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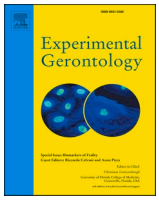
Experimental Gerontology 195 (2024) 112534

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero



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

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ARTICLE INFO

Section Editor: Marzetti Emanuele

Keywords:
COVID-19
Frailty
Ageing
Immunosenescence
Inflammation

ABSTRACT

Introduction: During the COVID19 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-19and 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 difference in inflammatory responses.

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https://doi.org/10.1016/j.exger.2024.112534
Received 25 June 2024; Received in revised form 29 July 2024; Accepted 31 July 2024
Available online 6 August 2024

1. Introduction

Older patients hospitalized with COVID-19 had a higher mortality risk during the pandemic compared to younger patients (Moens et al., 2022; Michels et al., 2023). 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 (Blomaard et al., 2021; Smits et al., 2022; Hewitt et al., 2020).

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-CoV2 virus (Smorenberg et al., 2021; Jia et al., 2022; Hussien et al., 2021; Tran Van Hoi et al., 2023). Immunosenescence and inflammageing contribute to frailty status and adverse health outcomes (Jia et al., 2022; Kravitz et al., 2009). 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 (Blomaard et al., 2021; Tran Van Hoi et al., 2023; Kravitz et al., 2009; Tilvis et al., 2004; Jylha et al., 2007; Buonacera et al., 2022; Appelman et al., 2023). These immune markers also have been associated with worse COVID-19 outcomes in hospitalized patients (Zhang et al., 2022). Interestingly, in the COVID-OLD study, higher CRP associated with increased risk of mortality, but CRP levels at admission were lower in frail patients compared with non-frail patients (Blomaard et al., 2021). This counterintuitive finding raises the question 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-CoV2 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.

2. Methods

2.1. Study design

Data were used from three multicenter cohorts in the Netherlands: COVID-OLD, Covid-Predict and CliniCo (Fig. 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.

2.2. Study participants

We included patient aged 70 years and older. Diagnosis of COVID-19 was defined by a positive reverse-transcriptase polymerase chain reaction (PCR) test for SARS-CoV2 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.

2.3. Cohorts

2.3.1. 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 (Fig. 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 $\geq$ 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 $\geq$ 24 h after admission were excluded) (Blomaard et al., 2021).

2.3.2. 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 (Appelman, n.d.). Data were collected from 9 Dutch hospitals (Fig. S1). The inclusion criteria were patients $\geq$ 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.

2.3.3. 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 (Fig. S1). The inclusion criteria were adult patients $\geq$18 years old, with PCR-confirmed infection with SARS-CoV-2, who were admitted for at least 24 h 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 family and documented in the electronic medical records.

2.4. 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.

2.5. Data collection

2.5.1. 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.

2.5.2. 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 (NVIC, n.d.). The CFS was determined prospectively during the first patient contact, or when a consultation of 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 (Kay et al., 2022; Davies et al., 2018; Shears et al., 2018; Stille et al., 2020). 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) (Specialisten, 2021).

2.5.3. 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 24 h 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 counts (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 (Zhang et al., 2022). 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 (Fig. 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.

2.6. 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.

2.7. Statistical analysis

Data of 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 was 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).

3. Results

3.1. 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 among 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]) (mm Hg)	137 [122–152]	135 [120–150]	137 [122–152]
Diastolic blood pressure (median [IQR]) (mm Hg)	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.

3.2. In-hospital outcomes and treatment medications

Length of hospital stay was 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 (Pouw et al., 2021). As more intervention studies were published, the focus shifted to corticosteroids as the cornerstone of the treatment of COVID-19 (Group RC et al., 2021). However, treatments did not vary across each frailty group.

3.3. 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 level in all three cohorts ($p < 0.01$).

3.4. 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.

3.5. 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 prior to admission. Fig. 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 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 immune suppressive medication compared to the initial analyses (Tables S2 and S3).

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			
Immune suppression 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.

4. 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 ratio 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 (Blomaard et al., 2021; Aw et al., 2020). 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 (Olivieri et al., 2022). Previous studies showed that high NLR, PLR, and low lymphocyte count were significant predictors of in-hospital mortality in COVID-19 patients (Olivieri et al., 2022). 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 (Guthrie et al., 2013; Azab et al., 2010), 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 to long term mortality in older patients, and could be indicative of inflammageing (Dugue et al., 2022; Huang et al., 2020; Stringer et al., 2021). In the acute phase of SARS-CoV-2 infection, however, frail patients had lower CRP levels compared to fit patients (Blomaard et al., 2021). 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 (Blomaard et al., 2020), 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, among others (Taylor et al., 2023; Lee et al., 2011). 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

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

Differences of inflammatory markers levels between the three groups of patients were assessed using Chi-square tests. 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.

	N	Stratum of inflammatory marker				
		Low	Middle	p-Value	High	p-Value
		OR (95 % CI)	OR (95 % CI)		OR (95 % CI)	
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
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	1.09 (0.78–1.53)	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

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.
Abbreviations: CRP, C-reactive protein; N, number; OR, odd ratio; NA, non-applicable.

reflection of immunosenescence may predispose older individuals to increased susceptibility to infections and a diminished response to vaccinations (Aspinall et al., 2007). Our study showed finally 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

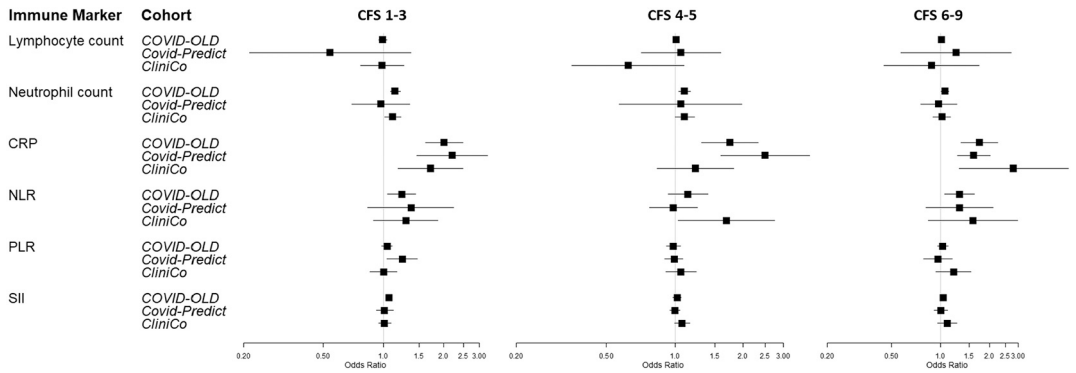

Fig. 1. Exploring the interaction between immune markers and frailty status in association with in-hospital mortality in older hospitalized COVID-19 patients. Multivariable logistic regression adjusted for age-gender-duration of symptoms till admission. Patients are stratified by CFS (CFS score 1–3 = 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 ratios PLR are presented per 100-units per increase. Inflammation ratio SII is presented per 500-units per increase. Inflammation ration NLR is presented per 10-units per increase.
Abbreviations: CRP, C-reactive protein; CFS, Clinical Frailty Scale.

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 (Stille et al., 2020). Additionally, we used the SOP to standardize the retrospective assignment process, minimizing variation in how CFS scores are adjudicated across different evaluator. 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 in future health problems or pandemic 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 the patients in three separate multicenter 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 difference in inflammatory responses.

Funding

This work was supported by Zorg Onderzoek Nederland en Medische Wetenschappen (ZonMw) to the COVID-19 Outcomes in Older People (COOP) study (project number 10430102110005) under the COVID-19 program. ZonMW had no role in data analysis or reporting.

CRediT authorship contribution statement

Estelle Tran Van Hoi: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Brent Appelman:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Simon Mooijaart:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Virgil A.S.H. Dalm:** Writing – review & editing. **Harmke A. Polinder Bos:** Writing – review & editing. **Diana van Heemst:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Bas F.M. van Raaij:** Writing – review & editing. **Raymond Noordam:** Writing – review & editing, Formal analysis. **Anna Kuranova:** Writing – review & editing, Formal analysis. **Jacobien J. Hoogerwerf:** Writing – review & editing. **Geeske Peeters:** Writing – review & editing, Methodology. **Annemieke Smorenberg:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Simon P. Mooijaart:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Harmke A. Polinder-Bos:** Writing – review & editing. **Geeske Peeters:** Writing – review & editing, Methodology. **Simon P. Mooijaart:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Harmke A. Polinder-Bos:** Writing – review & editing. **Brent Appelman:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Jacobien J. Hoogerwerf:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known conflict of interest.

Acknowledgement

The Covid-19 Outcomes in Older People (COOP)-consortium is a national collaboration in the Netherlands between stakeholders from different care settings (hospitals, primary care practices and nursing homes) and a Seniors Advisory Board (Ouderenraad). In particular, the researchers wish to acknowledge this Seniors Advisory Board (Ouderenraad) for their diverse participation as representatives of older persons and for their helpful feedback and insights throughout the entire project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2024.112534.

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