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Frailty is associated with elevated CRP trajectories and higher numbers of neutrophils and monocytes

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A B S T R A C T

Background: With aging, the human immune system undergoes several changes. The clinical relevance of these changes, however, is relatively unknown. We investigated immunological aspects of human aging in relation to frailty in the Doetinchem Cohort Study (DCS).

Methods: We calculated a frailty index score based on 36 health parameters for each individual in the DCS with data obtained in the period 2008–2016. The frailty index was used to define three health groups ('healthy', 'intermediate', and 'frail'), stratified by age and sex. In a subcohort (n = 289, 60–85 years, selected by balanced random sampling per frailty group), we collected blood samples between October 2016 and March 2017 to determine absolute numbers of leukocyte subsets. In addition, cytomegalovirus serostatus was assessed. C-reactive protein (CRP) levels were longitudinally assessed in four consecutive plasma samples per individual. These samples had been previously collected (1993–2013) as part of the DCS at regular time intervals and spanning a period of > 15 years.

Results: We observed higher numbers of myeloid derived neutrophils and monocytes in the frail group compared to the healthy group in both men and women, and, retrospectively, consistently higher CRP concentrations over a period of > 15 years. An increase in CRP concentration with age was found in women, but not in men. Frailty was not associated with cytomegalovirus serostatus or with changes in lymphoid derived T-, B-, or NK-cell numbers.

Conclusion: Frail elderly, compared to their age- and sex-matched peers, endure a chronic and stable low-grade inflammation, which is associated with a myeloid cell lineage expansion. These findings could help to monitor clinically significant immunological decline in the elderly.

1. Introduction

The human immune system is made up of sets of various specialized cells communicating through numerous proteins and other molecules. These immune cells, cytokines and chemokines work together in a tightly controlled manner to maintain a healthy balance allowing to clear infections and other threats, without causing damage to healthy tissues (Goldszmid and Trinchieri, 2012). However, as people age, this balance maybe lost, due to the aging process itself and to chronic environmental stressors (Goldszmid and Trinchieri, 2012; Weyand and Goronzy, 2016). This could lead, among others, to changes in the cellular composition of the immune system (Carr et al., 2016). In addition, a disturbed immunological balance with age may manifest as a state of chronic, low-grade inflammation without imminent signs of an infection ('inflammaging') (Franceschi and Campisi, 2014) which is evidenced by elevated inflammatory markers like c-reactive protein (CRP) and interleukin-6 (IL-6) (Franceschi and Campisi, 2014). This chronic, low-grade inflammation has been linked to diminished leukocyte cytokine responses and to various chronic diseases such as cardiovascular diseases, diabetes, Alzheimer, arthritis, and cancer (Shen-Orr et al., 2016; Rea et al., 2018; Liu et al., 2017).

Despite these insights, it is still poorly understood how and to what extent such immunological changes affect health and the odds of reaching old age in good health ("successful" aging). Furthermore, how frailty relates to the onset and development of chronic low-grade inflammation has hardly been studied.

The aim of the present study is to better understand how low-grade inflammation develops during aging, and how it influences the immune system and general health. In order to be able to answer such questions, an observational study with long-term follow-up is necessary. The Doetinchem Cohort Study (DCS) provides such a unique framework. As part of the population-based DCS, blood samples have been collected every five years in the same individuals since 1987 (Picavet et al., 2017; Verschuren et al., 2008) which were used to measure CRP concentrations. Therefore, a time series of CRP concentrations (CRP trajectories) was available, covering a timespan of > 15 years per individual. To be able to analyze the major human immune cell subsets (neutrophils, monocytes, lymphocytes, B cells, T cells, and NK cells), we collected

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additional peripheral blood samples from 289 participants of the Doetinchem cohort above 60 years old, randomly selected and stratified by age, sex, and health status. Latent cytomegalovirus CMV infection was taken into account since it could be a confounding factor when studying immune aging (Wertheimer et al., 2014; Apoil et al., 2017; Pawelec, 2014). Health status was defined by employing the concept of a frailty index (Mitnitski et al., 2001; Searle et al., 2008), which we also used in this study as an outcome measure. Thus, the study presented here aims to investigate the cross-sectional association of frailty with the numbers of innate and lymphocyte subsets in peripheral blood and, in addition, the association of frailty with time trends in CRP levels over the preceding 15 years in 289 elderly people. With this study, we intended to gain more insights into the development of low-grade inflammation and how it affects frailty and aging in both men and women.

2. Methods

2.1. Study design and population

The Doetinchem cohort study (DCS) started in 1987–1991 with a random sample of the population in and around Doetinchem, a provincial town in the Netherlands (n = 7768, aged 20–60 years). Since then, participants have been examined every five years (1993–1997, 1998–2002, 2003–2007, 2008–2012, 2013–2017). At every DCS examination round, participants answered questionnaires about general health, lifestyle, and incurred current or previous diseases (Picavet et al., 2017; Verschuren et al., 2008). In addition, physical examination was performed and blood samples were taken, as described in detail elsewhere (Picavet et al., 2017; Verschuren et al., 2008). We used the latest available data collected in the period 2008–2016 to construct a frailty index score as a measure of overall health status for each participant (see below). The participants were then stratified by age, sex, and health status (frailty), and thus, by stratified sampling, a DCS subcohort was selected in order to investigate cellular immune parameters. The timeline is provided in Fig. 1a, which shows the temporal relationships between the different measurements performed in this study.

2.2. Construction and validation of the frailty index

We constructed a frailty index to quantify health status based on a methodology developed in previous studies (Searle et al., 2008; Rockwood et al., 2011; Collerton et al., 2012; Schoufour et al., 2017). Details and validation of this frailty index can be found in the supplementary materials (Supplementary Methods, Supplementary Table 1 and Supplementary Fig. 1).

2.3. DCS subcohort selection

In order to select a representative subcohort for analyzing the blood immune cell composition, and to include sufficient numbers of individuals covering the full spectrum of health status, we first stratified all DCS participants aged ≥60 years by age category, sex, and health status based on frailty index score (see Supplementary Fig. 2 for the stratification scheme). Within each group, we randomly selected equal numbers of DCS participants for inclusion in our subcohort, aiming to include approximately 300 individuals, taking into account a number of exclusion criteria (see below). From each of these DCS subcohort participants, a blood sample (30 mL) was drawn for analysis of the immune cell composition.

For stratification, four age categories were used: 60–65 years, 65–70 years, 70–75 years, and > 75 years (75–85 years). Three 'health groups' were defined by using the frailty index score as follows: within every age category and for each sex separately, participants in the DCS with the 15% highest score on the frailty index were defined as being frail, those with the 15% lowest frailty index as being healthy and the remaining 70% as having an 'intermediate' health status.

2.4. Subcohort inclusion and exclusion criteria

As health related deficits tend to accumulate later in life, we only included participants ≥60 years old on September 1, 2016, as candidates for our study (n = 2860). From these candidates, we excluded participants without written consent for re-invitation (n = 483), with information available on fewer than 30 out of 36 health deficits due to missing measurements (n = 37), and participants who had missed two or more measurement rounds since the start of the DCS in 1987 (n = 53, Fig. 1b). From those remaining, we invited 390 people for inclusion in our subcohort, 300 of which were willing to participate. Finally, we excluded people with a severe immunological disease or those using strong immunosuppressant medications (n = 11), ending up with including 289 participants (Fig. 1b). Blood withdrawal was postponed if people had received antibiotics or vaccinations, or had a fever with temperatures > 38 °C, within two weeks of the scheduled blood withdrawal.

2.5. Ethical approval

The study was approved by the Medical Ethics Committee of the University Medical Center (Utrecht, The Netherlands). The participants gave written informed consent for every DCS round and for this subcohort study separately.

2.6. Blood immune cell composition

From the DCS subcohort participants, fresh whole blood samples were collected in the morning (non-fasted) in sodium heparin tubes in the period from September 1, 2016 until March 1, 2017. The samples were analyzed within 6 h for absolute numbers of leukocyte subsets (cell counts per μL), which were obtained with a lyse-no-wash protocol using TruCOUNT® tubes and using a 4-laser LSRII Fortessa X20 flow cytometer (BD Biosciences). Neutrophil, monocyte, and lymphocyte populations were defined by side scatter and the fluorochrome conjugated antibody CD45(GA90)-OC515 (Cytognos). In addition, lymphocyte subsets (T cell, NK cell, and B cell populations) were defined by CD3(UCHT1)-BV711, CD16(B73.1)-PE, CD56(B159)-APC (all BD Biosciences), and CD19(J3-119)-PE-Cy7 (Beckman Coulter). For gating strategy, see Supplementary Fig. 3. In six participants the monocytes and in seven participants the lymphocytes were not clearly distinguishable from debris and therefore these results were excluded from analysis. Compensation was performed automatically with BDComp beads (BD Biosciences). Gating of cellular subsets was performed in FlowJo V10 (FlowJo company, Ashland, OR).

2.7. C-reactive protein (CRP)

Repeated blood samples were taken with a five-year interval between 1993 and 2012 and frozen at -80 °C (resulting in a total of four samples per participant). In these samples, high sensitivity CRP levels had been measured previously by particle-enhanced immunological agglutination, as described elsewhere (Hulsegge et al., 2016). All samples from the same person were measured at the same time to reduce intra-individual between-assay variation. The entire CRP time series of four measurements was available for 239/289 participants. The remaining participants had three (n = 46) or two (n = 4) available CRP measurements.

2.8. Anti-cytomegalovirus antibodies

We quantified CMV antibody concentrations using an in-house developed multiplex immunoassay (MIA) based on a commercially available Cytomegalovirus IgG ELISA kit (EUROIMMUN, Germany)

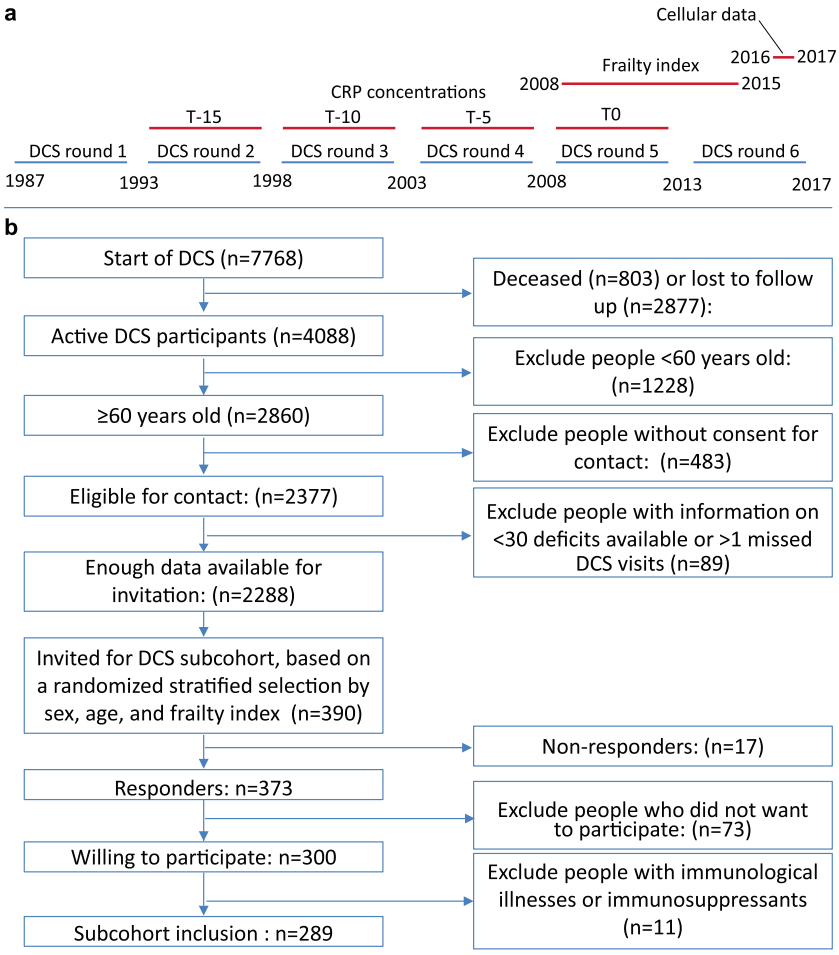

Fig. 1. (a) Timeline of the current study by using the Doetinchem study (DCS) subcohort; (b) selection of the DCS subcohort. Active DCS participants: participants who participated at least once since 2012. The DCS subcohort contains an equal selection of the participants in the frailty groups 'healthy', 'intermediate', and 'frail'. Reference time point T0 in (a) corresponds to DCS round 5. For 58% of the participants (n = 106), the frailty score was also assessed at T0. For the others (n = 183), it was assessed more recently (5 years after T0 and thus at DCS round 6). Immune cellular data was obtained from October 2016 until March 2017.

(Tcherniaeva et al., 2018). In accordance to the ELISA kit manufacturer's instructions, a concentration of < 16 relative units (RU) mL^{-1} was considered seronegative and > 22 RU mL^{-1} was considered seropositive. Two participants were excluded from analysis, since their concentration remained in the range of 16–22 RU mL^{-1} after two consecutive measurements, resulting in an inconclusive serostatus.

2.9. Statistical analysis

Leukocyte numbers were tested for associations with age category, health group, and sex using the Kruskal-Wallis test. In addition, they were tested for associations with the frailty index score after adjusting for age and CMV serostatus using a linear regression model for both sexes separately. All leukocyte numbers were log transformed in the regression model since this gave an approximately normal distribution for all subsets.

To investigate how differences in frailty index at the final time point were associated with different CRP trajectories over the preceding 15 years, we used a linear mixed model with log-transformed CRP concentrations as dependent variable and both frailty index and time elapsed since the first CRP measurement as independent variables. The model was evaluated for both sexes separately and adjusted for age at baseline (first CRP measurement). Next, a second model was further adjusted for education, BMI, and smoking. Additionally, we tested interactions between frailty index and time to analyze whether different CRP slopes could be observed with different frailty index scores. We used a random slope and intercept in the models, to take into account the correlation between repeated measurements within the same person. CRP concentrations were log transformed to reach an approximately normal distribution.

As a sensitivity analysis, the models were reevaluated after removing samples with CRP plasma concentrations > 10 mg L^{-1} (to exclude participants with possible acute inflammation), and after removing participants with joint inflammation and with cardiovascular diseases.

Finally, we investigated how CRP concentrations were associated with the cellular immune composition. For this purpose we transformed the cell numbers into z-scores and used these as independent variables in a linear mixed model, again with (log-transformed) CRP as the dependent variable.

All statistical analyses were performed in R version 3.5.1. The package lme4 (v1.1-21) was used for linear mixed effects modeling. The package sjPlot (v2.6.2) was used to produce tables for the regression models and to calculate p-values for the linear mixed models using the Kenward-Roger approximation. The package ggplot2 (v3.1.1) was used to create graphics. A p-value below 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

As shown in Table 1, age and sex distribution were similar between health categories due to the stratified random selection. In addition,

Table 1
Baseline characteristics of the DCS subcohort stratified by sex and frailty group.

	Men				Women			
	Healthy	Intermediate	Frail	p value	Healthy	Intermediate	Frail	p value
Age in years, mean (SD)	70.2 (6.6)	70.8 (6.8)	70.9 (6.8)	0.835	70.1 (6.6)	70.5 (5.9)	71.4 (0.12)	0.444
Frailty index, mean (SD)	0.01 (0.028)	0.08 (0.040)	0.24 (0.092)	*Not tested*	0.02 (0.020)	0.11 (0.065)	0.29 (0.12)	*Not tested*
BMI in kg m^{-2}, mean (SD)	25.7 (2.1)	27.1 (3.4)	29.3 (4.6)	< 0.001	25.3 (3.2)	27.5 (4.2)	29.9 (5.5)	< 0.001
Smoking, % (n)				0.037				0.087
Never	50 (26)	28 (12)	22 (11)		38 (20)	29 (12)	27 (13)	
Ex-smoker	48 (25)	58 (25)	58 (29)		60 (32)	57 (24)	57 (28)	
Current smoker	1.9 (1)	14 (6)	20 (10)		1.9 (1)	14 (6)	16 (8)	
Education				0.101				0.219
Low, % (n)	23 (12)	33 (14)	46 (23)		49 (26)	45 (19)	65 (32)	
Middle, % (n)	40 (21)	28 (12)	32 (16)		25 (13)	21 (9)	20 (10)	
High, % (n)	36.5 (19)	40 (17)	22 (11)		26 (14)	33 (14)	14 (7)	
CMV+, % (n)	42 (22)	47 (20)	54 (27)	0.522	62 (33)	55 (23)	55 (27)	0.643
Leukocytes (counts μL^{-1})								
Neutrophils	2447	2691	3374	< 0.001	2315	2744	3294	< 0.001
Monocytes	201	221	251	0.010	192	207	211	0.002
Lymphocytes	1593	1147	1405	0.855	1520	1516	1334	0.709
T cells	1053	1034	1130	0.587	1231	1241	1260	0.668
B cells	147	123	132	0.510	170	182	188	0.504
NK cells	256	221	222	0.026	233	227	216	0.857
Total, % (n)	36 (52)	30 (43)	34 (50)		37 (53)	29 (42)	34 (49)	

Note: Leukocyte numbers are geometric means per group. P-values show outcomes of either Kruskal-Wallis or chi-squared test between health groups and the baseline characteristic.

CMV serostatus was similar among health categories for both men and women (Table 1). Frail men smoked more often than healthy men. Furthermore, BMI was higher in the frail group in both men and women (Table 1).

3.2. The longitudinal associations between CRP concentrations and frailty, sex, and age

The average time span between the first CRP measurement and the frailty index assessment was 17.5 ± 2.6 years (mean ± sd). CRP levels in both men and women increased over the observed period from T-15 to T0, and hence with advancing age (paired *t*-test between log (CRP) at T-15 and T0, with a mean CRP level increase of 1.27 mg L^{-1} (95% conf. int: 1.04–1.55, p = 0.02) in men and 1.37 mg L^{-1} (95% conf. int: 1.12–1.66, p = 0.002) in women). CRP concentrations over time are shown in Fig. 2 for the three health groups separately. This Figure shows that the frail group had higher geometric mean CRP concentrations than the other groups for all four time points. Indeed, a higher frailty index was associated with higher CRP concentrations in both men and women in our linear mixed model (p = 0.001 and p < 0.001, respectively, Table 2). However, only in women this effect was also found after correcting for education, smoking, and BMI (p = 0.033, Table 2). CRP concentrations at the last time point (T0, see Fig. 1a) were positively correlated with frailty in both men and women (p = 0.005 and p < 0.001, respectively, Supplementary Fig. 4). In addition, CRP concentrations increased over time (and thus with age) when adjusted for frailty in women but no statistically significant effect was found in men (p = 0.008 and p = 0.062 respectively, Fig. 2 and Table 2). The slope of the CRP trajectories was not affected by the frailty index score (p value for the interaction between frailty index and time: 0.93 for men and 0.4 for women, data not shown).

When people with joint inflammation or with cardiovascular diseases were excluded from the analysis (as a sensitivity analysis), higher CRP concentrations were still associated with frailty in both men and women (p = 0.024 and p = 0.005, respectively, Supplementary Fig. 5 and Supplementary Table 2). Thus, the main outcome was not disproportionately affected by two specific conditions known to increase CRP concentrations. Furthermore, the outcome did not change after excluding CRP concentrations higher than 10 to account for possible acute infections (data not shown).

3.3. The associations between immune cell subsets and health group, age category, and sex

We compared cell numbers of both myeloid derived (neutrophils and monocytes) and lymphoid derived (B cells, T cells and NK cells) immune cell subsets (Table 1, Fig. 3) between the predefined health groups.

Frail participants had higher numbers of neutrophils compared to healthy participants (women: p < 0.001, men: p < 0.001) and higher numbers of monocytes (women: p = 0.002, men: p = 0.010, Table 1, Fig. 3a). In contrast, frailty was not associated with total lymphocyte, B cell, NK cell or T cell numbers in both sexes. Thus, the composition of the cellular immune repertoire showed an expansion of the myeloid lineage but not of the lymphoid lineage with frailty (Fig. 4).

Women had higher numbers of B cells (p < 0.001), T cells (p < 0.001), and lymphocytes (p = 0.008) compared to men, but lower numbers of monocytes (p = 0.003, Fig. 3a).

Older women had higher numbers of monocytes (p = 0.018) than younger women, but this age-related difference was not observed in men (p = 0.435, Fig. 3b). However, both older men and women had lower numbers of T cells than younger ones (women: p = 0.010, men: p = 0.019, Fig. 3b). Age was not associated with absolute numbers of neutrophils, which was in contrast to the strong association of frailty with neutrophil numbers. Furthermore, age was not associated with numbers of lymphocytes, B cells, or NK cells.

3.4. The associations of immune cell subsets with age, frailty, sex, and CMV serostatus

In the linear regression analyses with frailty and age as continuous variables and corrected for CMV serostatus, higher numbers of neutrophils corresponded with a higher frailty index in both men and women (Tables 3a and 3b). Similarly, monocyte numbers correlated positively with the frailty index but statistical significance was reached in women only (Tables 3a and 3b). Increasing age was associated with decreasing T cells numbers in both men and women (p = 0.005 and p = 0.015, respectively, Tables 3a and 3b), confirming the results of the analysis in which frailty and age were handled as categorical variables. In addition, age was associated with decreasing lymphocyte and B cell numbers in men (p = 0.010 and p = 0.004, respectively, Table 3a), but

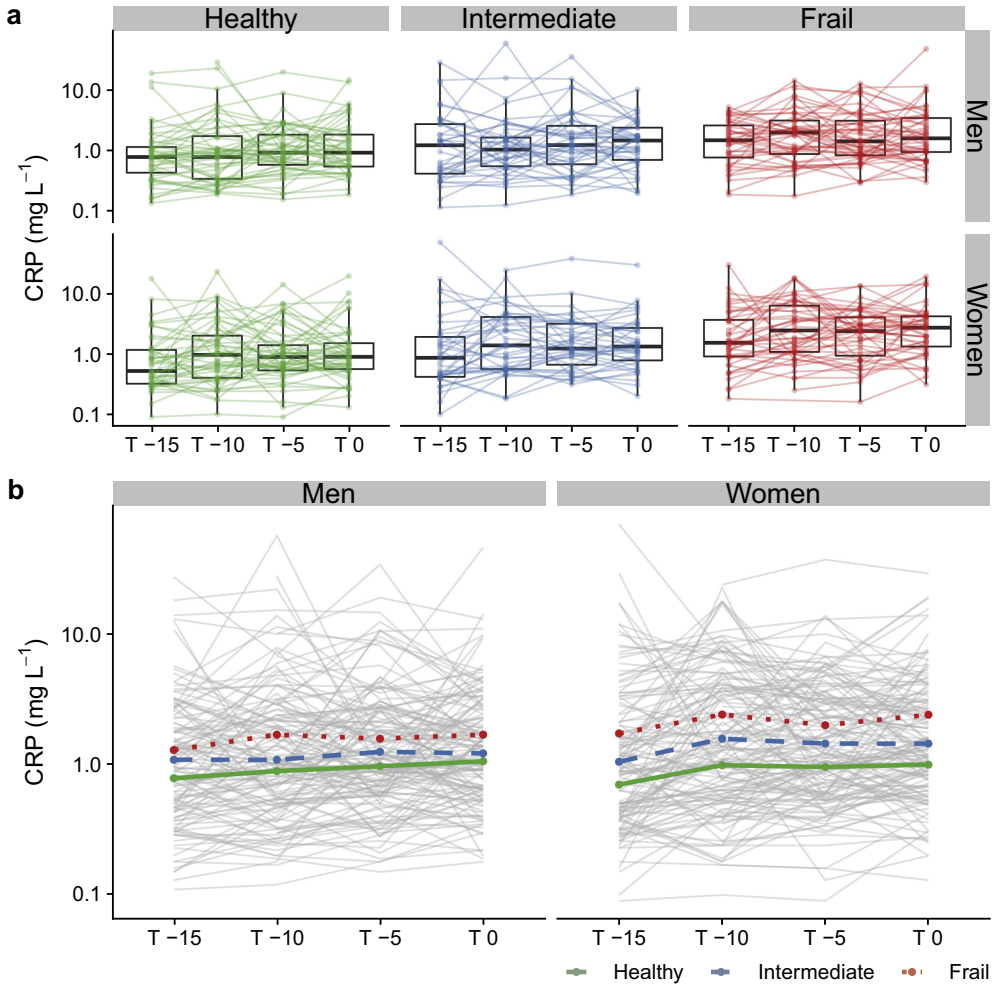

Fig. 2. CRP trajectories over a time period of approximately 15 years per frailty group, (a) for each health group and sex separately, and (b) the general pattern in geometric mean CRP concentration per health group and sex. The x-axis displays the time in years according to the reference time point (T0). This T0 is the moment of the most recent blood withdrawal for CRP concentration measurement. For 58% of the participants (n = 106), the frailty score was also assessed at T0. For the others (n = 183), it was assessed more recently (5 years after T0). The bold colored lines in (b) show the geometric mean values per measurement time point, stratified by sex and frailty group. The gray lines show the individual CRP trajectories. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for both sexes, age was not associated with neutrophil or monocyte numbers. T cell numbers were also higher in CMV seropositive people for both men and women ($p < 0.001$ and $p = 0.010$, respectively) and lymphocytes were higher in CMV seropositive men.

3.5. The associations of CRP concentrations over time with immune cell composition

CRP concentrations were associated with higher numbers of

Table 2
Linear mixed effects regression models of CRP concentrations (mg L^{-1}) over time for men and women.

Predictors	Log(CRP) in men						Log(CRP) in women					
	Model 1			Model 2			Model 1			Model 2		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
(Intercept)	−0.39	−1.37–0.59	0.434	−1.60	−3.06 to −0.14	**0.034**	−0.46	−1.53–0.61	0.400	−2.49	−3.93 to −1.05	**0.001**
Frailty index	2.20	0.95–3.45	**0.001**	0.51	−0.90–1.93	0.476	2.54	1.48–3.61	< **0.001**	1.27	0.11–2.43	**0.033**
Age at baseline	0.00	−0.02–0.03	0.634	0.01	−0.01–0.03	0.468	0.01	−0.02–0.03	0.593	0.01	−0.01–0.03	0.248
Time (years after baseline age)	0.01	−0.00–0.02	0.062	0.01	−0.00–0.02	0.061	0.02	0.00–0.03	**0.008**	0.02	0.00–0.03	**0.011**
Education: intermediate				−0.29	−0.60–0.02	0.072				0.27	−0.07–0.61	0.127
Education: high				−0.46	−0.79 to −0.13	**0.007**				−0.10	−0.44–0.24	0.567
Smoking: ex				0.31	0.02–0.60	**0.037**				−0.06	−0.35–0.24	0.710
Smoking: current				0.74	0.29–1.20	**0.002**				0.19	−0.30–0.67	0.450
BMI				0.05	0.01–0.09	**0.023**				0.07	0.04–0.10	< **0.001**
Observations	549			549			553			553		
Marginal R^2/ conditional R^2	0.061/0.608			0.142/0.611			0.106/0.608			0.181/0.610		

Note: CRP concentrations were log transformed before analysis. Four CRP concentration measurements per person were used, with approximately 5 years between measurements. Age at baseline: the age at which the first CRP measurement took place. Education: categorical variable with Education level 'Low' as reference. Smoking: categorical variable with smoking level 'None' as reference. CI = 95% confidence interval. *Note:* P-values < 0.05 are displayed in bold and were considered statistically significant.

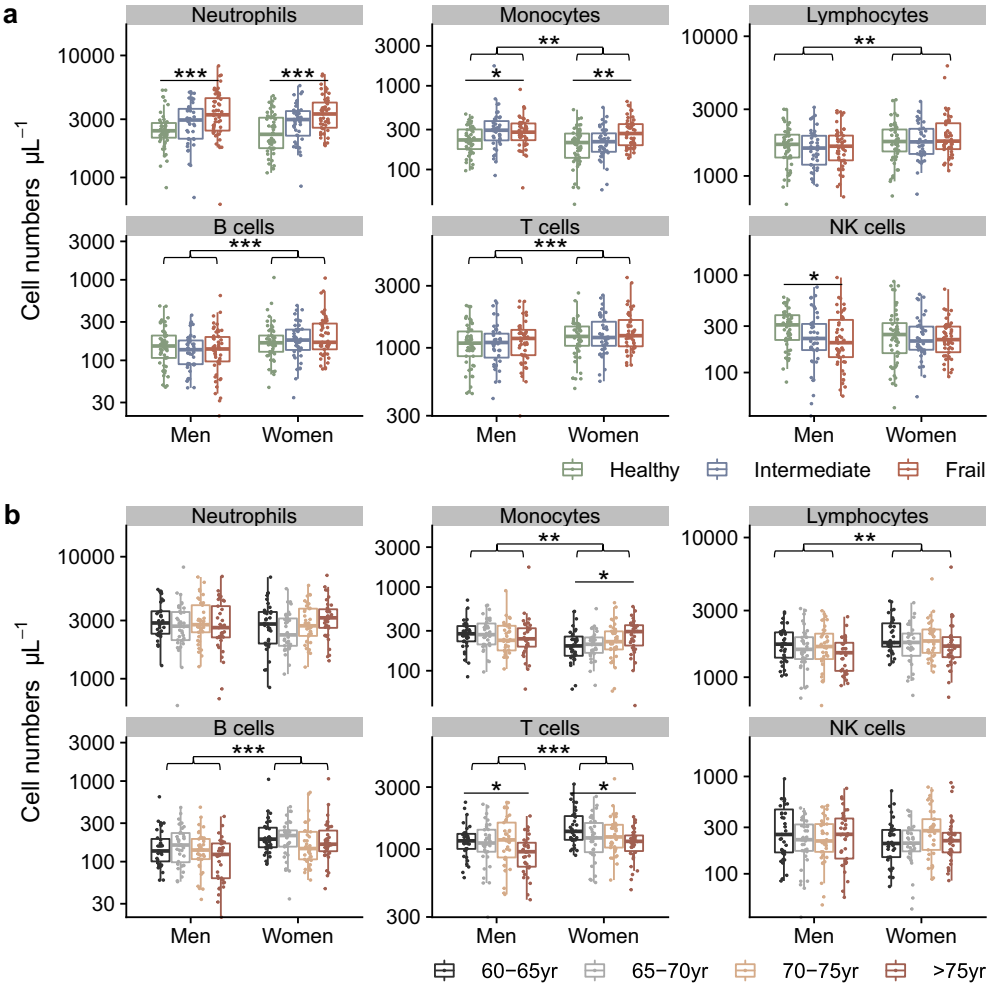

Fig. 3. Absolute numbers of leukocytes for men and women per (a) frailty group and (b) age group. Box plots showing median values and interquartile ranges. ***: $0 < p < 0.001$, **: $0.001 < p < 0.01$, *: $0.01 < p < 0.05$.

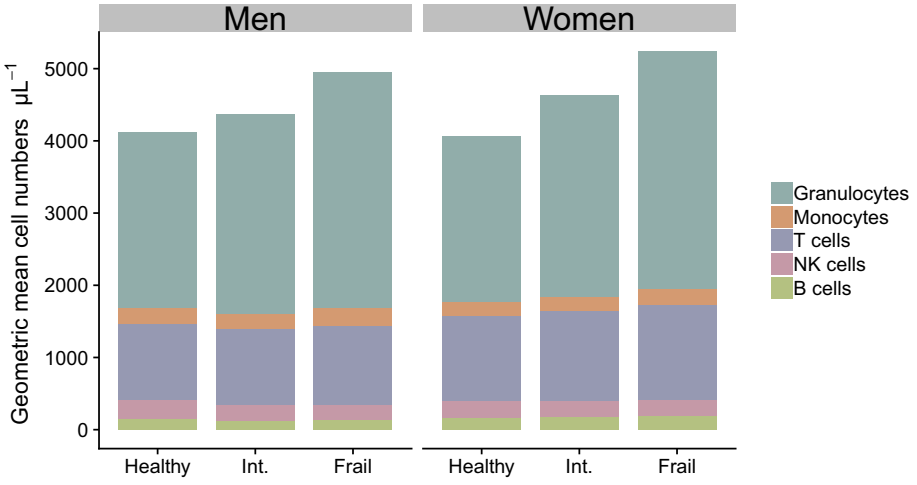

Fig. 4. A cumulative schematic overview of the immune cell composition by health group and sex. The bar chart shows the geometric mean concentration per cell subset as indicated.

Table 3a
Male leukocyte numbers and their association with age and frailty, adjusted for CMV serostatus.

Predictors	Neutrophils		Monocytes		Lymphocytes		B cells		T cells		NK cells	
	Estimates	p	Estimates	p	Estimates	p	Estimates	p	Estimates	p	Estimates	p
Frailty index	1.27 (0.67–1.86)	< **0.001**	0.59 (−0.09–1.27)	0.091	0.14 (−0.34–0.61)	0.576	−0.40 (−1.25–0.46)	0.365	0.32 (−0.21–0.85)	0.241	−0.64 (−1.54–0.25)	0.160
Age	−0.01 (−0.02–0.00)	0.077	−0.00 (−0.02–0.01)	0.440	−0.01 (−0.02 to −0.00)	**0.010**	−0.02 (−0.04 to −0.01)	**0.004**	−0.01 (−0.02 to −0.00)	**0.005**	-0.00 (−0.02–0.01)	0.649
CMV +	−0.11 (−0.25–0.02)	0.091	−0.14 (−0.29–0.01)	0.068	0.13 (0.03–0.24)	**0.014**	0.03 (−0.16–0.21)	0.791	0.22 (0.11–0.34)	< **0.001**	−0.14 (−0.34–0.06)	0.170
Observations	144		140		141		144		144		144	
R^2/adjusted R^2	0.123/0.104		0.042/0.021		0.086/0.066		0.079/0.059		0.139/0.121		0.036/0.015	

Note: P-values < 0.05 are displayed in bold and were considered statistically significant.

neutrophils in both sexes (both $p < 0.001$), but not with numbers of monocytes, T cells, B cells or NK cells (Supplementary Table 3).

4. Discussion

Our main finding is that in both sexes, an elderly frail population is characterized by higher numbers of circulating myeloid derived neutrophils and monocytes and higher CRP concentrations compared to an age matched healthy population. Moreover, this study revealed that CRP concentrations were consistently higher over a period of > 15 years in the frail population. Frailty was not associated with CMV serostatus or with changes in lymphoid lineage immune cells. The results suggest that in the frail elderly, chronic low-grade inflammation induces an expansion of the myeloid lineage. To the best of our knowledge, this is the first study showing associations between frailty and CRP trajectories with multiple consecutive CRP measurements covering a period of > 15 years.

The frailty index that we used to define health status was constructed using data from the Doetinchem Cohort Study (DCS). Its characteristics were found to be consistent with common frailty index characteristics reported in the literature (Searle et al., 2008; Rockwood et al., 2011; Collerton et al., 2012; Schoufour et al., 2017). Another method broadly used to characterize frailty is by defining a particular frailty phenotype (Fried et al., 2001). Both the frailty index and the frailty phenotype have been used extensively and while the frailty phenotype is easier to operationalize, the frailty index generally is superior in predicting adverse outcomes (Rockwood et al., 2007; Kulminski et al., 2008).

Our study confirms the results of previous studies showing that elevated CRP concentrations were associated with frailty (Soysal et al., 2016; Walker et al., 2018). In addition, our findings provide critical insight into the presence of chronic low-grade inflammation throughout a major part of the adult human lifetime, in relation to frailty. In a recent study, Walker et al. (2018) found that rising CRP levels at midlife, measured at two points in time, increased the risk of becoming frail later in life. Interestingly, we found that the CRP concentrations over time (CRP trajectories) evolved more or less at the same pace in the different health groups. This suggests that the quite stable difference in CRP trajectories between the frail and the healthy group must have developed before our first measurements and would thus have occurred > 15 years ago. In addition, we found that the effect of frailty on CRP levels over time was stronger in women than in men. In women, the effect also holds after correcting for several covariates that could influence CRP levels (education, smoking, BMI). This confirms that frailty manifests differently in men and women (Gordon et al., 2017) and shows the importance of investigating them separately when studying frailty.

The elevated CRP trajectories in the frail found in our study indicate that a process of long-term lingering inflammation is common in those who become frail. Lingering inflammation could lead to a skewing of the hematopoietic stem cell lineage towards the myeloid cell precursors, as suggested by Kovtonyuk et al. (2016). This is in line with our findings that myeloid lineage cell numbers were elevated in the frail population, while lymphoid lineage cell numbers were not. Another possibility is that with elevated CRP concentrations, monomeric CRP (mCRP) levels could increase as well. mCRP is a subunit of the pentameric native CRP (nCRP) that can be formed either directly in inflamed tissue or after dissociation of nCRP (Sproston and Ashworth, 2018; Thiele et al., 2014). mCRP has several pro-inflammatory effects and can delay apoptosis of neutrophils (Khreiss et al., 2002), which could explain the positive associations of CRP with neutrophils. Moreover, mCRP can also bind to monocytes and mediate pro-inflammatory effects on monocytes through FcγR binding and signaling (Thiele et al., 2014).

The observed chronic low-grade inflammation-induced expansion of the myeloid cell lineage in the frail population is in line with previous

Table 3b
Female leukocyte numbers and their association with age and frailty, adjusted for CMV serostatus.

Predictors	Neutrophils		Monocytes		Lymphocytes		B cells		T cells		NK cells	
	Estimates	p	Estimates	p	Estimates	p	Estimates	p	Estimates	p	Estimates	p
Frailty index	0.94 (0.48–1.41)	< **0.001**	0.99 (0.40–1.58)	**0.001**	0.17 (−0.25–0.59)	0.429	0.47 (−0.22–1.16)	0.187	0.13 (−0.28–0.54)	0.543	−0.62 (−1.30–0.05)	0.073
Age	0.00 (−0.01–0.01)	0.504	0.01 (−0.00–0.02)	0.147	−0.01 (−0.01–0.00)	0.210	−0.01 (−0.02–0.00)	0.187	−0.01 (−0.02 to −0.00)	**0.015**	0.01 (−0.00–0.03)	0.132
CMV+	−0.08 (−0.20–0.05)	0.223	−0.10 (−0.26–0.05)	0.184	0.09 (−0.02–0.20)	0.130	−0.08 (−0.27–0.10)	0.384	0.15 (0.04–0.26)	**0.010**	−0.09 (−0.27–0.09)	0.311
Observations	143		141		139		143		143		143	
R^2/adjusted R^2	0.137/0.118		0.131/0.112		0.031/0.010		0.024/0.003		0.092/0.072		0.037/0.016	

Note: P-values < 0.05 are displayed in bold and were considered statistically significant.

results (Baylis et al., 2013; Leng et al., 2009). In one study, the same association between high numbers of neutrophils and frailty was found, but not between monocytes and frailty (Collerton et al., 2012). An explanation for these differences could be that those findings pertained to a much older cohort (85+ years old).

While myeloid cell numbers were associated with frailty, lymphoid lineage cell numbers were not, which is consistent with previous studies (Marcos-Perez et al., 2018). These results were not affected by CMV seropositivity. However, CMV seropositivity was associated with higher T-cell numbers in both sexes, as expected (Collerton et al., 2012).

To identify frailty related immune differences between men and women, we performed analyses separately for each sex. This was inspired by the notion that auto-immune diseases are more prevalent in women than in men (Ngo et al., 2014) and that women tend to have a higher frailty index but generally live longer than men (Gordon et al., 2017). We observed the same chronic low-grade inflammation-induced myeloid cell lineage expansion in both sexes, albeit that these patterns were more pronounced in women than in men. Furthermore, we observed higher T- and B-cell numbers in women, which agrees with previously reported results (van der Heiden et al., 2016). In addition, in women we observed a clear increase in CRP concentrations with age, while in men we did not.

This study has some limitations. One limitation was that CRP levels were available retrospectively, while health status could only be assessed at around the time of inclusion in the study. Thus, it was not possible to study CRP levels as a predictor of the development of frailty. Furthermore, this was an observational study and as such potentially affected by unaccounted for confounding and selection bias, not allowing conclusions regarding causality. In addition, many components of our frailty index were self-reported, which could potentially induce some subjective bias in the results. Furthermore, diseases like cardiovascular disease and joint inflammation could potentially be confounders in evaluating the association between chronic low-grade inflammation and frailty. Our sensitivity analyses, however, showed that these did not change the main outcomes of this study. Lastly, we were only able to use CRP to quantify low-grade inflammation over time. Since inflammation is a multifactorial process, future studies should follow to confirm our findings with other important inflammatory biomarkers that have been associated with frailty, like IL-6 (Soysal et al., 2016).

It has been suggested that chronic low-grade inflammation may underlie immune dysfunction with aging and associated morbidities such as infection and cancer. Indeed, low-grade inflammation has been found to be associated with lower immune cell cytokine responses (Shen-Orr et al., 2016) and lower Delayed-type Hypersensitivity responses to varicella zoster virus (Vukmanovic-Stejic et al., 2018). Monitoring low-grade inflammation may thus be of use in identifying the development of immune frailty. Our study shows that this type of inflammation could possibly already be identified in a frail population of middle-aged adults. Longitudinal studies like ours could help to clarify the relationship between chronic low-grade inflammation, dysfunctional immunity, and 'successful' or 'unsuccessful' aging. This could open up the possibility to target this inflammatory state at an early stage for preventing its detrimental effects. Several interventions have already been proposed to target low-grade inflammation (Xia et al., 2016; Aspinall and Lang, 2018). Recent attempts to decrease low-grade inflammation have been successful in improving vaccine responses after specific anti-inflammatory therapy (Vukmanovic-Stejic et al., 2018).

In summary, both male and female frail elderly endure a chronic and remarkably stable low-grade inflammation, which is associated with a myeloid cell lineage expansion. These results may aid the monitoring of clinically significant immune decline in the elderly. Further research is needed on the functionality of the innate and adaptive immune cells and on additional inflammatory biomarkers over time to understand better the most relevant changes of the immune system with progressing age.

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Declaration of Competing Interest

Nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article, including Supplementary Figures 1-5, Supplementary Tables 1-3, and Supplementary Methods, can be found online at https://doi.org/10.1016/j.exger.2019.110674.

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