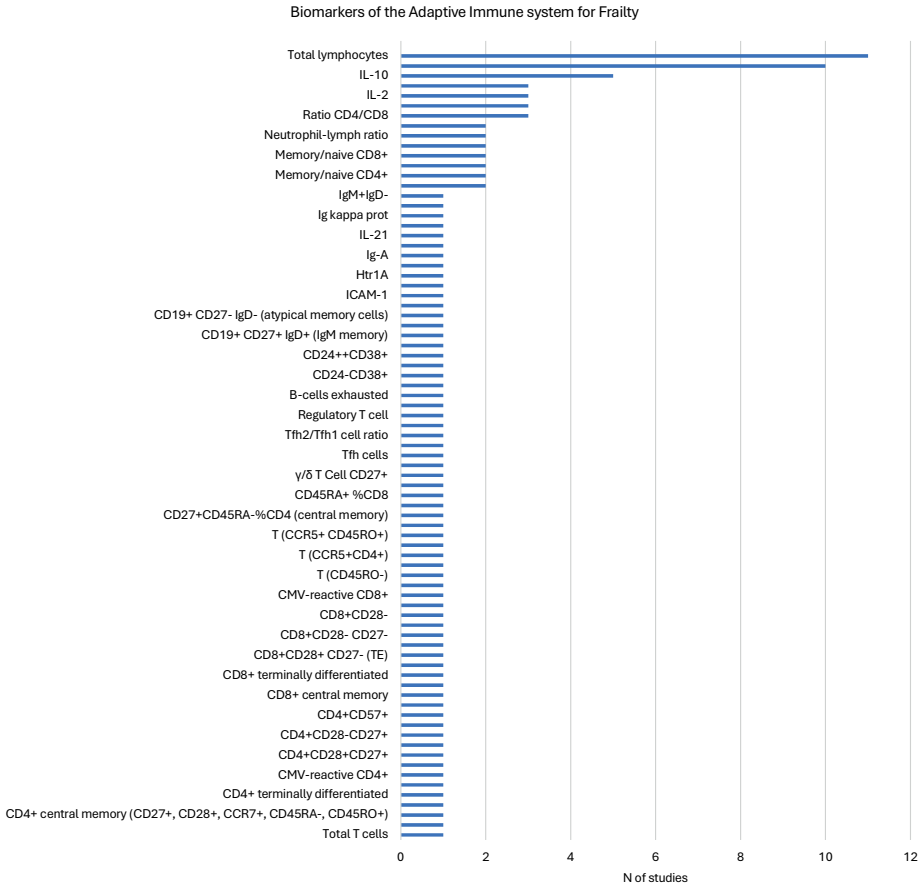

Figure S3. Graph describing the number of studies demonstrating significant associations between the most studied immune biomarkers and frailty.