THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2019, Vol. 74, No. 4, 480–488
doi:10.1093/gerona/gly135
Advance Access publication June 19, 2018

OXFORD

Original Article

Association Between Immunosenescence Phenotypes and Pre-frailty in Older Subjects: Does Cytomegalovirus Play a Role?

Hung Cao Dinh, MD,[1,2] Ivan Bautmans, PhD,[1,2,3] Ingo Beyer, PhD,[1,2,3] Tony Mets, PhD,[1,2,3] Oscar Okwudiri Onyema, PhD,[1] Louis Nuvagah Forti, PhD,[1] Wim Renmans, BSc,[4] Sam Vander Meeren, MD,[4] Kristin Jochmans, PhD,[4] Sofie Vermeiren, MSc,[1] Roberta Vella-Azzopardi, MD,[1] and Rose Njemini, PhD[1,2]; on behalf of the Gerontopole Brussels Study Group

[1]Frailty in Ageing Research Group and [2]Gerontology Department, Vrije Universiteit Brussel, Belgium. [3]Department of Geriatric Medicine and [4]Laboratory of Hematology, Universitair Ziekenhuis Brussel, Belgium.

Address correspondence to: Ivan Bautmans, PhD, Head Gerontology (GERO) & Frailty in Ageing Research (FRIA) Departments, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium. E-mail: ivan.bautmans@vub.be

Received: September 27, 2017; Editorial Decision Date: May 31, 2018

Decision Editor: Rafael de Cabo, PhD

Abstract

Frailty is highly prevalent in old age and confers an important mortality risk. Although the causes of frailty are multiple, immunosenescence (IS)—predominantly driven by cytomegalovirus (CMV)—has been implicated in its pathophysiology. Thus far, research examining the association between IS and frailty states is sparse and equivocal. On the other hand, evidence is mounting in support of the view that frailty can be reversed, especially for those in the pre-frail stage. Therefore, we aimed to clarify the impact of CMV on IS and its relevance to pre-frailty. One hundred seventy-three persons aged 80 to 99 years were enrolled. Pre-frailty was defined according to Fried's criteria. Anti-CMV IgG and serum IL-6 were measured using Architect iSystem and Luminex, respectively. T-cell phenotypes were determined using flow cytometry. The prevalence of pre-frailty was 52.6%, increased with age (p = .001), and was greater in men than women (p = .044). No relationship was found between pre-frailty and positive CMV serology. Further, CMV-seropositivity was significantly associated with less naïve cells, more memory and senescence-prone phenotypes (all p < .001). After adjusting for potential confounders, only IL-6, age and sex were predictive of pre-frailty. We conclude that the presence of pre-frailty is independent from CMV infection in very old subjects.

Keywords: Senescence, Inflammation, Lymphocytes, Robust

Frailty is a complex geriatric syndrome that results from a decreased physiologic reserve in multiple organ systems, to the extent that minor stress will put a number of physiological systems beyond the threshold of symptomatic clinical failure (1). It is very prevalent among older people—with estimated prevalence of up to one-third of those aged 80 years and over—and is associated with an increased risk of disability, falls, morbidity, hospitalization, institutionalization, and death (2–4). Given the expanding older adult population, the numbers of frail older people will increase, particularly as the current numbers of the oldest old are predicted to triple over the next 30 years (http://www.un.org/esa/population/publications/worldageing19502050/).

A pre-frail state has been described by several researchers as an incomplete physical frailty phenotype (5,6), and pre-frail older adults have more than twice the risk of becoming frail than robust ones (7). In a recent meta-analysis, pre-frailty was shown to be a high risk factor for mortality (odds ratio [OR] = 1.761 [1.359, 2.282], hazard ratio/relative risk [HR/RR] = 1.466 [1.323, 1.624]), disability in basic (OR = 1.855 [1.347, 2.556], HR/RR = 1.587 [1.442, 1.747]) and instrumental (OR = 2.302 [1.947, 2.721]) activities

of daily life, physical limitations (OR = 1.813 [1.412, 2.328], HR/RR = 1.484 [1.328, 1.658]), falls (HR/RR = 1.167 [1.049, 1.299]), and hospitalization (OR = 1.527 [1.191, 1.959], HR/RR = 1.148 [1.063, 1.239]) (4). Notwithstanding, it has been shown that frailty is a highly dynamic condition that can revert, particularly in pre-frail individuals (8). Scientific evidence suggests that pre-frail older adults respond more successfully to physical interventions than those who have already moved to a frail state (9). Therefore, pre-frail older persons can be considered as an important target group to counter frailty. However, the clinical and physiological profiles of pre-frail older adults are scarcely described in literature, especially for the oldest old.

Although the pathophysiology of (pre-)frailty needs further elucidation, given its complex and multifactorial nature, there is growing evidence for the involvement of immunosenescence (IS) and its associated conditions in the development of the syndrome (10). Inflammaging (11,12) and immune risk profile (IRP) (13) are two recent concepts regarding IS that are increasingly being recognized to be, at least in part, the cause of increased susceptibility to frailty and death in older subjects. Inflammaging refers to a chronic low-grade inflammatory profile (CLIP) with advancing age, and emerging studies have shown that this heightened inflammatory state may play a central role in the pathogenesis of pre-frailty and frailty, either by promoting protein degradation, or through its deregulation of other metabolic pathways (14). On the other hand, IRP is characterized by a shift in T-cell sub-population types manifested by decreased CD4+/CD8+ T-cell ratio, lower numbers and proportions of naïve and early-differentiated T-cells (defined as cells expressing the costimulatory molecule CD28 and lacking the cell surface receptor CD57), with a concomitant accumulation of highly differentiated memory and senescent T-cells, identified by the expression of CD57 and/or absence of CD28. IRP is strongly associated with seropositivity to chronic viral infections such as cytomegalovirus (CMV), suggesting that CMV infection may be a driving force behind the shifts in T-cell subsets. Indeed, age-related increase of memory CD8+ T-cells is paralleled by an increase in the proportion of CMV epitope-specific T-cells. Khan and colleagues portrayed that individual CMV epitope-specific CD8+ T-cells could represent up to 23% of the total CD8+ T-cells in older adults with CMV infection (15). This clonal expansion of CMV-specific CD8+ T-cells is thought to exacerbate human T-cell IS, and thereby increase the susceptibility to inflammatory processes (16). Also, high levels of CMV IgG antibodies have been inconsistently reported to be associated with an increased risk of pre-frailty. In women under 80 years of age, Wang and colleagues (17) reported an increased prevalence of pre-frailty in those with high CMV antibody concentrations compared to CMV-seronegative women. Therefore, cellular mechanisms—in concert with alterations in inflammatory processes—may be implicated in the (pre-)frailty syndrome.

Our understanding of the effects of multiple deregulations in the T-cell pool in mediating frailty with advancing age is imperfect. In a study of community-dwelling adults aged 55 years and over, frailty and pre-frailty were predicted by the frequency of terminal effector CD8+ T cells (18). However, in a large population-based study on persons older than 85, an inverse relationship of memory/naïve CD8 T-cell ratio with pre-frailty was observed, that was contrary to expectation (19). Additionally, in a large population-based study of 724 community-dwelling women, Schmaltz and colleagues (10) could not confirm the results of Wang and colleagues (17) indicating an increased prevalence of pre-frailty in subjects with higher levels of CMV antibodies.

In light of this ongoing controversy and limited available data, we sought to clarify the impact of CMV on the relationship between IS phenotypes and pre-frailty in community-dwelling older subjects.

Method

Participants and Study Design

The BrUssels sTudy on The Early pRedictors of FraiLtY (BUTTERFLY) is an ongoing longitudinal study—organized by the Vrije Universiteit Brussel, Universiteit Ziekenhuis Brussel, and Universiteit Gent—designed to identify the determinants for active and healthy aging and for early stages of frailty in the oldest old. Apparently, healthy older individuals (≥80 years old) who presented no acute pathology, able to walk, and living independently in the community were recruited for this observational study. Recruitment was done—between February 2015 and February 2017—by advertisement through day centers, health insurance companies, seniors associations, general practitioners, municipalities, and other public places. Participants were excluded if they met any of the following criteria: acute pathology, cognitive impairment (unable to understand instructions and/or mini-mental state examination score < 24/30); diagnosis of cancer during the past 6 months; undergone surgery, radiotherapy, or chemotherapy within the past 6 months or scheduled in the near future. When eligible, the subjects were examined by a team of MDs and researchers to determine whether they portrayed any sign of frailty. Frailty was operationalized using three well-known definitions: Fried's frailty phenotype focusing mostly on physical frailty, the Groningen Frailty Indicator with a mainly psychosocial approach, and the Rockwood Frailty Index, which focuses on the medical aspects of frailty. Potential participants were excluded if they were identified as frail based on the Fried's criteria. This paper was based on the baseline data of the first 173 included subjects—81 women and 92 men—and, for the purpose of the present report, only physical frailty was considered since Fried's frailty index is the only one that identifies pre-frail subjects. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki and each participant gave a written informed consent.

Flow Cytometry Analysis

Venous blood specimens were collected in the morning for serum (stored at −80°C until analysis) and for EDTA anticoagulated blood. Peripheral blood leucocytes were recovered as described previously (20). Briefly, EDTA blood was exposed to lysis buffer for 10 minutes. After lysing the red blood cells, the blood leucocytes were centrifuged at 2,800 rpm for 4 minutes. Thereafter, the cells were isolated, washed twice in PBS containing 1% BSA at 2,800 rpm for 3 minutes, and re-suspended in 200 µL PBS containing 1% BSA.

Antibodies were initially titrated to determine the optimal conditions for flow cytometry analysis before staining. About 5×10^5 cells were stained with 3 µL each of PE-CY5-labeled anti-CD8 (Becton Dickinson, San Jose, CA), PE-CY7-labeled anti-CD3 (Biolegend, San Diego, CA), FITC-labeled anti-CD28 (Biolegend), and PE-labeled anti-CD57 (Biolegend). After 20 minutes incubation at room temperature in the dark, cells were washed at 2,800 rpm for 3 minutes, and 500 µL of FACS flow solution (Becton Dickinson) were added.

The labeled samples were analyzed with a Coulter FC 500 flow cytometer (Beckman Coulter, Fullerton, CA). Data acquisition was performed using the Coulter CXP software (Epics). The lymphocyte subpopulation was gated according to size and granularity in

the forward versus side scattergram, and as such, dead cells were excluded. Fluorescence-minus-one controls were used to distinguish positive from negative events, and the various lymphocyte clusters were identified according to their expression or non-expression of a combination of surface markers (Supplementary Figure 1). As CD3+ T-cells almost exclusively express CD4 or CD8 (21) and because in a previous study—we found that at least 95% of CD3+ CD8– cells from our subjects were CD4+ (20)—we considered the CD8– T-cells to be largely CD4+ T-cells (20). In this perspective, CD8–/CD8+ T-cell ratio could represent an imperfect but acceptable approximation of CD4+/CD8+ T-cell ratio in our setup.

Serum CMV IgG and IL-6 Determination

Serum levels of CMV IgG were measured by a chemiluminescent microparticle immunoassay on the ARCHITECT iSystem (Abbott Diagnostics, Abbott Park, Ireland) with an assay sensitivity and specificity of 100% and 99%, respectively. Assays were regarded as positive if they had concentrations of 6.0 arbitrary units (AU)/mL or greater and negative if they had concentrations of less than 6.0 AU/mL. The detection limit of 6 AU/mL was based on the indications from the manufacturer of the CMV IgG kit. The intra-assay and inter-assay coefficients of variation ranged from 4.39% to 5.67% and from 4.87% to 6.17%, respectively. The serum levels of IL-6 were determined using an IL-6 ultrasensitive singleplex Bead kit (Lifetechnologies USA). For IL-6 determination, the limit of detection, intra-assay, and inter-assay coefficients of variation were < 0.05 pg/mL, 7.59%, and 9.99% respectively. All reagents were applied according to the manufacturers' instructions.

Frailty Indicators

Fried and colleagues (5) developed an operational definition of frailty containing five criteria: weight loss, exhaustion, physical activity, gait speed, and grip strength. Each item is dichotomized and a total score of 0 means robustness, a score of 1–2 refers to pre-frailty, while a score of 3 or more signifies the presence of frailty (5). This construct of frailty is widely used, with the originally proposed measures, as well as in modified constructs. Inspired by the operational definition of Fried, our approach was based on four frailty characteristics suggested in previous research: weight loss, exhaustion, gait speed, and grip strength (5). Weight loss was evaluated by the self-reported question: "In the last six months, have you lost more than 4.5 kg unintentionally?" which was answered by yes (1) or no (0). Exhaustion was measured similarly to the original Fried phenotype, questioning two statements from the CES-D Depression Scale (22): "I felt that everything I did was an effort" and "I could not get going". The participants were asked: "How often in the last week did you feel this way?" and were scored 0 for rarely or none of the time, 1 for some or a little of the time, 2 for a moderate amount of time, or 3 for most of the time. When participants scored a 2 or 3 on either of the two statements, they received a point on the frailty scale for exhaustion. Gait speed was measured by timing the walked distance of 4.5 m and was stratified for gender and height, as proposed by Fried (5). Participants were scored a point for slow walking if their walking time was ≥7 seconds in men ≤173 cm and women ≤159 cm, and if their time was ≥6 seconds in men >173 cm and women >159 cm. Grip strength was performed using the Martin Vigorimeter, a reliable and practical instrument which measures handgrip strength in kPa (23). Cut-offs were 42 kPa for women, and 71 kPa for men. Participants showing a lower grip strength received a point for this item (24). The frailty scale contained four items and in analogy with previous research, the following scoring system was put forward to assign the level of frailty: a score of 0/4 signifies robustness, 1–2/4 points means pre-frailty and with a score of 3 or 4/4 one is considered frail (25).

Anthropometric Measurements

Weight was measured using a SECA balance, which was regularly calibrated, to the nearest 0.1 kg. Height was determined using a SECA measuring rod to the nearest 0.1 cm. Body mass index (BMI) was calculated using the measurements of height and weight (weight [kg]/ height^2 [m^2]).

Medical History

Participants were asked whether a doctor had ever told them that they had any of the following conditions: hypertension, ischemic heart disease, heart failure, peripheral vascular disorders, cerebrovascular disorders, thyroid disorders, diabetes mellitus, cancer, respiratory disorders, musculoskeletal conditions, osteoporosis, eye disorders, falls, skin disorders, kidney problems, problems with urination, depression, or anxiety. Only two (1.16%) of the participants were current smokers. Smoking habit was evaluated as ever smoked versus never smoked.

Statistical Analyses

Statistical analysis was performed using IBM SPSS version 22.0. Data were tested for normality using the Kolmogorov-Smirnov goodness of fit test. Most of the data were not normally distributed even after log-transformation and as such, nonparametric tests were applied during analysis. The Wilcoxon's Signed Rank test, Kruskal–Wallis and Mann–Whitney U-tests were used for continuous variables. Comparisons between categorical variables were performed using the chi-square test or Fisher exact test, where appropriate. Spearman's rank correlations were used to determine associations between participants' characteristics and CMV titers. Also, because the relationship between T-cell differentiation markers and the presence of pre-frailty differed significantly by CMV serostatus (p for interaction terms < .05), data were analyzed for CMV-seropositive and CMV-negative subjects separately. A binary logistic regression was applied to explore the relationship between IL-6 and T-cell differentiation markers and the risk of pre-frailty. Participants were classified into three groups—of about the same number of subjects—according to the levels of IL-6 as low, <1.4 pg/mL; intermediate, 1.4 to 2.5 pg/mL; and high, > 2.5 pg/mL and the Low group was the reference group. This concentration range was chosen based on findings by other authors (26) indicating that subjects aged 65 years and older are at higher risk of functional decline if they have circulating levels of IL-6 greater than 2.5 pg/mL. Collinearity was assessed with the variance inflation factor, and the naïve/early-differentiated phenotypes were removed due to a significant collinearity with their more differentiated counterparts. Analyses were carried out with or without adjustment for BMI, and heart failure. In addition, the discriminatory power of IL-6 was evaluated by analyzing the receiver operating characteristic curve, along with its 95% confidence interval. The –2-log likelihood and Hosmer–Lemeshow Test of the model were also assessed. Statistical significance was set a priori at two-sided $p < .05$.

Results

Descriptive Statistics

As portrayed in Supplementary Table 1, the overall prevalence of pre-frailty was 52.6%. Pre-frail subjects were significantly older than

robust individuals (p = .001) and the prevalence of pre-frailty was greater in men than in women (p = .044). Pre-frailty was associated with increased BMI (p = .019) and low CD8+ counts (p = .021). The IgG for CMV was positive in 92 (53.2%) subjects, and no direct association was found between CMV seropositivity or CMV titer and pre-frailty. However, more pre-frail subjects tended to have a history of heart failure compared to robust (p < .05, Supplementary Table 2). Further, an increase in tertiles of IL-6 was significantly associated with increasing age (p = .012).

IS Phenotypes According to Pre-frailty and CMV Serostatus

Table 1 shows the IS phenotypes according to pre-frailty and CMV serostatus. No significant difference was found in the percentage of T-cell differentiation markers or CD8–/CD8+ T-cell ratio between pre-frail and robust individuals. The pro-inflammatory cytokine IL-6 was significantly higher in pre-frail subjects compared to robust (p < .001). The CMV-seropositive group was characterized by a significantly higher proportion of highly differentiated memory and senescence-like phenotypes, in both the CD8+ and the CD8– sub-populations of T-cells (all p < .001). On the other hand, CD28+CD57– expressing cells (mainly representing the naïve phenotype) in both lineage markers of the lymphocyte subset as well as CD8–/CD8+ T-cell ratio were significantly higher in subjects without CMV compared to their CMV-seropositive counterparts (all p < .001). No significant difference was found in IL-6 levels with respect to CMV serostatus.

Association Between IS Phenotypes and Pre-frailty Stratified by CMV Serostatus

Considering the significant impact of CMV on the proportion of various T-cell subsets, we investigated the T-cell differentiation phenotypes according to pre-frailty status and separately in CMV-seropositive and CMV-negative subjects (Table 2). In the CMV-seronegative population, we found a significantly higher proportion of the highly differentiated memory phenotypes and a lower proportion of the naïve cell subset—in the CD8– compartment—in pre-frail subjects compared to robust (all p < .05, see Table 2). A similar trend was found for the CD8+ compartment. Also, pre-frailty was associated with higher levels of IL-6 (p < .001) in CMV-negative subjects. No significant difference was recorded concerning the percentages of T-cell phenotypes, IL-6 or CD8–/CD8+ T-cell ratio between the robust and pre-frail groups in the seropositive CMV population.

Association Between IS Phenotypes and CD8–/CD8+ T-Cell Ratio Category

We further investigated the association between T-cell subsets and the CD8–/CD8+ T-cell ratio category in the whole cohort (Figure 1) as well as by CMV serostatus (Figure 2 and Supplementary Figure 2). 15 (8.7%), 122 (70.5%), and 36 (20.8%) subjects had a ratio < 1, ratio = 1 to 4 and ratio > 4, respectively. The frequency of cells expressing the highly differentiated memory phenotype was significantly higher in the ratio < 1 group compared to the other groups, both in the CD8– and CD8+ sub-populations of T-cells (all p < .01). Also, the ratio < 1 group was characterized by a significantly higher proportion of the senescence-like phenotypes (all p < .05) compared to the other groups, in the CD8– pool. On the other hand, the proportion of cells expressing the predominantly naïve phenotype was significantly higher in the ratio > 4 group compared to the other groups (p < .001). Figure 2 and Supplementary Figure 2 show the distribution of various T-cell sub-populations according to the CD8–/CD8+ ratio categories and by CMV serostatus. For the CMV-negative group, we found a significantly higher percentage of

Table 1. IS Phenotypes According to Pre-frailty and CMV Serostatus

Parameter	Robust (n = 82)	Pre-frail (n = 91)	CMV+ (n = 92)	CMV– (n = 81)
T-cell subset				
CD8+ T-cells				
CD8+CD28+CD57– (naïve)	51.05 (40.88)	55.50 (34.80)	42.80 (28.33)	68.30 (29.25)[b,**]
CD8+CD28–CD57– (memory)	29.65 (31.12)	31.40 (24.80)	36.60 (27.03)	22.50 (21.65)[b,**]
CD8+CD28–CD57+ (SPC)	9.25 (20.68)	8.40 (17.80)	14.70 (19.00)	6.30 (11.90)[b,**]
CD8+CD28+CD57+ (SPC)	0.50 (0.70)	0.50 (1.10)	0.60 (0.95)	0.50 (0.90)
CD8– T-cells				
CD8–CD28+CD57– (naïve)	96.95 (8.9)	97.40 (6.10)	94.80 (9.18)	99.20 (2.60)[b,**]
CD8–CD28–CD57– (memory)	1.85 (4.73)	2.00 (4.20)	3.05 (6.00)	0.50 (1.80)[b,**]
CD8–CD28–CD57+ (SPC)	0.55 (2.73)	0.30 (1.80)	1.15 (2.68)	0.00 (0.30)[b,**]
CD8–CD28+CD57+ (SPC)	0.10 (0.30)	0.10 (0.30)	0.20 (0.30)	0.10 (0.10)[b,**]
Inflammatory marker				
IL-6 tertiles (n, %)				
Low (< 1.4 pg/mL)	37 (45.12)	18 (19.78)[a,**]	29 (31.52)	26 (32.10)
Intermediate (1.4 to 2.5 pg/mL)	27 (32.93)	32 (35.16)	31 (33.70)	28 (34.57)
High (> 2.5 pg/mL)	18 (21.95)	41 (45.06)	32 (34.78)	27 (33.33)
IRP marker				
CD8–/CD8+ ratio (n, %)				
<1	8 (9.76)	7 (7.69)	12 (13.04)	3 (3.70)[b,**]
1–4	60 (73.17)	62 (68.13)	69 (75.00)	53 (65.43)
>4	14 (17.07)	22 (24.18)	11 (11.96)	25 (30.87)

Notes: The values denote median (Interquartile range), unless otherwise stated; CMV cytomegalovirus; IRP = immune risk profile; IS = immune-senescence; SPC= senescence-prone cells; Phenotype frequencies were expressed as percentages within the CD3+CD8+ or CD3+CD8– T-cells.

[a]Mann–Whitney U test difference between robust and pre-frail.

[b]Mann–Whitney U-test difference between CMV+ and CMV–.

*p < .05; **p < .01.

Table 2. Association Between IS Phenotypes and Pre-frailty Stratified by CMV Serostatus

	CMV+ (n = 92)		CMV− (n = 81)	
Parameter	Robust (n = 47)	Pre-frail (n = 45)	Robust (n = 35)	Pre-frail (n = 46)
T-cell subset				
CD8+ T-cells				
CD8+CD28+CD57− (naïve)	41.70 (28.00)	49.80 (33.30)	72.60 (34.00)	60.00 (29.13)*
CD8+CD28−CD57− (memory)	37.40 (21.10)	33.70 (22.75)	19.50 (25.40)	23.90 (25.90)
CD8+CD28−CD57+ (SPC)	16.40 (16.10)	12.80 (19.35)	1.70 (10.60)	7.50 (12.58)
CD8+CD28+CD57+ (SPC)	0.60 (0.60)	0.60 (1.20)	0.40 (0.70)	0.50 (1.13)
CD8− T-cells				
CD8−CD28+CD57− (naïve)	92.50 (10.10)	95.40 (8.80)	99.50 (0.70)	99.00 (4.00)*
CD8−CD28−CD57− (memory)	4.00 (7.00)	2.80 (5.35)	0.40 (0.50)	0.90 (2.83)**
CD8−CD28−CD57+ (SPC)	1.30 (4.20)	0.70 (2.25)	0.00 (0.20)	0.10 (0.45)
CD8−CD28+CD57+ (SPC)	0.30 (0.30)	0.20 (0.30)	0.10 (0.10)	0.10 (0.10)
Inflammatory marker				
IL-6 tertiles (n, %)				
Low (< 1.4 pg/mL)	18 (38.30)	11 (24.44)	19 (54.29)	7 (15.22)**
Intermediate (1.4 to 2.5 pg/mL)	16 (34.04)	15 (33.33)	11 (31.43)	17 (36.96)
High (> 2.5 pg/mL)	13 (27.66)	19 (42.23)	5 (14.28)	22 (47.82)
IRP marker				
CD8−/CD8+ ratio (n, %)				
<1	6 (12.77)	6 (13.33)	2 (5.71)	1 (2.17)
1–4	38 (80.85)	31 (68.89)	22 (62.86)	31 (67.39)
>4	3 (6.38)	8 (17.78)	11 (31.43)	14 (30.44)

Notes: The values denote median (Interquartile range), unless otherwise stated; CMV = cytomegalovirus; IRP = immune risk profile; IS = immunosenescence; SPC = senescence-prone cells; Phenotype frequencies were expressed as percentages within the CD3+CD8+ or CD3+CD8− T-cells.

*p < .05, **p < .01: Mann–Whitney U-test difference between robust and pre-frail.

the highly differentiated memory and senescence-like phenotypes in the ratio < 1 group compared to the other groups (all p < .05). Contrariwise, the frequency of the naïve phenotypes was significantly higher in the ratio > 4 group compared to the other groups (all p < .01, see Figure 2). For the CMV-positive group, we found a significantly higher percentage of the highly differentiated memory cells and lower percentage CD8+CD28+CD57+ cells in the ratio < 1 group compared to the other groups (all p < .05). The percentages of the other differentiation phenotypes did not differ with respect to CD8−/CD8+ T-cell ratio among the CMV-seropositive individuals (Supplementary Figure 2).

Association Between IS Phenotypes and Pre-frailty Stratified by CD8−/CD8+ T-Cell Ratio Category

The association between IS phenotypes and pre-frailty was not consistent among the various categories of CD8−/CD8+ T-cell ratio (Supplementary Table 3). In the CD8− compartment of T-cells, we found a significantly higher proportion of the highly differentiated memory and senescence-like phenotypes and lower proportion of the naïve phenotype in pre-frail compared to robust subjects in the CD8−/CD8+ T-cell ratio > 4 group (all p < .05). A similar trend was found in the CD8+ subset. It is noteworthy that more than two-thirds (69.4%) of the subjects in the CD8−/CD8+ T-cell ratio > 4 group was CMV-negative.

Predictors of Prefrailty

Finally, logistic regression was used to determine the predictors of the risk of pre-frailty for the cohort. Since significant correlations were found among the T-cell phenotypes within the CD8− and CD8+ T-cell compartments, we entered just the senescence-prone CD57+ phenotype of each T-cell compartment in the regression analyses. When parameters associated with inflammation, senescence, IRP, sex and age were included in the model—corrected for, BMI and history of heart failure—only changes in IL-6, sex and age predicted the risk of pre-frailty (Table 3). In a separate analysis, we did include the CD28− subset in the regression analysis. However, the frequency of CD28− T-cells was not predictive of the risk of pre-frailty. Moreover, the inclusion of CD28− phenotype did not influence the predictive ability of IL-6 in identifying pre-frailty (data not shown). The -2 log likelihood of the model was 187.718 and the Hosmer-Lemeshow Test was not significant (p = .846).

In addition to the regression analysis, a receiver operating characteristic curve was created for the identification of participants at risk of pre-frailty based on IL-6. The area under the curve, reflecting the discriminating power of IL-6, was 0.661 (confidence interval = 0.579–0.743, see Table 3). The cutoff-point for IL-6 based on the highest Youden's index was 1.66 pg/mL, and for this cutoff value the sensitivity, specificity, positive and negative predictive values were, respectively, 0.670, 0.622, 0.663, and 0.630.

Discussion

Exploring baseline data from the longitudinal BUTTERFLY study, we investigated the impact of CMV on IS and its relevance on pre-frailty in a very old population. The findings of the present study indicate that pre-frailty does not require the CMV infection as a necessary factor for its development in very old subjects. More so, our study put in doubt the predominant role of the CMV infection on the inflammatory profile of very old persons.

In this study on people older than 80 years, IgG for CMV was positive in 92 (53.2 %) subjects. Higher CMV prevalence has often been described in older adults. In a study of 549 community-dwelling persons aged 80 and older in Belgium—where the current study was performed—Matheï and colleagues (27) reported 74% positive CMV serology. However, in their study, they included patients unlike

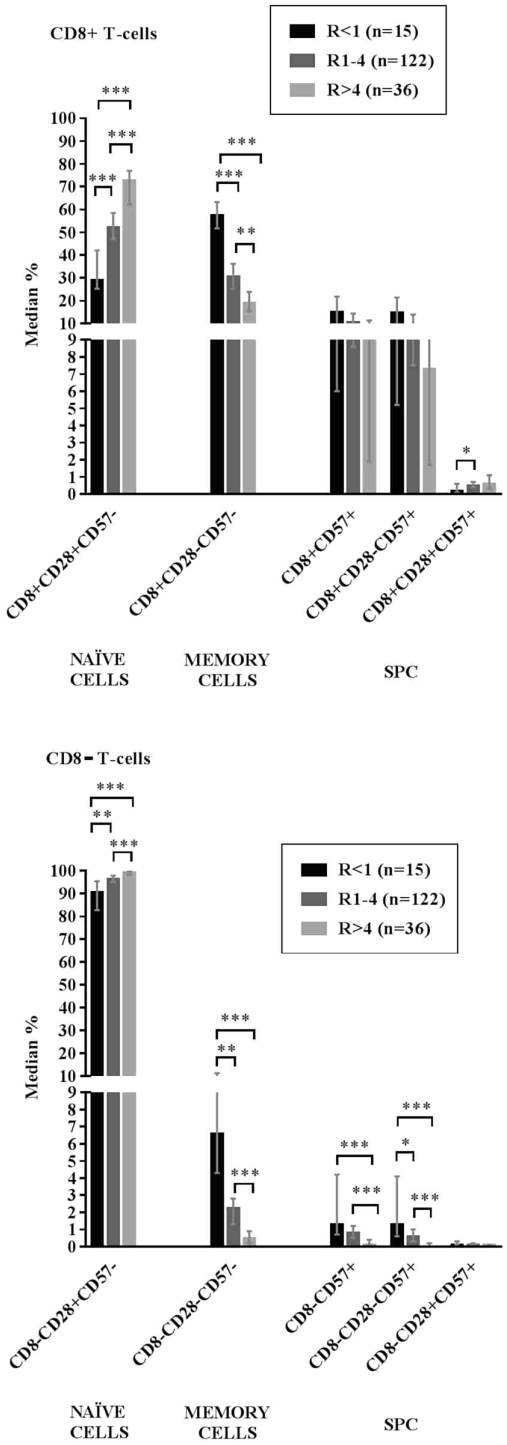

Figure 1. Association between T-cell differentiation markers and CD8–/CD8+ T-cell ratio in the cohort. Note: Data represent median percentage of cells within the CD3+CD8+ and CD3+CD8– T-cell subsets. SPC, senescence-prone cells. The error bars represent 95% confidence intervals. Results of Mann–Whitney U-test for between-group pairwise comparison *$p < .05$, **$p < .01$, ***$p < .001$.

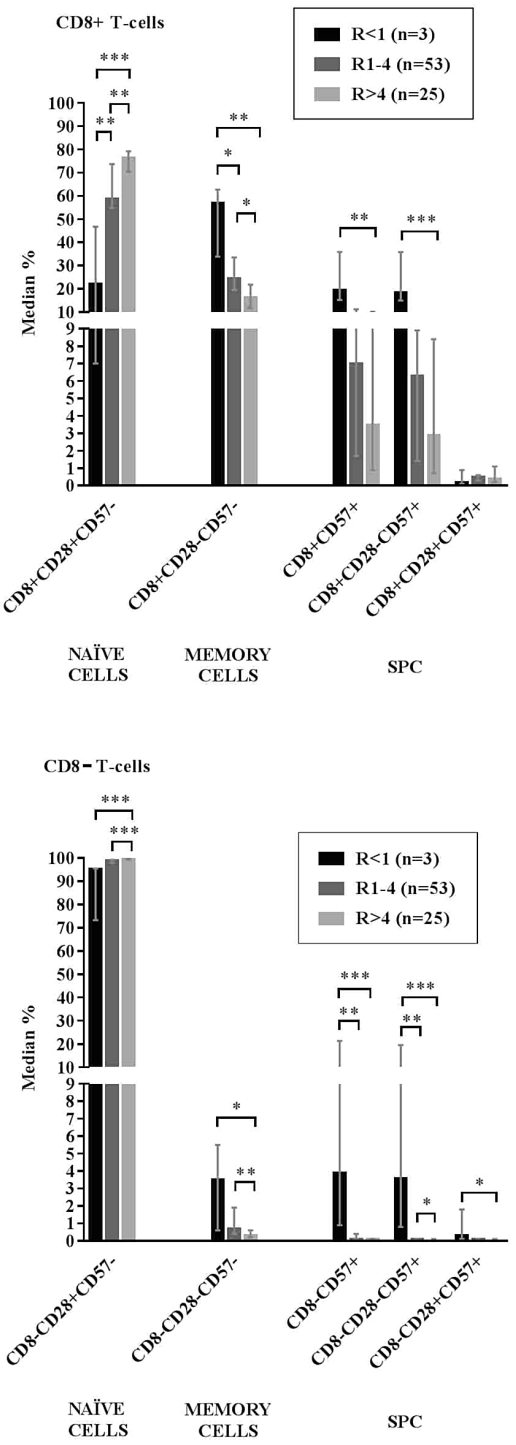

Figure 2. Association between T-cell differentiation markers and CD8–/CD8+ T-cell ratio in CMV-seronegative subjects. Note: Data represent median percentage of cells within the CD3+CD8+ and CD3+CD8– T-cell subsets. SPC, senescence-prone cells. The error bars represent 95% confidence intervals. Results of Mann–Whitney U-test for between-group pairwise comparison *$p < .05$, **$p < .01$, ***$p < .001$.

the apparently healthy population of the present study. Further, a Finnish study (28) showed that CMV seroprevalence was higher in Helsinki compared to a rural area in the southwest of the country (70.7% vs 56.3%, respectively). Therefore, it is conceivable that the overall CMV seropositivity can change over time as a result of changes in health status, age, and socio-economic situation (29).

We found no significant relationship between CMV seropositivity and the pro-inflammatory cytokine IL-6. Although positivity for IgG class anti-CMV antibodies cannot distinguish between participants with persistent and those with resolved infection (30), evidence for frequent age-related reactivation and increased viral load of

Table 3. Logistic Regression and ROC Curve Analyses of the Association Between Inflammatory and Senescence Parameters and Prevalent Pre-frailty

	Model 1		Model 2	
Parameter	OR (95% CI)	*p* value	OR (95% CI)	*p* value
IL-6				
Low	reference		reference	
Intermediate	3.29 (1.39–7.77)	**.007**	3.42 (1.41–8.26)	**.006**
High	5.08 (2.11–12.26)	**<.001**	4.64 (1.89–11.39)	**.001**
ROC curve analysis[a]	AUC 0.661 (0.579–0.743)	**<.001**		
CMV serostatus, positive vs negative	0.86 (0.40–1.85)	.705	0.90 (0.41–2.00)	.804
CD8–/CD8+ ratio	1.13 (0.93–1.38)	.215	1.14 (0.93–1.40)	.209
CD8+CD57+ (%)	1.01 (0.98–1.04)	.540	1.01 (0.98–1.05)	.405
CD8–CD57+ (%)	0.91 (0.81–1.02)	.102	0.90 (0.79–1.01)	.069
Sex, female vs male	0.48 (0.24–0.96)	**.038**	0.47 (0.23–0 .96)	**.038**
Age (y)	1.20 (1.06–1.36)	**.004**	1.21 (1.07–1.38)	**.003**

Notes: Unless otherwise specified, data are presented as odds ratio (OR) and 95% confidence interval (CI); CMV = cytomegalovirus; IL-6 = interleukin 6; low (< 1.4 pg/mL), intermediate (1.4–2.5 pg/mL), high (> 2.5 pg/mL); AUC = area under the curve; ROC = receiver operating characteristic. Model 1: Inflammatory and senescent parameters, as well as sex and age were included in the model; Model 2: Inflammatory and senescent parameters, as well as sex and age were included in the model with additional adjustment for heart failure and body mass index. The *p*-values that are statistically significant (i.e. <0.5) were formatted in bold in order to improve the readability of the table.

[a]Unadjusted AUC (95% CI) and *p*-values were calculated using ROC curve analysis.

CMV in individuals with positive CMV serology has been reported (31,32). More so, data indicating an age-related prevalence of CMV infection—15% vs 63%, for subjects <20 years and those >60 years, respectively—in CMV seropositive individuals has been reported (33). These findings support the hypothesis that CMV infection would be present in most, if not all, of our very old participants with positive CMV serology. In this light, our observation of no significant association between CMV serostatus and the key marker of inflammation IL-6 put in doubt the predominant role of CMV infection in sustaining chronic inflammation in very old persons.

The data portray that pre-frailty does not require the CMV infection as a necessary factor for its development in very old subjects. This finding supports the relatively few published works putting in doubt the predominant role of the CMV infection in frailty states (19,27). In a large population-based study on persons older than 85 years in England, no evidence was found to support the association of CMV seropositivity with pre-frailty or frailty (19). More strikingly, in another population-based study in the oldest old in Belgium, a negative association between positive CMV serology and frailty states was reported (27). These findings, regarding CMV-serostatus and frailty states in very old subjects, might reflect a survival effect as was proposed by Adriaensen and colleagues (34). From this perspective, individuals susceptible to the long-term deleterious effects of CMV exposure are more likely to die at a younger age (17) and thus be under-represented in a cohort of very old people like ours. Accordingly, Derhovanessian and colleagues (35) found that CMV-infected offspring from long-lived families had significantly lower levels of pro-inflammatory parameters than did their age-matched CMV-infected controls, hypothetically reflecting a better immunological control of the virus—with less contributing factors to frailty status—in the siblings of long-lived families (35). A better immunological control would imply less reactivation of the virus and perhaps up-regulation of the anti-inflammatory pathway (36). This reasoning might explain the lack of difference in IL-6 levels with respect to CMV serostatus and the absence of a relationship between IL-6 and pre-frailty among the CMV-seropositive subjects, in the perspective that the frailty status might depend on the balance between pro- and anti-inflammatory cytokines (37).

Pre-frailty was clearly associated with increased IL-6 independent of age, sex, BMI, history of heart failure, and CMV serostatus. This observation is consistent with the consensus that pro-inflammatory parameters, particularly IL-6, may inhibit the synthesis of IGF-1 and induce—through its catabolic effects on muscles—skeletal muscle mass loss (38,39). In this light, subjects may become less active, and express physical characteristics of frailty including low muscle strength, exhaustion, reduced physical activity, and unintentional weight loss. In agreement with the present study, many reports in both cross-sectional and longitudinal studies have consistently found elevated levels of various inflammatory markers among pre-frail as well as frail individuals (18,19,40). Considering the well-established burden of CLIP in the elderly, it is reasonable to think that CLIP would maintain and reinforce the frailty syndrome in older subjects. Notwithstanding, the absence of association between IL-6 and pre-frailty in CMV seropositive individuals deserves further investigation.

In our cohort of older adults, 15 (8.7%) subjects had a CD8–/CD8+ ratio <1, which was associated with CMV-seropositivity. Large increases in the proportion of memory and senescence-like phenotypes and decrease in naïve cell phenotypes were significantly associated with CMV seropositivity. Similar associations were found between T-cell subtypes and CD8–/CD8+ ratio <1, albeit in CMV negative subjects. Loss of CD28 marker and increase in the CD57 marker on T-cells of very old subjects with IRP was reported for Swedish OCTO and NONA cohorts (41). However, prudence should be exercised when drawing conclusions from the present results, since our CD8–/CD8+ parameter is a surrogate, which might differ from the originally used CD4+/CD8+ ratio.

Pre-frail subjects were more prone to have a history of heart failure compared to robust. This finding corroborates results from other authors indicating an increased risk of heart failure diagnosis in community-dwelling individuals with moderate and severe frailty (42,43). Although it is not clear how heart failure and pre-frailty may be linked, both phenomena are associated with inflammation (44). There is emerging evidence of NLRP3 activation in heart failure patients, with resulting inflammation (45). On the other hand, aged mice deficient in the NLRP3 inflammasome exhibit enhanced walk distance and running time as compared to their wild-type

controls, suggesting that NLRP3 may enhance inflammation and thereby lead to pre-frailty (46). Whether the NLRP3 inflammasome may represent a common pathway by which pre-frailty and heart failure interact requires future investigation.

Our data revealed that pre-frailty depends on sex and age. This finding corroborates reports from other studies (47,48), which portrayed a relation between pre-frailty and age. With regard to the association between sex and pre-frailty, equivocal results have been reported. Whereas several authors have reported a higher prevalence of pre-frailty in women than in men (5), others found no sex difference or even an increase in men compared to women (48,49). This sex dichotomy could be due to differences in age category of the participants in the various studies. In fact, García-González and colleagues (50) noticed that women showed significantly higher mean frailty index values than men in the age groups younger than 80 years and the reverse thereafter. Hence, our finding of higher prevalence of pre-frailty in men than women over 80 years is in accordance with the available literature (50) and calls for prudence when interpreting data across different age and sex categories.

Limitations

The findings of the present study should be interpreted within its limitations. First, this was a cross-sectional study, which precludes causal relationships. Therefore, caution should be exercised when making inferences about temporality. Second, since our intent was to focus on markers of IS with regards to pre-frailty, we did not investigate psycho-social factors, which are modifying factors for the development of pre-frailty. The authors also acknowledge the limitation that the selection of apparently healthy individuals might have masked other pre-frailty related patterns. More so, given the small sample size in some of the subsets of the population, it is possible that our study was not sufficiently powered to detect small differences between groups. Despite some limitations, this study adds a highly needed element in the context of frailty and associated characteristics. Our attempt to simultaneously investigate CMV, inflammatory, and IS markers in the same cohort offers insights into the impact of CMV on IS phenotypes and their relevance in the setting of pre-frailty, thus extending current knowledge on the frailty concept. Another strong point is that this study was performed in very old subjects with a distinctly different physiologic profile compared to the relatively younger adult participants in most literature reports. Our finding of no association between pre-frailty and CMV-seropositivity makes our study complementary to previous studies in younger populations. Moreover, the observation that subjects' CMV serostatus may define the association between IS phenotypes and pre-frailty could act as a guide for future research concerning IS phenotypes and their association with frailty status.

Conclusions

The findings of the current study indicate that the presence of pre-frailty is independent of CMV infection in very old subjects. Further, higher concentrations of the inflammatory cytokine IL-6, age and sex were independently predictive of pre-frailty. Whether IL-6 might facilitate the identification of people at risk of developing pre-frailty deserves further study.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Funding

This study was funded by a grant from the People's Committee of Hochiminh City, Vietnam (grant number 35-QĐ/BTCTU) to H.C.D. and an "Interdisciplinary Research Program" grant from the research council of the Vrije Universiteit Brussel (grant number IRP3).

Acknowledgments

The authors would like to thank all members of the Gerontopole Brussels Study group, comprising Ivan Bautmans, Dominque Verté, Ingo Beyer, Mirko Petrovic, Liesbeth De Donder, Tinie Kardol, Gina Rossi, Peter Clarys, Aldo Scafoglieri, Eric Cattrysse, Paul de Hert, and Bart Jansen for their inputs in the conception of the project.

Conflict of Interest

All authors certify that they comply with the ethical guidelines for publishing in the *Journal of Gerontology: Biological Sciences*. None of the authors have any conflict of interest with any entity with regard to this study. The authors have no other conflict of interest to declare.

References

1. Fried LP, Ferrucci L, Darer J, Williamson JD, Anderson G. Untangling the concepts of disability, frailty, and comorbidity: implications for improved targeting and care. *J Gerontol A Biol Sci Med Sci*. 2004;59:255–263. doi:10.1093/gerona/59.3.M255
2. Bandeen-Roche K, Xue QL, Ferrucci L, et al. Phenotype of frailty: characterization in the women's health and aging studies. *J Gerontol A Biol Sci Med Sci*. 2006;61:262–266. doi:10.1093/gerona/61.3.262
3. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. *J Gerontol A Biol Sci Med Sci*. 2007;62:722–727. doi:10.1093/gerona/62.7.722
4. Vermeiren S, Vella-Azzopardi R, Beckwée D, et al.; Gerontopole Brussels Study group. Frailty and the prediction of negative health outcomes: a meta-analysis. *J Am Med Dir Assoc*. 2016;17:1163.e1–1163.e17. doi:10.1016/j.jamda.2016.09.010.
5. Fried LP, Tangen CM, Walston J, et al.; Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56:M146–M156. doi:10.1093/gerona/56.3.M146
6. Kiely DK, Cupples LA, Lipsitz LA. Validation and comparison of two frailty indexes: the MOBILIZE Boston Study. *J Am Geriatr Soc*. 2009;57:1532–1539. doi:10.1111/j.1532-5415.2009.02394.x.
7. Gill TM, Gahbauer EA, Allore HG, Han L. Transitions between frailty states among community-living older persons. *Arch Intern Med*. 2006;166:418–423. doi:10.1001/archinte.166.4.418.
8. Trevisan C, Veronese N, Maggi S, et al. Factors influencing transitions between frailty states in elderly adults: the progetto veneto anziani longitudinal study. *J Am Geriatr Soc*. 2017;65:179–184. doi:10.1111/jgs.14515.
9. Faber MJ, Bosscher RJ, Chin A. Paw MJ, van Wieringen PC. Effects of exercise programs on falls and mobility in frail and pre-frail older adults: a multicenter randomized controlled trial. *Arch Phys Med Rehabil*. 2006;87:885–896. doi:10.1016/j.apmr.2006.04.005.
10. Schmaltz HN, Fried LP, Xue QL, Walston J, Leng SX, Semba RD. Chronic cytomegalovirus infection and inflammation are associated with prevalent frailty in community-dwelling older women. *J Am Geriatr Soc*. 2005;53:747–754. doi:10.1111/j.1532-5415.2005.53250.x.
11. Fougère B, Boulanger E, Nourhashémi F, Guyonnet S, Cesari M. Chronic inflammation: accelerator of biological aging. *J Gerontol A Biol Sci Med Sci*. 2017;72:1218–1225. doi:10.1093/gerona/glw240
12. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biomed Sci Med Sci*. 2014;69:S4–S9. doi:10.1093/gerona/glu057
13. Wikby A, Ferguson F, Forsey R, et al. An immune risk phenotype, cognitive impairment, and survival in very late life: impact of allostatic load in

Swedish octogenarian and nonagenarian humans. *J Gerontol A Biol Sci Med Sci*. 2005;60:556–565. doi:10.1093/gerona/60.5.556

14. Darvin K, Randolph A, Ovalles S, et al. Plasma protein biomarkers of the geriatric syndrome of frailty. *J Gerontol A Biol Sci Med Sci*. 2014;69:182–186. doi:10.1093/gerona/glt183.
15. Khan N, Shariff N, Cobbold M, et al. Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. *J Immunol*. 2002;169:1984–1992. doi:10.4049/jimmunol.169.4.1984
16. Kusunoki Y, Yamaoka M, Kubo Y, et al. T-cell immunosenescence and inflammatory response in atomic bomb survivors. *Radiat Res*. 2010;174:870–876. doi:10.1667/RR1847.1.
17. Wang GC, Kao WH, Murakami P, et al. Cytomegalovirus infection and the risk of mortality and frailty in older women: a prospective observational cohort study. *Am J Epidemiol*. 2010;171:1144–1152. doi:10.1093/aje/kwq062.
18. Lu Y, Tan CTY, Nyunt MSZ, et al. Inflammatory and immune markers associated with physical frailty syndrome: findings from Singapore longitudinal aging studies. *Oncotarget*. 2016;7:28783. doi:10.18632/oncotarget.8939
19. Collerton J, Martin-Ruiz C, Davies K, et al. Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: cross-sectional findings from the Newcastle 85+ Study. *Mech Ageing Dev*. 2012;133:456–466. doi:10.1016/j.mad.2012.05.005.
20. Onyema OO, Njemini R, Bautmans I, Renmans W, De Waele M, Mets T. Cellular aging and senescence characteristics of human T-lymphocytes. *Biogerontology*. 2012;13:169–181. doi:10.1007/s10522-011-9366-z.
21. Campbell JP, Guy K, Cosgrove C, Florida-James GD, Simpson RJ. Total lymphocyte CD8 expression is not a reliable marker of cytotoxic T-cell populations in human peripheral blood following an acute bout of high-intensity exercise. *Brain Behav Immun*. 2008;22:375–380. doi:10.1016/j.bbi.2007.09.001.
22. Orme JG, Reis J, Herz EJ. Factorial and discriminant validity of the Center for Epidemiological Studies Depression (CES-D) scale. *J Clin Psychol*. 1986;42:28–33. doi:10.1002/1097-4679(198601)42:1<28::AID-JCLP2270420104>3.0.CO;2-T
23. Sipers WM, Verdijk LB, Sipers SJ, Schols JM, van Loon LJ. The martin vigorimeter represents a reliable and more practical tool than the jamar dynamometer to assess handgrip strength in the geriatric patient. *J Am Med Dir Assoc*. 2016;17:466.e1–466.e7. doi:10.1016/j.jamda.2016.02.026.
24. Bautmans I, Onyema O, Van Puyvelde K, Pleck S, Mets T. Grip work estimation during sustained maximal contraction: validity and relationship with dependency and inflammation in elderly persons. *J Nutr Health Aging*. 2011;15:731–736. doi:10.1007/s12603-010-0317-1
25. Sirola J, Pitkala KH, Tilvis RS, Miettinen TA, Strandberg TE. Definition of frailty in older men according to questionnaire data (RAND-36/SF-36): the Helsinki Businessmen Study. *J Nutr Health Aging*. 2011;15:783–787. doi:10.1007/s12603-011-0131-4
26. Ferrucci L, Harris TB, Guralnik JM, et al. Serum IL-6 level and the development of disability in older persons. *J Am Geriatr Soc*. 1999;47:639–646. doi:10.1111/j.1532-5415.1999.tb01583.x
27. Matheï C, Vaes B, Wallemacq P, Degryse J. Associations between cytomegalovirus infection and functional impairment and frailty in the BELFRAIL Cohort. *J Am Geriatr Soc*. 2011;59:2201–2208. doi:10.1111/j.1532-5415.2011.03719.x.
28. Alanen A, Kahala K, Vahlberg T, Koskela P, Vainionpää R. Seroprevalence, incidence of prenatal infections and reliability of maternal history of varicella zoster virus, cytomegalovirus, herpes simplex virus and parvovirus B19 infection in South-Western Finland. *BJOG*. 2005;112:50–56. doi:10.1111/j.1471-0528.2004.00320.x.
29. de Ory F, Ramírez R, García Comas L, León P, Sagües MJ, Sanz JC. Is there a change in cytomegalovirus seroepidemiology in Spain? *Eur J Epidemiol*. 2004;19:85–89. doi:10.1023/B:EJEP.0000013253.56343.6f
30. Leng SX, Qu T, Semba RD, et al. Relationship between cytomegalovirus (CMV) IgG serology, detectable CMV DNA in peripheral monocytes, and CMV pp65(495-503)-specific CD8+ T cells in older adults. *Age (Dordr)*. 2011;33:607–614. doi:10.1007/s11357-011-9205-9.
31. Mehta SK, Stowe RP, Feiveson AH, Tyring SK, Pierson DL. Reactivation and shedding of cytomegalovirus in astronauts during spaceflight. *J Infect Dis*. 2000;182:1761–1764. doi:10.1086/317624.
32. Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R. Chronic herpesvirus reactivation occurs in aging. *Exp Gerontol*. 2007;42:563–570. doi:10.1016/j.exger.2007.01.005.
33. McVoy MA, Adler SP. Immunologic evidence for frequent age-related cytomegalovirus reactivation in seropositive immunocompetent individuals. *J Infect Dis*. 1989;160:1–10. doi:10.1093/infdis/160.1.1
34. Adriaensen W, Derhovanessian E, Vaes B, et al. CD4:8 ratio >5 is associated with a dominant naive T-cell phenotype and impaired physical functioning in CMV-seropositive very elderly people: results from the BELFRAIL study. *J Gerontol A Biol Sci Med Sci*. 2015;70:143–154. doi:10.1093/gerona/glu018.
35. Derhovanessian E, Maier AB, Beck R, et al. Hallmark features of immunosenescence are absent in familial longevity. *J Immunol*. 2010;185:4618–4624. doi:10.4049/jimmunol.1001629.
36. Ouyang Q, Wagner WM, Zheng W, Wikby A, Remarque EJ, Pawelec G. Dysfunctional CMV-specific CD8(+) T cells accumulate in the elderly. *Exp Gerontol*. 2004;39:607–613. doi:10.1016/j.exger.2003.11.016.
37. Minciullo PL, Catalano A, Mandraffino G, et al. Inflammaging and anti-inflammaging: the role of cytokines in extreme longevity. *Arch Immunol Ther Exp (Warsz)*. 2016;64:111–126. doi:10.1007/s00005-015-0377-3.
38. Barbieri M, Ferrucci L, Ragno E, et al. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. *Am J Physiol Endocrinol Metab*. 2003;284:E481–E487. doi:10.1152/ajpendo.00319.2002.
39. Doi T, Shimada H, Makizako H, et al. Insulin-like growth factor-1 related to disability among older adults. *J Gerontol A Biol Sci Med Sci*. 2016;71:797–802. doi:10.1093/gerona/glv167.
40. Soysal P, Stubbs B, Lucato P, et al. Inflammation and frailty in the elderly: a systematic review and meta-analysis. *Ageing Res Rev*. 2016;31:1–8. doi:10.1016/j.arr.2016.08.006.
41. Wikby A, Johansson B, Olsson J, Löfgren S, Nilsson BO, Ferguson F. Expansions of peripheral blood CD8 T-lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish NONA immune study. *Exp Gerontol*. 2002;37:445–453. doi:10.1016/S0531-5565(01)00212-1
42. Khan H, Kalogeropoulos AP, Georgiopoulou VV, et al. Frailty and risk for heart failure in older adults: the health, aging, and body composition study. *Am Heart J*. 2013;166:887–894. doi:10.1016/j.ahj.2013.07.032.
43. Nadruz W Jr, Kitzman D, Windham BG, et al. Cardiovascular dysfunction and frailty among older adults in the community: The ARIC Study. *J Gerontol A Biol Sci Med Sci*. 2017;72:958–964. doi:10.1093/gerona/glw199.
44. Fedarko NS. The biology of aging and frailty. *Clin Geriatr Med*. 2011;27:27–37. doi:10.1016/j.cger.2010.08.006.
45. Butts B, Gary RA, Dunbar SB, Butler J. The importance of NLRP3 inflammasome in heart failure. *J Card Fail*. 2015;21:586–593. doi:10.1016/j.cardfail.2015.04.014.
46. Youm YH, Grant RW, McCabe LR, et al. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab*. 2013;18:519–532. doi:10.1016/j.cmet.2013.09.010.
47. Saum KU, Dieffenbach AK, Müller H, Holleczek B, Hauer K, Brenner H. Frailty prevalence and 10-year survival in community-dwelling older adults: results from the ESTHER cohort study. *Eur J Epidemiol*. 2014;29:171–179. doi:10.1007/s10654-014-9891-6.
48. Kim HJ, Park S, Park SH, et al. The significance of frailty in the relationship between socioeconomic status and health-related quality of life in the Korean community-dwelling elderly population: mediation analysis with bootstrapping. *Qual Life Res*. 2017;26:3323–3330. doi:10.1007/s11136-017-1672-8.
49. Liu LK, Lee WJ, Chen LY, et al. Association between frailty, osteoporosis, falls and hip fractures among community-dwelling people aged 50 years and older in Taiwan: results from i-lan longitudinal aging study. *PLoS One*. 2015;10:e0136968. doi:10.1371/journal.pone.0136968.
50. García-González JJ, García-Peña C, Franco-Marina F, Gutiérrez-Robledo LM. A frailty index to predict the mortality risk in a population of senior Mexican adults. *BMC Geriatr*. 2009;9:47. doi:10.1186/1471-2318-9-47.