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Ageing and immunity: unraveling the association between immunosenescence and frailty

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AGEING AND IMMUNITY

Unraveling the Association between Immunosenescence and Frailty

Estelle Tran Van Hoi

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Ageing and Immunity

Unraveling the Association between Immunosenescence and Frailty

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À mes chers parents,

Má và Ba

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General introduction

GENERAL INTRODUCTION

People are living longer worldwide, leading to an unprecedented increase in the ageing population (1). By 2030, one out of every six individuals will be 60 years or older. The proportion of the population aged 60 years or older is expected to increase from 1 billion in 2020 to 1.4 billion by 2030. The global population of those aged 60 years and above will double by 2050, reaching 2.1 billion (1). The global population aged over 65 is growing more rapidly than any other age group (1, 2). As a result of this demographic shift, it is essential to explore strategies that enhance the quality of life of older adults and enable them to live independently (2).

Healthy ageing and biological age

In later life, chronic diseases become increasingly common, with most older adults experiencing one or more conditions (3). In countries where life expectancy is highest, the prevalence of chronic disease and disability is also greater (4). This suggests that a longer lifespan does not necessarily translate into a healthier life, but is often accompanied by a greater burden of disease.

While the risk of health problems increases with age, their manifestations are highly heterogeneous among older individuals. Some people over 70 may present with multiple comorbidities and frailty, whereas others remain healthy, physically active, and capable of independent living. Such variability is influenced by a wide range of factors, including lifestyle, environmental exposures, socioeconomic status, (epi) genetic predispositions, and encounters with infectious agents.

Given this heterogeneity, there is increasing recognition that chronological age alone is an insufficient variable to understand older individuals' health status. Instead, biological age provides a more accurate reflection of the ageing process, as it incorporates diverse physiological, molecular, and clinical factors. Biological age captures how an individual is actually ageing, beyond what is indicated by the calendar, thereby helping to explain the wide variability in health outcomes observed in later life. Frailty has emerged as a key concept in this context. Measuring frailty offers a practical means of identifying individuals who are most vulnerable to adverse outcomes and in need of targeted interventions and/or treatment. Classifying people according to frailty status enables a more accurate classification of health, supporting the delivery of more tailored and effective care within an ageing and heterogeneous population.

Frailty

Frailty, which is highly prevalent and observed in 20% to 30% of the older population over 75 years (5), 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 (6). Frailty has been shown to significantly elevate the risk of adverse outcomes of infectious diseases such as COVID-19 in older individuals. Consequently, it is crucial to elucidate which mechanisms may underlie the increased susceptibility to adverse outcomes to chronic and infectious disease in older patients with frailty.

There are many approaches to identifying frailty. Frailty may present as physical or psychological, or a combination of both (7). Common definitions of physical frailty include a specific phenotype model constructed from five components developed by Fried, comprising exhaustion, unintentional weight loss, low muscle strength, slow walking speed, and low physical activity, which is used as a dichotomous scale where individuals are categorized as frail or not based on specific criteria thresholds (6). Although a number of frailty definitions have been developed, Fried et al's description of the frailty phenotype remains the reference frame in geriatric medicine. Studies have demonstrated that the frailty phenotype was associated with negative health outcomes, including mortality and morbidity (8). However, there are no standardized definitions of how frailty should be measured, and studies use many different methods to measure and define frailty generally. A commonly used tool is the Clinical Frailty Scale (CFS), with a continuous scoring system, enhancing its utility in clinical settings by enabling the quantification of frailty along a spectrum rather than as a binary state. Moreover, the CFS is well-validated and has been used in various settings (9).

Immunosenescence and frailty

It has been well established that the immune system becomes compromised with age and is further altered with frailty. Age-related changes occur in both arms of immunity, innate and adaptive, a phenomenon collectively known as immune senescence (10). Studies have revealed this phenomenon to result in an increased frequency and severity of infection, as well as in lower immune surveillance of malignant cells, a reduced discrimination between self and non-self, and a decreased efficacy of vaccination in older individuals (11, 12).

The innate immune system, serving as the primary defense mechanism against injuries and infections, provides an immediate reaction to external stressors. It plays a crucial role in shaping immune responses against infections and inducing inflammation. The innate immunity is an antigen-independent (non-specific) defense system that is triggered when a pathogen intrudes (13). The rapid recruitment of immune cells occurs through the production of cytokines and chemokines. Cytokine production

during innate immunity induces many defense mechanisms while also activating local cellular responses to infection or injury, through the mobilization and activation of antigen-presenting cells (APCs). Dysregulation of the inflammatory cytokines results in a state of chronic, low-grade, sterile inflammation, also called inflammageing, which is often associated with chronic inflammatory or autoimmune diseases. In addition, ageing affects key cellular functions of macrophages, neutrophils, and natural killer cells, reducing their phagocytic and cytotoxic activities (14, 15). This diminished capacity not only slows the initial immune response to pathogens but also affects the regulation of inflammatory processes. Inflammageing is also 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 (11).

Unlike the components of the innate immune system that are present before the onset of infection, in adaptive immunity, the immune response is triggered by antigen recognition and results in the formation of memory cells (16). The adaptive immunity is critical when innate immunity is insufficient in eliminating infectious agents. It is based on the recognition of “non-self” antigens, inducing pathogen-specific immunologic effector pathways that eliminate specific pathogens. This process results in the development of an immunologic memory that can eliminate a specific pathogen rapidly upon subsequent infections. The cells of the adaptive immune system include antigen-specific T cells, which are activated to proliferate through the action of APCs, and B cells, which produce antibodies. The function and regulation of adaptive immunity rely on the interaction between fully matured dendritic cells, T cells, cytokines, and various signaling molecules (10).

Immunosenescence reflects age-related changes in the innate and adaptive immune system. The adaptive immune response is notably compromised due to thymic atrophy that occurs with increasing age. The thymus is critical for the maturation of T-cells, and its diminished function results in a reduced output of naïve T-cells. This leads to a decreased diversity in the T-cell receptor repertoire, impairing the body’s ability to respond to new antigens (17). Moreover, the existing T-cells often exhibit a phenomenon known as replicative senescence, whereby repeated stimulation causes them to lose their proliferative capacity and enter a state of functional exhaustion. The B-cell lineage also undergoes significant alterations with age. There is a shift in B-cell subsets, with a noticeable decrease in naïve B-cells and an increase in memory B-cells. Consequently, older adults often show a poorer response to new infections and vaccines, and a higher prevalence of autoantibody production (18).

The relationship between frailty and the phenomena of immunosenescence and inflammageing remains a significant area of research, with many aspects still unclear.

While ageing influences the immune system, leading to both phenotypical and functional changes, how these alterations specifically contribute to the development of frailty is not well-defined. The intersection of these processes is crucial for understanding why some individuals age more successfully than others and what role the immune system plays in this divergence. Despite the recognized link between a declining immunity and increased vulnerability to age-related diseases, the direct pathways connecting immunosenescence and inflammageing to the physiological state of frailty have yet to be fully elucidated. Addressing these knowledge gaps is essential not only for advancing therapeutic strategies to enhance healthy ageing and reducing frailty-related complications but also for guiding treatment decisions, as frail patients may respond differently to therapies in terms of both effectiveness and risk of side effects.

The role of immunosenescence and frailty in COVID-19 and cancer

This thesis focuses on two significant diseases of modern times: COVID-19 and cancer, both of which are highly influenced by the age-related immune decline.

The COVID-19 pandemic has disproportionately affected older adults, who not only face heightened susceptibility to SARS-CoV-2 but also exhibit reduced vaccine responsiveness (19-21). Previous studies discussed immunosenescence and inflammageing as key factors influencing vulnerability to novel pathogens such as the SARS-CoV-2 virus (22, 23), highlighting the critical interplay between age-related frailty and the body's inflammatory response to the virus.

This reduced immune responsiveness is particularly problematic in older individuals who face higher mortality rates. The severe disease progressions and poorer outcomes observed in the ageing population underscore the need for research into how ageing-related changes in the immune system and frailty contribute to the severity of infections like COVID-19.

Among chronic diseases, cancer remains one of the principal causes of death in older populations. In cancer, the relationship between immunosenescence and disease progression is similarly critical. The ageing immune system not only becomes less efficient at surveilling and eliminating cancer cells but may also respond less effectively to cancer treatments that rely on immune activation (24). Moreover, processes related to defective wound repair may trigger carcinogenesis (25). Immunosenescence may thus compromise the effectiveness of these therapies, which are designed to harness the body's immune response to target and kill cancer cells.

Aim of this thesis

The overall aim of this thesis is to uncover how age-related changes in the immune system are associated with frailty and disease outcomes of two prevalent health challenge paradigms: cancer (more specifically, melanoma) and COVID-19. These conditions are particularly relevant as they represent significant health challenges in the older population, cancer being the leading cause of death, and COVID-19 exemplifying the high vulnerability of older adults to infectious diseases. This exploration is important for developing targeted interventions that could improve the quality of life in older patients.

Study populations

To address our research question within the COVID-19 paradigm, we used data from three cohorts in the Netherlands: COVID-OLD, COVID-Predict and CliniCo.

The **COVID-OLD study** is a retrospective multicenter cohort study that included patients aged 70 years and older who were hospitalized with COVID-19 from 27 February to 14 May 2020 in the Netherlands. Data were collected from 19 Dutch hospitals. The **Covid-Predict study** is a consortium of hospitals that aims to understand and predict COVID-19-related outcomes and to evaluate treatment options. Data were collected from 9 Dutch hospitals. The **CliniCo study** is a multicenter prospective cohort study that aims to describe clinical characteristics, disease course, and outcome of patients with COVID-19 and aims to develop diagnostic and prognostic prediction models for COVID-19. Data were collected from 6 Dutch hospitals.

To address our research questions within the cancer paradigm, we first used the prospective tumor-specific **T-Cell IMMunity in patients with solid tumors study** (TCIMM study). This prospective observational cohort study aimed to understand the immune factors related to the efficacy and side effects of immunotherapy in treated cancer patients by performing an in-depth analysis of systemic and intra-tumoral immune parameters using blood, tumor, intestinal and faecal samples.

Finally, we used three ongoing studies: 1) **The Triage of Elderly Needing Treatment study** (TENT study), 2) **IMmunotherapy in AGINg patiEnts study** (IMAGINE study), and 3) **The Tolerability and safety of immunotherapy study** (ImToSa study).

The **TENT study** is a prospective study. Patients who were candidates for intensive treatments (chemotherapy, (chemo-)radiation therapy, or major surgery) underwent frailty screening based on the Geriatric 8 (G-8) questionnaire and the Six-Item Cognitive Impairment Test (6CIT). If screening revealed potential frailty, a conventional geriatric assessment was performed. The study aimed to investigate

associations between geriatric characteristics and outcomes of treatment that are relevant to older patients.

The **IMAGINE study** is a prospective cohort study in patients who were treated in the LUMC with immunotherapy. Outcomes, including toxicity and hospitalization, were prospectively registered from medical charts.

The **ImToSa study** is a partly retrospective cohort study; some patients were included after treatment, and others before treatment. All patients treated with immunotherapy were included in our study population. The initial aim of the study was to assess age-related differences in side-effects and predictors of toxicity, including sex, geriatric characteristics, and previous treatments.

Outline of this thesis

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. In **Chapter 2**, a systematic review of current literature was conducted to scope current understanding of the immunological determinants of frailty, potentially leading to the development and delivery of more effective care for older individuals.

During the COVID-19 pandemic, older hospitalized patients faced a significant mortality risk, emphasizing the importance of understanding the interplay between frailty and inflammatory response to the SARS-CoV-2 virus. In **Chapter 3**, the association of frailty with inflammatory markers and its role in the relationship between inflammatory markers and in-hospital mortality among older patients hospitalized for COVID-19 was investigated.

Immunotherapy with checkpoint inhibition (ICI) is increasingly prescribed in older patients with cancer. High age, especially in combination with frailty, has been associated with immune senescence, thereby possibly hindering ICI effectiveness. In **Chapter 4**, the association between blood cell immune senescence markers and age, frailty and response to anti-PD-1 immunotherapy was investigated in older patients with metastatic melanoma.

Although ICI has been established as a promising treatment strategy for patients with cancer, older cancer patients represent a heterogeneous group as they can vary widely in frailty, cognition and physical status. In **Chapter 5**, the association between clinical frailty and immune-related treatment toxicity (IrTox), hospitalization and treatment discontinuation due to IrTox in older patients treated with checkpoint inhibitors was investigated.

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2

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.

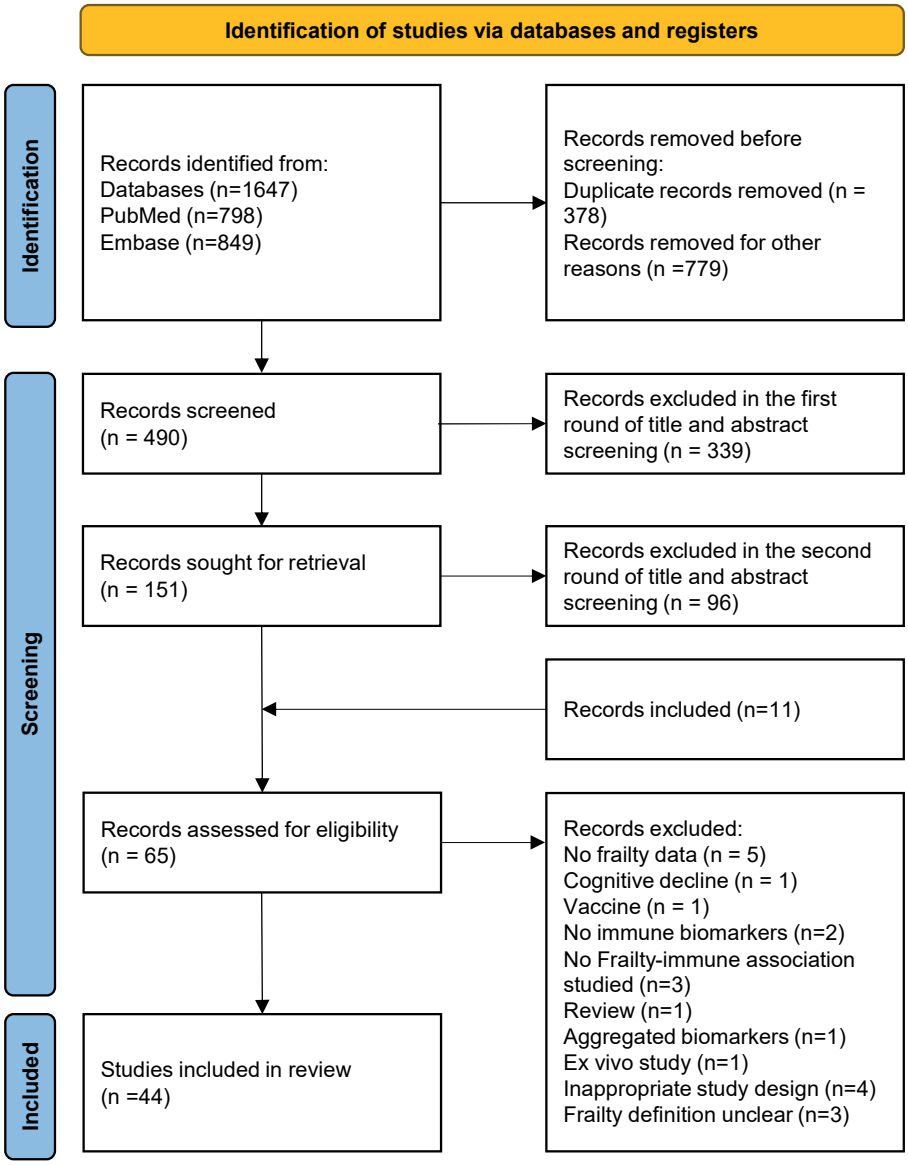
RESULTS

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.

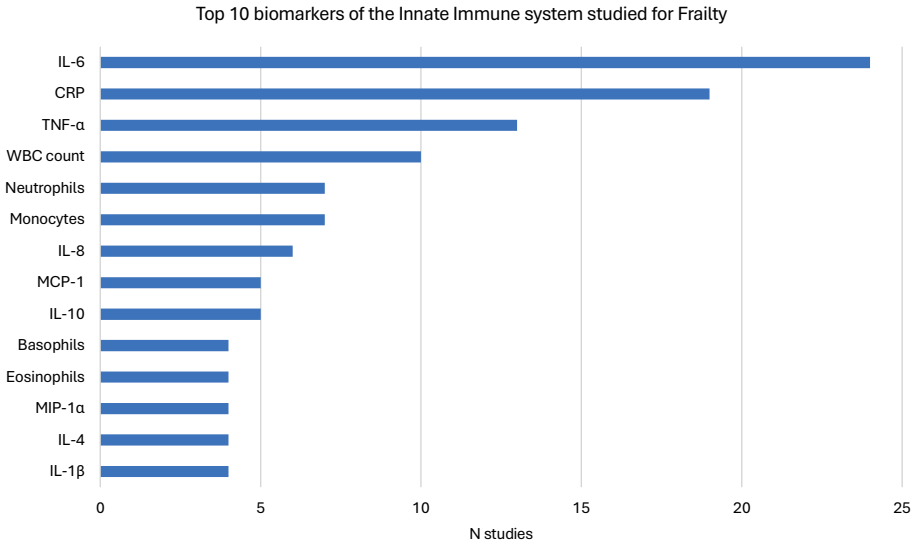
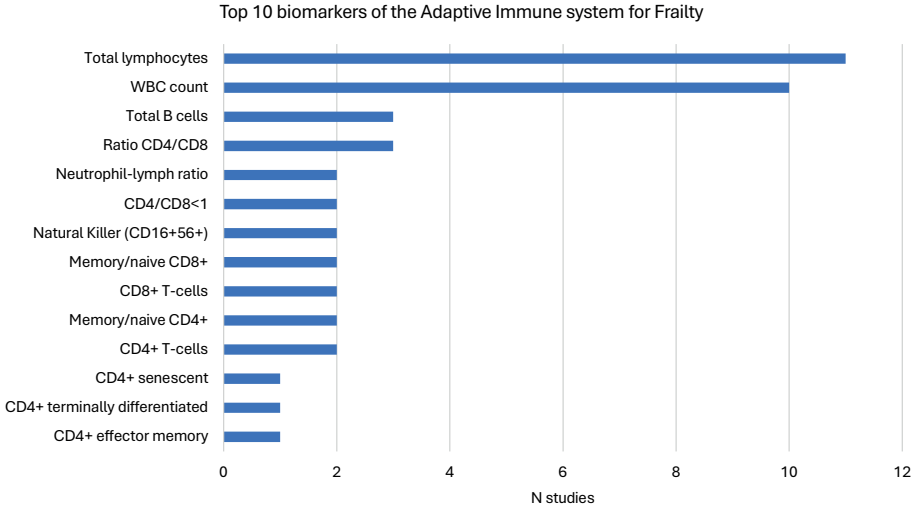


Figure 3. Graphic describing the most studied biomarkers of the adaptive immune system for frailty.



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

Acknowledgments

We would like to thank José W. M Plevier, librarian at Leiden University Medical Center, for her assistance in the search strategy.

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.

Funding

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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.

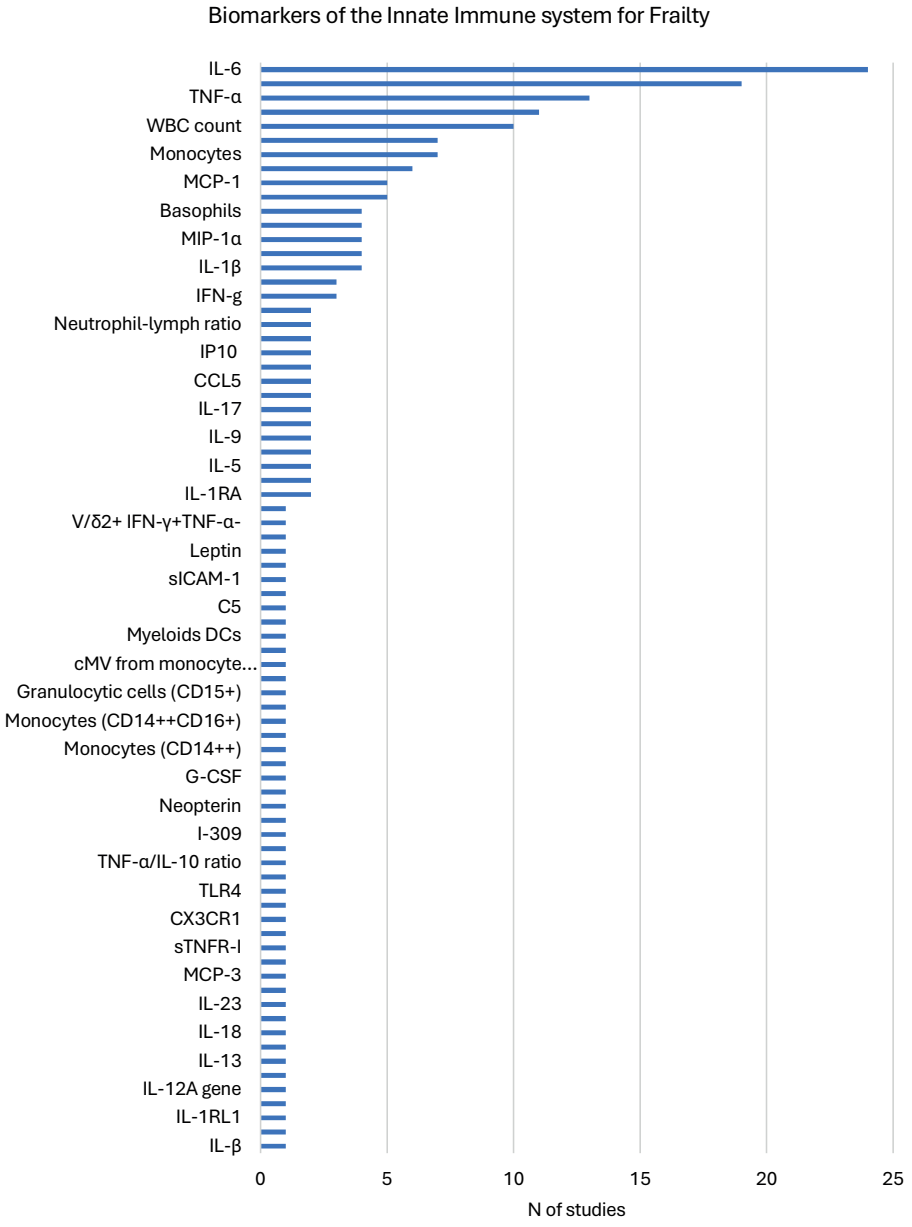


Figure S2. Graph describing the number of studies investigating the association between biomarkers of the adaptive immune system and frailty.

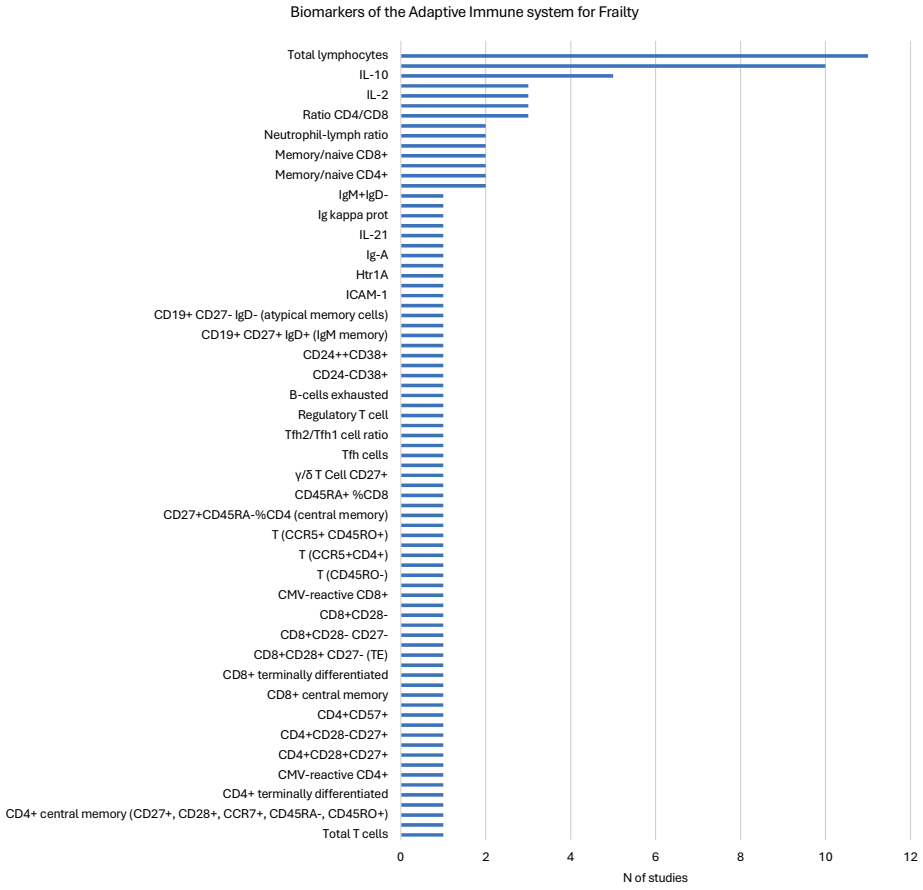
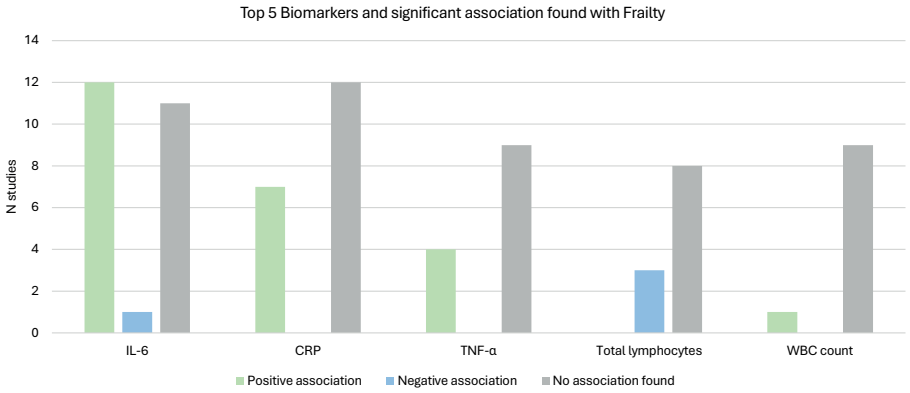


Figure S3. Graph describing the number of studies demonstrating significant associations between the most studied immune biomarkers and frailty.



3

The association of inflammatory markers with frailty and in-hospital mortality in older COVID-19 patients

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ABSTRACT

Introduction

During the COVID-19 pandemic, older patients hospitalized for COVID-19 exhibited an increased mortality risk compared to younger patients. While ageing is associated with compromised immune responses and frailty, their contributions and interplay remain understudied. This study investigated the association between inflammatory markers and mortality and potential modification by frailty among older patients hospitalized for COVID-19.

Methods

Data were from three multicenter Dutch cohorts (COVID-OLD, CliniCo, Covid-Predict). Patients were 70 years or older, hospitalized for COVID-19 and categorized into three frailty groups: fit (Clinical Frailty Score (CFS) 1-3), pre-frail (CFS 4-5), and frail (CFS 6-9). Immunological markers (lymphocyte count, neutrophil count, C-reactive protein, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic inflammation index (SII)) were measured at baseline. Associations with in-hospital mortality were examined using logistic regression.

Results

A total of 1697 patients were included from COVID-OLD, 656 from Covid-Predict, and 574 from CliniCo. The median age was 79, 77, and 78 years for each cohort. Hospital mortality rates were 33%, 27% and 39% in the three cohorts, respectively. A lower CRP was associated with a higher frailty score in all three cohorts (all $p<0.01$). Lymphocyte count, neutrophil count, NLR, PLR, or SII, were similar across frailty groups. Higher CRP levels were associated with increased in-hospital mortality risk across all frailty groups, across all cohorts (OR (95% CI), 2.88 (2.20 - 3.78), 3.15 (1.95 - 5.16), and 3.28 (1.87 - 5.92)), and frailty did not modify the association between inflammatory markers and in-hospital mortality (all p-interaction>0.05).

Conclusion

While frailty is a significant factor in determining overall outcomes in older patients, our study suggests that the elevated risk of mortality in older patients with frailty compared to fit patients is likely not explained by differences in inflammatory responses.

INTRODUCTION

Older patients hospitalized with COVID-19 had a higher mortality risk during the pandemic compared to younger patients (1, 2). Numerous studies showed that age and frailty were independently associated with adverse disease outcomes in older patients, with those assessed as frail on the Clinical Frailty Scale (CFS) experiencing the highest mortality rates in the initial pandemic waves compared to the non-frail patients. (3-5).

Frailty is associated with age-induced immunosenescence that leads to reduced immune response and chronic low-grade inflammation, also called inflammageing. This increases the vulnerability to novel infections, like SARS-CoV-2 virus (6-9). Immunosenescence and inflammageing contribute to frailty status and adverse health outcomes (7,10). Frailty and mortality in the general out-patient population have been associated with higher levels of pro-inflammatory markers, including CRP and the systemic immune-inflammation index (SII), which is a ratio based on neutrophil, lymphocyte, and platelet counts (3, 9-14). These immune markers have also been associated with worse COVID-19 outcomes in hospitalized patients (15). Interestingly, in the COVID-OLD study, higher CRP was associated with increased risk of mortality, but CRP levels at admission were lower in frail patients compared with non-frail patients (3). This counterintuitive finding raises the question of whether a lower CRP in frail patients indicates a lower inflammatory response to acute infection compared to fit patients, potentially due to compromised immune responses. Given this contradiction in previous findings, along with the complex interplay between frailty and the immune system's response to infection, the precise mechanisms by which frailty influences these dynamics remain unclear. Thus, there is a crucial need for an understanding of the dynamic interplay between frailty and inflammatory response to the SARS-CoV-2 virus. Addressing this knowledge gap is essential for tailoring more effective clinical assessment and interventions for this vulnerable population.

Therefore, this study aims to investigate the association of frailty with various inflammatory markers in patients hospitalized for COVID-19 aged 70 years and over. Additionally, it aims to elucidate the role of frailty in the relationship between these markers and in-hospital mortality.

METHODS

Study design

Data were used from three multicenter cohorts in the Netherlands: COVID-OLD, Covid-Predict and CliniCo (Figures S1). All databases included data collected retrospectively or in real-time from patients hospitalized for COVID-19. No additional diagnostic test or intervention was studied, and treatment followed national and/or local guidelines.

Study participants

We included patients aged 70 years and older. Diagnosis of COVID-19 was defined by a positive reverse-transcriptase polymerase chain reaction (PCR) test for SARS-CoV-2 from an oropharyngeal and/or nasal swab (CliniCo), or diagnosis based on symptoms and typical radiological findings (Covid-Predict and COVID-OLD). Patients transferred from and to other hospitals were excluded to prevent overlap in cohorts and because admission data could be limited or missing. One hospital was excluded from the Covid-Predict study dataset due to missing data and not enough investigators available to complete the dataset. Patients were included from 27th February 2020 (first case of COVID-19) till the 8th of January 2021 (first SARS-CoV-2 vaccination in the Netherlands) to exclude the effect of vaccination on primary outcomes. Data from the three cohorts were analyzed separately.

Cohorts

COVID-OLD

The COVID-OLD study is a partly prospective and retrospective multicenter cohort study that included patients aged 70 years and older who were hospitalized with COVID-19. Data were collected from 19 Dutch hospitals (Figure S1). The medical ethics committees of all hospitals waived the necessity for formal approval of the study, as data collection followed routine practice. The inclusion criteria were patients aged ≥70 years and hospitalized with diagnosed COVID-19. Patients were excluded if they were not initially admitted for COVID-19 symptoms, but were infected in the hospital during admission for another illness (patients with positive PCR ≥ 24 hours after admission were excluded) (3).

Covid-Predict

The Covid-Predict study is a consortium of hospitals that aims to understand and predict COVID-19-related outcomes and to evaluate treatment options (16). Data were collected from 9 Dutch hospitals (Figure S1). The inclusion criteria were

patients ≥18 years old, hospitalized with COVID-19. The medical ethics committee approved the study protocol (AUMC 20.131). The need for informed consent was waived; an opt-out procedure was communicated through written information in accordance with national guidelines and the European privacy law, meaning that chart data were available unless a patient explicitly objected.

CliniCo

The CliniCo study is a multicenter prospective cohort study that aims to describe clinical characteristics, disease course and outcomes of patients with COVID-19, and aims to develop diagnostic and prognostic prediction models for COVID-19. Data were collected from 6 Dutch hospitals (Figure S1). The inclusion criteria were adult patients ≥18 years old, with PCR-confirmed infection with SARS-CoV-2, who were admitted for at least 24 hours between March and May 2020. This study was not subject to the Medical Research Involving Human Subjects Act (WMO) in the Netherlands, and was approved by the institutional review board (IRB) of the Radboud University Medical Center (number 2020-2923 and 2020-6344). According to the IRB, only oral consent was required. Oral consent was obtained from all patients or their families and documented in the electronic medical records.

Setting

Patient data, including biochemical data, from the initial medical assessment in the hospital, was used. Patient data included data collected during the emergency department visit, both from the primary evaluation and any subsequent assessments, as well as from direct admissions to the hospital ward for patients coming from an outpatient setting.

Data collection

Demographic Data

In all cohorts, data were partly prospectively and retrospectively collected from electronic health records. Patient characteristics included age, sex, height, weight, BMI, medical history and comorbidities.

Clinical Frailty Scale

During the first COVID-19 wave, national guidelines were developed to optimize the use of Intensive Care Unit (ICU) capacity and prevent scarcity. These guidelines promoted the use of the Clinical Frailty Scale (CFS) to assess the physiological reserve capacity of a patient to better estimate the potential impact of intensive care treatment, including survival and quality of life (17). The CFS was determined prospectively

during the first patient contact, or when a consultation with a geriatrician/internist geriatrician was performed. However, in a number of patients, the CFS scores were retrospectively added according to a Standard Operating Procedure (SOP) (Table S4). The SOP aims to standardize the retrospective assignment process and is crucial for minimizing variation in how CFS scores are adjudicated across different evaluators. The use of the SOP was particularly targeted at those patients for whom direct assessment was unfeasible, thereby supporting the consistency of frailty evaluations across the entire cohort. Previous studies have validated the reliability of retrospective CFS assignment (18-21). Through these measures, we aimed to maintain the integrity of our frailty assessments, ensuring that they reflect a true cross-section of the COVID-19 patient population and not just those who are visibly more severely affected or more likely to require imminent ICU admission. According to the Dutch guidelines, three CFS groups were categorized: fit (CFS 1-3), pre-frail (CFS 4-5), and frail (CFS 6-9) (22).

Clinical and laboratory Data

COVID-19-related parameters were registered, including day of admittance since start of symptoms, vital signs, temperature, use of oxygen and invasive ventilation. Laboratory results were collected within 24h of admission, including blood cell counts, CRP, and parameters including renal and liver function. The neutrophile/lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by the lymphocyte blood count (x10^9/L). The platelet/lymphocyte ratio (PLR) was calculated for the absolute peripheral platelet and lymphocyte blood counts. The systemic immune-inflammation index (SII) was calculated by multiplying platelets and NLR (15). Information was retrieved from the electronic health records on whether a patient received antimicrobial treatment (suggestive of bacterial superinfection) during hospital admission, was admitted to the ICU, received invasive mechanical ventilation treatment and on length of hospital stay, ventilation and ICU stay. In-hospital mortality was registered.

Three hospitals were included in multiple cohorts (Figure S1). To prevent double inclusion, hospitals were included in the cohort analyses from Covid-Predict where the analyses were conducted first, and excluded from the other cohort.

Outcome

The primary outcome was in-hospital mortality, defined as patients who were deceased during admission. The secondary outcome was frailty, measured with the CFS.

Statistical analysis

Data from each cohort were analyzed separately. Continuous data are presented as median (IQR) and categorical data by number (percentage). Since clinical reference ranges are unknown for inflammation ratios, we used tertiles to classify the inflammation markers into three categories (low/middle/high). The tertiles were determined in the Covid-Predict cohort, and the same cut-off values were used in the other datasets. Multivariable logistic regression analysis was used with in-hospital mortality as the dependent variable. The multivariable model included age, sex, and duration of symptoms as covariates. In the COVID-OLD cohort, data on immunosuppressive medication use were not recorded. As the use of immunosuppressive medication can influence inflammation and biochemical parameters, a sensitivity analyses were additionally performed in Covid-Predict and CliniCo, adjusting the model for immunosuppressive medication. Results are presented as median with interquartile range (IQR), number and percentage, or ORs with 95% confidence intervals (CIs). Variables with missing values to a limit of 10% were complemented with imputed data using multiple imputation. Statistical analyses were performed using R (v3.6.1).

RESULTS

Patient baseline characteristics

A total of 2927 patients were included (Table 1). 1697 patients from COVID-OLD, 656 from Covid-Predict, and 574 from CliniCo. Median ages were similar across three cohorts, with COVID-OLD patients being 79 years (IQR: 75 – 84 years), Covid-Predict being 77 years (IQR: 73-81 years), and CliniCo being 78 years (IQR: 74-82 years). Each cohort had a majority of male patients. COVID-OLD 44% fit, 26% pre-frail, and 30% frail patients. Covid-Predict had a distribution of 42% fit, 34% pre-frail and 24% frail patients. CliniCo presented a similar pattern with 42% fit, 37% pre-frail and 22% frail patients. Baseline characteristics were comparable amongst all three cohorts.

Table 1. Baseline characteristics

	COVID-OLD	Covid-Predict	CliniCo
	n=1697	n=656	n=574
Demographics			
Age (median [IQR])	79 [75-84]	77 [73-81]	78 [74-82]
Sex male (n, (%))	1013 (60)	417 (63.6)	372 (64.8)
BMI (median [IQR])	26.3 [23.7-29.5]	26.71 [24.0-30.1]	26.88 [24.2-30.3]
Days since onset disease (median [IQR])	6 [3-10]	7 [4-10]	7 [4-10]
SarsCov19 PCR positive (n, (%))	1655 (97.5)	631 (96.2)	555 (96.7)
Clinical Frailty Scale			
Fit (CFS 1-3) (n, (%))	745 (44.0)	276 (42.1)	239 (41.6)
Pre-frail (CFS 4-5) (n, (%))	447 (26.3)	223 (34.0)	210 (36.6)
Frail (CFS 6-9) (n, (%))	505 (29.8)	157 (23.9)	125 (21.8)
Comorbidities			
Hypertension (n, (%))	923 (54.4)	395 (60.2)	299 (52.1)
Chronic pulmonary disease (n, (%))	328 (19.4)	127 (19.4)	82 (14.3)
Diabetes (n, (%))	530 (31.2)	242 (36.9)	156 (27.2)
Chronic cardiac disease (n, (%))	56 (3.3)	241 (36.7)	353 (61.5)
Malignancy (n, (%))	238 (14.0)	66 (10.1)	22 (3.8)
Vital signs			
Systolic blood pressure (median [IQR]) (mmHg)	137 [122-152]	135 [120-150]	137 [122-152]
Diastolic blood pressure (median [IQR]) (mmHg)	74 [65-84]	76 [66-85]	75 [65-84]
Respiratory rate (median [IQR]) (breaths/min)	21 [18-26]	23 [18-27]	23 [19-27]
Oxygen saturation (median [IQR]) (L/min)	96 [94-98]	94 [91-96]	94 [92-96]
Regular laboratory measurements			
Haemoglobin (median [IQR])	8.2 [7.4-8.8]	8.1 [7.4-8.8]	8.3 [7.5-9.0]
Platelet count (median [IQR]) (10^9/L)	190 [150-250]	203 [162.3-277]	199 [157-266]
Creatinine (median [IQR]) (µmol/L)	94 [74-131]	91 [71-121]	93 [70-123]
LDH (median [IQR]) (U/L)	309 [242-405]	337 [270-439]	353 [276-454]

Abbreviations: BMI, Body Mass Index; PCR positive, Polymerase chain reaction positive; CFS, Clinical Frailty Scale; LDH, Lactate Dehydrogenase; N, number; NR, non-recorded

In-hospital outcomes and treatment medications

The length of hospital stay was a median 6 days across all cohorts (Table 2). Hospital mortality rates were 33% in COVID-OLD, 27% in Covid-Predict, and 39% in CliniCo.

During the period of inclusion, the national COVID-19 treatment guidelines changed. First, (hydroxy-)chloroquine was opted as a potential treatment during the first COVID-19 wave. CliniCo included patients during this period, thus most patients received (hydroxy-)chloroquine as treatment (23). As more intervention studies were published, the focus shifted to corticosteroids as the cornerstone of the treatment of COVID-19 (24). However, treatments did not vary across each frailty group.

Table 2. In-hospital outcomes and treatment medication for older hospitalized COVID-19 patients in cohorts COVID-OLD, Covid-Predict and CliniCo

	COVID-OLD	Covid-Predict	CliniCo
	n=1697	n=656	n=574
Outcomes			
In-hospital mortality (n, (%))	560 (33.0)	180 (27.4)	222 (38.7)
Length of hospitalization in days (median [IQR])	6 [4-11]	7 [4-13]	6 [3-10]
ICU or medium care admission (n, (%))	150 (8.8)	132 (20.1)	65 (11.3)
Treatment medication			
Immunosuppression medication (n, (%))	NR	50 (7.6)	62 (10.8)
Corticosteroids (n, (%))	NR	374 (57.0)	75 (13.1)
Remdesivir (n, (%))	136 (8.0)	113 (18.0)	2 (0.3)
Chloroquine (n, (%))	470 (27.7)	64 (9.8)	313 (54.5)
Antibiotic use in the first seven days (n, (%))	1245 (73.4)	384 (58.5)	433 (75.4)

Abbreviations: N, number; NR, non-recorded; IQR, interquartile range

Levels of inflammatory markers between frailty patient groups

For all three cohorts, lymphocyte count, neutrophil count and NLR were comparable between fit, pre-frail and frail patients (Table 3, all $p>0.10$). PLR and SII values also did not differ across frailty groups in COVID-OLD and COVID-predict, but in CliniCo, values were lower in the pre-frail patients ($p<0.05$). CRP levels were consistently lower in patients with higher frailty levels in all three cohorts ($p <0.01$).

Association between inflammatory markers and risk of in-hospital mortality

The association between tertiles of inflammatory markers and risk of in-hospital mortality are displayed in Table 4, corrected for age, sex, duration of symptoms till admission, and CFS groups (fit, pre-frail, frail). In the highest tertile of CRP, risk of in-hospital mortality was increased in all three cohorts (all p-values <0.01). No association was observed with lymphocyte count, PLR or SII and in-hospital mortality in all cohorts.

Table 3. Levels of inflammatory markers of older hospitalized COVID-19 patients stratified by Clinical Frailty Scale

Inflammatory markers (median [IQR])	N	Fit (CFS 1-3)	N	Pre-frail (CFS 4-5)	N	Frail (CFS 6-9)	P-value
Lymphocyte count (10^9/L)							
COVID-OLD	745	0.82 [0.59-1.30]	447	0.90 [0.60-1.40]	505	0.84 [0.58-1.30]	*0.33*
Covid-Predict	276	0.90 [0.60-1.24]	223	0.80 [0.52-1.15]	157	0.84 [0.51-1.14]	*0.1*
CliniCo	239	0.80 [0.51-1.00]	210	0.80 [0.60-1.20]	125	0.80 [0.50-1.10]	*0.35*
Neutrophil count (10^9/L)							
COVID-OLD	745	5.00 [3.55-6.87]	447	4.81 [3.30-6.68]	505	4.77 [3.40-6.65]	*0.60*
Covid-Predict	276	5.50 [4.10-8.36]	223	5.23 [3.50-7.22]	157	5.40 [3.64-7.90]	*0.17*
CliniCo	239	5.90 [3.95-8.10]	210	5.10 [3.68-7.03]	125	5.93 [3.80-8.40]	*0.12*
C-reactive protein (mg/L)							
COVID-OLD	745	79.00 [42.00-142.00]	447	69.50 [36.00-122.75]	505	63.00 [27.00-111.00]	*<0.01*
Covid-Predict	276	101.50 [54.48-158.25]	223	82.00 [40.85-132.40]	157	75.20 [38.00-128.00]	*<0.01*
CliniCo	239	109.00 [54.00-174.00]	210	81.00 [44.00 -140.00]	125	65.50 [31.00-118.00]	*<0.01*
Neutrophil-to-lymphocyte ratio							
COVID-OLD	745	6.20 [3.83-10.16]	447	5.80 [3.54-9.63]	505	6.23 [3.42-10.35]	*0.67*
Covid-Predict	276	7.13 [3.93-10.54]	223	6.36 [4.08-10.31]	157	6.86 [3.69-12.23]	*0.96*
CliniCo	239	7.18 [4.29-12.24]	210	6.16 [3.81-9.77]	125	6.46 [4.00-11.75]	*0.10*
Platelet-to-lymphocyte ratio							
COVID-OLD	745	231.25 [143.48-354.79]	447	217.78 [120.63-328.00]	505	220.95 [133.33-340.43]	*0.16*
Covid-Predict	276	266.13 [170.21-387.50]	223	268.00 [159.47-396.65]	157	264.91 [170.42-398.08]	*0.97*
CliniCo	239	291.60 [188.85-390.15]	210	229.09 [153.67-370.14]	125	241.62 [168.52-415.54]	*0.03*
Systemic immune-inflammation index							
COVID-OLD	745	1155.17 [655.07-2191.57]	447	1126.29 [612.72-2029.27]	505	1159.42 [601.30-2305.38]	*0.41*
Covid-Predict	276	1629.36 [757.74-2805.90]	223	1334.39 [673.62-2613.75]	157	1393.06 [744.55-2757.04]	*0.50*
CliniCo	239	1488.90 [830.69-2850.15]	210	1155.00 [631.07-2167.45]	125	1390.75 [717.71-2991.87]	*0.03*

Abbreviations: CRP, C-reactive Protein; N, number; IQR, interquartile range

Table 4. Risk of in-hospital mortality dependent on stratum of inflammatory markers in older hospitalized COVID-19 patients

		Stratum of inflammatory marker				
		Low	Middle		High	
	N	OR (95% CI)	OR (95% CI)	P-value	OR (95% CI)	P-value
Lymphocyte count (10^9/L)						
COVID-OLD	1697	Reference	0.73 (0.56-0.94)	*0.02*	0.83 (0.64-1.07)	*0.14*
Covid-Predict	461	Reference	0.63 (0.38-1.05)	*0.08*	0.54 (0.32-0.92)	*0.02*
CliniCo	469	Reference	1.03 (0.66-1.60)	*0.91*	0.67 (0.39-1.12)	*0.13*
Neutrophil count (10^9/L)						
COVID-OLD	1697	Reference	1.64 (1.27-2.11)	*<0.01*	2.27 (1.75-2.96)	*<0.01*
Covid-Predict	441	Reference	0.57 (0.33-0.99)	*0.05*	0.85 (0.50-1.43)	*0.54*
CliniCo	469	Reference	1.47 (0.92-2.34)	*0.11*	1.74 (1.07-2.82)	*0.03*
CRP (mg/L)						
COVID-OLD	1697	Reference	1.59 (1.23-2.07)	*<0.01*	2.88 (2.20-3.78)	*<0.01*
Covid-Predict	589	Reference	1.64 (1.00-2.74)	*0.05*	3.15 (1.95-5.16)	*<0.01*
CliniCo	501	Reference	1.86 (1.06-3.34)	*0.03*	3.28 (1.87-5.92)	*<0.01*
Neutrophil/lymphocyte ratio (NLR)						
COVID-OLD	1697	Reference	1.27 (0.99-1.64)	*0.06*	1.64 (1.28-2.12)	*<0.01*
Covid-Predict	431	Reference	0.90 (0.51-1.57)	*0.71*	1.22 (0.71-2.09)	*0.47*
CliniCo	469	Reference	1.02 (0.63-1.66)	0.93	1.48 (0.92-2.39)	*0.11*
Platelet/lymphocyte ratio (PLR)						
COVID-OLD	1697	Reference	0.77 (0.59-0.99)	*0.04*	1.02 (0.79-1.32)	*0.88*
Covid-Predict	461	Reference	1.22 (0.72-2.06)	*0.46*	1.10 (0.65-1.85)	*0.72*

		Stratum of inflammatory marker				
		Low	Middle		High	
	N	OR (95% CI)	OR (95% CI)	P-value	OR (95% CI)	P-value
CliniCo	467	Reference	0.88 (0.54-1.44)	*0.62*	0.98 (0.60-1.62)	*0.95*
Systemic immune-inflammation index (P*N/L, SII)						
COVID-OLD	1697	Reference	1.05 (0.82-1.34)	*0.69*		*0.60*
Covid-Predict	431	Reference	0.85 (0.49-1.48)	*0.57*	1.01 (0.59-1.75)	*0.96*
CliniCo	467	Reference	1.03 (0.64-1.64)	*0.91*	1.61 (0.99-2.65)	*0.06*

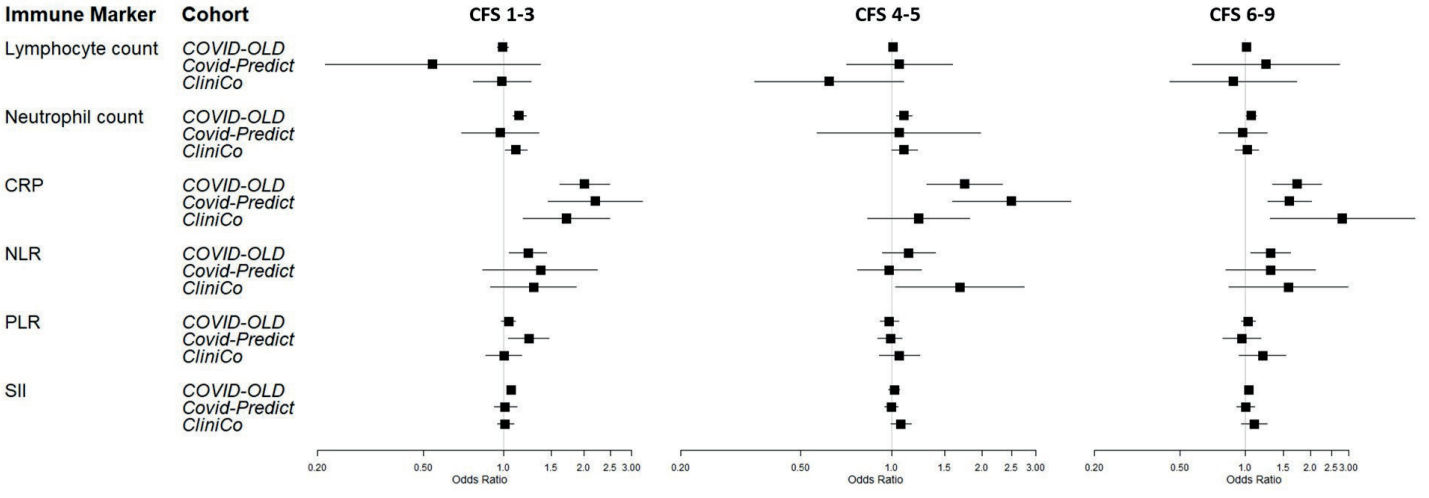
Abbreviations: CRP, C-reactive Protein; N, number; OR, odds ratio; NA, non-applicable

Table 4. Inflammatory markers were stratified using tertiles. P-values indicate difference compared to the reference category. Multivariable logistic regression adjusted for age, gender, duration of symptoms till admission, CFS (fit, pre-frail, frail). Inflammatory markers are displayed in tertiles with the following cut-off: CRP <57.3 = low tertile, 57.3 ≤ CRP ≤ 121 = mid tertile, CRP > 121 = high tertile; Lymphocyte count <0.66 = low tertile, 0.66 ≤ Lymphocyte count ≤ 1.06 = mid tertile, Lymphocyte count > 1.06 = high tertile; Neutrophil count < 4.38 = low tertile, 4.38 ≤ Neutrophil count ≤6.7 = mid tertile, Neutrophil count > 6.7 = high tertile; NLR < 4.8 = low tertile, 4.8 ≤ NLR ≤ 8.76 = mid tertile, NLR > 8.76 = high tertile; PLR < 203.7 = low tertile, 203.7 ≤ PLR ≤ 345 = mid tertile, PLR > 345 = high tertile; SII < 938.6 = low tertile, 938.6 ≤ SII ≤ 2169.4 = mid tertile, SII > 2169.4 = high tertile.

Association between inflammatory markers and risk of in-hospital mortality between frailty patient groups

To analyze the effect of frailty on the association between inflammatory markers and in-hospital mortality, we adjusted our models for age, sex, and duration of symptoms till admission. Figure 1 presents these associations, stratified by Clinical Frailty Scale (CFS), illustrating how different levels of frailty may modify the risk of in-hospital mortality associated with inflammatory markers. Higher levels of CRP were associated with an increased risk of in-hospital mortality in fit patients in all cohorts ($p<0.01$), and in pre-frail patients in COVID-OLD and Covid-Predict ($p<0.01$) and in frail patients only in the COVID-OLD cohort ($p<0.01$). A higher neutrophil count was associated with an increased risk of in-hospital mortality in fit and pre-frail patients compared to frail patients in the COVID-OLD cohort ($p<0.01$). In all frailty strata across the cohorts, lymphocyte count, NLR, PLR, and the SII demonstrated no association with in-hospital mortality risk. Furthermore, there was no interaction between inflammatory markers and frailty in all cohorts (all p-values for interaction > 0.05, table S1). Additional sensitivity analyses were performed in Covid-Predict and CliniCo. Results did not substantially differ after adjusting for the use of immunosuppressive medication compared to the initial analyses (Table S2 and S3).

Figure 1. Exploring the interaction between immune markers and frailty status in association with in-hospital mortality in older hospitalized COVID-19 patients



Abbreviations: CRP, C-reactive Protein; CFS, Clinical Frailty Scale

Multivariable logistic regression adjusted for age, gender, and duration of symptoms till admission. Patients are stratified by CFS (CFS score 1-3=fit patients, CFS score 4-5= pre-frail patients, CFS score 6-9= frail patients). Lymphocyte count (10^9/L) and Neutrophil count (10^9/L) are presented in one-unit per increase. CRP (mg/L) and inflammation ratio PLR are presented per 100-units per increase. Inflammation ratio SII is presented per 500-units per increase. Inflammation ratio NLR is presented per 10-units per increase.

DISCUSSION

In this study, we found an association of frailty with lower CRP levels compared to fit and pre-frail patients. Higher CRP levels were associated with an increased in-hospital mortality risk in all patients. Inflammation ratios NLR, PLR, SII and neutrophil and lymphocyte count were not consistently associated with frailty and in-hospital mortality across all cohorts. There was no interaction observed, indicating that the association of immune markers with mortality did not differ over strata of frailty.

During the initial stages of the COVID-19 pandemic, reports showed a correlation between frailty and increased mortality rates among older patients upon hospital admission (3, 25). In a recent study, researchers showed that routine laboratory parameters, including inflammation parameters such as lymphocyte count, PLR and NLR, predicted results of COVID-19 outcomes (26). Previous studies showed that high NLR, PLR, and low lymphocyte count were significant predictors of in-hospital mortality in COVID-19 patients (26). In the present study, these associations did not consistently extend across all cohorts of our study, but this discrepancy could be attributed to the higher age and greater frailty of the patients compared to those in our study. Previous research has linked these markers to various age-related diseases, including cancer and cardiovascular diseases (27, 28), yet their normal values remain undefined, complicating their clinical application.

Previous research in an outpatient setting showed that an elevated CRP was found to be prognostic of long-term mortality in older patients, and could be indicative of inflammageing (29-31). In the acute phase of SARS-CoV-2 infection, however, frail patients had lower CRP levels compared to fit patients (3). In frail patients subject to inflammageing, elevated levels of CRP and other inflammatory markers may be anticipated during an acute viral infection and may lead to uncontrolled inflammation (previously known as cytokine storm). However, due to the reduced physiological reserves characteristic of frailty, even minimal inflammation, with lower CRP compared to fit patients, could lead to significant damage. These minimal elevations may still precipitate adverse outcomes by initiating a cascade of inflammatory responses leading to death. On the other hand, frail patients tend to experience adverse outcomes, such as hospitalization and mortality, at lower levels of disease severity compared to their fitter counterparts. This observation, seen in the general emergency department population (32), suggests that frailty is associated with poorer outcomes even at milder stages of illness. Thus, it may not directly relate to the underlying biology of the immune system. This phenomenon should be considered as a possible explanation for the earlier hospital admission of frail patients. These findings highlight the complexities of frailty beyond inflammation-related factors, encompassing other determinants, including cognitive function, lung volumes,

atherosclerosis, sarcopenia, nutritional status, amongst others (33, 34). Also, CRP levels at the time of hospital admission might not yet reflect the peak levels of inflammation. Alternatively, lower CRP levels during infection could be a sign of immune paralysis in older frail patients. This reflection of immunosenescence may predispose older individuals to increased susceptibility to infections and a diminished response to vaccinations (35). Finally, our study showed that the association between CRP and mortality is similar for fit, pre-frail and frail patients.

This study has several limitations. First, administration of immunosuppressive medication was not documented in COVID-OLD, precluding adjustment in our analyses. However, a sensitivity analysis was conducted by adjusting for immunosuppressive medication in the Covid-Predict and CliniCo cohorts, which yielded results consistent with the analyses that did not adjust for immunosuppressive medication. Second, the CFS was determined in two ways: pro- and retrospectively. Literature has shown a strong correlation between retrospectively and prospectively assessed CFS scores, suggesting minimal impact on our findings (21). Additionally, we used the SOP to standardize the retrospective assignment process, minimizing variation in how CFS scores are adjudicated across different evaluators. Lastly, an individual patient data (IPD) meta-analysis was not performed, and the cohorts were not pooled, which could have enhanced the power and depth of our analysis. Preparation of standardized study design and data collection for future health problems or pandemics across all (academic) hospitals may facilitate future data sharing and studies, especially in novel infectious agents. The present study has several strengths. The study comprised a large number of patients in three separate multi-center studies from the first and second waves of the COVID-19 pandemic across the Netherlands, offering a comprehensive representation. The patients included in the three cohorts were admitted to various hospitals in the Netherlands, along with an extensive range of variables collected - including demographics, comorbidities, frailty, diseases, medications, and ICU admissions – which enhances the generalizability of the study's findings. The three cohorts allowed cross-validation of the findings.

While frailty is a significant factor in determining overall outcomes in older patients, our study suggests that the elevated risk of mortality in older patients with frailty compared to fit patients is likely not explained by differences in inflammatory responses.

DECLARATIONS

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Authors contribution

BFMR, BA, GP were involved in data collection and data management, which included direct access to and verification of the study data. Statistical analysis was performed by ETVH, BA, AK. All authors were involved in the interpretation of the study findings and critically revised the manuscript. ETVH, BA, SM, DVH and AS had final responsibility for the decision to submit for publication.

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SUPPLEMENTAL MATERIAL

Table S1. Exploring the interaction between immune markers and frailty status in association with in-hospital mortality in older hospitalized COVID-19 patients

	Fit (CFS 1-3)			Pre-frail (CFS 4-5)			Frail (CFS 6-9)				
	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	N	P for interaction with CFS
Lymphocyte count (10^9/L)											
COVID-OLD	745	0.99 (0.95-1.04)	*0.72*	447	1.01 (0.98-1.04)	*0.60*	505	1.01 (0.98-1.05)	*0.35*	1697	*0.67*
Covid-Predict	203	0.54 (0.21-1.37)	*0.16*	145	1.06 (0.71-1.59)	*0.83*	113	1.25 (0.57-2.73)	*0.51*	461	*0.04*
CliniCo	204	0.98 (0.77-1.26)	*0.88*	171	0.62 (0.35-1.09)	*0.12*	94	0.88 (0.45-1.73)	*0.70*	516	*0.84*
Neutrophil count (10^9/L)											
COVID-OLD	745	1.14 (1.08-1.21)	*<0.01*	447	1.10 (1.04-1.17)	*<0.01*	505	1.06 (1.01-1.12)	*0.02*	1697	*0.08*
Covid-Predict	197	0.97 (0.69-1.35)	*0.56*	134	1.06 (0.57-1.97)	*0.34*	110	0.97 (0.76-1.26)	*0.65*	441	*0.68*
CliniCo	204	1.11 (1.01-1.22)	*0.03*	171	1.10 (1.00-1.22)	*0.04*	94	1.02 (0.90-1.16)	*0.76*	516	*0.06*
CRP (mg/L)											
COVID-OLD	745	2.01 (1.62 -2.49)	*<0.01*	447	1.75 (1.31-2.33)	*<0.01*	505	1.73 (1.33-2.25)	*<0.01*	1697	*0.93*
Covid-Predict	256	2.20 (1.46-3.30)	*<0.01*	200	2.50 (1.59-3.93)	*<0.01*	133	1.60 (1.28-2.01)	*0.17*	589	*0.39*
CliniCo	217	1.72 (1.62-2.82)	*<0.01*	180	1.23 (0.83-1.81)	*0.30*	104	2.81 (1.30-6.09)	*0.01*	559	*0.25*
Neutrophil/ lymphocyte ratio											
COVID-OLD	745	1.23 (1.05-1.45)	*0.01*	447	1.14 (0.93-1.40)	*0.19*	505	1.31 (1.06-1.62)	*0.01*	1697	*0.82*
Covid-Predict	193	1.37 (0.83-2.24)	*0.20*	130	0.98 (0.77-1.25)	*0.88*	108	1.31 (0.81-2.10)	*0.25*	431	*0.57*

	Fit (CFS 1-3)			Pre-frail (CFS 4-5)			Frail (CFS 6-9)				
	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	N	P for interaction with CFS
CliniCo	204	1.29 (0.89-1.87)	*0.15*	171	1.68 (1.03-2.74)	*0.03*	94	1.58 (0.84-2.98)	*0.15*	516	*0.59*
Platelet/lymphocyte ratio											
COVID-OLD	745	1.04 (0.98-1.10)	*0.24*	447	0.98 (0.92-1.06)	*0.65*	505	1.03 (0.96-1.11)	*0.39*	1697	*0.81*
Covid-Predict	203	1.24 (1.04-1.47)	*0.20*	145	0.99 (0.90-1.08)	*0.75*	113	0.96 (0.79-1.18)	*0.71*	461	*<0.01*
CliniCo	203	1.00 (0.86-1.17)	*0.99*	171	1.06 (0.91-1.24)	*0.42*	93	1.20 (0.93-1.53)	*0.15*	513	*0.33*
Systemic immune-inflammation index											
COVID-OLD	745	1.01 (1.00-1.02)	*<0.01*	447	1.00 (1.00-1.01)	*0.33*	505	1.01 (1.00-1.01)	*0.06*	1697	*0.36*
Covid-Predict	193	1.01 (0.92-1.12)	*0.79*	130	1.00 (0.95-1.05)	*0.87*	108	1.00 (0.91-1.10)	*0.96*	431	*0.44*
CliniCo	203	1.01 (0.94-1.09)	*0.69*	171	1.07 (0.99-1.16)	*0.06*	93	1.10 (0.96-1.26)	*0.17*	513	*0.56*

Abbreviations: CRP, C-reactive Protein; N, number; OR, odds ratio

Table S1. Multivariable logistic regression adjusted for age, gender, duration of symptoms till admission. Lymphocyte count (10^9/L) and Neutrophil count (10^9/L) are presented in one-unit per increase. CRP and inflammation ratio PLR and SII are presented per 100-units per increase. Inflammation ratio NLR is presented per 10-units per increase. P-values of the interaction term between each inflammatory marker and CFS (score 1-9).

Table S2. Risk of in-hospital mortality depending on tertiles of inflammatory markers in older patients in Covid-Predict and CliniCo adjusted for immunosuppressive medication

		Stratum of inflammatory marker				
		Low tertile	Mid tertile		High tertile	
	N	OR (95% CI)	OR (95% CI)	P-value	OR (95% CI)	P-value
Lymphocyte count (10^9/L)						
Covid-Predict	461	Reference	0.64 (0.38-1.06)	*0.08*	0.55 (0.32-0.92)	*0.02*
CliniCo	457	Reference	0.79 (0.49-1.25)	*0.91*	0.65 (0.39-1.08)	*0.10*
Neutrophil count (10^9/L)						
Covid-Predict	441	Reference	0.56 (0.32-0.98)	*0.04*	0.82 (0.49-1.40)	*0.47*
CliniCo	457	Reference	1.44 (0.88–2.38)	*0.15*	1.76 (1.08-2.90)	*0.02*
CRP (mg/L)						
Covid-Predict	589	Reference	1.65 (1.00-2.75)	*0.05*	3.04 (1.88-5.00)	*<0.01*
CliniCo	487	Reference	1.48 (0.90-2.47)	*0.13*	2.86 (1.74-4.75)	*<0.01*
Neutrophil/lymphocyte ratio						
Covid-Predict	431	Reference	0.90 (0.51-1.58)	*0.71*	1.20 (0.70-2.06)	*0.51*
CliniCo	457	Reference	1.03 (0.62-1.70)	*0.92*	1.54 (0.97-2.48)	*0.07*
Platelet/lymphocyte ratio						
Covid-Predict	461	Reference	1.21 (0.72-2.06)	*0.47*	1.08 (0.64-1.83)	*0.77*
CliniCo	455	Reference	1.12 (0.69-1.80)	*0.65*	1.13 (0.69-1.86)	*0.62*
Systemic immune-inflammation index (P*N/L)						
Covid-Predict	431	Reference	0.84 (0.48-1.46)	*0.54*	0.99 (0.57-1.71)	*0.97*
CliniCo	455	Reference	1.09 (0.67-1.77)	*0.72*	1.59 (0.97-2.62)	*0.07*

Abbreviations: CRP, C-reactive Protein; N, number; OR, odds ratio

Table S2. Multivariable logistic regression adjusted for age, gender, CFS (fit, pre-frail, frail), duration of symptoms till admission and immunosuppressive medication in Covid-Predict and CliniCo. Inflammatory markers are displayed in tertiles with the following cut-off: CRP <57.3 = low tertile, 57.3 ≤ CRP ≤ 121 = mid tertile, CRP > 121 = high tertile; Lymphocyte count <0.66 = low tertile, 0.66 ≤ Lymphocyte count ≤ 1.06 = mid tertile, Lymphocyte count > 1.06 = high tertile; Neutrophil count < 4.38 = low tertile, 4.38 ≤ Neutrophil count ≤6.7 = mid tertile, Neutrophil count > 6.7 = high tertile; NLR < 4.8 = low tertile, 4.8 ≤ NLR ≤ 8.76 = mid tertile, NLR > 8.76 = high tertile; PLR < 203.7 = low tertile, 203.7 ≤ PLR ≤ 345 = mid tertile, PLR > 345 = high tertile; SII < 938.6 = low tertile, 938.6 ≤ SII ≤ 2169.4 = mid tertile, SII > 2169.4 = high tertile.

Table S3. Risk of in-hospital mortality depending on inflammatory markers in older patients stratified by Clinical Frailty Scale in Covid-Predict and CliniCo adjusted for immunosuppressive medication

	Fit (CFS 1-3)			Pre-frail (CFS 4-5)			Frail (CFS 6-9)			
	N	OR	P-value	N	OR	P-value	N	OR	P-value	P for interaction with CFS
Lymphocyte count (10^9/L)										
Covid-Predict	203	0.54 (0.25-1.16)	*0.15*	145	1.11 (0.62-1.99)	*0.70*	113	1.22 (0.61-2.44)	*0.56*	*0.03*
CliniCo	199	1.11 (0.72-1.60)	*0.55*	164	0.66 (0.34-1.15)	*0.17*	91	0.89 (0.44-0.72)	*0. 72*	*0.63*
Neutrophil count (10^9/L)										
Covid-Predict	197	0.96 (0.86-1.08)	*0.52*	134	1.06 (0.95-1.20)	*0.28*	110	0.97 (0.87-1.09)	*0.64*	*0.63*
CliniCo	195	1.12 (0.99-1.27)	*0.08*	160	1.09 (0.97-1.24)	*0.15*	90	1.06 (0.92-0.21)	*0.43*	*0.20*
CRP (mg/L)										
Covid-Predict	256	2.19 (1.46-3.29)	*<0.01*	200	2.47 (1.56-3.89)	*<0.01*	133	1.31 (0.82-2.07)	*0.25*	*0.28*
CliniCo	214	1.01 (1.00-1.01)	*<0.01*	174	1.00 (1.00-1.01)	*0. 37*	99	1.01 (1.00-1.02)	*<0.01*	*0.20*
Neutrophil/lymphocyte ratio										
Covid-Predict	193	1.35 (0.82-2.22)	*0.22*	130	1.00 (0.79-1.28)	*0.98*	108	1.34 (0.82-2.19)	*0.22*	*0.57*
CliniCo	201	1.02 (0.99-1.06)	*0.18*	165	1.05 (1.00-1.10)	*0.08*	91	1.06 (1.00-1.13)	*0.97*	*0.60*
Platelet/lymphocyte ratio										
Covid-Predict	203	1.23 (1.04-1.47)	*0.02*	145	0.98 (0.89-1.09)	*0.70*	113	0.97 (0.79-1.19)	*0.75*	*<0.01*
CliniCo	200	1.00 (1.00-1.00)	*0.94*	165	1.00 (1.00-1.00)	*0. 60*	90	1.00 (1.00-1.00)	*0.12*	*0.31*
Systemic immune-inflammation index (P*N/L)										
Covid-Predict	193	1.00 (0.98-1.02)	*0.82*	130	1.00 (0.99-1.01)	*0.95*	108	1.00 (0.98-1.02)	*0.94*	*0.44*
CliniCo	200	1.00 (1.00-1.00)	*0.81*	165	1.00 (1.00-1.00)	*0. 12*	90	1.00 (1.00-1.00)	*0. 13*	*0.53*

Abbreviations: CRP, C-reactive Protein; N, number; OR, odds ratio

Table S3. Multivariable logistic regression adjusted for age, gender, duration of symptoms till admission and immunosuppressive medication in Covid-Predict and CliniCo. Lymphocyte count and neutrophil count are presented per one-unit increase. CRP and inflammation ratios PLR and SII are presented per 100-units per increase. Inflammation ratio NLR is presented per 10-units per increase. P-values of the interaction term between each inflammatory marker and CFS (score 1-9).

Figure S1. Figure of the Netherlands with the hospitals included per cohort

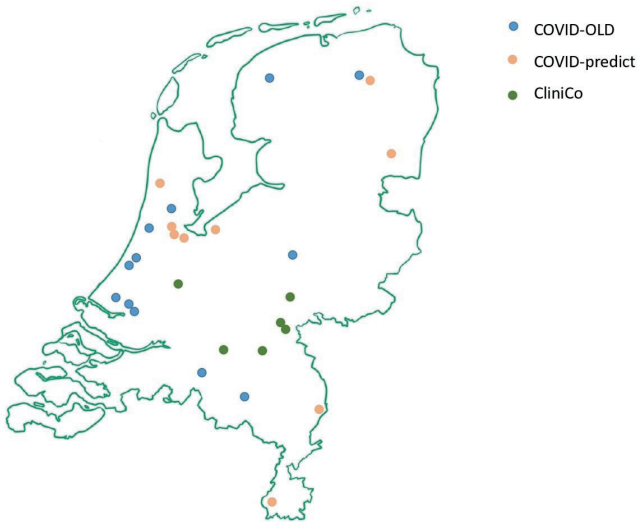

Figure S1. COVID-OLD: Alrijne Ziekenhuis (Leiderdorp), Catharina Ziekenhuis (Eindhoven), Deventer Ziekenhuis (Deventer), Erasmus Medical Center (Rotterdam), Elisabeth Tweesteden Ziekenhuis (Tilburg), Gelre Ziekenhuizen (Apeldoorn and Zutphen), Isala (Zwolle), Leiden University Medical Center (Leiden), Maasstad Ziekenhuis (Rotterdam), Medische Centrum Leeuwaarden (Leeuwarden), Reinier de Graaf Gasthuis (Delft), Spaarne Gasthuis (Haarlem), Sint Jansdal (Harderwijk), University Medical Center Groningen (Groningen), ZGT (Almelo) , Zaans Medisch Centrum (Zaandam). **CliniCo:** Bernhoven (Uden), Canisius Wilhelmina Hospital (Nijmegen), Jeroen Bosch Ziekenhuis (Den Bosch), Radboudumc (Nijmegen), Rijnstate (Arnhem), St Maartensklinie (Nijmegen). **Covid-Predict:** Amsterdam Medical Center (Amsterdam), VU University Medical Center (Amsterdam), Flevo Hospital (Almere), Martini Hospital (Groningen), Noordwest Ziekenhuisgroep (Alkmaar), OLVG (Amsterdam), TREANT (Drenthe-zuidoost Groningen), VieCurie Medical Center (Venlo-Venray).

Table S4. Standard operating procedure for frailty assessment

It concerns the frailty assessment of the patient before COVID-19, approximately in the two weeks before hospital admittance.

Clinical Frailty Scale (CFS)	Definition
1: Very fit	People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.
2: Well	People who have no active disease symptoms but are less fit than category 1. Often, they exercise or are very active occasionally, e.g. seasonally.
3. Managing well	People whose medical problems are well controlled, but are not regularly active beyond routine walking.
4. Vulnerable	While not dependent on others for daily help, often symptoms limit activities. A common complaint is being 'slowed up, and/or being tired during the day.
5. Mildly frail	These people often have more evident slowing, and need help in high order IADLs (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation, and housework.
6. Moderately frail	People need help with all outside activities and with keeping the house. Inside, they often have problems with stairs and need help with bathing and might need minimal assistance (cuing, standby) with dressing.
7. Severely frail	Completely dependent on personal care from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~6 months)
8. Very severely frail	Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.
9. Terminally ill	Approaching the end of life. This category applies to people with a life expectancy < 6 months who are not otherwise evidently frail.

The referral letter of the general practitioner often gives more information.
- use of a walking aid can be a clue for a CFS ≥ 4.
- presence of professional home care can be a clue for a CFS ≥ 5

- polypharmacy (5 or more different medication) can indicate a CFS ≥ 3
- the use of an in advance prepped medication roll (Baxter) indicates a CFS ≥ 5
- when family participates in informal care, this indicates a CFS ≥ 4
- when living in a nursing home, CFS ≥ 6.
- use of professional daytime activities can indicate a CFS ≥ 5
- a Body Mass Index indicating underweight indicates CFS ≥ 4
- dementia in medical history indicates a CFS ≥ 5

Concomitantly, the e-learning Radboudumc Clinical Frailty Scale (CFS) by Julian Lieverse was provided to train investigators to categorize patients according to the CFS. https://ru.h5p.com/content/1291038501098015477

4

Blood-based immune biomarkers associated with Clinical Frailty Scale in older patients with melanoma receiving checkpoint inhibitor immunotherapy

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ABSTRACT

Introduction

Immunotherapy with checkpoint inhibition (ICI) is increasingly prescribed to older patients with cancer. High age, especially in combination with frailty, has been associated with immune senescence, which is the age-related decline in immune function, thereby possibly hindering ICI effectiveness. This cross-sectional study aimed to assess whether blood cell immune senescence markers are associated with age, frailty and response to anti-PD-1 treatment in older patients with metastatic melanoma.

Methods

In a prospective observational study, sixty patients with stage IIIC or IV melanoma undergoing anti-PD1 treatment were categorized into young (<65 years; n=22), old (>65 years) without frailty (n=19), and old with frailty (n=19). In-depth immune cell phenotyping was performed in baseline blood samples (prior to treatment) using multispectral flow cytometry and compared between groups and with immunotherapy treatment response. Antigen-presenting cell capacity was evaluated using mixed lymphocyte reaction and T cell proliferative potential was assessed using PHA proliferation assay.

Results

No significant differences in treatment response rates were observed across age groups. Older patients, irrespective of frailty, showed lower levels of naïve CD8+ T cells, with the old and frail group also exhibiting reduced tissue-resident effector memory CD8+ T cells and CD8+ mucosal-associated invariant T (MAIT) cells. These differences were not associated with treatment outcomes. T cell proliferation and antigen-presenting cell capacities did not differ across groups.

Conclusion

Several ageing and frailty-associated changes were detected among circulating immune cells in blood, but were not associated with response to immunotherapy in our study. While these findings suggest that the level of frailty and ageing may not necessarily preclude the efficacy of ICI therapy, further investigation is needed to fully understand the impact of frailty and ageing on immunotherapy.

INTRODUCTION

The incidence of melanoma has rapidly increased over the past decades. In 2022, there were 331 722 cases worldwide and over 8000 cases in the Netherlands (1, 2). Of the worldwide newly diagnosed melanoma patients, 66% is 60 and 29% is 75 years or older, respectively (2). In recent years, immunotherapy with immune checkpoint inhibition (ICI) has become a promising treatment for various cancers, including advanced melanoma. Immunotherapy has significantly extended patient survival, particularly with the use of ipilimumab and nivolumab combination (3). Recent studies demonstrated that high age is associated with deterioration of the immune system, called immune senescence (4-6). One of the contributors to immune senescence is the shrinkage of the thymus and bone marrow, as well as skewing of immune cells to the myeloid lineage, resulting in substantial changes in diverse immune compartments. This is reflected by a progressive decline in the frequency of naïve T cells along with the accumulation of terminally-differentiated memory T cells, which was shown to induce melanoma growth and metastasis. Moreover, T cells express an immunosenescent phenotype characterized by reduced expression of CD27 and CD28 and higher expression of CD57, a major marker of immunosenescence. This immunosenescent phenotype has been associated with resistance to immunotherapy treatment (7) (Figure S1). Additionally, there is a decline in the transition from stem cell to subsequent pro-B and pre-B cell stages, resulting in decreased numbers of peripheral B cells exported from the bone marrow, which may compromise the humoral immunity in older patients (8). Simultaneously, myeloid-derived suppressor cell (MDSCs) numbers increase with ageing (9, 10). These cells may suppress the priming and reactivation of antigen-specific immune cell responses.

Importantly, several studies suggest that immune senescence is linked with frailty, a clinical state characterized by a decline in functioning across multiple physiological systems, accompanied by increased vulnerability to stressors resulting in high risk of poor health outcomes, incident disability, hospitalization and mortality (11). Individuals can be categorized as robust, pre-frail, or frail, based on the extent of their physiological and functional impairments. Frailty can result from multiple factors, including socio-demographic aspects (such as poverty, living alone, and low educational levels), psychological issues (like depression), nutritional deficiencies (such as malnutrition), polypharmacy, and chronic diseases (including inflammatory conditions, cancer, endocrine disorders, and dementia), as well as low levels of physical activity. Frailty is recognized as a dynamic condition that fluctuates over time. As frailty exists on a continuum, minor differences in frailty scores can impact patient outcomes. Emerging research shows that an elevated lymphocyte count has

been associated with frailty and especially with low physical activity and grip strength (4). Additionally, it was shown that lower frequencies of naïve CD4+ T cells and higher proportion of central memory CD8+ T cells were predictive of higher scores of the frailty index (12). It is possible that the efficacy of ICI, which relies on the initiation and reactivation of tumor-specific immunity, may be hampered due to frailty (13). Only a few studies have investigated the relation between immunological ageing, frailty and immunotherapy efficacy (14-18). These studies suggest that the ageing of the immune system compromises the adaptive immune response, particularly affecting T cells, which may reduce the effectiveness of immunotherapy (19). While previous studies showed no significant differences in efficacy or side effects of ICIs between young and older patients, it is important to note that these studies did not take differences between patients in terms of frailty or immunological ageing into account (20). Balancing the potential benefits of treatment, such as an increase in survival or a reduction of symptoms, against potential harms, including adverse side effects and risk of complications for older patients, is crucial during the decision-making process. Thus, it is important to identify better biomarkers of response to immunotherapy in older adults, as frailty can increase the impact of potential adverse events and hamper the eligibility of patients for immunotherapy. The aim of the exploratory study is to identify markers of immune senescence in older patients with metastatic melanoma, their association with calendar age and frailty, and to determine whether these markers of immune senescence are associated with clinical response to anti-PD-1 treatment.

METHODS

Study population

The present cohort included a selection of patients from the prospective tumor-specific T-Cell IMMunity in patients with solid tumors study (TCIMM study). This prospective observational cohort study aimed to understand the immune factors related to the efficacy and side effects of immunotherapy in treated cancer patients by performing an in-depth analysis of systemic and intra-tumoral immune parameters using blood, tumor, intestinal and faecal samples.

The TCIMM study included patients aged 18 years or older, with a histological or cytological confirmed solid tumor, who received immunotherapy between 2015 and 2023 and had a WHO score of 0-2 at the time of study entry. Written informed consent was obtained from all participants. Patients presenting with severe anaemia (Hb < 6.0 mmol/L), human immunodeficiency virus (HIV) or chronic hepatitis B or C infection were excluded for safety reasons. Peripheral blood samples were drawn

at baseline (prior to treatment), during treatment and after treatment at 1 month, 3 months and 6 months. The study was approved by the Medical Ethics Committee of Leiden University Medical Center (Committee of Medical Ethics; NL59959.058.17). All patients signed informed consent.

For the current study, patients were eligible if they had irresectable stage IIIC or stage IV melanoma and started anti-PD1 treatment as first line monotherapy. In total, 60 patients were included. For the immunological analyses, patients were randomly selected, to avoid any potential sources of bias, and divided in 3 almost equally large groups of patients composed of young patients (<65 years) (n=22), old patients without frailty (n=19) and older patients with frailty (both >65 years) (n=19).

Frailty definition

The Clinical Frailty Scale (CFS) was used to define frailty in the cohort and was collected retrospectively. The clinical frailty scale is a 9-point scale that quantifies frailty based on function in individual patients. It is complemented by a visual chart to assist with the classification of frailty. Higher scores indicate increased frailty and associated risk (21). The validity of retrospective CFS assignment has been demonstrated in various studies (22-25). Patients with a CFS score of 4 or higher were classified as pre-frail or frail, thus categorized in the “older patients with frailty” group. Considering the relatively fit nature of the cohort with only few patients with a CFS of 5 or higher, a CFS cut-off of 4 instead of 5 was used to define frailty. However, previous studies have employed a CFS cut-off of 4 to define frailty, with the frail patients experiencing poorer outcomes, supporting the validity of our approach (26). Patients with a score of 0-3 were classified as non-frail.

Clinical data

All clinical data were registered from medical charts. This information about patient and tumor characteristics included comorbidity, defined by the Charlson Comorbidity Index (CCI) (27), tumor characteristics (superficially spreading, acro lentiginous, desmoplastic, nodular, lentigo maligna), stage of disease, and detailed information on ICI treatment as well as previous treatment (with a BRAF inhibitor, type of anti-PD-1 treatment) and outcome.

Response outcomes

Imaging assessment, including CT or PET CT or MRI (in case of cerebral metastases), was performed after 3 months and 6 months of treatment, or in some cases earlier if there were clinical signs of progression before these timepoints. Responses were evaluated according to the RECIST 1.1. For the current study, the radiological

response to treatment was categorized as follows: a partial or complete response observed at 3 months. Should the disease remain stable or present a mixed response at the 3-month time point, assessment was deferred to the 6-month mark. A partial or complete response at 6 months, or the persistence of stable disease at this time point, was classified as a response to treatment. In case progression occurred after either 3 or 6 months, the patient was classified as a non-responder to treatment.

Sample collection

The blood sampling of patients on anti-PD-1 immunotherapy was done at baseline as part of the study "Longitudinal analysis of tumor-specific T-cell immunity in patients with solid tumors" (NL59959.058.17). 100mL of blood from patients prior to immunotherapy was collected in sodium heparin tubes (BD Bioscience, Drachten, the Netherlands) and processed within 6 hours upon withdrawal. Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved. Buffy coats from three healthy donors, after informed consent, was obtained from Sanquin (Leiden, the Netherlands) and the isolated PBMCs were used as third parties for the mixed lymphocyte reaction (MLR) assay (28).

Isolation of white blood cells

Viable PBMCs were purified by Ficoll (LUMC pharmacy, Leiden, the Netherlands) density gradient separation, washed with Phosphate buffered saline (PBS; Fresenius Kabi, Huis ter Heide, the Netherlands), cryopreserved in 80% fetal calf serum (FCS; Serana Europe, Pessin, Germany) and 20% dimethyl sulfoxide (DMSO; WAK-Chemie Medical, Steinbach, Germany) and stored in the vapor phase of liquid nitrogen until further use (28). The handling, immune assays and analysis of the PBMCs were done according to the standard operation procedures (SOPs) of the Leiden department of Medical Oncology by trained personnel (29).

Immune assays

The immune profiles between the 3 groups of patients in blood samples taken at baseline were compared, and it was investigated whether the obtained immune cell populations were associated with a response to the applied immunotherapy. We performed the following analyses.

Immunophenotyping of PBMCs

The cryopreserved PBMC samples were thawed in IMDM + 10% FCS, washed with Iscove's Modified Dulbecco's *Medium* (IMDM, Thermo Fisher Scientific, Eindhoven, the Netherlands) + 10% FCS and counted according to standard SOP, as published

previously (28). The samples of the 3 above-mentioned patient groups were divided equally between the 3 staining runs.

Immunophenotype of the PBMC was assessed by multispectral flow cytometry (AURORA, Cytek Biosciences, Amsterdam, the Netherlands) staining using our previously described 40-marker panel (28). The PBMCs were first stained with 1:2400 diluted LIVE-DEAD zombie UV fixable amine-reactive dye (Biolegend Europe, Amsterdam, the Netherlands) at room temperature (RT) for 20 minutes, after which the cells were washed with FACS buffer consisting of PBS+0.5% Bovine serum albumin (BSA, Sigma, St Louis, USA), and subsequently incubated with 50 µl PBS/0.5%BSA/5%Trustain FcX blocking solution (Biolegend) for 10 minutes on ice to block Fc receptors. Next, the cells were stained for 30 minutes at RT and in the dark with the cell surface antibodies in two consecutive rounds with three times washing with FACS buffer in between. Intracytoplasmic/nuclear staining was performed using the True-nuclear Transcription Factor Buffer set (Biolegend) according to manufacturers' instruction. Details on antibodies, titers and unmixing are listed in Table S1. After staining the cells were washed twice and stored in FACS buffer. Acquisition was done within 24 hours on a 5-laser Aurora CytekTM spectral analyzer (Cytek Biosciences). High-dimensional single cell data analysis was performed by opt-Distributed Stochastic Neighbor Embedding (optSNE) dimensionality reduction followed by FLOWSOM consensus metaclustering using the cloud-based OMIQ data analysis software (OMIQ, Boston, USA). OptSNE/FLOWSOM analysis were performed on the total CD45+ immune cell population, as well as on cellular subsets (T cells, natural killer (NK) cells, B cells and myeloid cells after gating on CD3+, CD3-CD56+, CD19+ and CD3-CD56-CD19-remaining cells, respectively). The different cell populations were visualized and quantified. Expression levels of each of the indicated markers were depicted for the individual cell populations and frequencies of CD3 T cells, NK cells, B cells and myeloid cells, and remaining cells were shown as percentage of total CD45.

Mixed Lymphocytes Reaction assay

The antigen-presenting capacity of PBMCs was determined in a Mixed Lymphocyte Reaction (MLR) assay as published previously (28, 30). The MLR assay is based on a third-party (allogeneic) T cell proliferation response. PBMCs from three healthy donors served as third parties for the MLR assay. The MLR assays were carried out in triplicate wells in round-bottomed 96-well plates to ensure efficient third-party PBMCs/patient APC (within PBMCs) contact. Irradiated PBMCs alone, as well as third-party PBMCs alone, were used as negative controls. Proliferation of cells was measured by the addition of ^{3}H-thymidine (50ul/ well, stock 10 µCi/mL, Perkin Elmer, Boston, USA) for 16-18 hours at 37°C, whereafter the cells were harvested on

MicroBeta glass fiber filter paper (Perkin Elmer). Incorporation of ^{3}H-thymidine was determined on a Wallac MicroBeta TRILUX 1450 LSC & Luminescence counter (Perkin Elmer). The proliferation of the third-party PBMCs is expressed as the stimulation index (SI) calculated as the ratio of the counts per minute of ^{3}H-thymidine in MLR co-culture to that in the third-party only (control) culture. A threshold of at least 3 is defined as a positive response. The number of positive responses out of the tested three third parties, as well as the strength of response (SI), were determined.

Phytohemagglutinin (PHA) proliferation assay

The proliferative potential of PBMCs was evaluated in a proliferation assay [26], using PHA stimulation and ^{3}H-thymidine incorporation (as described above). PBMCs from patients were cultured in quadruplicate wells in the presence or absence of PHA (1 µg/ml). The SI was calculated as the ratio of lymphocytes cultured with PHA over those of the unstimulated control cultured lymphocytes. To discard the outliers, we determined the mean (m) of the value of the replicate wells, then determined the most distant value (data point x). Next, we calculated the mean (m) and the standard deviation (s) of the replicate wells, excluding this data point x. We considered x an outlier when $x > m + 3s$ or $x < m - 3s$. Finally, the mean was recalculated excluding the outlier point x.

Statistical analysis

Statistical evaluation was performed using the statistical package SPSS version 25.0 and Graphpad version 9.3.1. First, the baseline clinical characteristics between the three groups of patients (young, old without frailty and old with frailty) were compared using chi-square tests. Second, the differences between the cell populations as identified by OMIQ analyses between the three groups were assessed. Cell population data were not normally distributed. All cell populations were presented as a percentage of the total CD45. For this, as well as for the PHA assay data, the Kruskal-Wallis tests were used. The MLR assay data were analyzed using Chi-square tests. Third, the association between the presence of frailty and response to treatment was assessed using the Chi-square for trend tests. Finally, the association between the cell populations and response to treatment was determined using Mann-Whitney tests. A p-value of <0.05 was defined as statistically significant.

Laboratory environment

Immunomonitoring of patients' PBMCs was performed in the laboratory of the department of Medical Oncology (LUMC) that operates under research conditions but uses standard operation procedures for all tests, with pre-established definitions of positive responses and trained personnel. This laboratory has been externally

and internally audited according to the reflection paper for laboratories that perform immunomonitoring and participated in all proficiency panels of the CIMT Immunoguiding Program (CIP; of which SHvdB and MJPW are

steering committee members; http://www.cimt.eu/workgroups/cip/) as well as many of the proficiency panels (including ICS gating and ELISPOT plate reading panels) of the USA-based Cancer Immunotherapy Consortium (CIC of the Cancer Research Institute) to validate its standard operating procedures (SOPs) (29).

RESULTS

Patient characteristics are summarized in Table 1. Sixty patients were enrolled in this study, including 22 patients < 65 years (young patients), 19 patients ≥ 65 years (old patients) without frailty and 19 patients ≥ 65 years and frail (old with frailty). The distribution of the CFS score in patients with frailty is displayed in Figure S2. The median age of young patients was 61 (interquartile range IQR 55-64), of old patients without frailty 73 (IQR 70-77) and of old patients with frailty 76 (IQR 72-81) years. Forty-three patients were male (71.7%). Fifty-nine patients had stage IV melanoma (98.3%) and one patient had an irresectable stage IIIC melanoma (1.7%). Sixteen patients had brain metastases (26.7%). Among the thirty-two patients with a BRAF mutation (53.3%), eight had previously received BRAF treatment. They were distributed equally across the three groups (n=2 in the young patient group, n=3 in the older patient group without frailty, and n=3 in the older patient group with frailty).

Twenty-four patients had a WHO score of 0 (40%), and twenty-nine had a WHO score of 1 (48.3%). Forty-five patients had a Charlson Comorbidity Index (CCI) of 0 (75%), thirteen patients had a score of 1 (21.7%), and three patients had a score of 3 (3.3%). Except for the WHO score and age, no major differences were observed between the three patient groups.

Table 1. Baseline patient characteristics

	All patients		<65 years		≥65 non-frail		≥65 (pre)-frail		p-value
Age, median (IQR)	70	(63-75.8)	61	(55-64)	73	(70-77)	76	(72-81)	
Sex, n (%)									
Male	43	(71.7)	15	(25)	15	(25)	13	(21.7)	0.696
Female	17	(28.3)	7	(11.7)	4	(6.7)	6	(10)	
WHO-performance status, n (%)									
0	24	(40)	13	(21.7)	9	(15)	2	(3.3)	0.008
1	29	(48.3)	8	(13.3)	6	(10)	15	(25)	
2	1	(1.7)	0	0	0	0	1	(1.7)	
Unknown	6	(10)	1	(1.7)	4	(6.7)	1	(1.7)	
Charlson score, n (%)									
0	45	(75)	19	(31.7)	12	(20)	14	(23.3)	0.491
1	13	(21.7)	3	(5)	6	(10)	4	(6.7)	
2	0	0	0	0	0	0		0	
3	2	(3.3)	0	0	1	(1.7)	1	(1.7)	
Stage, n (%)									
IIIC	1	(1.7)	0	0	0	0	1	(1.7)	0.334
IV	59	(98.3)	22	(36.7)	19	(31.7)	18	(30)	
BRAF mutation, n (%)									
No	26	(43.3)	7	(11.7)	11	(18.3)	8	(13.3)	0.072
Yes	32	(53.3)	15	(25)	6	(10)	11	(18.3)	
Unknown	2	(3.3)	0	0	2	(3.3)	0	0	
Brain metastases, n (%)									
No	44	(73.3)	17	(28.3)	14	(23.3)	13	(21.6)	0.815
Yes	16	(26.7)	5	(8.3)	5	(8.3)	6	(10)	

	All patients		<65 years		≥65 non-frail		≥65 (pre)-frail		p-value
Previous treatment BRAF inhibitor, n (%)									
No	52	(86.7)	20	(33.3)	16	(26.7)	16	(26.7)	0.763
Yes	8	(13.3)	2	(3.3)	3	(5)	3	(5)	
LDH (U/L), n (%)									
Normal (<250 U/L)	39	(65)	17	(28.3)	11	(18.3)	11	(18.3)	0.299
Elevated (>250 U/L)	18	(30)	4	(6.7)	7	(11.7)	7	(11.7)	
Missing	3	(5)	1	(1.7)	1	(1.7)	1	(1.7)	

Table 1. Patients baseline characteristics stratified into 3 groups: <65 years, young patient group; > 65 years non-frail, old patients without frailty group; > 65 years (pre)-frail, old patients with frailty group. Baseline characteristics differences between the three groups of patients were assessed using Chi-square tests.

Association between immune cell composition and calendar age and frailty

To study potential age- and/or frailty-related changes in the immune cell composition in metastatic melanoma patients, an in-depth immunophenotyping of pre-therapy isolated PBMCs was performed for 60 patients using a 40-marker spectral flow cytometry panel (Figure S3, Table S1). Simple optSNE dimensionality reduction analysis of the total CD45 population with the lineage markers CD3, CD19, CD56, and CD14 (Figure 1A-B) revealed the lowest frequencies of total B cells in old patients with frailty (p=0.0477) and a trend towards higher frequencies of total NK cells in old patients irrespective of frailty. No significant differences in the total frequency of CD3+ T cells and myeloid cells among the 3 patient groups were observed (Figure 1C).

To get insight into the phenotype and subsets of the circulating immune cell lineages and their association with age and frailty, pre-gated T cells, NK cells, B cells and myeloid cells were subjected to further detailed optSNE-FlowSOM cluster segmentation.

Analysis of the CD3+ T cells revealed 19 different subpopulations (Figure 2A), of which populations 10 and 17 were reduced in old patients with frailty (p=0.0234 and p=0.0008, respectively) and population 11 in old patients without and with frailty (p=0.0031 and p=0.0009, respectively) (Figure 2B). Population 11 comprises naïve CD8+CD45RA+CCR7+CD27+CD127+CD28+ T cells, and populations 10 and 17 comprise CD8+CD45RA-CCR7-CD45RO+CD103+ tissue resident-like effector memory T cells and CD8+CD45RO+KLRG-1+CD161++ mucosal-associated invariant T (MAIT) cells, respectively (Figure 2C).

Figure 1. CD45 cell populations

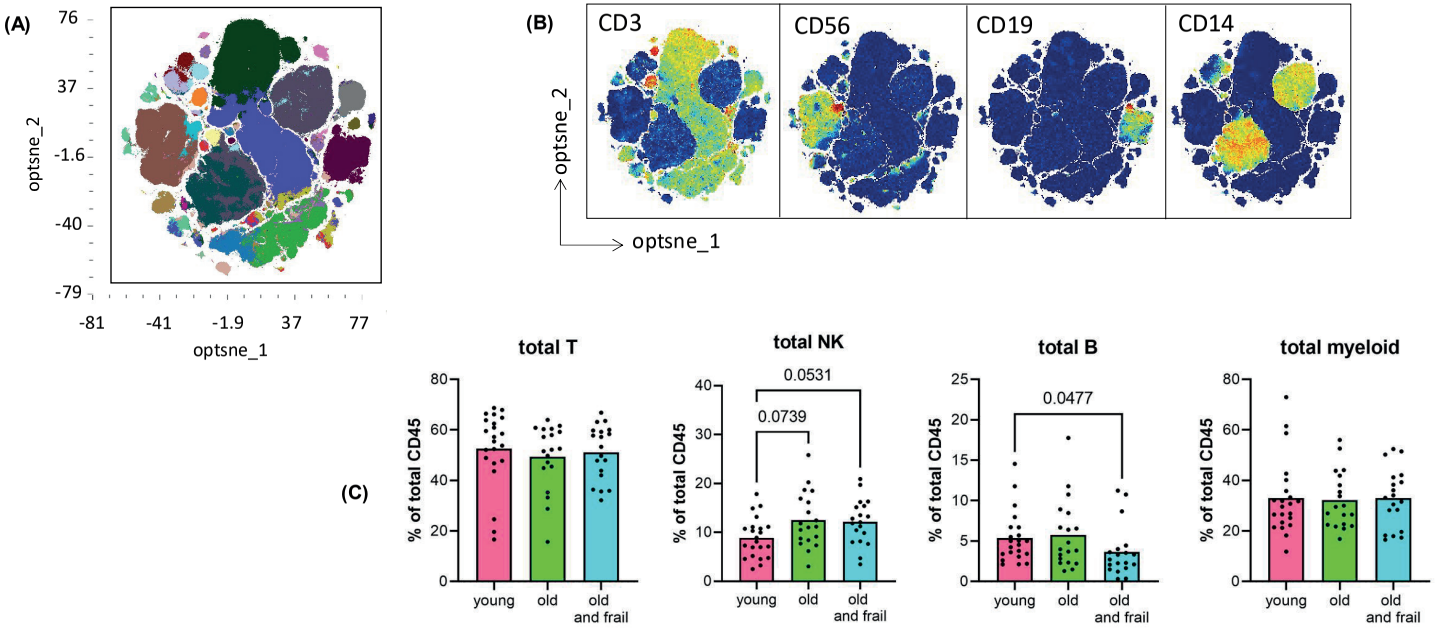
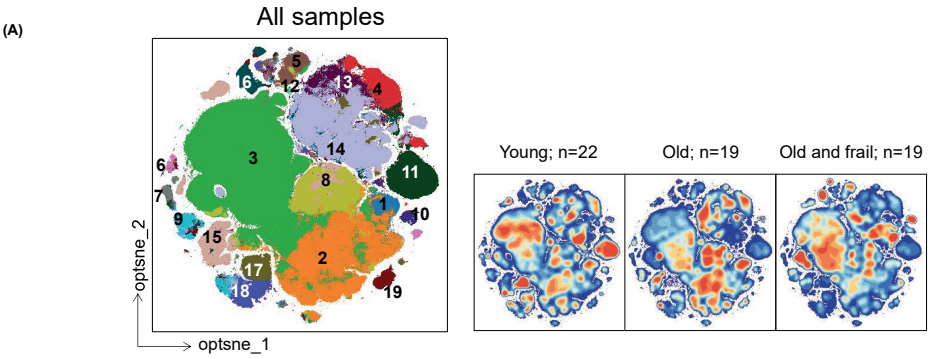


Figure 1. (A) Cluster partitions by FLOWSOM. (B) OptSNE plots visualizing contour plots. Contour plots show the staining intensity of individual cell markers used. (C) Frequencies of total T-cells, total NK cells, total B-cells, and total myeloid cells in young, old and old-frail patient groups. Cell populations are presented as a percentage of the total CD45 cells. Statistical differences were assessed with Kruskal-Wallis tests and $p<0.05$. Young, young patient group; old, old patient without frailty group; old and frail, old patients with frailty group.

Figure 2. CD3+ T cell populations



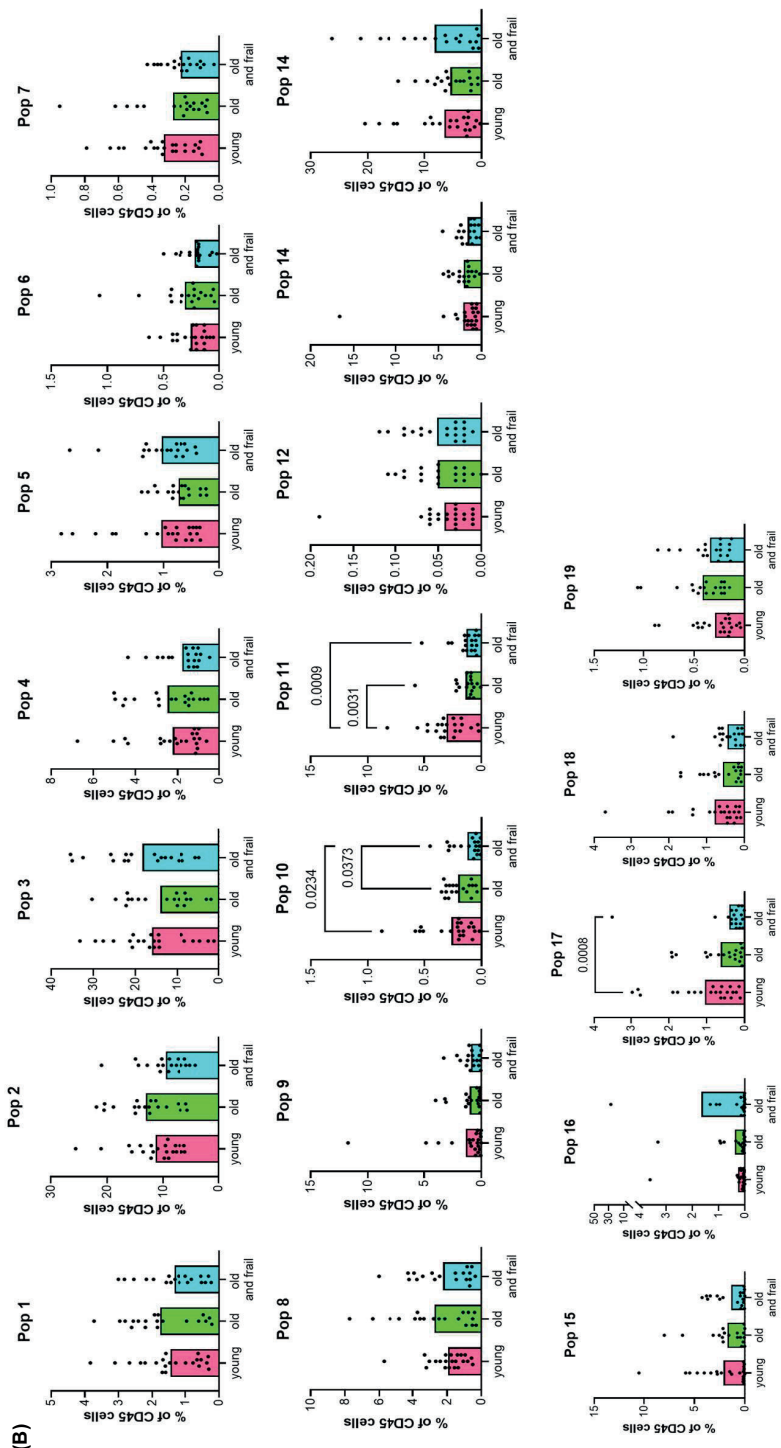


Figure 2. Continued

Figure 2. Continued

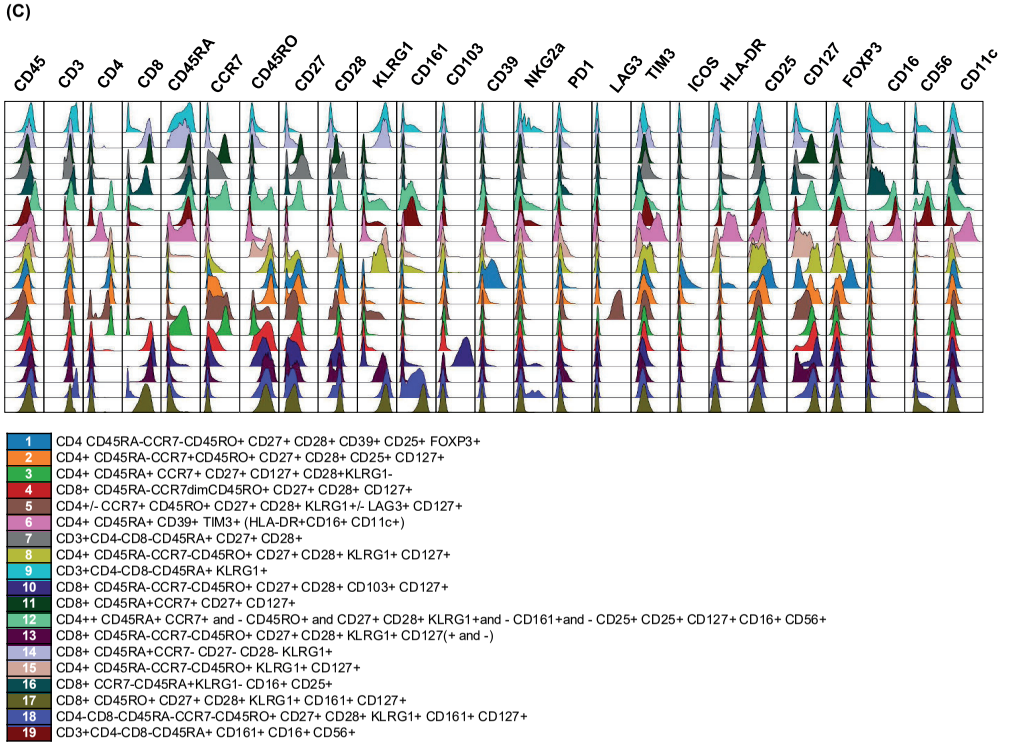


Figure 2. (A) Cluster partitions by FLOWSOM of PBMCs stained with antibodies for CD3+ T-cell markers. In total, 19 different clusters were defined (left). OptSNE plots visualizing contour plots of the 3 patient groups (right). (B) Frequencies of T cell populations in young, old and old-frail patient groups. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Kruskal-Wallis tests and $p<0.05$. (C) Expression levels of each of the indicated markers are depicted for the individual immune cell populations.

Sub-clustering of the NK cell, myeloid cell, and B cell populations revealed differences between the 3 patient groups only for the B cell subpopulations (Figures S4-S6). The CD19+ B cells comprised 8 different subpopulations (Figure S6A), of which populations 31 and 34 were reduced in both old and frail patients (Figure S6B). Population 31 (CD19+ HLADR+ CD27- CD86- CCR7+ CD45RA+ CD39dim) and population 34 (CD19+ HLADR+ CD27- CD86- CCR7+ CD45RA+ CD39dim CD1c+) both expressed CCR7, which in previous studies was shown to be expressed during B cell development [27,28], suggesting that these are naïve B cells.

No clear difference in antigen-presenting cells (APC) capacity upon ageing and frailty.

To evaluate the capacity of APCs to stimulate T cell responses upon ageing and frailty, a mixed lymphocyte reaction (MLR) assay was performed (Figure 3 and Table 2). In the young patient group, the APCs of 3 out of 22 patients (13.6%) were not able to induce T cell proliferation of any of the third-party donor PBMCs compared to 5 out of 19 (26.3%) in the old patient group without frailty and 6 out of 19 (31.6%) in the old patient group with frailty. Yet, these differences were not statistically significant (p=0.37). Also, not when the number of positive proliferation responses against the three different allogeneic PBMC donors was compared (p=0.73). In the young patient group, 15 (68.2%) patients had a proliferation response to ≥ 2 donors, compared to 11 (57.9%) patients in both old patient groups.

Figure 3. Antigen-presenting cell responses in young, old non-frail and old-frail patients

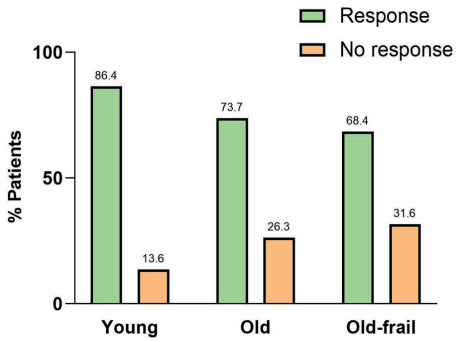


Figure 3. Mixed Lymphocytes Reaction assays were performed to evaluate the antigen-presenting cells (APCs) capacity based on a T-cell proliferative response of healthy donor PBMCs. The results for the MLR were expressed in terms of cell proliferation quantified by the stimulation index (SI). SI≥3 indicated a positive response and SI<3 indicated no response. Statistical differences were assessed using Chi-square tests and p<0.05.

Table 2. Antigen-presenting cells responses in young, old non-frail and old-frail patients

	All patients	Young	Old	Old-frail	p-value
Age (median, IQR)	70 (63-75.8)	61 (55-64)	73 (70-77)	76 (72-81)	
Stimulation Index, n (%)					
Yes	46 (76.7)	19 (86.4)	14 (73.7)	13 (68.4)	0.37
No	14 (23.3)	3 (13.6)	5 (26.3)	6 (31.6)	
Number of responses, n (%)					
≥2	37 (61.7)	15 (68.2)	11 (57.9)	11 (57.9)	0.73
<2	23 (38.3)	7 (31.8)	8 (42.1)	8 (42.1)	

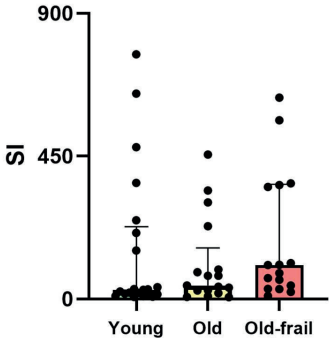
Abbreviations: SI, stimulation index; n, number; IQR: interquartile range

Table 2. Mixed Lymphocytes Reaction assays were performed to evaluate the antigen-presenting cells (APCs) capacity based on a T-cell proliferative response of healthy donor PBMCs. The results for the MLR were expressed in terms of cell proliferation quantified by the stimulation index (SI). SI≥3 indicated a positive response and SI<3 indicated no response. Statistical differences were assessed using Chi-square tests and p<0.05.

No clear difference in PBMCs proliferative capacity upon ageing and frailty.

To evaluate the proliferative capacity of T cells, PBMCs were stimulated with PHA (Figure 4). No significant differences were observed between the three patient groups, although a trend was observed of a stronger proliferation from young to old and frail old patients. The median (IQR) SI in the young patient group was 29 (95% C.I. 12-228) compared to 42 (95% C.I. 22-161) in the old patient group without frailty and 107 (95% C.I. 37-361) in the old patient group with frailty (p=0.09).

Figure 4. Antigen-presenting cells responses in young, old non-frail and old frail patients



SI PHA	Young	Old	Old-frail	p-value
Median (IQR)	28.51 (12.23-227.93)	42.47 (22.44-160.89)	107.09 (37.18-361.37)	
Mean (SD)	28.51 (228.13)	109.5 (136.43)	260.04 (370.09)	0.09

Abbreviations: SI, stimulation index; IQR, interquartile range; SD, standard deviation

Figure 4. PHA proliferation assays were performed to evaluate the patients' lymphocytes' proliferative potential. Statistical differences were assessed using Kruskal-Wallis tests and p<0.05.

Immune correlates to clinical response

Since there was no difference in the clinical response of young and old (frail or not) patients, we divided the patients into two groups based only on their clinical outcome in order to determine whether there is a difference in the immune profile between clinical responders and non-responders. While the different T, B, and NK cell populations did not vary substantially between the responders and non-responders, we did observe a correlation between clinical response and the myeloid subpopulations (Figures S7-S10).

In the T cell subpopulations, population 6 was significantly associated with treatment response (p=0.0368) (Figure S7). This cell population comprises both T cell and myeloid markers and was found to be CD4+CD45RA+ CD39+ CD68+ TIM3+ (HLA-DR+CD16+CD11c+). Emerging studies have demonstrated the expression of CD4 in myeloid progenitor cells, and therefore, this cell population is defined as a CD4-expressing myeloid cell subset [29].

Analysis of the frequencies of myeloid cell populations in relation to treatment responses revealed significant differences in cell population 40, with lower frequencies in patients with a clinical response (p=0.0491). Both cell populations 46 (p=0.0461) and 47 (p=0.017) were higher in these responding patients. Population 40 expressed HLA-DR, but could not be further defined. Population 46 comprised HLA-DR+ CD14dim/+CD11c+ CD16++ CD86+ TIM3+ dendritic cells. The frequency of population 47 was lower in the old patient group without frailty compared to the young patient group (p= 0.04), but only expressed CD123+, and thus could not be further defined (Figure S5).

Frailty may not impact the clinical response to checkpoint therapy in older patients

Figure 5 shows the clinical response to treatment between the young, old patients without frailty and the old patients with frailty. The clinical response was a bit higher in the young patient group (13 out of 22 patients, 59.1%) when compared to the old patient group with frailty (9 out of 19 patients, 47.4%), but there were no statistically significant differences between the three groups of patients. In addition, no

association with treatment response and ageing or frailty was observed (p=0.45). This demonstrates that frailty in older patients does not preclude them from a successful immunotherapeutic treatment.

Figure 5. Response treatment between young, old, and old-frail groups

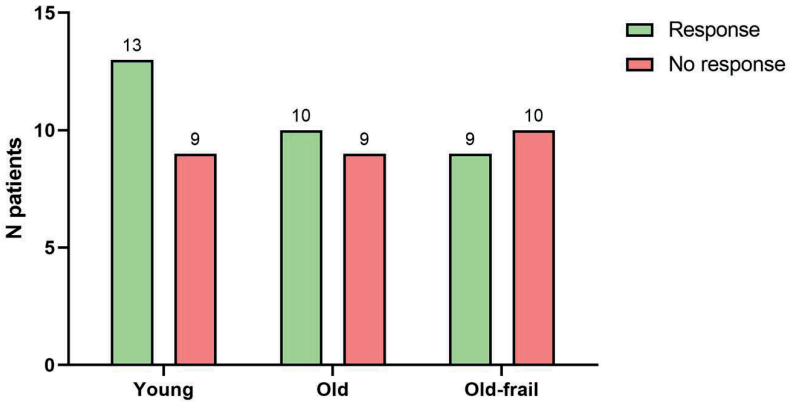


Figure 5. Comparison of response to treatment between young, old, and old-frail patient groups. No statistical differences were observed using the Chi-square for trend tests.

DISCUSSION

In this study, we observed lower frequencies of CD8+ naïve T cells in older compared to young patients, irrespective of the presence of frailty, confirming the loss of naïve T cells with age as observed in other groups focusing on immune senescence in older patients (4, 5). In addition, we observed a frailty-associated loss of effector CD8+ tissue resident-like memory (CD8+ TRM) T cells, CD8+ MAIT cell populations and of B cells, the latter of which is in line with earlier observations on declining B cells during ageing, but not with that of increasing MDSCs with ageing (8). Moreover, we detected higher frequencies of NK cells in older patients, irrespective of clinical frailty. However, none of these age and frailty-related immune cell differences were associated with differences in the clinical response to immunotherapy.

In contrast to previous studies in old non-cancer patients that showed a correlation between frailty and decreased levels of total T cells (5, 31), our study in melanoma patients did not reveal significant differences in total T cell frequencies between the young and older patients, regardless of frailty status. Age-dependent changes in the naïve and memory T cell pools have been widely reported in frail patients without cancer (32). Consistent with previous research, we observed lower numbers

of CD8+ naïve T cells in older melanoma patients compared to young patients, irrespective of the presence of frailty. Moreover, prior studies have shown lower frequencies of circulating naïve CD4+ T cells in old patients with frailty from nursing homes and in the general practice (4, 5, 12), results that we did not observe in our study. Our results revealed a significant decrease in CD8+ MAIT cells with age independent of frailty, which is in line with a previous study that demonstrated a gradual decline in percentage and number of CD8+ MAIT cells from young to older individuals (33). In our study, we have identified a subpopulation of senescent CD8+ T cells, characterized by the phenotype CD8+CD45RA+CCR7-CD27-CD28-KLRG-1+ (cell population 14); however, no significant differences were observed between the patient groups, diverging from previous findings that have shown a correlation with frailty and loss of CD28 and CD27 markers in older patients (34-36). The failure to detect senescence-linked changes in immune cell populations in older patients can potentially be attributed to the relatively small age difference between young and old patients. Plus, the sample size of our cohort was relatively small, which may have resulted in underpowering of the study. Another explanation may be that patient inclusion in this observational cohort led to a relatively healthy cohort, as the frailest patients may be underrepresented in the study, which might be the reason for the uniform distribution of the senescence-associated cell frequencies across these age groups. Additionally, no significant differences were observed between population 14 and the response rates. Hui et al. demonstrated that CD28 was the primary target for PD-1-mediated inhibition, suggesting that the abundance of T cells lacking CD28 expression may correlate with the absence of efficacy of anti-PD-1/PD-L1 therapy (37). The absence of observed differences in response rates among our study groups may be attributed to the uniform distribution of the senescent cell frequencies across the groups, which may have resulted in only minor differences in immunological profiles between patients, thereby hampering the associations with response to treatment. The lack of correlation between immune senescence markers and response may also suggest that immune senescence per se does not interact with the effectiveness of immune checkpoint inhibitors (38).

Our results did not reveal statistically significant differences in response rates among the study participants. However, there appears to be a trend of declining responses with advancing age and increasing frailty. Although this trend could hold clinical significance, the small participant numbers might not allow for statistical confirmation. The observed lack of correlation may result from the study's insufficient power or the cross-sectional design at baseline, which did not capture the dynamic changes in frailty over time. Although categorizing patients into three groups (young, old fit, and old frail) allowed clear comparison across distinct age and fitness profiles, longitudinal assessments and treating the Clinical Frailty Scale as a continuous

variable could have provided more nuanced insights into the relationship between frailty and treatment outcomes.

We did not observe age- or frailty-dependent differences in myeloid-derived suppressor cells (MDSCs). MDSCs potently suppress T cell activity, leading to the immune escape of malignant tumors, thus promoting resistance to checkpoint inhibitor treatments (39, 40). Verschoor et al. reported a significant increase in number of circulating myeloid cells, especially in MDSCs, in older and frail patients compared to young patients (41). Our results of the MLR did not show significant differences in relation to ageing and frailty, suggesting no problems with APC function, as would have been the case when higher frequencies of MDSC would be present (42). This supports our observation that they were not prominently present among our patients. However, a trend of impaired function of APCs in the old patients without and with frailty in comparison to the young patients can be observed, implying that immune cells, in the older and frail individuals, may be less efficient at capturing and presenting antigens.

The main strengths of this paper include the use of validated methods according to standard operating procedures (SOPs) and of a large panel of markers for flow cytometry. Moreover, analyses included frailty, rather than only using calendar age.

However, this study also has its limitations. First, the lack of specific antibodies for subpopulations of myeloid cells, as well as for NK cells and B cells, did not allow us to identify all the cell subsets. Therefore, it is important to remain cautious regarding the naming of cell populations. Marker expression can overlap and vary within different degrees, which cannot be nuanced when naming those subpopulations. Secondly, the sample size was low, decreasing statistical power. For this reason, we chose not to correct for multiple testing. Additionally, we included patients pre-treated with BRAF inhibitors; however, their distribution was nearly uniform across the three groups, and they were not outliers with respect to the identified immune cell populations, ensuring they did not skew the study's outcomes. Finally, there was a relatively small age difference between young and old patients, potentially leading to a lack of power in our study. Moreover, a CFS score of 4 or higher was used to include both pre-frail and frail patients. This decision was made in light of the cohort's relatively fit nature, with only few patients with a CFS of 5 or higher, to ensure adequate sample sizes and comparability across groups. Although a CFS score of 5 or higher is typically used to define frailty, our approach is supported by precedents in the literature that have utilized similar cut-offs in comparable populations (25). We acknowledge that this may impact the study's power and the robustness of its conclusions. Additionally, incorporating measures of plasma cytokines could have potentially strengthened the classification of frail and non-frail older adults; however, the resources did not

allow for further experimentation. Considering the variation and importance of the tumor microenvironment, investigating immune senescence markers in relation to immunotherapy responses should be further studied at the tissue level.

Several ageing- and frailty-associated changes were detected among circulating immune cells in blood, but were not associated with response to immunotherapy in our study. While these findings suggest that the level of frailty and ageing may not necessarily preclude the efficacy of ICI therapy, further investigation is needed to fully understand the impact of frailty and ageing on immunotherapy.

DECLARATIONS

Clinical trial number

Not applicable.

Ethics approval and consent to participate

The study conducted in accordance to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Leiden University Medical Center (Committee of Medical Ethics; NL59959.058.17). All patients signed informed consent.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

No conflicts of interest to disclose.

Authors contributions

Estelle Tran Van Hoi carried out the research and analysis under the supervision of Johanneke E.A. Portielje. Simon P. Mooijaart, Diana Van Heemst and Nienke A. de Glas. Saskia J. Santegoets performed analysis and contributed to interpretation of the results. Asli Özkan contributed to the patients follow-up. Marije Slingerland, Elizabeth M.E. Verdegaal, Ellen Kapiteijn, Marij J.P. Welters and Sjoerd H. van der Burg contributed in the design and implementation of the TCIMM study. Marije J.P. Welters, and Nienke A. de Glas conducted the present research.

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Single stain	Laser	Detector	Fluor	Antigen	Characteristics of markers in immunosenescence field [1-3]		Clone	Lot 1	Unmixing
s1	UltraViolet laser	UV2	BUV395	CD45RA	Naïve and terminally differentiated effector T cells marker	Lost as T cells become central or effector memory cells; ratio of naïve to these memory T cells is a hallmark of immunosenescence	HI100	2077243	with beads
s2		UV4	Zombie UV	L/D	Distinguishes live from dead cells	(to be able to only analyse viable cells as dead cells can non-specifically bind antibodies)	NA		with cells
s3		UV7	BUV496	CD16	NK cells marker and is expressed on monocytes and some macrophages	Changes in expression levels can indicate altered immune functions in ageing	3G8	1099494	with cells
s4		UV9	BUV563	CD39	Exhausted T cells marker, also expressed in activated antigen-specific CD4 T cells in the tumour [4]	Can lead to increased immunosuppression with age	TU66	2069477	with beads
s5		UV10	BUV615	ICOS	A costimulatory molecule for T cell activation and function	May be involved in the functional decline and activation of T cells in ageing	DX29	2077268	with beads
s6		UV11	BUV661	CD1c	Marker of dendritic cells	Involved in antigen presentation; initiation of immune responses can decline with age	F10/21A3	2189988	with cells
s7		UV14	BUV737	CD86	Costimulatory molecule on antigen-presenting cells	Essential for effective T cell activation, which can decline with age	2331 (FUN-1)	1228590	with beads
s8		UV16	BUV805	CD8	Cytotoxic T cells marker	Accumulation of senescent CD8+ T cells is a hallmark of immunosenescence	SK1/HIT8a	1200765	with cells
s9	violet laser	V1	BV421	CD161	Expressed on a subset of T cells and NK cells	CD161 is involved in regulating immune responses[5], potentially implicated in age-related immune alterations	HP-3G10	B334269	with beads
s10		V2	SB436	CD123	Plasmacytoid dendritic cells marker	Numerical and functional decline in pDC is associated with ageing	6H6	2305276	with beads
s11		V3	PacBlue	CD15	Neutrophils marker	Neutrophil functions may decline with ageing, although the neutrophils are hardly present after gradient isolation of blood sample	W6D3	B273508	with beads
s12		V4	BV480	CD33	Early myeloid cells marker	Increased proportions are associated with ageing	P67.6	276608	with beads
s13		V6	BV510	CD11c	Dendritic cells marker	Involved in antigen presentation, initiation of immune responses can decline with age	B-Ly6	1344885	with cells
s14		V7	PacOrange	CD3	T cells marker	Ratio of different T cell subsets changes with age, can indicate immunosenescence	UCHT1	539078	with cells
s15		V8	BV570	CD45RO	Memory T cells marker	Increased proportions suggest an aged immune repertoire	UCHL1	B354623	with beads
s16		V10	BV605	CD163	M2-like macrophages marker	M2 macrophages may increase with age, can promote tumour [6, 7]	GHI/61	B339795	with beads
s17		V11	BV650	PD1	Immune checkpoint inhibitor marker	Increases on T cells as they become activated and exhausted, a hallmark of immunosenescence	EH12.2H7	B341605	with beads
s18		V13	BV711	CD103	Integrin expressed on tissue resident memory T cells	Tissue resident marker, can be upregulated upon activation[8]	Ber-ACT8	B328658	with beads
s19		V14	BV750	CD56	NK cells marker (can also be expressed in T cells)	Changes in NK cell numbers and function are associated with ageing	5.1H11	B348832	with beads
s20		V15	BV785	CD28	Costimulatory molecule for T cells activation	Loss of CD28 in T cells is a hallmark of immunosenescence	CD28.2	B344136	with cells
s21	blue laser	B1	BB515	CD141	Expressed in subset of dendritic cells	CD141+ DC may decrease in number with age	1A4	1166028	with beads
s22		B2	AF488	Foxp3	Transcription factor for Treg cells	Treg Foxp3+ cells may increase in number with age	259D	B315166	with beads
s23		B3	Spark Blue 550	CD14	Monocytes/macrophages marker	Phenotype and function of monocytes and macrophages change with age	63D6	B321401	with cells
s24		B8	PerCP	CD45	Leukocytes marker	Decreased proportions with age	Hi30	B331249	with beads
s25		B9	PerCP/Cy5.5	CD11b	Myeloid cells marker	Changes in expression might reflect shifts in innate immunity with age	ICRF44	B328101	with cells
s26		B10	PerCP/eF710	CD274/PD-L1	PD-1 Ligand marker	Its expression on tumor cells and tumor associated macrophages can contribute to the immunosuppressive environment seen in ageing	MIH5	2246625	with beads

Table S1. Panel markers for flow cytometry

For each antibody, the conjugated fluorochrome, clone name and supplier are given.

s27	yellow green laser	YG1	PE	CLec9a	Expressed in subset of dendritic cells	CLEC9A+ DC (cDC1) may decrease in number with age [6]	8F9	B309940	with cells
s28		YG2	CF568	CD4	Helper T cells marker	Changes in the CD4+ T cell compartment are associated with immunosenescence	C4-206	21C0304	with cells
s29		YG3	PE/Dazzle 594	CD206	Activated macrophages marker	Activated macrophages increase with age	15-Feb	B329923	with beads
s30		YG4	PE/Fire640	CD25	Expressed on activated T cells and Treg cells	Involved in cell proliferation and regulatory functions that may change with age	M-A251	B332511	with cells
s31		YG5	PE/Cy5	Tim3	Immune checkpoint molecule	Increased expression is associated with T cell exhaustion in ageing	F38-2E2	B353724	with beads
s32		YG6	PE/Fire700	CD127	Memory T cells and effector T cells marker, also expressed in a subset of Treg cells	Memory and effector T cells increase in number with age	AO19D5	B348589	with beads
s33		YG9	PE/Cy7	KLRG1	Expressed on senescent T cells	Marker of terminal differentiation and senescence in T cells	SA231A2	B330891	with cells
s34		YG10	PE/Fire810	HLA-DR	MHC class II molecule marker	Its expression on T cells can mean activated T cells. Its increased expression can be associated with inflammation and ageing	L243	B341939	with cells
s35	red laser	R1	APC	NKG2a	NK cells marker and immune checkpoint molecule on T cells	Increased proportions with age	Z199	200056	with beads
s36		R2	Alexa647	CD68	Macrophages marker	Decreased proportions with age	Y1/82A	B311503	with beads
s37		R3	Spark NIR 685	CD19	B cells marker	Changes in B cell subsets are hallmarks of immunosenescence	HIB19	B324543	with beads
s38		R4	APC/R700	Lag3	Immune checkpoint molecule	Contributes to immune exhaustion and increases with age	T47-530	1114707	with cells
s39		R7	APC/Fire750	CCR7	Involved in cell migration to lymph nodes, expressed in various cell types	Loss of CCR7 expression on T cells is associated with immunosenescence	G043H7	B338294	with cells
s40		R8	APC/Fire810	CD27	Costimulatory molecule	Loss of CD27 expression on T and B cells is a hallmark of immunosenescence	QA17A18	B332527	with beads

Continued Table S1. Panel markers for flow cytometry

For each antibody, the conjugated fluorochrome, clone name and supplier are given.

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Figure S1. Conceptual overview of the interplay between immune cell response and melanoma growth with immunosenescence

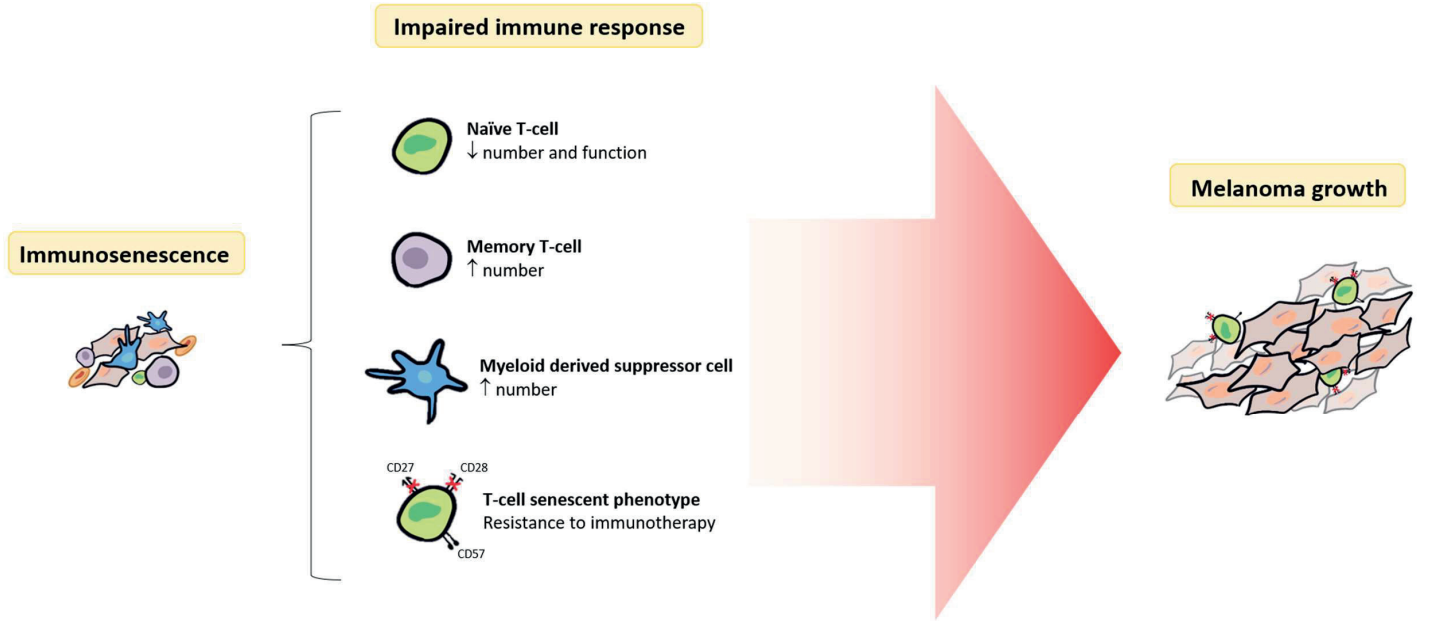


Figure S1. In melanoma, immunosenescence leads to reduced cell number and function of naïve T cells and memory T cells, inducing melanoma growth and metastasizing process. Moreover, T cells express an immunosenescent phenotype characterized by reduced expression of CD27 and CD28 and higher expression of CD57, a major marker of immunosenescence. This immunosenescent phenotype has been associated with resistance to immunotherapy treatment.

Figure S2. Distribution of Clinical Frailty Scale score in patients with frailty

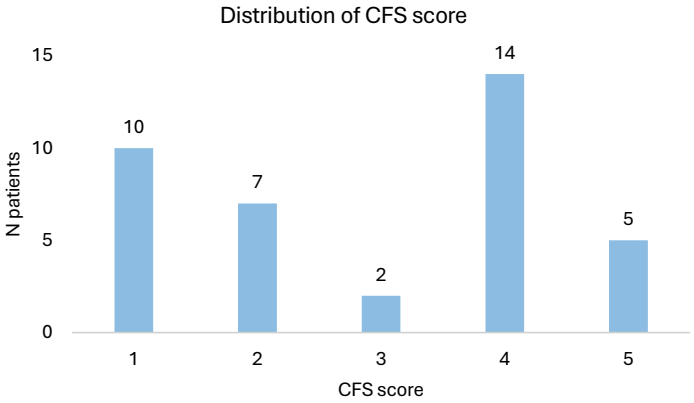


Figure S3. Frequencies of CD3+ T cell populations between patients having response/no response to treatment

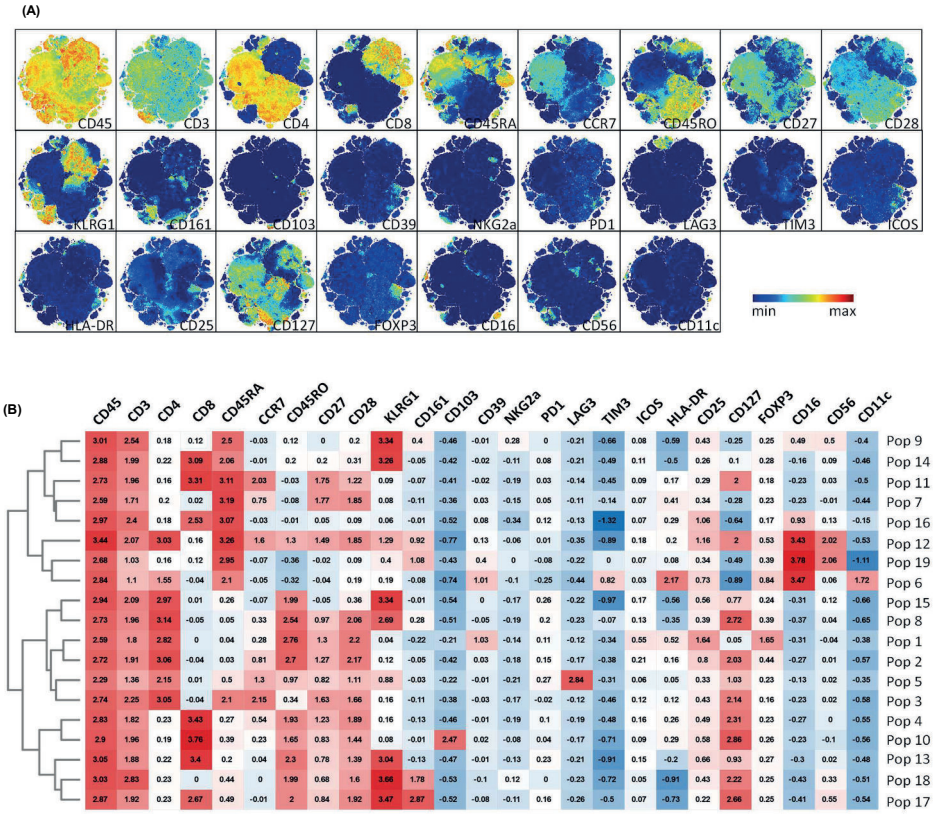


Figure S3. (A) OptSNE plots visualizing contour plots with the staining intensity of the individual markers used. (B) Heat map (FlowSOM plugin output) of the relative fluorescent intensity for the markers associated with the identified T cell subpopulations.

Figure S4. NK cell populations

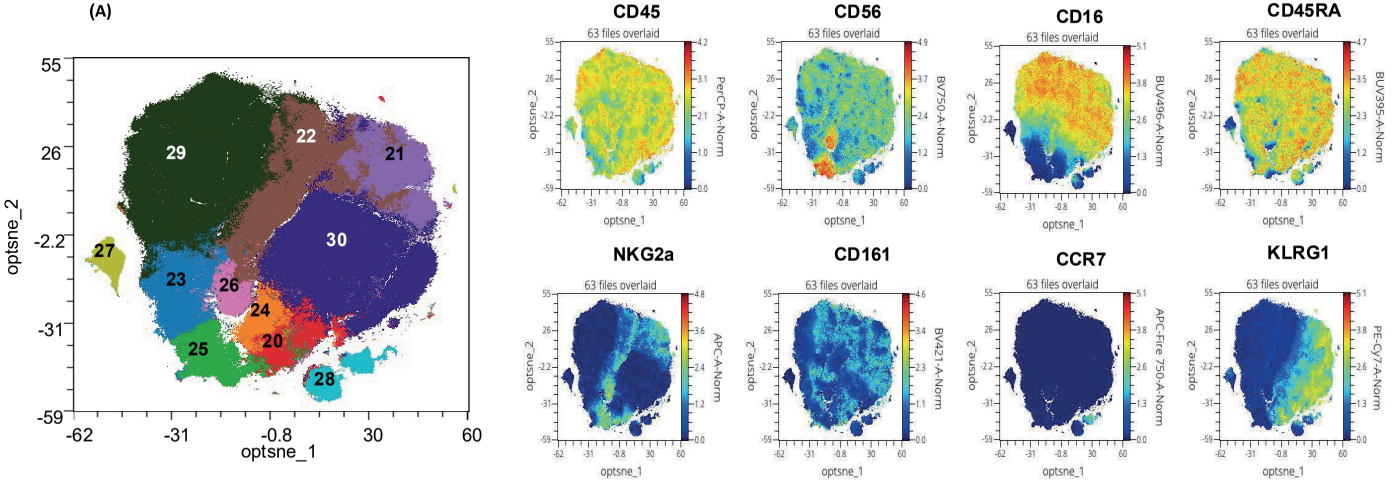
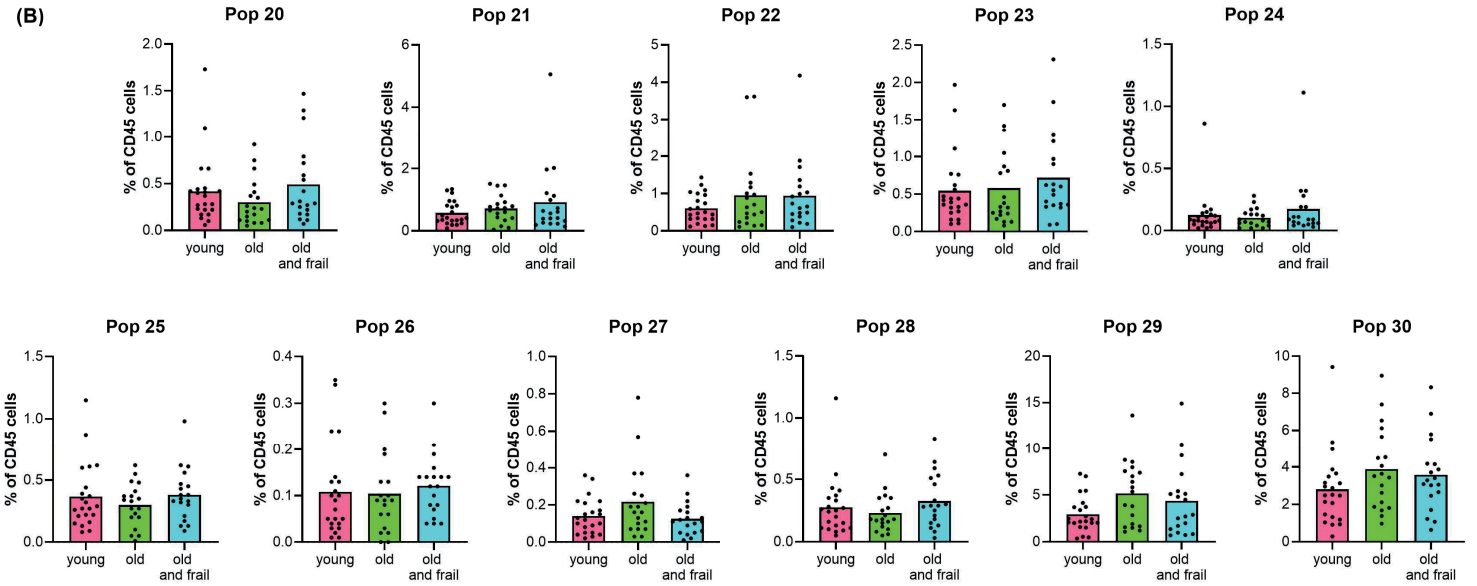


Figure S4. (A) Cluster partitions by FLOWSOM of PBMCs stained with antibodies for CD56+ NK cell markers. In total, 11 different clusters were defined (left). OptSNE plots visualizing contour plots of the 3 patient groups (right).

Figure S4. Continued



(B) Frequencies of NK cell populations in young, old and old-frail patient groups. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Kruskal-Wallis tests and $p<0.05$.

Figure S4. Continued

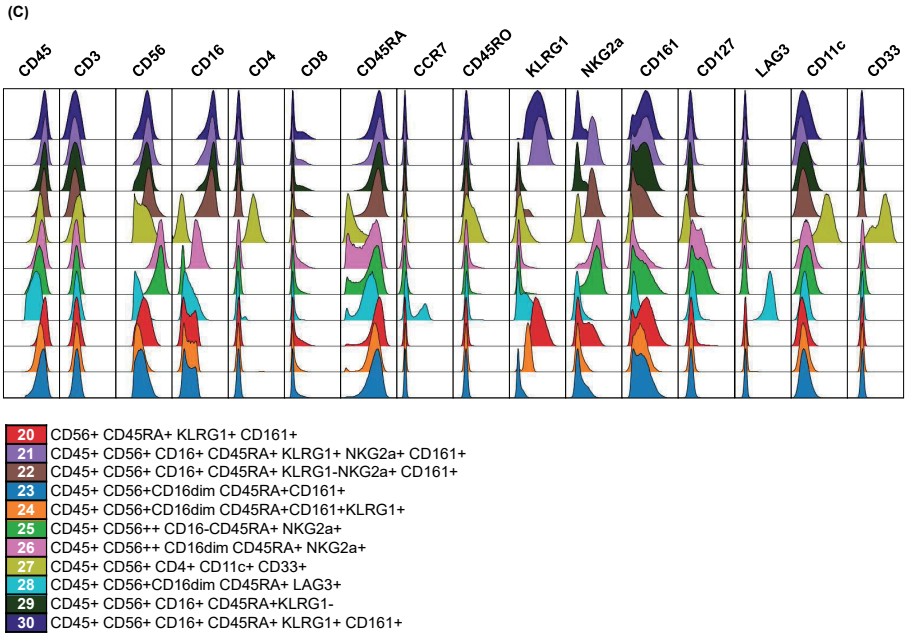


Figure S4. (C) Expression levels of each of the indicated markers are depicted for the individual cell populations.

Figure S5. Myeloid cell populations

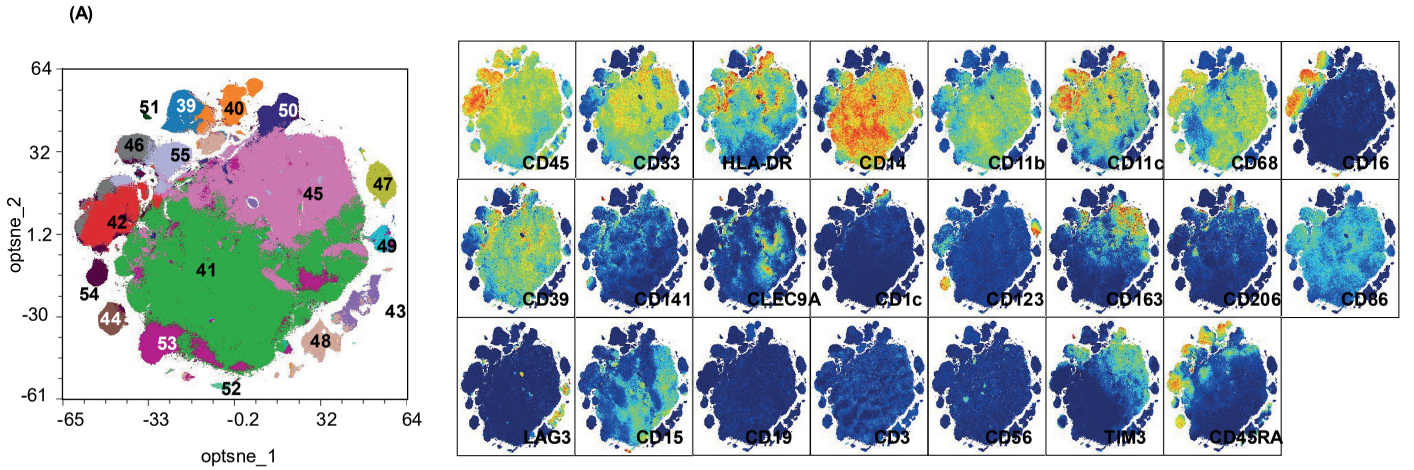


Figure S5. Continued

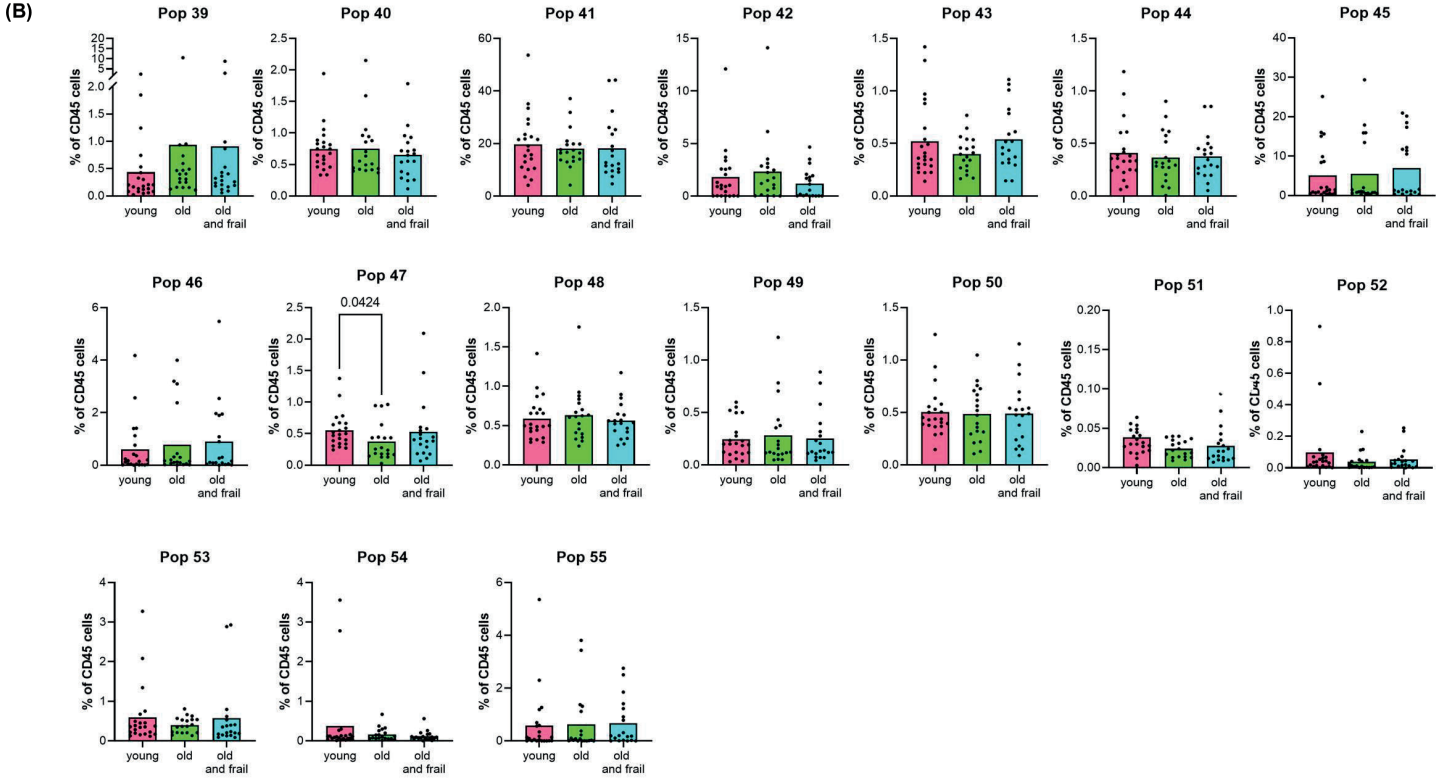


Figure S5. (A) Cluster partitions by FLOWSOM of PBMCs stained with antibodies for myeloid cell markers. In total, 17 different clusters were defined (left). OptSNE plots visualizing contour plots of the 3 patient groups (right). (B) Frequencies of myeloid cell populations in young, old and old-frail patient groups. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Kruskal-Wallis tests and $p<0.05$.

Figure S5. Continued

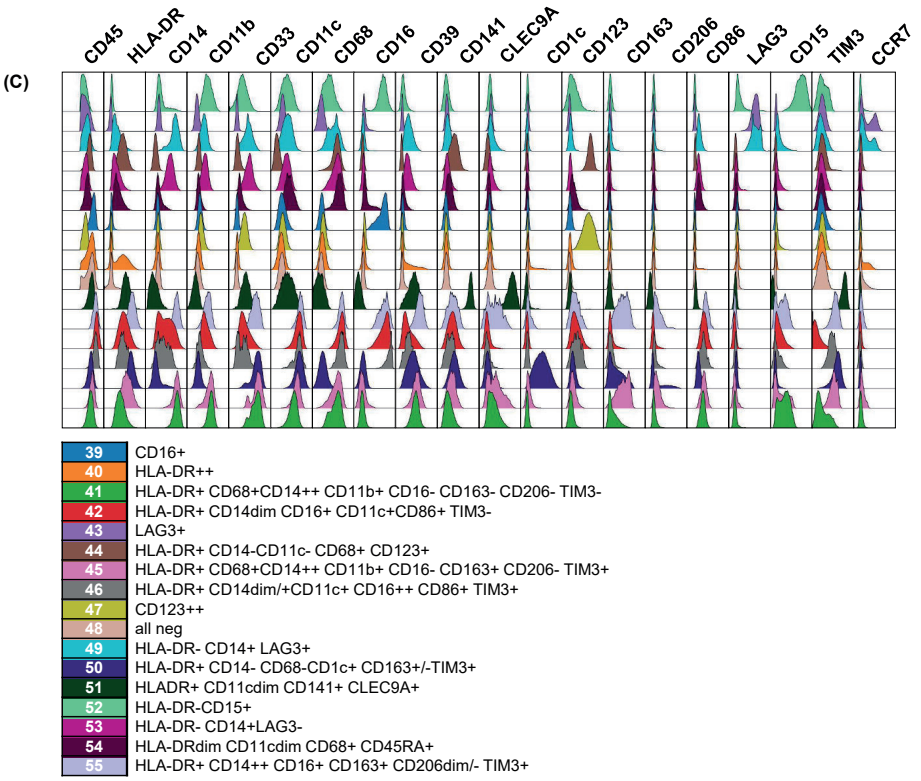


Figure S5. (C) Expression levels of each of the indicated markers are depicted for the individual cell populations.

Figure S6. B cell populations

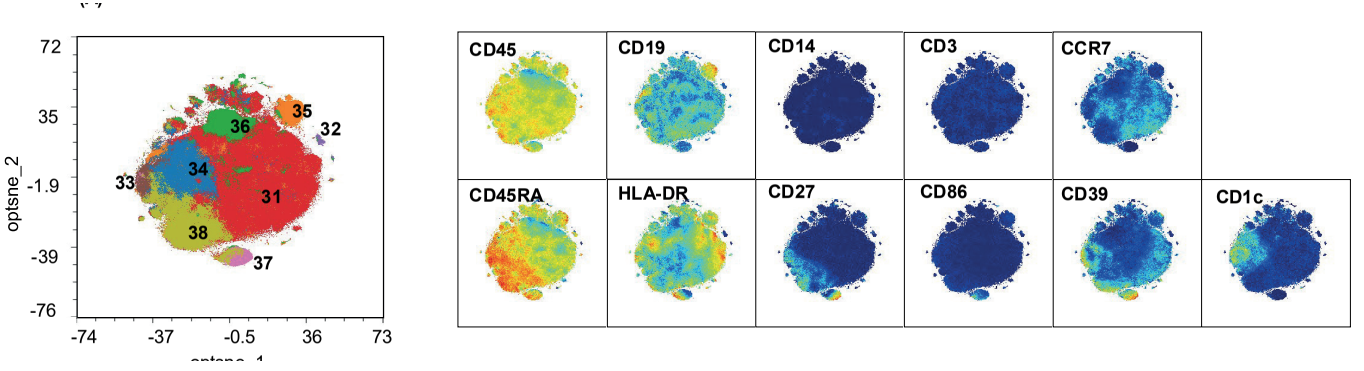
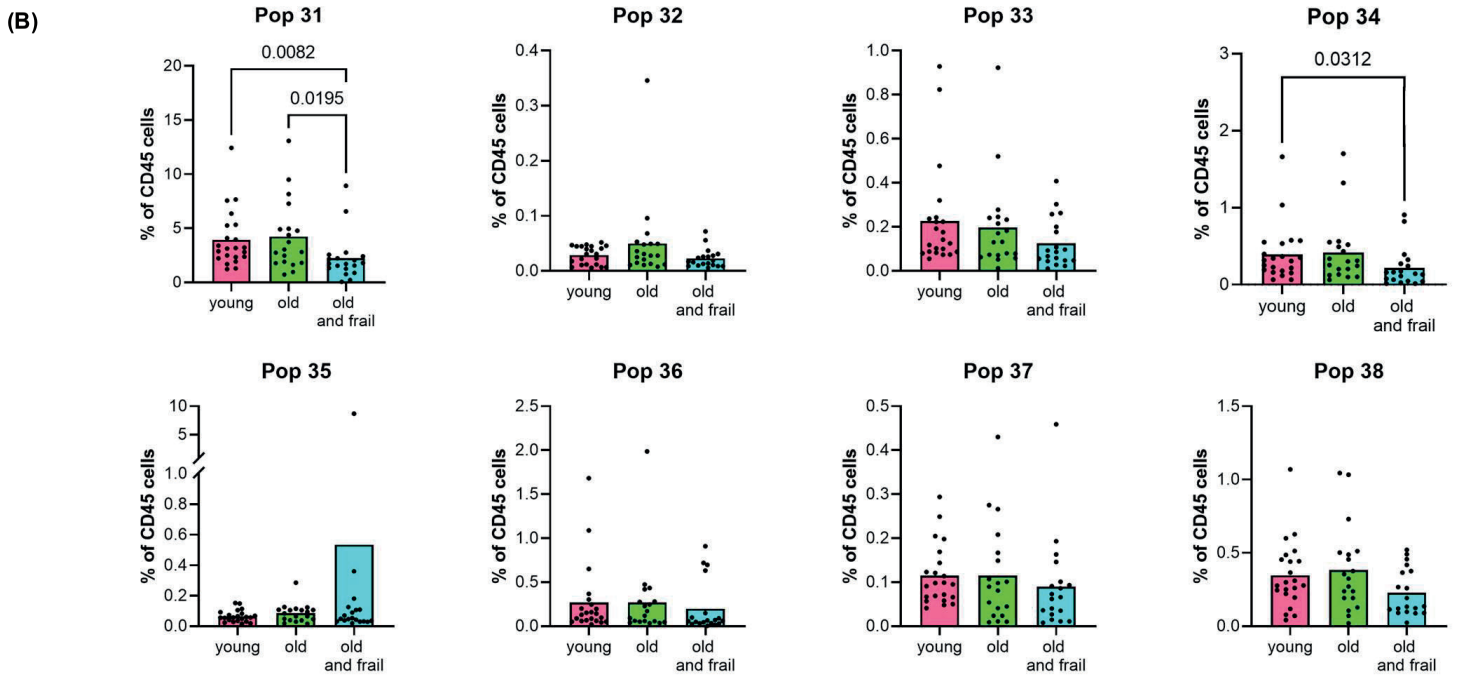


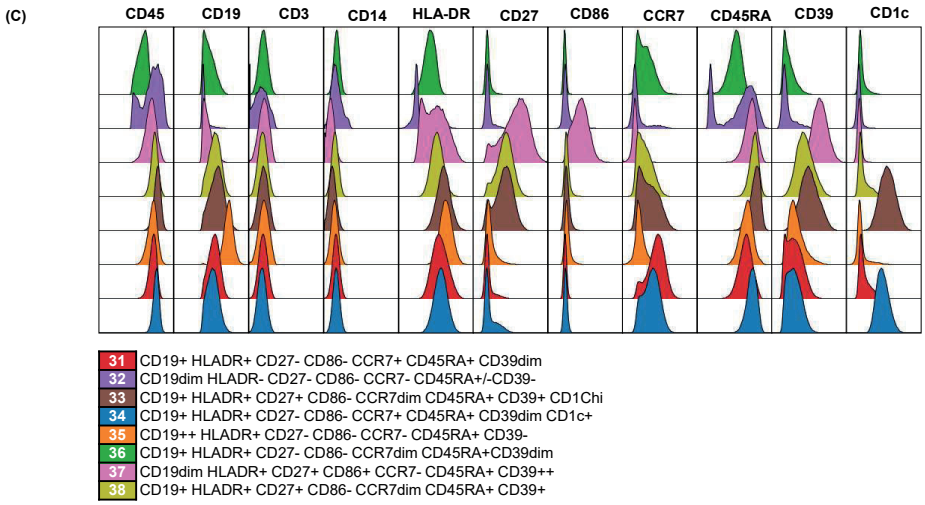
Figure S6. (A) Cluster partitions by FLOWSOM of PBMCs stained with antibodies for CD19+ B cell markers. In total, 8 different clusters were defined (left). OptSNE plots visualizing contour plots of the 3 patient groups (right).

Figure S6. Continued



(B) Frequencies of B cell populations in young, old and old-frail patient groups. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Kruskal-Wallis tests and p<0.05.

Figure S6. Continued



(C) Expression levels of each of the indicated markers are depicted for the individual cell populations.

Figure S7. Frequencies of CD3+ T cell populations between patients having a response/no response to treatment

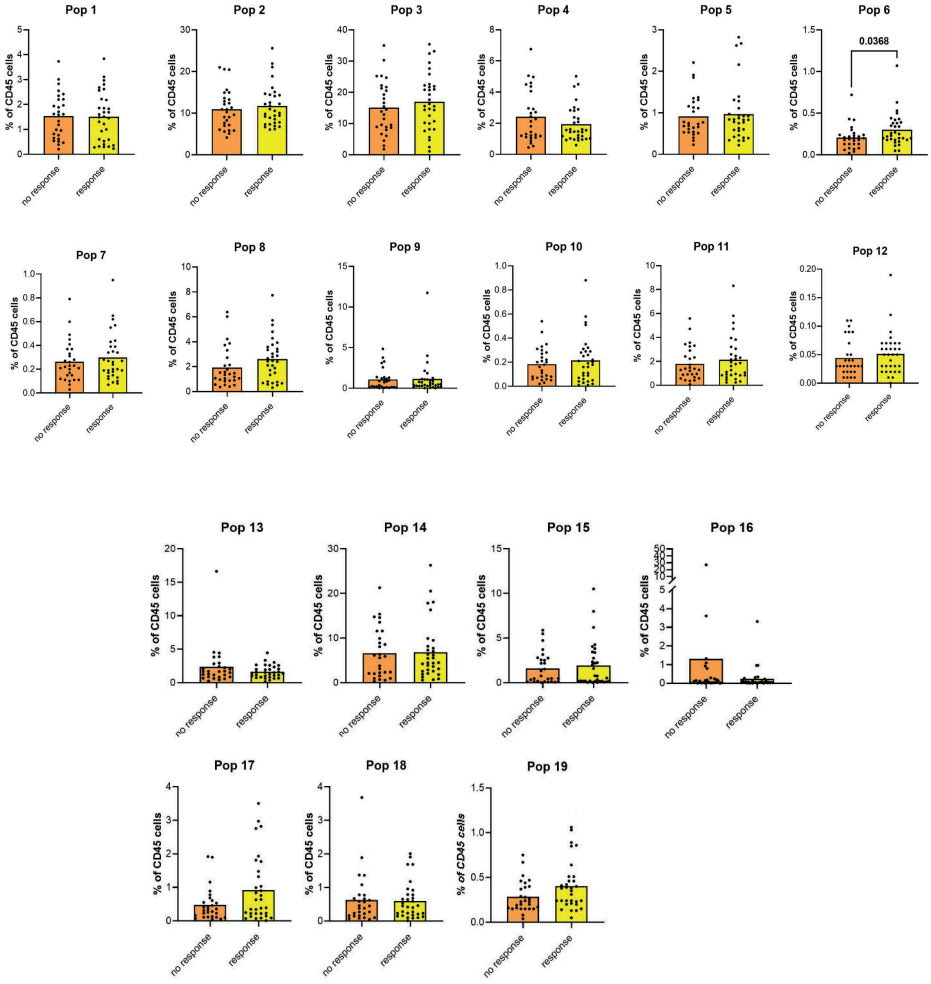


Figure S7. Frequencies of CD3+ T cell populations between the different responses to treatment. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Mann-Whitney tests and p<0.05.

Figure S8. Frequencies of NK cell populations between patients having a response/no response to treatment

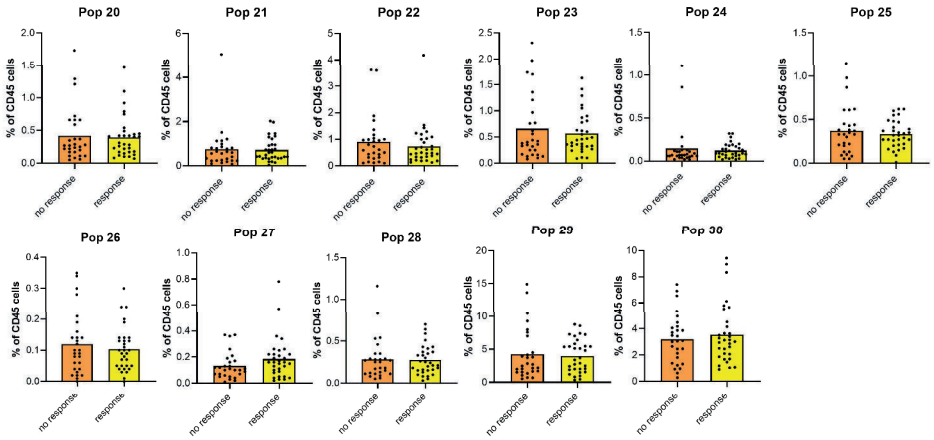


Figure S8. Frequencies of NK cell populations between the different responses to treatment. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Mann-Whitney tests and p<0.05.

Figure S9. Frequencies of myeloid cell populations between patients having a response/no response to treatment

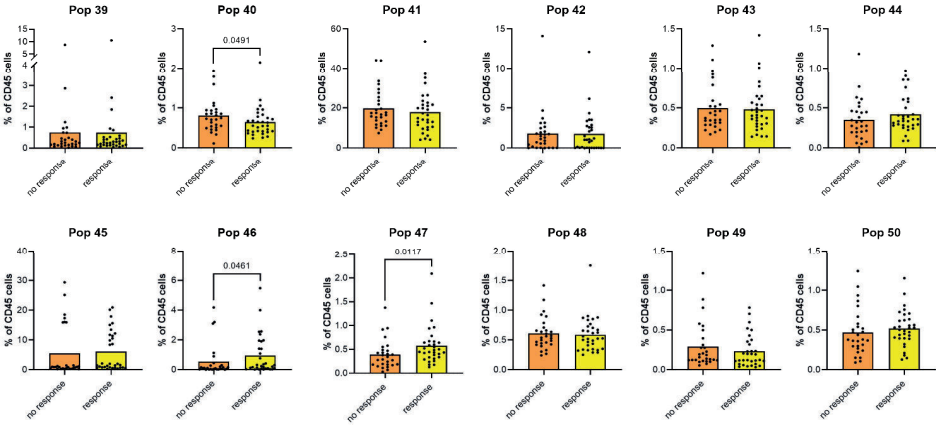


Figure S9. Frequencies of myeloid cell populations between the different responses to treatment. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Mann-Whitney tests and p<0.05.

Figure S10. Frequencies of B cell populations between patients having a response/no response to treatment

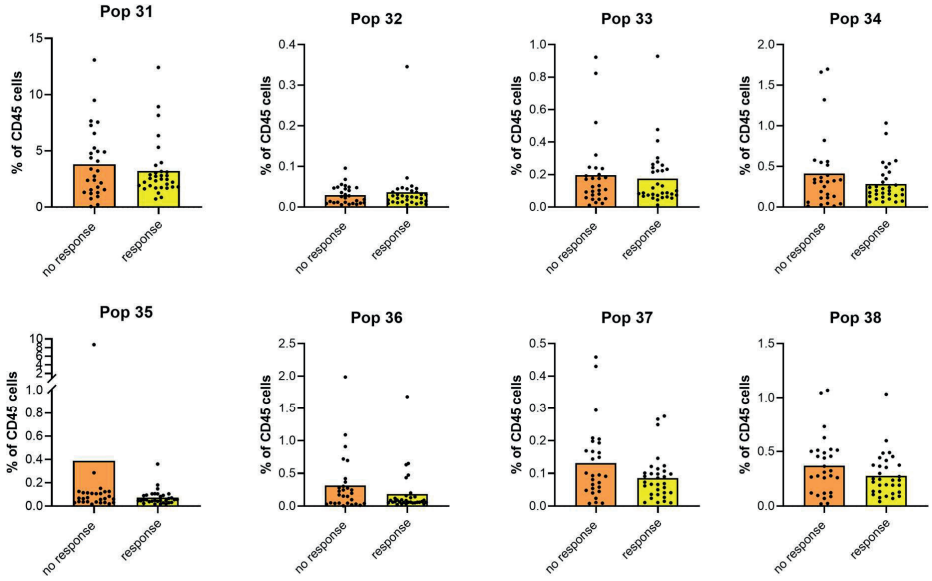


Figure S10. Frequencies of B cell populations between the different responses to treatment. Cell populations are presented as a percentage of the total CD45+ cells. Statistical differences were assessed with Mann-Whitney tests and p<0.05.

5

Toxicity in older cancer patients receiving immunotherapy – an observational study

Estelle Tran Van Hoi, Stella Trompet, Yara Van Holstein, Frederiek Van Den Bos, Diana Van Heemst, Henrik Codrington, Geert Labots, Suzanne Lohman, Asli Ozkan, Johanneke Portielje, Simon P. Mooijaart, Nienke A. De Glas, Marloes Derks

Drugs Aging. 2024 May;41(5):431-441.

ABSTRACT

Background

Immunotherapy by checkpoint inhibition has been established as an effective treatment strategy for a variety of diseases affecting the care of all patients with cancer, including older adults. However, older cancer patients represent a heterogeneous group as they can vary widely in frailty, cognition and physical status.

Objective

This study aims to investigate the association between clinical frailty and immune-related treatment toxicity (IrTox), hospitalization and treatment discontinuation due to IrTox in older patients treated with checkpoint inhibitors.

Patients and Methods

Patients aged 70 years and older, treated with checkpoint inhibitors, were selected from the TENT study, IMAGINE study and "Tolerability and safety of immunotherapy study". Clinical Frailty was assessed by Geriatric-8 (G8) score and WHO status. Outcomes were grades 3-5 toxicity, hospitalization and treatment discontinuation due to toxicity during treatment.

Results

Of the 99 patients included, 22% had comorbidities. While 33% of the patients were considered frail based on an abnormal G8 score of < 15, physical impairments were considered absent in 51% (WHO score of 0) and mild in 40% (WHO score of 1). Despite the limited sample size of the cohort, consistent trends were observed with patients with an abnormal G8 score of < 15 or higher WHO score of 1 for having higher odds of toxicity (OR 2.32 (95% 0.41-13.02); OR 1.33 (95% 0.45-4.17)), treatment discontinuation due to IrTox (OR 2.25 (95% 0.61-8.31); OR 2.18 (95% 0.7-6.73)); and hospitalization due to IrTox (OR 3.72 (95% 0. 39-35.4); OR 1.31 (95% 0.35-4.9)). Moreover, in a sub-analysis, we observed that the treatment discontinuation due to IrTox occurred often in patients with a grades 1-2 toxicity as well.

Conclusion

Although not statistically significant, in older patients treated with immunotherapy in a real-life population with cancer, we observed consistent trends towards increased toxicity, hospitalization and treatment discontinuation with increasing frailty. Larger studies are needed to confirm these exploratory results. Moreover, older patients with lower toxicity grade 1-2 experienced early treatment discontinuation frequently, suggesting a lower tolerance of toxicity.

INTRODUCTION

Cancer is highly prevalent in old age, and it is estimated that 55% of new cases are diagnosed in people aged over 65 years (1). Older patients are a heterogeneous group with large variation in geriatric problems such as impaired cognitive and physical functioning, comorbid diseases, polypharmacy or frailty. Treatment selection for older patients with cancer can be challenging in the setting of various impairments. Balancing potential benefits of treatment, such as increased survival or reduction of symptoms against potential harms like adverse side effects or risk of complications) can be difficult as the varying complexity in health issues among older patients can affect the ability to endure toxic cancer treatments.

Geriatric deficits have been associated with increased risk of toxicity due to chemotherapy, and with increased mortality (2, 3). Moreover, in the GAP-70 and GAIN randomized control trials, it was demonstrated that when treatment decisions were based on geriatric assessment (GA), toxicity was decreased due to dose reduction, while survival was unaffected, thereby supporting the implementation of GA-guided processes for cancer treatment (4-6). Therefore, a personalized treatment plan adjusted to the specific geriatric characteristics could lead to improved treatment care.

Immunotherapy with immune checkpoint inhibitors (ICI) has recently become a promising treatment for various types of cancer. For patients with advanced lung cancer, immunotherapy has improved overall survival with an acceptable toxicity profile (7). The most frequently used immunotherapies are pembrolizumab and nivolumab, which both target the programmed cell death protein 1 (PD-1) (8, 9). However, immunotherapy can have important side effects that may limit the ability to endure this treatment, especially in patients with additional geriatric deficits, considering the ageing immune system (10, 11). As prior studies showed no difference in the efficacy and toxicity of ICI based on calendar age, investigating frailty seems relevant. However, data on outcomes of immunotherapy in older people and their association with frailty are very scarce. For instance, Bruijnen et al. demonstrated that frail patients had higher irAE-related hospitalizations, longer stays, and more ICI discontinuations. Few studies demonstrated that geriatric impairments may increase the risk of immune-related adverse events in older patients treated with ICIs; however, association did not reach statistical significance (12, 13).

Therefore, the aim of the present study is to investigate the association between clinical frailty, measured by G8 score and WHO status, and treatment toxicity, hospitalization and treatment discontinuation due to toxicity in older patients treated with immunotherapy in a real-life population with cancer.

METHODS

Study population

For the present analyses, we selected patients from three ongoing studies: 1) TENT study, 2) IMAGINE study, 3) "Tolerability and safety of immunotherapy study", as described below. Patients were included between June 2011 and October 2020. We included patients with cancer of 70 years and older who were treated with immunotherapy regardless of treatment line and duration. Patients could be included both if they received only immunotherapy, the combination of immunotherapy and chemotherapy or the combination of immunotherapy and targeted therapy. All patients that were included in this cohort were treated in the Hagaziekenhuis (Den Haag, The Netherlands) or the Leiden University Medical Center (LUMC; Leiden, The Netherlands).

The Triage of Elderly Needing Treatment (TENT) study is a multicenter prospective study (14). All patients aged 70 years and older presenting in the outpatient department and needing an invasive medical intervention or treatment (surgery, chemotherapy or radiotherapy) in one of the participating hospitals undergo a short geriatric screening before their intervention. Prior to an invasive medical intervention, patients underwent a geriatric screening with the Geriatric-eight (G8) test and the 6-item Cognitive Impairment Test (6-CIT). Patients who scored abnormally on at least one of the two screening instruments received a comprehensive geriatric assessment (CGA) before starting treatment. The CGA is used to assess frailty or vulnerability in older patients, a clinical state characterized by a decline in functioning across multiple physiological systems, accompanied by increased vulnerability to stressors resulting in high risk of adverse health outcomes and mortality. CGA is a multidimensional assessment that takes into account medical diagnoses, psychological, somatic and functional impairments and social issues affecting patients' well-being (15). During follow-up, patients were assessed on mobility, independence and patients' self-rated health, mortality, toxicity and hospitalization. Participating patients were followed for 12 months and reassessed at 6 months and 12 months after the start of treatment. Ethical approval and consent to participate in the TENT study protocol were approved by the Medical Ethics Committee (METC) at Leiden University Medical Center. All participants or a proxy provided written informed consent.

The IMAGINE study (IMmunotherapy in AGINg patiEnts) is a prospective cohort study in patients who were treated in the LUMC with immunotherapy. Geriatric characteristics were gathered during the CGA and patients were followed over 24 months. Outcomes, including toxicity and hospitalization, were prospectively registered from medical charts. Ethics approval and consent to participate in The IMAGINE study protocol

were approved by the Medical Ethics Committee (METC) at Leiden University Medical Center. All participants or a proxy provided written informed consent.

The Tolerability and safety of immunotherapy study (ImToSa) is a partly retrospective cohort study; some patients were included after treatment, and others before treatment. All patients treated with immunotherapy were included in our study population. In the majority of patients, the G8 was performed as part of the standard of care. The initial aim of the study was to assess age-related differences in side-effects and predictors of toxicity, including gender, geriatric characteristics, and previous treatments. Patients were followed for 24 months. The METC Southwest Holland has issued that the study was not subject to Medical Research Involving Human Subjects Act (WMO) declaration. Patients consent was therefore not requested.

Clinical parameters

Tumor-specific information was extracted from the medical record or pathology report and consisted

of tumor type and tumor stage, which we eventually stratified into advanced or metastatic cancer.

Baseline parameters

For all 3 studies, demographic information was collected at baseline within 3 months before starting immunotherapy, including age, sex and living situation. Additionally, history of smoking and WHO performance score were assessed. The WHO performance score is classified with a score between 0 and 5, in which a higher score indicates a lower physical performance. A WHO score of 0 corresponds with an asymptomatic, fully active patient, a score of 1 corresponds to a symptomatic but ambulatory patient who can do light physical work, a score of 2 corresponds to a symptomatic patient that is <50% in bed during the day, and fully capable of self-care, a score of 3 corresponds to a symptomatic patient that is >50% in bed and capable of limited self-care, a score of 4 corresponds to a bedbound patient who is not able to perform any self-care and a score of 5 corresponds to a diseased patient (16) . The WHO status was extracted from the medical record.

Geriatric parameters

In the present study, we mainly focus on frailty using the G8 questionnaire, which was recorded before the start of treatment in all patients. The Geriatric-8 (G8) questionnaire is a frailty screening instrument and is used to assess if a CGA is further needed in older cancer patients. A G8 score < 15 (also “abnormal”) is considered to be potentially frail.

The presence of comorbidities was assessed. Comorbidities present at the time of diagnosis were classified into the following categories: 1) cardiovascular disease, 2) chronic lung disease, 3) neuropsychiatric, 4) rheumatoid arthritis, 5) ulcer, 6) other malignancy, 7) diabetes mellitus.

Outcomes

Endpoints were (i) immunotherapy-related toxicity (IrTox), (ii) hospitalization and (iii) treatment discontinuation due to toxicity. Severe toxicity was defined as grades 3 or higher immunotherapy-related side-effects according to the CTCAE criteria version 5 (17). In order to investigate the combined impact of treatment toxicity in (frail) older adults, we further defined "disadvantageous outcome" as having ≥1 of the 3 outcomes (IrTox, hospitalization and treatment discontinuation due to toxicity).

While the TENT and IMAGINE cohorts only included grade ≥3 toxicity, the Tolerability and Safety of Immunotherapy Study registered the full range of toxicity, including lower grades (grades 1-5). We performed a sub-analysis of associations between geriatric characteristics, all grades of toxicity and outcomes (immunotherapy-related toxicity, hospitalization and treatment discontinuation due to toxicity) in the Tolerability and Safety of Immunotherapy Study.

Data management

Data was recorded on Case Record Forms, encrypted, and stored in an electronic data management system (Castor EDC), in accordance with General Data Protection Regulations (GDPR).

Statistical analysis

Normal and skewed distributed continuous data were presented as respectively mean with standard deviation (SD) and median with interquartile range (IQR). Numbers with percentages were used to present categorical data. To investigate the association between baseline geriatric characteristics and toxicity, hospitalization and treatment discontinuation due to toxicity, we used a logistic regression model. Due to the relatively small number of events, we decided to perform univariate analyses only in order to avoid overfitting. Odds ratios with 95% confidence intervals (CI) were calculated, and a P-value of <0.05 was considered significant. All analyses were performed using SPSS (IBM version 25). All figures were graphically depicted using Microsoft Excel version 2019.

RESULTS

A total of 99 patients were included in this study: 21 from the TENT study, 68 from the ImToSa study and 10 from the IMAGINE study. Median follow-up was 15.8 months (IQR 11.2-20.4).

Patient characteristics are described in Table 1. We included 56 male patients and 43 female patients with a median age of 74 years (IQR 71.0-77.0 years). All patients had either stage III (advanced) or stage IV (metastatic) disease. Patients presented with lung cancer (71%), melanoma (11%), renal cell carcinoma (4%), breast cancer (8%) or urothelial cancer (6%). In the patient population with lung cancer, 39% had >50% PD-L1 expression. All patients were treated with immunotherapy, either as monotherapy (75%) or in combination with chemotherapy (19%) or targeted therapy (n=5, 5%). The majority of patients received either pembrolizumab (50%) or nivolumab (27%). Of all patients, 33% had an abnormal G8, 28% patients had a normal G8, and for 38% patients G8 score was not known. 78% of the patients had one or more comorbidities, 57% of whom had cardiovascular and 21% diabetes mellitus related.

Grade ≥3 toxicity outcomes

Types of grades ≥ 3 toxicity are presented in Figure 1. We observed 14% patients with Grade ≥3 toxicity due to immunotherapy. The most frequently observed types of toxicity were rash (18%) and pneumonitis (17%).

Hospitalization due to toxicity was seen in 10% of patients, and 21% of patients did not continue their treatment due to all-grade toxicity (Table 2).

Table 1. Patient and geriatric characteristics at baseline (N=99)

	N	%
Age (years), median (IQR)	99	74.0 (71.0-77.0)
Gender		
Male	56	56.6
BMI (median, IQR)	99	24.5 (22.3-27.1)
Healthy weight (18.5-24.9)	52	52.5
Underweight (<18.5)	7	7.1
Overweight (25-29.9)	31	31.3
Obese (>30)	9	9.1
History of smoking		
Yes	69	69.7
No	10	10.1
Unknown	20	20.2
Tumor type		
Non-small cell Lung carcinoma	70	70.7
Melanoma	11	11.1
Renal carcinoma	4	4.0
Urothelial carcinoma	6	6.1
Breast carcinoma	8	8.1
Tumor stage		
Locally Advanced	13	13.1
Metastatic	77	77.8
Unknown	9	9.1
Treatment		
Immunotherapy	75	75.8
Immuno- and chemotherapy	19	19.2
Immuno- and targeted therapy	5	5.1
Type of immunotherapy		
Ipilimumab	5	5.1
Nivolumab	27	27.3
Pembrolizumab	49	49.5
Atezolizumab	11	11.1
Durvalumab	6	6.1
Other	1	1.0

	N	%
G8 score		
Normal	28	28.3
Abnormal (< 15)	33	33.3
Unknown	38	38.4
WHO score		
Score 0	51	51.5
Score 1	40	40.4
Score 2-3	4	4.0
Unknown	4	4.0
Comorbidities		
Yes	77	77.8
No	22	22.2
Type of comorbidities		
Cardiovascular	56	56.6
Diabetes Mellitus	21	21.2
Asthma/COPD	21	21.2
Neuropsychiatric	4	4.0
Rheumatologic disease	3	3.0
Gastric ulcer	3	3.0
Other malignancy	11	11.1
More than 3 medications		
Yes	19	19.2
No	6	6.1
Unknown	74	74.7
Corticosteroids use[a]		
Yes	14	14.1
No	56	56.6
Unknown	29	29.3

[a] *In patients with grade III-V toxicity*

Abbreviations: N, number of patients; BMI, body mass index; COPD, chronic obstructive pulmonary disorder; G8, Geriatric-8 test; IQR, interquartile range; WHO score, Eastern Cooperative Oncology Group (ECOG) score

Table 2. Hospitalization and treatment discontinuation

	N	%
Hospitalization due to toxicity		
Yes	10	10.1
No	89	89.9
Treatment discontinuation		
Yes	78	78.8
No	19	19.2
Unknown	2	2.0
Treatment discontinuation reason (N=78)		
Due to toxicity	16	20.5
Due to severe toxicity (grade 3-5)	6	7.6
Due to disease progression	40	51.2
Other	22	28.2

Abbreviations: N, number of patients

Figure 1. Incidence of toxicity and types of toxicity (grade 3-5)

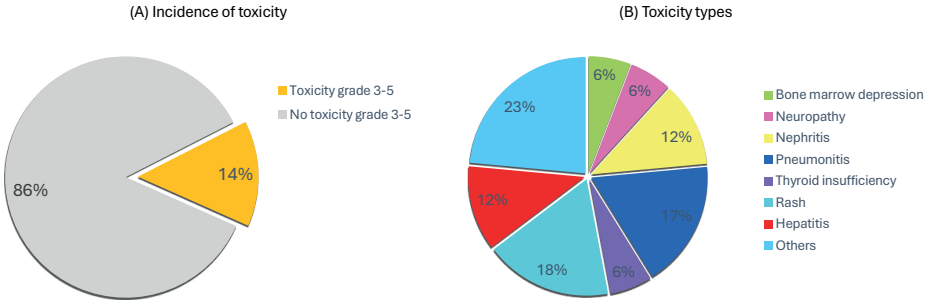


Figure 1. (A) Pie chart describing the incidence of toxicity grade 3-5. (B) Pie chart describing the toxicity types among patients with toxicity grade 3-5.

Results of the univariate analysis of toxicity are described in Table 3A, 3B. Although none of the associations with grade ≥ 3 toxicity were statistically significant, observed effect estimates showed consistent directions of effect as described below. Higher risk of Grade ≥*3* toxicity tended to be positively associated with higher age (OR 1.07 (95% 0.95-1.21)) and being overweight (OR 2.74 (95% 0.79-9.56)). As per treatment type, patients receiving immune- and chemotherapy showed a tendency towards higher risk of Grade ≥3 toxicity (OR 2.62 (95% 0.72-9.01)) compared to patients receiving immunotherapy alone. A tendency towards increased risk of toxicity

was observed in patients with an abnormal G8 score of < 15 (OR 2.32 (95% 0.41-13.02)) and a higher WHO score of 1 (OR 1.33 (95% 0.43-4.17)). Moreover, increased comorbidities tended to be associated with increased risk of toxicity (OR 1.85 (95% 0.38-9.0). The effect estimates were highest for cardiovascular comorbidities (OR 3.26 (95% 0.85-12.52)) and diabetes (OR 1.6 (95% 0.45-5.73)).

Comparable results were observed for treatment discontinuation and hospitalization due to toxicity (supplementary table 2-3).

Figure 2 reports the overlap of toxicity risk, treatment discontinuation and hospitalization due to IrTox. All patients who were hospitalized had toxicity and/or discontinued their treatments.

Figure S1 reports the incidence of comorbidity in patients with ≥ 1 disadvantageous outcome. 25% of the patients (n=24) had ≥1 disadvantageous outcome (toxicity grade ≥3 or hospitalization due to IrTox or treatment discontinuation due to IrTox). Of the patients with comorbidities (n=77), 29% (n=22) had ≥1 disadvantageous outcome, while of the patients without comorbidities (n=22), 9% (n=2) had ≥1 disadvantageous outcome.

Table 3. Determinants of toxicity risk (grade 3-5)

(A)		N	N events	OR	95% CI		p-value
					Lower	Upper	
Age		99	14	1.073	0.952	1.208	0.248
BMI							
	Healthy weight (18.5-24.9)	52	5	Reference			
	Underweight (<18.5)	7	1	1.567	0.156	15.768	0.703
	Overweight (25-29.9)	31	7	2.742	0.787	9.555	0.113
	Obese (>30)	9	1	1.175	0.121	11.420	0.889
Tumor type							
	Breast cancer	8	1	Reference			
	Non-small cell lung cancer	70	8	0.903	0.098	8.324	0.928
	Melanoma	11	5	5.833	0.525	64.823	0.151
	Renal carcinoma	4	0	NA			
	Urothelial carcinoma	6	0	NA			
Treatment type							
	Immunotherapy	75	9	reference			
	Immuno- and chemotherapy	19	5	2.619	0.716	9.014	0.127
	Immuno- and targeted therapy	5	0	NA			
Type of immunotherapy							
	Pembrolizumab	49	7	Reference			
	Nivolumab	27	4	1.043	0.276	3.944	0.95
	Ipilimumab	5	2	4.0	0.563	28.396	0.166
	Atezolizumab	11	1	0.6	0.066	5.447	0.650
	Durvalumab	6	0	NA			
	Other	1	0	NA			

(B)	N	N events	OR	95% CI		p-value
				Lower	Upper	
G8 score						
Normal	28	2	reference			
Abnormal (< 15)	33	5	2.321	0.414	13.023	0.338
Unknown	38	7	2.935	0.561	15.372	0.202
WHO score						
Score 0	51	7	reference			
Score 1	40	7	1.333	0.426	4.172	0.621
Score 2-3	4	0	NA			
Unknown	4	0	NA			
Comorbidities						
No	22	2	reference			
Yes	77	12	1.846	0.381	8.951	0.447
Type of comorbidities						
Cardiovascular	56	11	3.259	0.849	12.519	0.085
Diabetes Mellitus	21	4	1.6	0.447	5.729	0.470
Asthma/COPD	21	2	0.579	0.119	2.815	0.498
Neuropsychiatric	4	1	2.103	0.203	21.774	0.533
Rheumatologic disease	3	0	NA			
Gastric ulcer	3	0	NA			
Other malignancy	11	0	NA			

Abbreviations: N, number of patients; N events, number of patients with toxicity; OR, odds ratio for univariable logistic regression; BMI, body mass index; COPD, chronic obstructive pulmonary disorder; G8, Geriatric-8 test; WHO score, Eastern Cooperative Oncology Group (ECOG) score; NA, not applicable

Figure 2. Overlap of toxicity risk, treatment discontinuation and hospitalization due to IrTox.

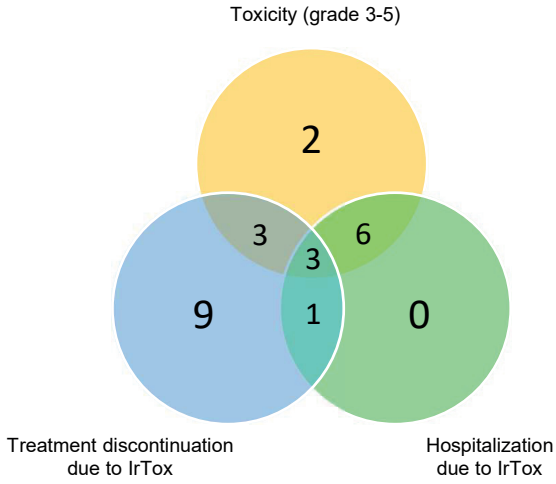


Figure 2. Venn Diagram describing the overlap of the different disadvantageous outcomes due to IrTox: risk of severe toxicity (grade 3-5), treatment discontinuation due to IrTox and hospitalization due to IrTox.

All grade toxicity in the Tolerability and Safety of Immunotherapy study

Figure 3 reports the incidence of all grades of toxicity and types of toxicity in the sub-analysis of the Tolerability and Safety of Immunotherapy study. Patient characteristics are described in Supplementary Table 3. Included patients (n=68) had a median age of 75 years (IQR 71.0-77.0 years). Most patients had lung cancer (n=53, 78%). All patients were treated with immunotherapy, either as monotherapy (n=62, 91%) or in combination with chemotherapy (n=6, 9%); the majority of patients received either pembrolizumab (n=30, 44%) or nivolumab (n=25, 37%). 38.2% patients had a G8 < 15, 22% patients had a G8>14 and for 40% patients, G8 score was not known.

Of the group of 68 patients, 81% of all patients had toxicity (any grade). Of these, 68% of toxicities were grade 1, 49% grade 2 and 9% grade 3. Fatigue (24%), rash (21%) and pruritis (17%) were the most common types of toxicity. In this subgroup, 9% of the patients have been hospitalized due to toxicity, and 16% of the patients had a treatment discontinuation due to toxicity (Table 4). Results of the univariable analysis of toxicity of the sub-cohort did not show predictors for toxicity (Supplementary Table 4).

Figure 3. Incidence of toxicity and toxicity types in the Tolerability and Safety of Immunotherapy study (N=68)

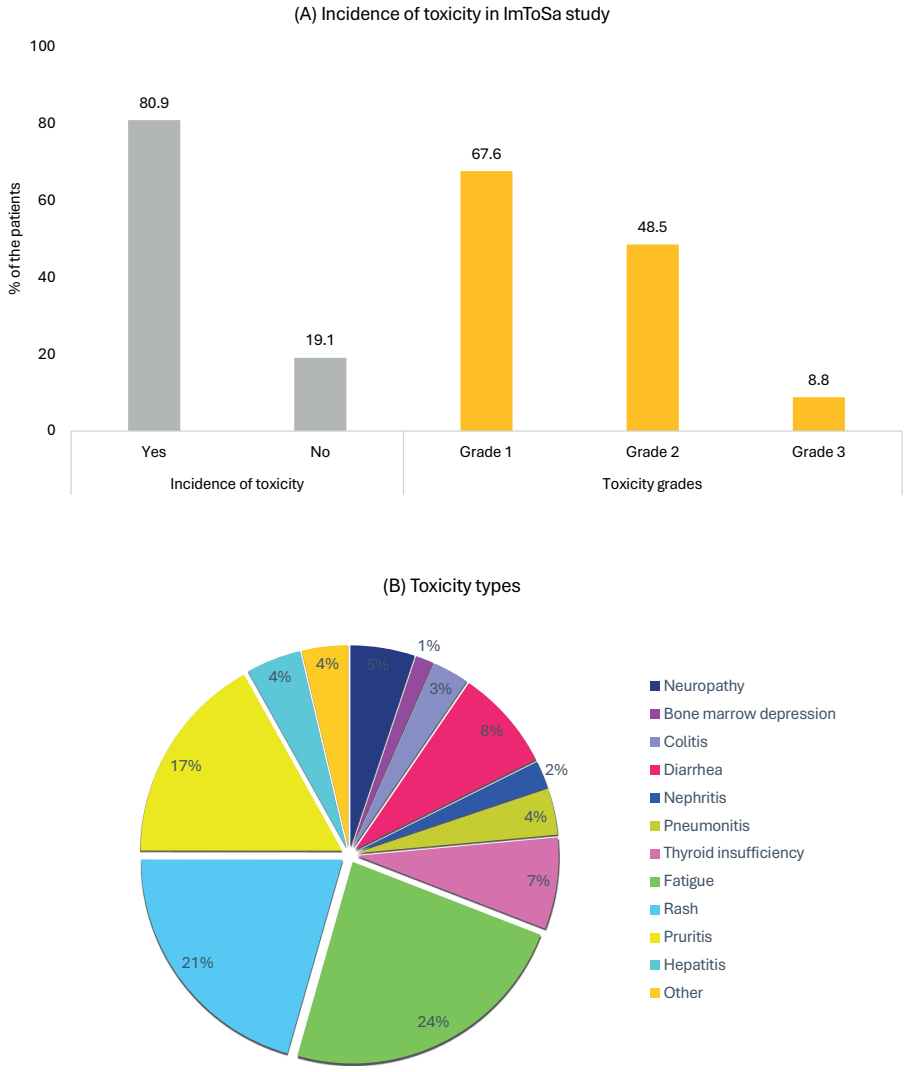


Figure 3. (A) Graphic describing the incidence of all grades of toxicity and the incidence of each grade of toxicity. (B) Pie chart describing toxicity type in patients with all grade toxicity.

Table 4. Incidence of hospitalization and treatment discontinuation due to toxicity (grade 1-5) in the ImToSa study

	N	%
Hospitalization due to toxicity		
Yes	6	8.8
No	62	91.2
Corticosteroids use		
Yes	10	14.7
No	50	73.5
Unknown	8	11.8
Treatment discontinuation		
Yes	54	79.4
No	14	20.6
Unknown		
Treatment discontinuation reason		
Due to toxicity (grade 1-5)	11	16.2
Due to disease progression	26	38.2
Other	17	25
Total	54	79.4
Missing	14	20.6

Abbreviations: N, number of patients

DISCUSSION

The present study observed consistent trends towards increased toxicity-related outcomes with frailty measured by the G8 score and comorbidities, although none of the observed associations were statistically significant. Both an abnormal G8 score of < 15 and a higher WHO score of 1 tended to be associated with a higher risk of toxicity, hospitalization, and treatment discontinuation due to toxicity. Additionally, severe toxicity tended to be more prevalent in patients with comorbidities, especially with cardiovascular comorbidities. Association of comorbidities showed trends towards higher odds of treatment discontinuation and hospitalization due to toxicity. Moreover, older frail patients were more at risk of experiencing a disadvantageous outcome. We observed that a considerable proportion of older patients had grade 1 or 2 toxicity with fatigue, rash and pruritis most often experienced. Of these patients, 16% discontinued treatment. These results suggest that grades 1 to 2 toxicities in older adults can lead to early immunotherapy discontinuation.

Numerous studies have confirmed the role of GA in predicting chemotherapy-related adverse events, but the association of frailty with ICI-related adverse events has not been extensively studied (18, 19). Bruijnen et al showed that significantly more frail patients were admitted to the hospital because of immune-related adverse events (irAEs) and, in addition, observed a trend toward increased length of hospitalization and ICI discontinuation for irAEs (20). Similarly, the ELDERS study demonstrated that an abnormal G8 score < 15 was a predictor of higher risk of irAEs such as comorbidity-related hospital admissions (p=0.031) and risk of death (p=0.01) (21). Comparable to our study, this suggests that lower-grade irAE in older patients may challenge tolerance due to comorbidity burden, reduced organ function and reduced reserve capacities. Less severe toxicities can obviously severely affect older patients in their daily functioning and quality of life. Moreover, a recent study revealed that higher comorbidity burden was associated with shorter overall survival (OS), suggesting that these conditions may limit the benefits of the treatment. Specifically, cardiovascular disease may predict shorter OS in patients experiencing irAEs (22), indicating the need for careful evaluation in this patient group. These findings align with our data, showing a trend toward immune-related toxicity in patients with comorbidities, especially with cardiovascular comorbidities. The present results highlight the importance of studies of patient-reported outcomes such as quality of life and functionality.

In randomized clinical trials, ICIs have been shown to have similar efficacy in older patients compared to younger patients with various types of cancer (23). In pivotal RCTs, however, only selected, more fit, older patients have participated, and little is known about the effects of ICI in older patients with frailty or comorbidities. Although initially proposed that checkpoint inhibitors could be less effective in

older patients due to ageing of the immune system (immunosenescence), this has never been demonstrated in real-world populations (24, 25). Activation of PD-1 results in decreased T cell effector activity, proliferation and survival, hence leading to an immunosuppressive function. When this immunosuppressive function is exploited by cancer cells, a tumor immune escape is enhanced. Therefore, the immunosenescence phenomenon may induce different efficacy and/or toxicity patterns of immunotherapies in older patients. Another concern is that older cancer patients may benefit less from immunotherapy because they are less resilient to endure toxic treatments (26, 27). This makes it even more important to incorporate predictive factors for toxicity in treatment decisions in older adults. Polypharmacy may serve as a significant predictive factor for toxicity in treatment decision-making. While our study did not examine polypharmacy due to limited data availability, it is critical to acknowledge its high prevalence in older cancer patients. Existing preliminary evidence demonstrates the correlation between polypharmacy and various health outcomes, such as adverse drug events, falls, frailty, hospitalization, postoperative complications, and mortality (28, 29). For instance, research by Hakozaki et al. in older patients with advanced non-small-cell lung cancer undergoing ICI therapy found an association between polypharmacy and an elevated rate of unexpected hospitalizations (30). Similarly, another study reported a high incidence of polypharmacy alongside increased rates of post-chemotherapy hospitalization. These studies collectively indicate that the concurrent use of multiple medications can significantly impact the treatment outcomes (31).

The main strengths of the present study include the incorporation of detailed toxicity outcome measures, including all-grade toxicity in the majority of the cohort. To the best of our knowledge, this is the first study that included all-grade toxicity and its consequences in relation to frailty. However, the study has several limitations in its scope and statistical power to detect significant associations. First, the most important limitation was the low sample size with low numbers of events, with substantial missing data. Second, this study included three different data sources (from two prospective studies and one partly retrospective study), affecting the uniformity of the results. Additionally, it can be noted that our study primarily focuses on a cohort with a significant representation of NSCLC, alongside other cancers. While our dataset comprises different tumor types, it is indeed weighted towards NSCLC. Nevertheless, our results suggesting a trend towards an association between frailty and immunotherapy toxicity are consistent with the current literature encompassing various forms of cancer (12). Moreover, our dataset lacked detailed information regarding pre-treatments that could have influenced the baseline characteristics of the patients and subsequent outcomes. Similarly, we did not have data on delirium, an increasing concern in older patients receiving immunotherapy.

Third, in the retrospective part of the ImTosa, one third of the patients had a missing G8-score, resulting in a lack of power of the study. Finally, we did not have data available of a full geriatric assessment, but were only able to study G8-score, comorbidity and WHO status as predictors of toxicity-related outcomes.

The present study supports the hypothesis of a possible association between frailty measured by the G8 and risk of immunotherapy toxicity, treatment discontinuation and hospitalization due to toxicity, but larger prospective real-life studies of patients are needed to confirm these results.

In conclusion, in patients treated with immunotherapy in a real-life population with cancer, we observed consistent trends towards increased toxicity, hospitalization and treatment discontinuation with increasing frailty, although not statistically significant (potentially) due to low numbers. Larger studies should be performed to confirm these associations. Moreover, many patients with lower toxicity grade 1-2 experienced early treatment discontinuation, suggesting a lower tolerance of toxicity in frail older patients.

DECLARATIONS

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Conflict of interest

Estelle Tran Van Hoi, Johanneke Portielje, Diana van Heemst, Nienke De Glas, Simon Mooijaart and Marloes Derks declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Data availability

Not applicable.

Authors contributions

S. Trompet, Y. Van Holstein, F. Van Den Bos contributed in the design and implementation of the TENT study. H. Codrington and G. Labots contributed in the implementation of the ImToSa study. A. Ozkan contributed to the patients follow-up and managed the IMAGINE study database. S. Lohman contributed in preparing the database of the present study. E. Tran Van Hoi carried the research and analysis with the supervision of N.A. De Glas, J. Portielje, S.P. Mooijaart and M. Derks. M. Derks conducted the present research.

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SUPPLEMENTARY MATERIAL

Table S1. Determinants of treatment discontinuation due to toxicity (TENT-IMAGINE-ImToSa study)

	N	N events	OR	95% CI		p-value
				Lower	Upper	
Age	99	17	0.991	0.879	1.117	0.878
BMI						
Healthy weight (18.5-24.9)	52	8	Reference			
Underweight (<18.5)	7	0	NA			
Overweight (25-29.9)	31	6	1.320	0.411	4.239	0.641
Obese (>30)	9	2	1.571	0.275	8.977	0.611
Tumor type						
Breast cancer	8	1	Reference			
Non-small cell lung cancer	70	13	1.596	0.180	14.125	0.674
Melanoma	11	2	1.556	0.116	20.854	0.739
Renal carcinoma	4	0	NA			
Urothelial carcinoma	6	0	NA			
Treatment type						
Immunotherapy	75	14	reference			
Immuno- and chemotherapy	19	2	0.513	0.106	2.479	0.406
Immuno- and targeted therapy	5	0	NA			
Type of immunotherapy						
Pembrolizumab	49	9	Reference			
Nivolumab	27	7	1.556	0.505	4.787	0.441
Ipilimumab	5	0	NA			
Atezolizumab	11	0	NA			
Durvalumab	6	0	NA			
Other	1	0	NA			
G8 score						
Normal	28	4	reference			
Abnormal (< 15)	33	9	2.250	0.609	8.311	0.224
Unknown	38	3	0.514	0.105	2.508	0.411

	N	N events	OR	95% CI		p-value
				Lower	Upper	
WHO score						
Score 0	51	6	reference			
Score 1	40	9	2.177	0.704	6.739	0.177
Score 2	3	0	NA			
Score 3	1	1	1.212	0		
Unknown	4	0	NA			
Comorbidities						
No	22	1	Reference			
Yes	77	15	5.081	0.632	40.825	0.126
Types of comorbidities						
Cardiovascular	56	13	4.031	1.069	15.198	0.04
Diabetes Mellitus	21	3	0.833	0.214	3.246	0.793
Asthma/COPD	22	2	0.481	0.1	2.307	0.36
Neuropsychiatric	4	0	NA			
Rheumatologic disease	3	1	2.7	0.230	31.694	0.429
Gastric ulcer	3	0	NA			
Other malignancy	11	2	1.175	0.229	6.026	0.847

Abbreviations: N, number of patients; N events, number of patients with toxicity; OR, odds ratio for univariable logistic regression; BMI, body mass index; COPD, chronic obstructive pulmonary disorder; G8, Geriatric-8 test; WHO score, Eastern Cooperative Oncology Group (ECOG) score; NA, not applicable

Table S2. Determinants of hospitalization due to toxicity (TENT-IMAGINE-ImToSa study)

	N	N events	OR	95% CI		p-value
				Lower	Upper	
Age	99	10	0.994	0.859	1.1150	0.936
BMI						
Healthy weight (18.5-24.9)	52	3	Reference			
Underweight (<18.5)	7	0	NA			0.999
Overweight (25-29.9)	31	6	3.920	0.904	17.002	0.068
Obese (>30)	9	1	2.042	0.188	22.135	0.557
Tumor type						
Breast cancer	8	1	Reference			
Non-small cell lung cancer	70	6	0.656	0.069	0.069	0.714
Melanoma	11	3	2.625	0.220	31.349	0.446
Renal carcinoma	4	0	NA			
Urothelial carcinoma	6	0	NA			
Treatment type						
Immunotherapy	75	7	reference			
Immuno- and chemotherapy	19	3	1.821	0.424	7.828	0.420
Immuno- and targeted therapy	5	0	NA			
Type of immunotherapy						
Pembrolizumab	49	4	Reference			
Nivolumab	27	4	1.957	0.448	8.545	0.372
Ipilimumab	5	1	2.813	0.251	31.571	0.402
Atezolizumab	11	1	1.125	0.113	11.175	0.920
Durvalumab	6	0	NA			
Other	1	0	NA			
G8 score						
Normal	28	1	reference			
Abnormal (< 15)	33	4	3.724	0.391	35.444	0.253
Unknown	38	5	4.091	0.450	37.160	0.211
WHO score						
Score 0	51	5	reference			

	N	N events	OR	95% CI		p-value
				Lower	Upper	
Score 1	40	5	1.314	0.353	4.897	0.684
Score 2-3	4	0	NA			
Unknown	4	0	NA			
Comorbidities						
No	22	0	reference			
Yes	77	10	NA			
Types of comorbidities						
Cardiovascular	56	8	3.417	0.687	17.0	0.133
Diabetes Mellitus	21	3	1.690	0.397	7.192	0.477
Asthma/COPD	21	2	0.921	0.180	4.702	0.921
Neuropsychiatric	4	1	3.185	0.299	33.905	0.337
Rheumatologic disease	3	0	NA			
Gastric ulcer	3	0	NA			
Other malignancy	11	0	NA			

Abbreviations: N, number of patients; N events, number of patients with toxicity; OR, odds ratio for univariable logistic regression; BMI, body mass index; COPD, chronic obstructive pulmonary disorder; G8, Geriatric-8 test; WHO score, Eastern Cooperative Oncology Group (ECOG) score; NA, not applicable

Sub-analysis of the Tolerability and Safety of Immunotherapy study

Table S3. Patient and geriatric characteristics at baseline of the ImToSa study

	N	%
Age (years), median (IQR)	68	74.7 (71.0-77.0)
Gender		
Male	39	57.4
BMI (median, IQR)		
Healthy weight (18.5-24.9)	39	57.4
Underweight (<18.5)	4	5.9
Overweight (25-29.9)	19	27.9
Obese (>30)	6	8.8
History of smoking		
Yes	49	72.1
No	9	13.2
Unknown	10	14.7
Tumor type		
Non-small cell Lung carcinoma	53	77.9
Renal carcinoma	2	2.9
Urothelial carcinoma	5	7.2
Breast carcinoma	8	11.8
Tumor stage		
Locally Advanced	10	14.7
Metastatic	51	75
Unknown	7	10.3
Treatment		
Immunotherapy	62	91.2
Immuno- and chemotherapy	6	8.8
Type of immunotherapy		
Nivolumab	25	36.8
Pembrolizumab	30	44.1
Atezolizumab	9	13.2
Durvalumab	4	5.9
G8 score		
Normal	15	22.1
Abnormal (< 15)	26	38.2
Unknown	27	39.7

	N	%
WHO score		
Score 0	36	52.9
Score 1	28	41.2
Score 2-3	3	4.4
Unknown	1	1.5
Comorbidities		
Yes	59	86.8
No	9	13.2
Type of comorbidities		
Cardiovascular	44	64.7
Diabetes Mellitus	13	19.1
Asthma/COPD	20	29.4
Psychosocial disease	4	5.9
Rheumatologic disease	3	4.4
Gastric ulcer	2	2.9
Other malignancy	11	16.2

Abbreviations: N, number of patients; BMI, body mass index

Table S4. Determinants of toxicity risk in the ImToSa study

	N	N events	OR	95% CI		p-value
				Lower	Upper	
Age	68	55	0.861	0.742	0.998	0.048
BMI						
Healthy weight (18.5-24.9)	39	33	Reference			
Underweight (<18.5)	4	3	0.545	0.048	6.162	0.624
Overweight (25-29.9)	19	14	0.509	0.133	1.947	0.324
Obese (>30)	6	5	0.090	0.090	9.219	0.936
Tumor type						
Non-small cell Lung carcinoma	53	43	Reference			
Renal carcinoma	2	1	0.233	0.013	4.044	0.317
Urothelial carcinoma	5	3	0.349	0.051	2.372	0.282
Breast carcinoma	8	8	NA			
Treatment type						
Immunotherapy	62	50	Reference			
Immuno- and chemotherapy	6	5	1.2	0.128	11.245	0.873
Type of immunotherapy						
Pembrolizumab	30	22	Reference			
Nivolumab	25	20	1.455	0.408	5.184	0.563
Durvalumab	4	4	NA			
Atezolizumab	9	9	NA			
G8 score						
Normal	15	11	Reference			
Abnormal (< 15)	26	21	1.527	0.340	6.869	0.581
Unknown	27	23	2.091	0.439	9.961	0.354
WHO score						
Score 0	36	31	Reference			
Score 1	28	22	0.591	0.160	2.184	0.431
Score 2	2	0	NA			
Score 3	1	1	NA			
Unknown	1	1	NA			

	N	N events	OR	95% CI		p-value
				Lower	Upper	
Comorbidities						
No	9	9	Reference			
Yes	59	46	NA			
Types of comorbidities						
Cardiovascular	18	10	Reference			
Asthma/COPD	15	12	1.491	0.364	6.116	0.579
Psychosocial disease	4	4	NA			
Rheumatologic disease	3	3	NA			
Gastric Ulcer	1	1	NA			
Other malignancy	11	10	2.667	0.310	22.939	0.372
Diabetes Mellitus	7	6	1.375	0.265	7.125	0.704

Abbreviations: N, number of patients; N events, number of patients with toxicity; OR, odds ratio for univariable logistic regression; BMI, body mass index; COPD, chronic obstructive pulmonary disorder; G8, Geriatric-8 test; WHO score, Eastern Cooperative Oncology Group (ECOG) score; NA, not applicable

Figure S1. Incidence of comorbidity in patients with ≥ 1 disadvantageous outcome in the IMAGINE, TENT, ImToSa studies combined.

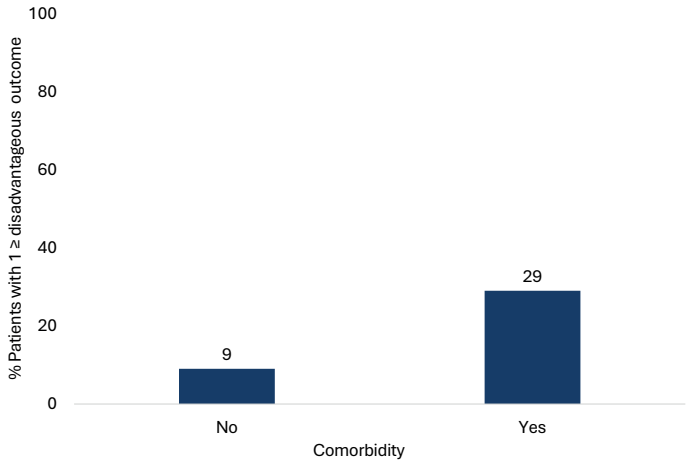

Figure S1. Graphic describing the incidence of comorbidity among patients with ≥ 1 disadvantageous outcome (toxicity risk, treatment discontinuation and hospitalization due to IrTox) in the IMAGINE, TENT, ImToSa studies combined.

6

General discussion

GENERAL DISCUSSION

The complex association between immunosenescence and frailty

Collectively, research on immunosenescence, frailty, and age-related diseases highlights the existence of a self-reinforcing cycle that reduces the chances of healthy ageing. Frailty and age-related diseases are closely linked, each increasing the risk of the other. A similar bidirectional relationship exists between immunosenescence and age-related diseases. However, the relationship between frailty and immunosenescence remains unclear. Ageing is marked by biomolecular changes that affect multiple systems, including the immune system, contributing to immunosenescence, which appears to underlie frailty. These overlapping mechanisms suggest that frailty, immunosenescence, and biological ageing are interdependent processes (Figure 1).

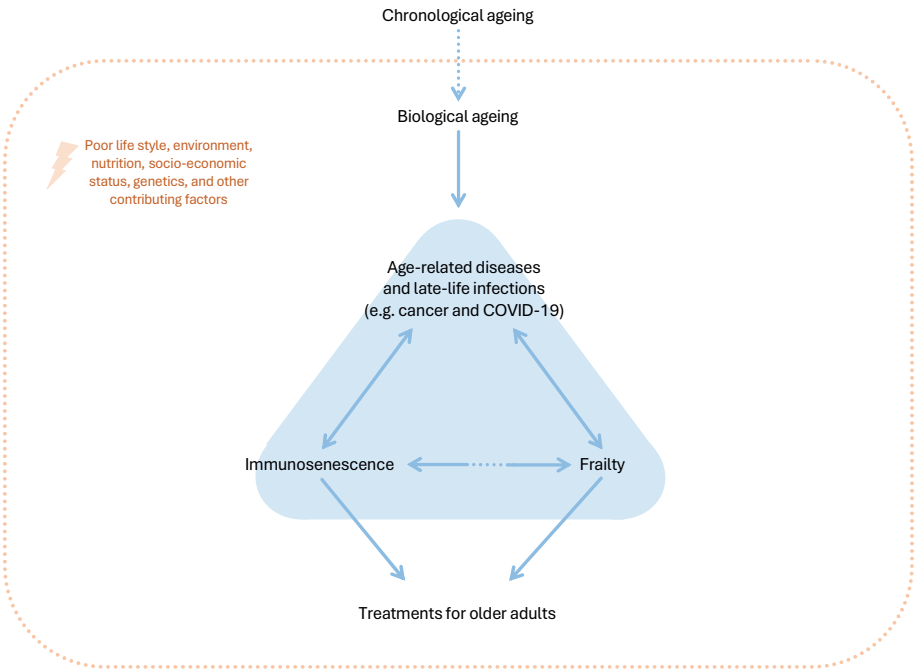

Figure 1. The complex association between immunosenescence and frailty

Several mechanisms potentially linking frailty and immunosenescence have been proposed, but they remain insufficiently studied and lack consistent evidence. In **Chapters 2-4**, we identified several immunological markers related to frailty from prior literature and our research. The most frequently reported markers include

C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α).

Evidence suggests that elevated CRP levels are associated with an increased risk of sarcopenia, cardiovascular diseases, disability, and cognitive decline in older adults. Elevated CRP levels were also associated with increased risk of mortality in frail older patients (1-3). Walker et al. (2017) demonstrated that higher midlife CRP levels predict frailty in later life, emphasizing its role as an early indicator (4). However, the 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, in which case CRP levels are much higher (>10 mg/L). Therefore, CRP seems to be a rather nonspecific biomarker of the ageing immune system. IL-6 and TNF-α also play central roles in frailty, particularly in the pathogenesis of chronic and age-related conditions. In acute inflammation, IL-6 facilitates T-cell expansion, B-cell differentiation, and the synthesis of acute-phase reactants such as CRP (5). Studies have linked elevated IL-6 and TNF-α levels with age-related diseases such as dementia and Parkinson's disease, which are commonly seen in frail patients (6, 7).

Immunosenescence involves the remodeling of the T cell compartment, driven by the thymic involution and bone marrow, skewing of immune cells to the myeloid lineage and resulting in substantial changes in the T cell populations. In **Chapter 2**, we also observed that only a limited number of T lymphocyte subpopulations had been studied previously. Among these, lower levels of naïve CD4+ T-cells and effector memory CD8+ T-cells were associated with increased frailty. However, the studies on T lymphocytes were limited in both size and scope. Although initial findings appeared promising, the lack of available data as well as standardized naming and measurement methods for biomarkers complicates comparisons across studies, making it difficult to draw definitive conclusions on their association with frailty. Nevertheless, **in Chapter 4**, when examining melanoma patients receiving anti-PD1 immunotherapy, we observed some of these age- and frailty-associated immune changes, including lower frequencies of CD8+ naïve T cells in older patients and a frailty-associated loss of CD8+ effector tissue-resident-like memory T cells and CD8+ mucosal-associated invariant T cells. These findings highlight that age- and frailty-driven immune remodeling, particularly of T-cell populations, is complex and can extend across diverse clinical settings.

Despite these associations, evidence on useful biomarkers in the clinic to identify frailty remains limited, due to the scarcity of studies and the small patient sample sizes, leading to inconsistent results. Uncovering shared biological pathways between frailty and immunosenescence is particularly challenging, given the complex

interplay with chronic low-grade inflammation, comorbidities and disease-specific factors. For example, elevated levels of inflammatory markers can be triggered by a wide range of conditions, such as diabetes, depression, age-related pain, endocrine disorders and nutritional deficiencies, which can modulate immune responses in diverse ways. The considerable variability in older adults' immune profiles makes it difficult to establish whether these markers are contributors to frailty or are byproducts of underlying disease conditions. The immune profile associated with frailty becomes more complex when influenced by acute or subacute infections, highlighting the importance of investigating the association in the context of specific diseases (Figure 1). Critically, disentangling the causal relationship between immunosenescence and frailty is essential for designing effective interventions, developing appropriate therapeutic strategies and identifying individuals at risk early. Without clarity on causality, there is a risk of misinterpreting association, which could lead to inappropriate clinical decisions.

Immunosenescence in COVID-19

In **Chapter 3**, frail patients paradoxically exhibited lower CRP levels upon hospital admission compared to their non-frail counterparts, even though elevated CRP was associated with increased frailty and mortality risk. These findings challenge conventional understanding. While frailty is a significant factor in determining overall outcomes in older patients, the elevated risk of mortality in older patients with frailty compared to fit patients may not be explained solely by the difference in inflammatory responses.

Indeed, it is also important to take into account that frail patients experience more comorbidities. Some studies suggest that chronic underlying conditions predict mortality risk in COVID-19 infections, which include hypertension, cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, cerebrovascular diseases, renal diseases, obesity, neurodegeneration and highly inflammatory conditions such as cancer and immunosuppression (8-13). Additionally, a systematic review of seven studies showed that patients with COPD, cardiovascular disease and hypertension were at higher risk of severe illness and intensive care unit admission (14). Furthermore, hypertension commonly coexists with Alzheimer's disease, which has also been recognized as a significant comorbidity influencing COVID-19 prognosis (15-17). These conditions predominantly affect older adults, potentially contributing to frailty and heightening their vulnerability to COVID-19 infection (18). As a result, affected patients may present at earlier stages of the disease, hence exhibiting lower CRP levels upon admission.

Although differences in inflammatory responses may not fully explain the increased mortality risk among frail older patients, CRP remains a valuable, rapid, and cost-effective biomarker for its prognostic value in assessing the risk of disease severity. Elevated serum CRP concentrations have been associated with COVID-19 disease progression and poor clinical outcomes (19, 20). However, we should be cautious when interpreting admission CRP levels in frail patients; clinicians must consider clinical history and perform geriatric assessments rather than relying solely on isolated markers. Studies often lack a detailed investigation into the immune and metabolic pathways underlying frailty. COVID-19 involves complex immune and vascular responses, including increased inflammatory markers, cytokine release, coagulation abnormalities, and endothelial dysfunction. Most prior studies have analyzed these biomarkers individually, with few investigating these interconnected pathways collectively, particularly within the context of frailty. Future investigations should include an extensive evaluation of biomarkers related to endothelial dysfunction, coagulation response, systemic inflammation, cytokine and chemokine release, and organ damage. Hence, CRP (trajectories) should be evaluated over time in conjunction with (those of) other biomarkers.

Notably, the dysregulated secretion of cytokines, known as a "cytokine storm," has been recognized as a critical contributor to poor outcomes in COVID-19. This cytokine storm involves an exaggerated inflammatory and immune response, predominantly affecting the pulmonary system and resulting in acute respiratory distress syndrome, pulmonary oedema, and multi-organ failure (21). Alleviating this inflammatory state may improve patients' prognosis. Older patients with comorbidities are particularly susceptible to developing hyperinflammation in the course of the disease, due to their impaired immune function and chronic inflammatory states (22-24).

One signalling pathway that plays a key role in mediating this inflammatory response is the JAK-STAT pathway. In particular, STAT3 activation has been shown to contribute to the development of severe symptoms like cytokine release syndrome in patients with COVID-19. Preclinical evidence suggested that JAK inhibitors may reduce low-grade inflammation in older adults. However, their immunosuppressive effects limit their use in immunocompromised patients or those with cancer (25). JAK inhibitors are also being explored for preventing severe COVID-19 cytokine storms, though results remain inconsistent (26, 27). Several therapeutic agents targeting this pathway, including IL-6 receptor antagonists (e.g., sarilumab), IL-1 receptor antagonists (e.g., anakinra), and JAK inhibitors (e.g., baricitinib, fedratinib, and ruxolitinib), are under investigation (26). So far, vaccination has been the best strategy to stop the viral spread and control the pandemic. However, vaccination might not provide full

protection to the older population due to the age-related decline in immune function (28).

Immunosenescence in melanoma cancer and toxicity of immunotherapy

Several studies suggest that ageing of the immune system compromises the adaptive immune response, particularly T cells, potentially reducing the effectiveness of such treatments. In **Chapter 4**, we found several ageing- and frailty-associated changes among circulating immune cells in blood, but these were not associated with response to immunotherapy in our study. A recent study also showed that aged and younger patients had derived similar clinical outcomes, despite a clear age-related divergence in immune phenotypes (29). It is also possible that the absence of a correlation between immune senescence markers and the treatment response indicates that immune senescence and frailty may not affect the effectiveness of immune checkpoint inhibitors, allowing older adults to respond to immunotherapy comparably to their younger counterparts despite diminished immune function. In fact, initial studies on older patients are quite reassuring. Retrospective studies and meta-analyses have investigated ICI effectiveness in older adults and found no difference in overall survival (OS) or response rate by age (30, 31). Similarly, observational data have shown no differences in treatment response and survival between younger and older patients (32).

However, older patients with geriatric impairments seem to be more likely to experience immune-related adverse event sequelae, and this patient group is usually underrepresented in clinical trials (33, 34). In **Chapter 5**, our findings revealed consistent trends between patients with an abnormal Geriatric-8 test score (of < 15, suggesting significant frailty) or a high WHO score (of ≤ 1, indicating some degree of functional impairment or reduced ability to perform daily activities) for having higher odds of toxicity, treatment discontinuation due to immune-related treatment toxicity, and hospitalization due to immune-related treatment toxicity. Both an abnormal G8 score and a higher WHO performance status were associated with a higher risk of toxicity, hospitalization, and treatment discontinuation due to toxicity. Additionally, severe toxicities were more common in patients with comorbidities, particularly cardiovascular conditions, which showed trends toward higher odds of hospitalization and treatment discontinuation. Similarly, the ELDERS study demonstrated that an abnormal G8 test score < 15 was a predictor of a significantly higher risk of adverse outcomes, such as comorbidity-related hospital admissions and risk of death(35), highlighting that while older patients and those with underlying frailty may experience more severe outcomes during treatment, much of this risk could be tied to their preexisting comorbidities and overall vulnerability, rather than strictly to immune-

related adverse events. In a Dutch national registry study of melanoma patients treated with ICIs, while response rates and grade 3 or higher toxicity rates were not influenced by age or comorbidities, patients aged ≥75 experienced higher rates of treatment discontinuation due to toxicities (36). This suggests that older and frail patients tolerate toxicity less well compared to younger patients and are more likely to discontinue treatment due to side effects, even if they are not necessarily experiencing more toxicities than their younger counterparts. Older patients may lack the physiological reserves needed to fully recover from toxicities, as they tend to be more frail than younger individuals and more likely to have multiple chronic conditions. Additionally, the reduced immune responsiveness in frail patients likely contributes to their decreased capacity to tolerate and recover from mild toxicities. This may suggest a role of frailty as a functional manifestation of immunosenescence.

Although current evidence suggests that older patients respond to immunotherapy at least as well as younger patients, it remains important to develop a more comprehensive panel of biomarkers to better predict treatment response and clinical outcomes. Studies have reported associations between immune-related blood markers, such as elevated neutrophil-to-lymphocyte ratio (NLR) and reduced albumin levels, and worse clinical outcomes across various cancer types (37). However, the findings were limited due to heterogeneous methodologies and small sample sizes. Alterations in immune parameters, including neutrophils, lymphocytes, total white blood cell counts (WBC), and particularly NLR, have been implicated in tumor-promoting and immunosuppressive roles associated with poor outcomes in solid tumors (38-43). Elevated NLR has also been linked to increased frailty in cancer survivors, individuals with cardiovascular disease, and community-dwelling older (44). Dilorom proposed that elevated levels of NLR, total WBC, and neutrophils may reflect an acute inflammatory response driven by cancer pathology and treatment-related stress (38). Additionally, increased circulating myeloid cells, particularly myeloid-derived suppressor cells, have been reported in older and frail patients relative to younger counterparts (45).

Recent laboratory and clinical studies showed that the timing of immunotherapy infusion plays a key role in the treatment outcomes for cancer patients. Multiple studies involving over 6000 patients across several tumor types (melanoma, lung, renal, bladder, liver, etc) consistently report better outcomes when infusions are given in the morning. For example, a melanoma cohort found that morning administration nearly doubled overall survival versus afternoon dosing, and a meta-analysis by Landré et al (2024) reported that early-day administration of immunotherapy doubled progression-free and overall survival compared to later administration. The immune system follows the body's circadian rhythms, with peak activity in the morning. These rhythms modulate immune-cell trafficking, cytokine secretion and T-cell function,

processes targeted by many immunotherapies. In our study, the timing of infusion was not recorded, representing a limitation of the study. Given the accumulating evidence, adequate randomized controlled trials comparing morning, afternoon, and evening administration are warranted to define optimal infusion time and to enable more personalized treatment strategies in oncology.

Challenges and future directions in immunology and frailty research

One of the main challenges in this research is the multifactorial nature of frailty, given the important heterogeneity of the older population and the lack of a uniformly accepted definition.

There remains ongoing debate within the geriatric field regarding the most appropriate instrument for measuring frailty. In associative research and in our studies, the Clinical Frailty Scale (CFS) appeared to be the most suitable tool, not only because it is time- and cost-efficient, but also because its continuous scoring system allows frailty to be quantified along a spectrum rather than dichotomized into frail versus non-frail, enabling stratified analysis that yields insights into underlying mechanistic pathways. This gradation enhances its clinical applicability, enabling a more nuanced assessment of health status. Furthermore, the CFS incorporates both physical and cognitive domains of frailty, in contrast to the Fried phenotype, which focuses exclusively on physical criteria. The clear cut-offs within the CFS (from fit to pre-frail to frail) capture individual variability while remaining practical and resource-efficient for use in clinical and research settings. The CFS has been validated and used across many settings.

Another relevant instrument, which we did not apply but which merits consideration, is the Frailty Index (FI) (46). The FI measures frailty as the accumulation of health deficits, including 30 or more symptoms, signs, disabilities, or diseases (47). Unlike phenotype-based measures, the FI includes a broad range of health-related parameters, many of which are correlated, reflecting the interconnected nature of ageing-related decline. The FI has been shown to behave consistently across diverse study settings, and its continuous nature enables robust prediction of adverse outcomes, including morbidity and mortality (48).

On the other hand, immunological variability is influenced by factors such as genetics, lifestyle, comorbidities and disease exposures, making it difficult to identify consistent immune senescence markers associated with frailty. In addition, standardization in the identification and validation of immune biomarkers is lacking, as differences in assays, methodologies, and marker definitions hinder cross-study comparisons. Future research should prioritize the development of standardized

protocols for immune cell profiling to enhance reproducibility and comparability across studies.

To clarify the relationship between immunosenescence and frailty, longitudinal studies are essential. Following older adults, with repeated measurements of immune markers and frailty levels, would help determine the time course and directionality, as well as the potential causality of immune changes in relation to frailty.

In practice, researchers often begin with cross-sectional analyses to identify candidate biomarkers associated with frailty, and then test these markers in subsequent prospective cohorts to confirm their predictive value. Usually, studies of ageing biomarkers have been hypothesis-driven and single-omic, for example, comparing one or few proteins or cell types between frail and fit individuals. These approaches can be laborious and may miss broader patterns, because ageing is a complex and context-dependent process. The next steps in research would be an in-depth analysis of frailty with multi-omics approaches. Multi-omics strategies use high-throughput, unbiased profiling across many molecular layers (genomics, transcriptomics, proteomics, metabolomics, etc) simultaneously. For example, one large study analyzed thousands of plasma proteins in tens of thousands of UK biobank participants and found that a “youthful” immune-system proteomic profile was uniquely associated with greater longevity (49). Similarly, another group used blood immune markers to train a deep-learning “inflammatory age” clock (iAge) that tracked multimorbidity, immunosenescence and frailty (50). These studies illustrate how combining multiple omics layers can identify novel biomarkers or composite indices of healthy vs unhealthy ageing. Omics-based techniques can offer a more holistic view of biological ageing by integrating inflammatory, immune-metabolic, and cellular ageing markers, preferably at multiple time points.

Such integrative studies of frailty and immunosenescence require rich datasets and careful design. This approach needs a comprehensive clinical and lifestyle data collection, which includes comorbidities, prior treatment, quality of life, nutrition, activity levels, etc. However, the extended follow-up required and the absence of a clear immunological definition for the onset of frailty present methodological challenges. Additionally, studies are often biased due to small sample sizes and non-representative cohorts. Very frail individuals are more likely to drop out due to illness or death, leading to the underrepresentation of frailty, compared to fitter participants, limiting the generalizability. The challenges in this research also lie in the complex interplay of chronic low-grade inflammation, comorbidities, and other age-related conditions that should be taken into account. Moreover, how immune markers are modulated by disease severity remains an important but underexplored question.

In COVID-19, measuring inflammation biomarkers frequently over time may be an optimal strategy to characterize the evolution of the patients' immune response and the progression of the disease. Continuous monitoring of the biomarkers could guide both the timing and dosing of anti-inflammatory interventions, as well as evaluate their efficacy. Older adults with comorbidities or chronic conditions face a higher risk of mortality from COVID-19, underscoring the need for systematic frailty screening and comprehensive clinical history documentation.

Additionally, careful monitoring of comorbidities during hospitalization could help to assess their influence on disease progression and outcomes. This could be addressed by a prospective cohort study that tracks disease severity (e.g. use of mechanical ventilation, admission to intensive care unit) or in-hospital mortality in COVID-19 patients stratified by the type and number of comorbidities and frailty level. Another idea would be assessing the level of protection vaccines provide to frail individuals to enable more accurate predictions of post-vaccination infection rates and morbidity in this vulnerable population. To date, no studies have specifically examined the incidence of infections in frail compared to non-frail individuals.

In melanoma, expanding blood-based multi-biomarker panels are needed to better capture the immune changes. Longitudinal studies in larger cohorts could monitor markers such as NLR, total WBC and myeloid-derived suppressor cells, which have been associated with frailty and poor outcomes in solid tumors, at baseline, during treatment and post-treatment, in relation to frailty, to clarify their clinical significance. Moreover, evaluating the tumor microenvironment should also be considered; however, its characteristics can vary significantly depending on tumor location. Furthermore, special attention must be given to monitoring toxicity in older individuals receiving immunotherapy. Developing immune profiling tools to track immune-related adverse events and tolerance in real time could help optimize safety and efficacy in this population. Considering the heterogeneity in older adults, biomarker strategies could incorporate personalized screening approaches that also account for key clinical parameters such as frailty, comorbidities, polypharmacy, functional status, etc. Assessing frailty over time can also guide decisions regarding treatment intensity or dosage and help identify potential supportive measures for patients (51). Indeed, preemptive dose adjustments, close monitoring, and early initiation of supportive care should be considered, even for toxicities of lower severity. The identification of immune-related adverse events can be particularly challenging due to mental impairment, social issues, or devious symptoms, which could be underreported or assigned to ageing (52). An idea could be to stratify patients, for example, by their (multi-)comorbidities, and monitor them by classification. The comorbidities and frequent polypharmacy make the management of immune-related adverse events challenging. Additionally, immunosuppressive treatments required to manage

immune-related adverse events must be carefully balanced with the patient's existing medication regimen and comorbidities to avoid exacerbating other health issues. Therefore, geriatric assessment could help to identify older patients who are more likely to benefit from immunotherapy with a reduced risk of immune-related adverse events, thereby preventing early treatment discontinuation. Additionally, patient-reported outcomes could be valuable for monitoring and assessing both the toxicities and the impact of immunotherapy on the quality of life in older patients (51).

Potential interventions and broader considerations

Interventions targeting frailty can occur at multiple levels. At a preventive stage, promoting healthy lifestyle behavior, including regular physical activity and balanced nutrition, may delay or mitigate the onset of frailty. Lifestyle factors influence biological ageing and may contribute to building physiological resilience.

At a molecular level, emerging studies are investigating therapeutic strategies that target senescent cells, which are more prevalent in ageing populations and contribute to chronic inflammation through the senescence-associated secretory phenotype (SASP). In preclinical studies, senolytic drugs have shown potential to mitigate SASP-driven inflammation or the “cytokine storm”, also observed in severe COVID-19 cases among older patients (53, 54).

On a societal level, research must become more inclusive. Many existing studies disproportionately represent Caucasian populations, and historically, biomedical research has been predominantly conducted on male subjects (55, 56). Yet emerging studies show that sex- and ethnicity-specific differences exist. For instance, females and individuals from different backgrounds may present different comorbidities burden and social determinants of health that interact with the ageing of the immune system (57-59). Greater inclusion of underrepresented populations is essential to capture the full spectrum of immune ageing and frailty.

Conclusion

A better understanding of the roles of the immune system in ageing and frailty is essential for developing effective therapeutic strategies for age-related diseases, enhancing preparedness for future infectious pandemics, and promoting healthy ageing. Current treatment approaches and decision-making tools remain inadequate for effectively monitoring frail individuals across various disease contexts. Findings from our studies on COVID-19 and melanoma suggest a common need for a personalized approach to treatment in older adults. Integrating geriatric assessment with a broader immune marker panel, rather than relying on isolated markers, may improve the treatment decision process and the prediction of treatment response and adverse events.

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A

ENGLISH SUMMARY

The overall aim of this thesis was to uncover how age-related changes in the immune system are associated with frailty and risk for adverse outcomes of two prevalent age-related disease paradigms: cancer (more specifically, melanoma) and COVID-19.

As age-related changes in the innate and adaptive immune system are thought to contribute to frailty, in **Chapter 2**, we explored the potential immunological determinants correlated to frailty in the literature. The most frequently studied inflammageing biomarkers were IL-6, CRP and TNF-α. Different types of T-lymphocyte subpopulations were studied, but each subset was studied only once. The findings revealed IL-6 and CRP to be consistently associated with frailty. Some studies showed an association between different T-lymphocyte subpopulations and frailty, including lower levels of naïve CD4+ T-cells and effector memory CD8+ T-cells with higher frailty; however, the number of investigations per cell subpopulation was too few, preventing us from drawing strong conclusions.

In **Chapter 3**, we examined the association of frailty with inflammatory markers and the role of frailty in the relationship between these markers and in-hospital mortality among older patients hospitalized for COVID-19. In all cohorts studied, lower CRP levels were associated with higher frailty scores, whereas higher CRP levels were associated with increased in-hospital mortality risk across frailty groups. The association of low CRP with higher frailty may be explained by patients with high frailty tending to present themselves at earlier stages of disease. In the interaction term analysis, frailty did not modify the association between inflammatory markers and in-hospital mortality. While frailty was a significant factor in determining overall outcomes in older patients, the findings suggest that the elevated risk of mortality in older patients with frailty compared to fit patients is likely not explained by differences in inflammatory responses.

In **Chapter 4**, we shifted our focus to melanoma, particularly in the context of immune checkpoint inhibitors (ICIs), which are increasingly used in older cancer patients. Several studies suggest that ageing of the immune system compromises the adaptive immune response, particularly T cells, potentially reducing the effectiveness of ICI treatment. To explore this hypothesis, we investigated the association between immune senescence markers in blood cells and age, frailty, and response to anti-PD-1 therapy in older patients with metastatic melanoma. Older patients, irrespective of frailty, showed lower levels of naïve CD8+ T cells, with the old and frail group also exhibiting reduced tissue-resident effector memory CD8+ T cells and CD8+ mucosal-associated invariant T (MAIT) cells. These differences were not associated with treatment outcomes. T cell proliferation and antigen-presenting cell capacities

did not differ across groups. These findings suggest that the level of frailty and ageing may not necessarily compromise the efficacy of ICI therapy.

Chapter 5 addressed the broader challenges faced by older patients with cancer undergoing ICI treatment. We investigated the association between frailty and immune-related treatment toxicity, hospitalization and treatment discontinuation due to immune-related treatment toxicity in older patients treated with checkpoint inhibitors. The findings revealed consistent trends between patients with an abnormal Geriatric-8 test score (of < 15, suggesting significant frailty) or a high WHO score (of ≤ 1, indicating some degree of functional impairment or reduced ability to perform daily activities) for having higher risk of toxicity, treatment discontinuation due to immune-related treatment toxicity, and hospitalization due to immune-related treatment toxicity, although not statistically significant. Moreover, in a sub-analysis, we observed that the treatment discontinuation due to immune-related treatment toxicity occurred often in frail patients with grade 1-2 toxicity, suggesting a lower tolerance of toxicity in frail older patients.

NEDERLANDSE SAMENVATTING

Het algemene doel van dit proefschrift was om te onderzoeken hoe leeftijdsgebonden veranderingen in het immuunsysteem samenhangen met kwetsbaarheid frailty en het risico op ongunstige uitkomsten bij twee veelvoorkomende leeftijdsgerelateerde ziektebeelden: kanker (in het bijzonder melanoom) en COVID-19.

Aangezien wordt aangenomen dat leeftijdsgebonden veranderingen in het aangeboren en adaptieve immuunsysteem bijdragen aan kwetsbaarheid, hebben we in **Hoofdstuk 2** de mogelijke immunologische determinanten die in de literatuur met kwetsbaarheid in verband zijn gebracht, onderzocht. De meest bestudeerde biomarkers van "inflammaging" waren IL-6, CRP en TNF-α. Verschillende subpopulaties van T-lymfocyten zijn onderzocht, maar elke subset werd slechts één keer bestudeerd. De resultaten toonden aan dat IL-6 en CRP consequent geassocieerd waren met kwetsbaarheid. Enkele studies lieten ook een verband zien tussen verschillende T-lymfocytensubpopulaties en kwetsbaarheid, waaronder lagere niveaus van naïeve CD4+ T-cellen en effectorgeheugen CD8+ T-cellen bij hogere kwetsbaarheid. Het aantal studies per celtype was echter te beperkt om sterke conclusies te kunnen trekken.

In **Hoofdstuk** 3 onderzochten we de relatie tussen kwetsbaarheid en ontstekingsmarkers, evenals de rol van kwetsbaarheid in de relatie tussen deze markers en sterfte in het ziekenhuis bij oudere patiënten die wegens COVID-19 waren opgenomen. In alle onderzochte cohorten waren lagere CRP-waarden geassocieerd met hogere kwetsbaarheidsscores, terwijl hogere CRP-waarden geassocieerd waren met een verhoogd risico op sterfte in het ziekenhuis, ongeacht de mate van kwetsbaarheid. De associatie tussen lage CRP en hogere kwetsbaarheid kan worden verklaard doordat kwetsbare patiënten zich mogelijk in een vroeger stadium van de ziekte presenteren. In de interactieanalyse bleek kwetsbaarheid de relatie tussen ontstekingsmarkers en sterfte in het ziekenhuis niet te beïnvloeden. Hoewel kwetsbaarheid een belangrijke factor was in het bepalen van de algehele uitkomst bij oudere patiënten, suggereren de bevindingen dat het verhoogde sterfterisico bij kwetsbare ouderen ten opzichte van fitte ouderen waarschijnlijk niet verklaard wordt door verschillen in ontstekingsreacties.

In **Hoofdstuk 4** richtten we ons op melanoom, met name in de context van immuuncheckpointremmers (ICI's), die steeds vaker worden toegepast bij oudere kankerpatiënten. Verschillende studies suggereren dat veroudering van het immuunsysteem de adaptieve immuunrespons, met name die van T-cellen, vermindert, wat mogelijk de effectiviteit van ICI-behandeling beperkt. Om deze hypothese te onderzoeken, bestudeerden we de relatie tussen markers van immuunsenescentie in bloedcellen en leeftijd, kwetsbaarheid en respons op

anti-PD-1-therapie bij oudere patiënten met gemetastaseerd melanoom. Oudere patiënten, ongeacht kwetsbaarheid, vertoonden lagere niveaus van naïeve CD8+ T-cellen. De groep van oude en kwetsbare patiënten vertoonde bovendien lagere niveaus van weefsel-residente effectorgeheugen CD8+ T-cellen en CD8+ mucosaal-geassocieerde invariant T (MAIT)-cellen. Deze verschillen waren niet geassocieerd met behandeluitkomsten. T-celproliferatie en de capaciteit van antigeen-presenterende cellen verschilden niet tussen de groepen. Deze bevindingen suggereren dat leeftijd en kwetsbaarheid de effectiviteit van ICI-therapie niet noodzakelijkerwijs verminderen.

Hoofdstuk 5 ging in op de bredere uitdagingen waarmee oudere kankerpatiënten die ICI-behandeling ondergaan te maken hebben. We onderzochten de relatie tussen kwetsbaarheid en immuungerelateerde behandeltoxiciteit, ziekenhuisopname en het staken van de behandeling als gevolg van immuungerelateerde toxiciteit bij oudere patiënten behandeld met checkpointremmers. De resultaten lieten consistente trends zien: patiënten met een afwijkende Geriatric-8-score (< 15, wat wijst op aanzienlijke kwetsbaarheid) of een hoge WHO-score (≤ 1, wat duidt op enige functionele beperking of verminderde dagelijkse zelfstandigheid) hadden een hoger risico op toxiciteit, op het staken van de behandeling wegens immuungerelateerde toxiciteit, en op ziekenhuisopname door immuungerelateerde toxiciteit, hoewel deze resultaten niet statistisch significant waren. Bovendien bleek uit een subanalyse dat het staken van de behandeling door immuungerelateerde toxiciteit vaak voorkwam bij kwetsbare patiënten met graad 1–2 toxiciteit, wat wijst op een lagere tolerantie voor toxiciteit bij kwetsbare oudere patiënten.

LIST OF PUBLICATIONS

Tran Van Hoi E, De Glas NA, Portielje JEA, Van Heemst D, Van Den Bos F, Jochems SP, Mooijaart SP. Biomarkers of the ageing immune system and their association with frailty - A systematic review. Exp Gerontol. 2023 Jun 1;176:112163. doi: 10.1016/j.exger.2023.112163. Epub 2023 Apr 15. PMID: 37028607.

Tran Van Hoi E, Trompet S, Van Holstein Y, Van Den Bos F, Van Heemst D, Codrington H, Labots G, Lohman S, Ozkan A, Portielje J, Mooijaart SP, De Glas NA, Derks M. Toxicity in Older Patients with Cancer Receiving Immunotherapy: An Observational Study. Drugs Aging. 2024 May;41(5):431-441. doi: 10.1007/s40266-024-01114-z. Epub 2024 May 10. PMID: 38727992; PMCID: PMC11093836.

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Tran Van Hoi E*, Santegoets SJ*, Mooijaart SP, Van Heemst D, Özkan A, Verdegaal EME, Slingerland M, Kapiteijn E, van der Burg SH, Portielje JEA, Welters MJP, de Glas NA. Blood based immune biomarkers associated with clinical frailty scale in older patients with melanoma receiving checkpoint inhibitor immunotherapy. Immun Ageing. 2024 Nov 27;21(1):83. doi: 10.1186/s12979-024-00463-y. PMID: 39593063; PMCID: PMC11600645.

CURRICULUM VITAE

Estelle Tran Van Hoi was born on December 3rd, 1996, in Châtenay-Malabry, France. After graduating from Lycée Notre Dame de Sion in Evry, France, in 2014, she pursued a Bachelor's degree in Life Sciences at La Sorbonne (Paris, France), which she completed in 2018. During her Bachelor's program, she completed an internship at Hôpital Cochin in the Physiology department under the supervision of Prof. Dr. Dinh Xuan. She then continued with a Master of Science in Cellular and Molecular Biology, specializing in the Biology and Pharmacology of Ageing, at La Sorbonne. In 2020, she participated in an exchange program at Yale University School of Medicine, where she worked in the Pharmacology Department under the supervision of Prof. Dr. Cheng.

In 2021, Estelle began her PhD at the Leiden University Medical Center, within the Department of Geriatrics and Gerontology, under the supervision of Prof. Dr. S.P. Mooijaart, Dr. N. A. de Glas, Prof. Dr. J.E.A. Portielje and Dr. Ir. D. van Heemst. Her research focuses on the association between immunosenescence and frailty.

Beyond her scientific work, Estelle is passionate about Art. In her free time, she enjoys painting and music. She currently lives in Paris.

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