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Ageing and immunity: unraveling the association between immunosenescence and frailty

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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 inflammaging. This increases the vulnerability to novel infections, like SARS-CoV-2 virus (6-9). Immunosenescence and inflammaging 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 ($\times 10^9/L$). 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 n=1697	Covid-Predict n=656	CliniCo 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 (10⁹/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 (10⁹/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]	0.70
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						
	N	Low		Middle		High	
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Lymphocyte count (10⁹/L)							
COVID-OLD	1697	Reference	0.02	0.73 (0.56-0.94)	0.83 (0.64-1.07)	0.14	
Covid-Predict	461	Reference	0.08	0.63 (0.38-1.05)	0.54 (0.32-0.92)	0.02	
CliniCo	469	Reference	0.91	1.03 (0.66-1.60)	0.67 (0.39-1.12)	0.13	
Neutrophil count (10⁹/L)							
COVID-OLD	1697	Reference	<0.01	1.64 (1.27-2.11)	2.27 (1.75-2.96)	<0.01	
Covid-Predict	441	Reference	0.05	0.57 (0.33-0.99)	0.85 (0.50-1.43)	0.54	
CliniCo	469	Reference	0.11	1.47 (0.92-2.34)	1.74 (1.07-2.82)	0.03	
CRP (mg/L)							
COVID-OLD	1697	Reference	<0.01	1.59 (1.23-2.07)	2.88 (2.20-3.78)	<0.01	
Covid-Predict	589	Reference	0.05	1.64 (1.00-2.74)	3.15 (1.95-5.16)	<0.01	
CliniCo	501	Reference	0.03	1.86 (1.06-3.34)	3.28 (1.87-5.92)	<0.01	
Neutrophil/lymphocyte ratio (NLR)							
COVID-OLD	1697	Reference	0.06	1.27 (0.99-1.64)	1.64 (1.28-2.12)	<0.01	
Covid-Predict	431	Reference	0.71	0.90 (0.51-1.57)	1.22 (0.71-2.09)	0.47	
CliniCo	469	Reference	0.93	1.02 (0.63-1.66)	1.48 (0.92-2.39)	0.11	
Platelet/lymphocyte ratio (PLR)							
COVID-OLD	1697	Reference	0.04	0.77 (0.59-0.99)	1.02 (0.79-1.32)	0.88	
Covid-Predict	461	Reference	0.46	1.22 (0.72-2.06)	1.10 (0.65-1.85)	0.72	

	Stratum of inflammatory marker							
	N	Low		Middle		High		P-value
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	
CliniCo	467	Reference	0.62	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	0.69	1.05 (0.82-1.34)	0.69		0.60	
Covid-Predict	431	Reference	0.57	0.85 (0.49-1.48)	0.57	1.01 (0.59-1.75)	0.96	
CliniCo	467	Reference	0.91	1.03 (0.64-1.64)	0.91	1.61 (0.99-2.65)	0.06	

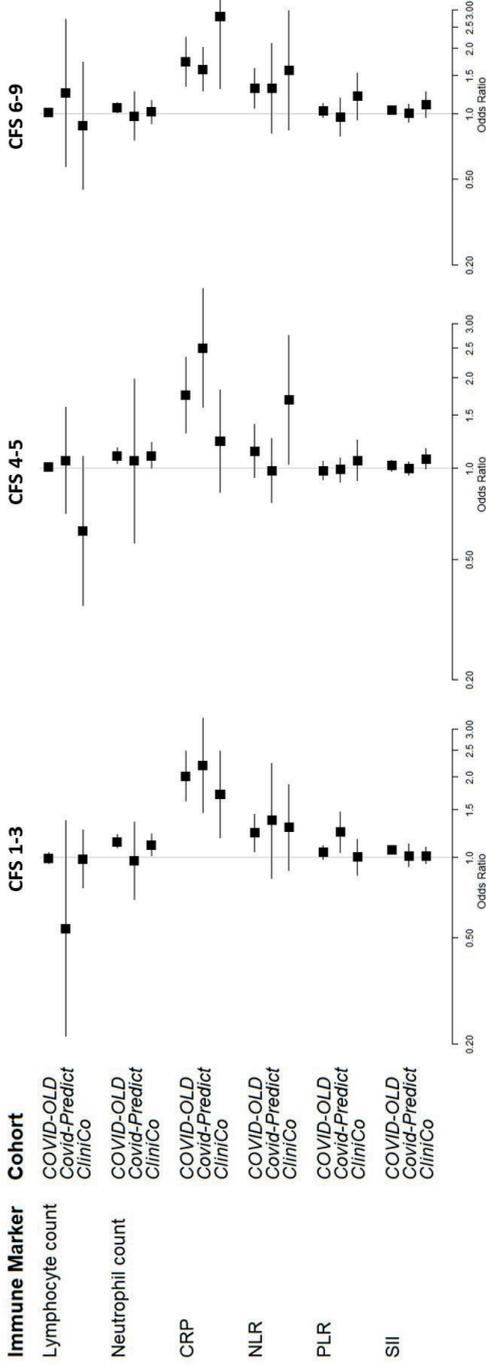
Abbreviations: CRP, C-reactive Protein; N, number; OR, odds ratio; NA, non-applicable

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 (10⁹/L) and Neutrophil count (10⁹/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 inflammaging (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 inflammaging, 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)			P for interaction with CFS	
	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value		
Lymphocyte count (10⁹/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 (10⁹/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)				P for interaction with CFS
	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	N	OR (95% CI)	P-value	
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						
	N	Low tertile		Mid tertile		High tertile	
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-value	P-value
Lymphocyte count (10⁹/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)	0.70	
Neutrophil count (10⁹/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)			P for interaction with CFS
	N	OR	P-value	N	OR	P-value	N	OR	P-value	
Lymphocyte count (10⁹/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 (10⁹/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

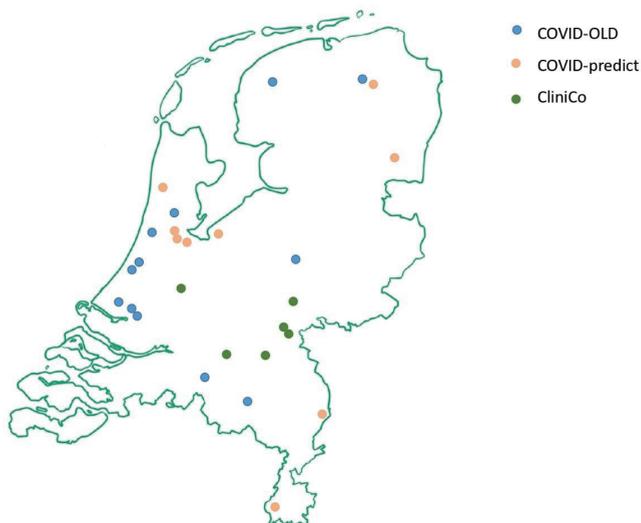


Figure S1. COVID-OLD: Alrijne Ziekenhuis (Leiderdorp), Catharina Ziekenhuis (Eindhoven), Deventer Ziekenhuis (Deventer), Erasmus Medical Center (Rotterdam), Elisabeth Tweesteden Ziekenhuis (Tilburg), Gelre Ziekenhuizen (Apeldoorn and Zutphen), Isala (Zwolle), Leiden University Medical Center (Leiden), Maasstad Ziekenhuis (Rotterdam), Medische Centrum Leeuwarden (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 Lieveise was provided to train investigators to categorize patients according to the CFS. <https://ru.h5p.com/content/1291038501098015477>

